

HTA Diagnostic Moléculaire en Belgique

KCE reports vol. 20B

Le Centre Fédéral d'Expertise des Soins de Santé

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Avant-propos

Le développement de la Polymerase Chain Reaction (PCR) a entraîné une évolution accélérée dans le domaine du diagnostic moléculaire. Il arrive souvent que la technique moléculaire soit plus sensible que la méthode de référence existante, comme la culture microbiologique. La haute sensibilité de la technique présente également des désavantages, à savoir que la technique laisse la porte largement ouverte à la pollution. Des précautions spécifiques et une infrastructure de laboratoire adaptée sont nécessaires. L'automatisation totale de l'exécution des tests est proche, mais n'est certainement pas encore une réalité à l'heure actuelle. Entre-temps, le coût des tests tend à diminuer. Afin d'encadrer l'introduction des applications de cette nouvelle technologie, les autorités ont créé en 1998 les Centres de Diagnostic Moléculaire (CDM). La mission des CDM, telle qu'elle était décrite dans l'AR du 24 septembre 1998, était très prometteuse et ambitieuse. Outre l'exécution du test, le programme des tâches comprenait également une mission éducative, l'exécution de contrôles de qualité internes et externes et une évaluation continue de la valeur diagnostique des tests moléculaires. Ce rapport évalue dans quelle mesure les 18 centres ont réalisé les objectifs de l'expérience CDM. Une décision de justice a, au début de l'année 2005, mis fin brutalement à la convention.

Pour les tests moléculaires et des tests diagnostiques en général, on distingue l'efficacité analytique et diagnostique, l'impact sur l'établissement du diagnostic ou le traitement du patient, l'effet sur l'évolution du patient et les aspects de coûts et d'efficacité. Cependant, de nombreuses applications du diagnostic moléculaire se limitent encore à l'étude des caractéristiques analytiques ou diagnostiques. De plus en plus de tests moléculaires font appel à une variété de méthodes « maison » qui reposent sur une validation minimale de la technique utilisée. Ceci ne favorise pas une validation clinique robuste. Sans validation, le test ne peut être considéré comme fiable dans la pratique clinique puisqu'il expose le patient à des résultats faussement positifs ou faussement négatifs avec de sérieuses conséquences possibles.

Cette convention fut, d'un point de vue financier, particulièrement intéressante puisqu'un nombre relativement restreint de centres a reçu une enveloppe budgétaire fermée. Dans cette étude, les données comptables des CDM ont été passées en revue puis analysées. Elles constituent la base de calcul du coût par test.

La question suivante reste en suspens: comment l'Assurance Maladie doit-elle à l'avenir approcher les technologies innovantes particulièrement prometteuses lorsque les évidences sont encore embryonnaires ou inexistantes ? Une attitude attentiste peut priver les patients de progrès médicaux. Une introduction précipitée peut exposer le patient à des risques importants et obérer le budget de l'Assurance Maladie. De nouveaux mécanismes de financement qui permettraient une introduction encadrée et échelonnée des technologies émergentes constituent une alternative idéale. Nous proposons un modèle d'évaluation des nouveaux tests moléculaires. Il y a beaucoup à apprendre de l'histoire des CDM, en négatif comme en positif.

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Résumé du rapport Diagnostic Moléculaire

I. Introduction

Au cours de ces 10 dernières années, le diagnostic moléculaire a connu une forte expansion tant en ce qui concerne le nombre de tests que son utilisation. Comme pour tout technologie diagnostique « émergente », l'introduction du diagnostic moléculaire dans les soins de santé se doit d'être sûre et coût-efficace. On peut distinguer trois niveaux à atteindre : développer des tests moléculaires qui soient fiables et exacts (niveau scientifique), utiles (niveau clinique) et enfin abordables (niveau financier).

L'INAMI a, lors de l'introduction du diagnostic moléculaire dans les soins de santé en 1998, opté pour la reconnaissance d'un certain nombre de Centres de Diagnostic Moléculaire (CDM). Par là les autorités ont eu à la fois un objectif scientifique (la garantie de la qualité des tests), un objectif clinique (l'exécution de tests moléculaires pour supporter l'activité clinique) et enfin un objectif financier (la limitation du nombre de centres et un budget total fermé pour l'assurance maladie).

Ce projet du Centre Fédéral d'Expertise s'attache à évaluer ces objectifs initiaux. Le rapport propose également une solution scientifiquement étayée pour la poursuite de l'introduction des tests de diagnostic moléculaire. Enfin, un modèle d'évaluation généralisable est développé et appliqué à quelques tests moléculaires existants.

Différents experts en recherche diagnostique et autres domaines spécifiques ont suivi pas à pas les différentes étapes de ce projet. Ce rapport a de surcroît fait l'objet d'une validation externe par un groupe d'autres experts. Le département de biologie clinique de l'Institut Scientifique de Santé Publique a prêté son concours pour l'évaluation de la qualité des tests.

Ne font pas l'objet de ce rapport les tests moléculaires effectués au sein des laboratoires de référence pour le SIDA, les tests sur les dérivés sanguins, le typage tissulaire, les tests à des fins épidémiologiques, industrielles ou de médecine légale. De même, le vaste domaine des tests pour les affections génétiques tombe en grande partie en dehors du champ d'investigation de ce projet. La pertinence du dépistage de l'Human Papilloma Virus (HPV) fait l'objet d'un projet distinct et n'est pas davantage étudiée.

I.1 Qu'est ce qu'un test moléculaire ?

Les tests moléculaires se basent sur l'analyse de l'ADN ou de l'ARN. La mise au point de la Polymerase Chain Reaction (PCR) en 1983 a permis une évolution accélérée du diagnostic et de la biologie moléculaire. Les tests basés sur l'amplification de l'ADN (ou de l'ARN via la RT-PCR) sont extrêmement sensibles. Ceci a aussi des désavantages, à savoir que la technique est extrêmement sensible à la pollution. Des précautions spécifiques et une infrastructure de laboratoire adaptée sont nécessaires. De plus en plus de trousse de diagnostic moléculaire DIV sont disponibles dans le commerce bien qu'un large éventail de méthodes "maison" soit encore utilisé. La robotisation de l'extraction de l'ADN et de l'ARN et le développement de techniques PCR en temps réel permettent aujourd'hui une véritable automatisation du diagnostic moléculaire.

A côté des techniques d'amplification de l'ADN par lesquelles un segment de l'ADN est copié de manière exponentielle, il existe également des techniques basées sur le simple couplage de l'ADN cible à une sonde ADN complémentaire fluorescente (FISH pour fluorescent in situ hybridisation). Cette dernière technique est toutefois moins sensible.

I.1.1 Applications en microbiologie

Dans de nombreux cas, la PCR est plus sensible que l'isolation du virus ou la culture bactérienne. La prudence et une gestion permanente de la qualité restent nécessaires lors

de l'introduction (précipitée) de nouvelles techniques très sensibles. C'est ainsi qu'une fausse épidémie de coqueluche dans l'Etat de New York a trouvé son explication dans un problème de résultats PCR faussement positifs. En microbiologie, la technique PCR s'est surtout révélée utile pour la sélection et le suivi (PCR quantitative) du traitement antiviral en cas d'affections virales chroniques comme par exemple le HIV, ainsi que les hépatites B et C chroniques. Pour la surveillance du Cytomégalo virus (CMV) en cas de suppression immunitaire, une PCR quantitative en temps réel pourrait représenter une alternative possible au test de l'antigène pp65. La sensibilité analytique élevée de la réaction PCR nécessite cependant de définir une valeur seuil cliniquement pertinente. Cette étude est encore en cours.

Pour l'identification d'infections bactériennes (et la résistance aux antibiotiques), la technique PCR n'est une alternative judicieuse que si elle fournit un résultat plus rapide (et aussi précis) que les méthodes meilleur marché basées sur la culture. Ici aussi, il sera peut-être nécessaire de définir une valeur seuil PCR, par exemple en cas de cathéter infecté. Les dispositifs automatiques récents permettent désormais d'identifier la colonisation par le staphylococcus *aureus* résistant à la methicilline (MRSA) en quelques heures et non plus en jours, ce qui peut entraîner une amélioration du contrôle de l'infection dans l'hôpital.

L'avancée majeure du diagnostic moléculaire en microbiologie réside donc dans la sensibilité supérieure et dans un résultat plus rapide qu'avec les techniques existantes. Toutefois, ces avancées ne se sont pas encore concrétisées dans de nombreux cas.

I.1.2 Applications en hémato-oncologie et en oncologie

Les anomalies chromosomiques comme les translocations sont normalement explorées par les techniques cytogénétiques (caryotypage). Des techniques cytogénétiques plus spécifiques (FISH) ainsi que la PCR (et la RT-PCR) complètent ces premiers outils de diagnostic. L'interprétation de ces tests demeure complexe.

Au moment de poser le diagnostic en hémato-oncologie, il faut parfois recourir à des tests PCR qui mettront en évidence un réarrangement du récepteur de la cellule T ou des gènes d'immunoglobuline pour établir le caractère monoclonal d'une prolifération cellulaire. Des marqueurs moléculaires particuliers permettent de cibler le traitement de certaines tumeurs. C'est le cas de l'imatinib (GLIVEC) pour la leucémie myéloïde chronique et du trastuzumab (HERCEPTIN) pour le traitement du cancer mammaire métastaté positif à HER2. Dans le même registre, on peut s'attendre à l'introduction de davantage de traitements ciblés grâce aux résultats de tests de diagnostic moléculaire parfois complexes.

Lors de la surveillance du traitement, par exemple en cas de leucémie, il est important de pouvoir détecter de faibles quantités de cellules tumorales. Des techniques sensibles de PCR / RT-PCR s'indiquent ici.

Messages clés

- L'avancée majeure du diagnostic moléculaire en microbiologie réside dans la sensibilité supérieure et dans un résultat plus rapide qu'avec les techniques existantes. Ces avancées ne se sont toutefois pas encore concrétisées dans de nombreux cas.
- Les forces et faiblesses de techniques concurrentes en hémato-oncologie (caryotypage, FISH et PCR) doivent être répertoriées afin de les implémenter de la manière la plus efficiente.

I.2 Historique des Centres de Diagnostic Moléculaire en Belgique

Pendant longtemps, la mesure de l'ADN et de l'ARN à des fins cliniques de routine s'est limitée aux Centres de Génétique Humaine (CGH). Le nombre d'applications cliniques basées sur l'analyse de l'acide nucléique a fortement augmenté après la découverte de la polymérase chain reaction (PCR), ce qui a notamment débouché sur la création d'un certain nombre de CDM (AR du 24 septembre 1998). De cette façon, une grande partie du diagnostic moléculaire est aujourd'hui financée par l'INAMI. L'objectif de la structure CDM était de lier l'expertise présente dans les laboratoires de microbiologie, d'héματο-oncologie et de pathologie. En outre, chaque CDM devait s'associer à un CGH. Outre le Comité National, des groupes de travail distincts ont été constitués pour la microbiologie, l'héματο-oncologie et la pathologie. La liste la plus récente de tests, dressée par les groupes de travail CDM, comporte 94 tests (tableau 2).

Selon l'AR du 24 septembre 1998, les CDM devaient :

- informer les hôpitaux sur les tests moléculaires proposés et leurs indications (avec adaptation annuelle de la liste des tests proposés),
- exécuter les tests moléculaires,
- orienter la formation des biologistes et des pathologistes cliniques concernant le diagnostic moléculaire,
- procéder à une évaluation permanente des techniques moléculaires, y compris des trousse de diagnostic in vitro (DIV).

En outre, le Comité National avait pour tâche :

- de mettre en œuvre le contrôle de qualité interne et externe et de l'optimiser, en ce compris la participation à des programmes de contrôle de qualité externes internationaux,
- d'organiser le contrôle de qualité des tests moléculaires dans la nomenclature ou qui ont été repris dans la nomenclature,
- de proposer l'introduction de certains tests moléculaires dans la nomenclature,
- d'élaborer des recommandations pour les indications des tests et leur interprétation dans le cadre d'une évaluation de la valeur diagnostique de ceux-ci.

Les CDM candidats devaient prouver leur spécialisation en diagnostic moléculaire et disposer de l'infrastructure nécessaire pour éviter la pollution des échantillons. Etant donné le caractère expérimental de la structure des CDM, des contrats de financement furent passés pour une période de deux ans. Ces contrats furent renouvelés par la suite. Au départ, en 1999, 10 CDM étaient impliqués. Sur la base du statut juridique, leur nombre progressa jusqu'à un total de 18 CDM au cours des premières années, ce nombre restant égal à partir du 3 juillet 2000. Tous les CDM ne sont pas liés à un hôpital universitaire. Le budget total des CDM s'élève à 6,53 millions d'euros par an. Ce budget annuel fixe a été distribué entre les CDM sur la base du coût du personnel, des réactifs et des investissements en fonction des factures introduites auprès de l'INAMI.

Cette convention entre l'INAMI et les CDM a été renouvelée jusqu'au 31 janvier 2006. Le 27 janvier 2005, le Conseil d'Etat rejetait la base légale relative aux CDM et donc aussi leur financement. Ces dernières années, 5 tests moléculaires ont été repris à l'article 24 de la nomenclature INAMI et peuvent être réalisés par tout laboratoire de biologie clinique. Il s'agit de la détection de *N. gonorrhoeae*, de *C. trachomatis*, du virus de l'hépatite C (HCV qualitatif), de *M. tuberculosis* et de *M. avium* intracellulaire (AR des 29 avril 1999 et 16 juillet 2001). Les volumes les plus importants en 2003 concernaient *C. trachomatis* et *N. gonorrhoeae* avec, respectivement, 30 000 et 16 000 tests, et ont été exécutés dans respectivement 30 et 19 laboratoires (tableau 1). Il convient de remarquer que la plupart de ces tests ont été exécutés dans des laboratoires non CDM. Outre le

financement via la nomenclature, les tests HCV qualitatifs et *M. tuberculosis* ont parfois aussi été inclus dans l'activité CDM (tableau 10).

Un certain nombre de CGH exécutent, en sus des tests cytogénétiques, un large éventail de tests moléculaires en hémato-oncologie et anatomo-pathologie. Les tests exécutés au sein des CGH sont remboursés via une nomenclature générique (INAMI article 33) qui a été établie pour des maladies héréditaires et non pas pour des maladies acquises (AR du 22 juillet 1988). Cette nomenclature ne distingue pas les tests simples des tests plus complexes basés sur l'hybridation de l'ADN, et accorde un remboursement de $\pm$ 300 euros par test. Contrairement au financement fermé des laboratoires de biologie clinique, les tests repris à l'article 33 sont totalement financés à l'acte quelque soit leur volume. Le coût total du remboursement des tests s'est élevé à 30,8 millions d'euros en 2003. Le montant pour les tests basés sur l'hybridation de l'ADN s'est élevé à 15,7 millions d'euros en 2003 et à 8,5 millions pour la première moitié de 2004. Ni les codes de nomenclature ni les rapports d'activité existants des CGH ne permettent d'estimer le volume et les coûts des tests individuels.

2. Etude de l'expérience CDM

2.1 Méthodes utilisées et sources d'information

Les volumes rapportés pour les tests CDM ont été tirés du rapport d'activité des CDM (annexe 1). Outre les informations disponibles via les rapports de contrôle CDM (site web : <http://webhost.ua.ac.be/cmd/index.html>), chaque CDM a également complété un questionnaire succinct par méthode de test utilisée (annexe 3). Les caractéristiques analytiques et diagnostiques des tests CDM ont été documentées à l'aide d'un questionnaire rempli par des experts au sein des groupes de travail CDM (annexe 4), et parfois complétées par une recherche bibliographique très limitée. Les factures introduites par les CDM ont permis une approche du coût par test. Le besoin clinique de tests moléculaires et l'évaluation du service rendu par les CDM au médecin demandeur ont été documentés pour 6 hôpitaux non CDM.

2.2 Résultats du questionnaire des Centres de Diagnostic Moléculaire

2.2.1 Les méthodes moléculaires utilisées

La réaction des CDM au questionnaire diffusé sur les méthodes moléculaires utilisées a été excellente. Les centres ont rempli un questionnaire méthodologique individuel pour chacun des tests réalisés. Pas moins de 594 questionnaires complétés sont revenus.

Même s'il existe sur le marché des trousse de diagnostic DIV CE pour la quasi-totalité des tests étudiés, les méthodes "maison" représentent encore 79% des méthodes rapportées. La méthode "maison" est dite validée dans 33% des cas seulement.

Une procédure pour l'exécution du test existe seulement pour 60% des méthodes non validées. Les raisons invoquées pour l'utilisation de tests "maison" en lieu et place des trousse de diagnostic DIV disponibles sont une performance insuffisante des trousse diagnostiques existantes et leur coût élevé. Le coût des tests "maison" ne prend toutefois pas en compte les coûts de validation ni de licence éventuelle.

La grande diversité des méthodes "maison" pour un seul et même test est également frappante. Les initiatives communautaires "Actions Concertées BIOMED" et les programmes "Europe Against Cancer" ont débouché sur des recommandations concrètes concernant la normalisation des méthodes PCR en hématologie-oncologie. Toutefois, ici aussi les CDM arguent du coût pour justifier l'adhérence partielle à ces recommandations.

Cette absence de normalisation des méthodes et l'absence d'études comparatives rendent difficile la comparaison de la valeur diagnostique entre les centres. Il n'existe pas de rapports d'évaluation CDM concernant la valeur diagnostique des tests qui figurent dans la liste des tests CDM ou qui ont été retenus dans les propositions de nomenclature.

Le temps moyen rapporté entre la demande et la communication du résultat du test variait largement selon le test, allant de 1,8 jour pour *B. pertussis* à plus de 7 jours pour HPV et HCV quantitatifs. La valeur médiane était de 3 jours. Dans 80% des cas, le laboratoire se charge également de l'interprétation du test. L'échange d'informations entre le demandeur et le centre dépendait beaucoup plus du CDM que du test.

2.2.2 Les caractéristiques des tests utilisés

Pour chacun des 94 tests CDM, un questionnaire a été complété par un expert CDM avec référence à la bibliographie, dans la quasi-totalité des cas. Pour la plupart des tests, on ne dispose pas d'évaluations de la reproductibilité du test entre plusieurs centres (en dehors des circuits de qualité externes) sauf pour les tests réalisés au moyen de trousse de diagnostic approuvées par la FDA.

En ce qui concerne les volumes de tests microbiologiques (tableau 4a), les nombres sont probablement représentatifs pour le pays (sauf *M. tuberculosis* et HCV quantitatif, parfois facturés via la nomenclature, et HPV, parfois exécuté dans des laboratoires non CDM sans

intervention de l'INAMI). En ce qui concerne l'hémo-oncologie et l'oncologie (tableau 4b), le nombre de tests rapporté via la structure CDM représente seulement une fraction du volume total des tests, puisqu'une partie non négligeable de ces tests a lieu dans les CGH.

Tests moléculaires en microbiologie

La précision diagnostique d'un test se définit typiquement par rapport à un test de référence, souvent qualifié aussi d'étalon or. Par test de référence il faut entendre ici le test simple ou composé (la clinique, les tests de labo et l'imagerie) qui identifie l'affection de la manière la plus fiable actuellement.

Parfois, le test de référence ne se positive que bien plus tard, comme la pathologie du col de l'utérus consécutive à l'HPV ou l'examen post mortem décisif pour la détection d'*Aspergillus*. Il convient de remarquer que pour un même microorganisme, le test de référence peut différer d'après l'indication du test. Le test de référence pour la détection prénatale d'infection à CMV et de toxoplasmose repose sur la mise en évidence du virus ou de la réponse immunitaire après la naissance de l'enfant. Pour l'encéphalite à Herpes Simplex Virus (HSV), on mentionne la PCR comme test de référence alors que pour les autres pathologies à HSV la culture du virus est la référence. Le type d'échantillon peut également déterminer la précision diagnostique, par exemple la détermination d'*Aspergillus* sur expectoration donne plus de faux positifs qu'une détermination sur le sang. Pour le virus de l'herpes humain du type 8 décrit plus récemment, la détection moléculaire a été la norme dès le départ. Pour un certain nombre d'autres tests (HCV, HBV, EBV et encéphalite HSV), le test moléculaire constitue le test de référence indiqué. Pour les tests de génotypage des virus, bactéries ou champignons, le test de référence est une détermination de la séquence des acides nucléiques.

Pour la majorité des tests microbiologiques, le test de référence est basé sur la culture virale ou bactérienne. Ce test de référence est toutefois souvent peu sensible ou difficile à exécuter. Dans la quasi-totalité des cas, la PCR (ou un autre test d'amplification) est plus sensible.

L'absence d'un test de référence fiable entraîne des estimations inexactes de la sensibilité et de la spécificité. C'est ainsi que la clinique, la sérologie et d'autres tests diagnostiques ne permettent pas toujours de classer des échantillons qui sont négatifs à la culture et positifs sur base de la PCR. La proportion des tests PCR faussement positifs n'est donc pas toujours connue de manière évidente et est donc difficile à distinguer de la contamination.

Les résultats des études rapportées ne sont pas toujours extrapolables aux résultats de la méthode PCR "maison". On note souvent l'absence des études nécessaires montrant une équivalence. Dans ces cas, les références invoquées ne peuvent donc pas conforter la méthode utilisée localement.

Pour certains microorganismes, la détection par PCR en temps réel est tellement sensible qu'il est nécessaire d'avoir une valeur seuil qui reste à déterminer, valeur sous laquelle la détection n'est pas considérée comme cliniquement pertinente dans l'indication étudiée. C'est ainsi qu'il est nécessaire d'avoir une valeur seuil pour le CMV lors du suivi de l'immunosuppression ou pour l'HBV lors de l'évaluation du traitement antiviral. Le même problème se pose pour le parvovirus dans le sérum, le polyomavirus dans l'urine et le pneumocyste dans les échantillons respiratoires.

La proportion de tests de microbiologie moléculaire qui ne fournissent pas de résultats interprétables reste toujours inférieure à 10%. Pour la moitié des tests étudiés, ces résultats s'accompagnent de coûts de santé supplémentaires. La proportion de faux positifs pour les tests de microbiologie moléculaire pour lesquels la valeur seuil est indiscutable est toujours rapportée comme <10%. Selon les experts CDM, pour deux tiers des tests étudiés, de tels faux positifs entraînent un impact négatif sur la santé du patient ainsi qu'une augmentation des coûts médicaux. La proportion de faux négatifs peut parfois être plus élevée. Pour la plupart des tests, ces faux négatifs entraînent des coûts médicaux supplémentaires.

Pour la plupart des tests moléculaires en microbiologie, on s'attend dans les 5 prochaines années à une évolution continue vers l'automatisation (de l'extraction, de la PCR en temps réel et de l'utilisation des trousses de diagnostic). De même, une augmentation du volume ou des indications est rapportée pour *B. pertussis*, *L. pneumophila*, *M. tuberculosis*, MRSA, VZV et *T. gondii*, de même qu'une augmentation temporaire du nombre de tests HCV.

Tests moléculaires en héματο-oncologie

La contribution des tests moléculaires au diagnostic est ici souvent complexe. Les experts signalent que l'hématologue moléculaire est le mieux placé pour sélectionner le test moléculaire adapté sur la base des observations cliniques et diagnostiques.

En règle générale, on dispose de très peu de données sur la précision diagnostique des tests RT-PCR en héματο-oncologie. Les tests diagnostiques de référence reposent soit sur les critères OMS soit sur la mise en évidence d'une translocation par la cytogénétique. Certaines translocations sont déjà reprises dans la définition OMS, comme t(9 ; 22) pour la leucémie myéloïde chronique (LMC). Cependant la sensibilité diagnostique de la plupart des translocations dans le cas de leucémie ou de lymphome est inférieure à 50%.

La détection des translocations est surtout utile en tant que variable pronostique ou comme marqueur de maladie résiduelle minimale (MRD) lors du suivi du traitement. Les techniques cytogénétiques comprennent le caryotypage et le FISH. Pour la leucémie lymphoïde chronique à cellules B (B-CLL), le caryotypage n'est toutefois possible que dans 50% des cas car aucune métaphase ne peut être induite. Le FISH est alors opportun sur cellules en interphase. Toutefois la PCR est mentionnée comme un test nettement meilleur marché que les tests cytogénétiques. Pour les translocations t(1;14), t(1;19), t(12;21), et les translocations MLL t(11;18), il n'existe pas, selon les experts, d'études comparatives entre RT-PCR et cytogénétique et donc pas de données concernant la précision diagnostique. La PCR pour t(11;14) et t(14;18) est donc comparée à la cytogénétique, mais présente une sensibilité diagnostique faible en raison de la dissémination des points de rupture chromosomiques. Pour t(11 ; 14), la RT-PCR pour l'expression de cycline-D représente donc une alternative. Dans le lymphome de Burkitt, cette dispersion des points de rupture dans 8q24 est même tellement étendue que le développement d'une PCR n'est pas réalisable. La mise en évidence par immunohistochimie de la protéine ALK est fréquemment utilisée comme test pour la mise en évidence de la translocation t(2 ; 5).

On considère souvent la RT-PCR comme la norme dans le suivi et la surveillance de maladie résiduelle minimale et on ne mentionne donc aucune donnée concernant la précision diagnostique. Le FISH est parfois utilisé, bien que pour le suivi thérapeutique et la surveillance de MRD, sa sensibilité analytique soit inférieure à la RT-PCR. Ici aussi, on manque d'études comparatives entre FISH et RT-PCR pour t(8 ; 21) et t(15 ; 17). Il est important de souligner que le changement de laboratoire ou de technique lors du dosage de BCR-ABL est à éviter dans le suivi de MRD. La prudence est également de mise lors de l'utilisation de tests PCR sensibles pour t(14 ; 18), ou de tests RT-PCR pour les transcriptions de t(2 ; 5), t(8 ; 21) et t(15 ; 17) étant donné que ces anomalies peuvent également être observées chez des sujets sains.

La PCR suivie par des enzymes de restriction ou l'analyse séquentielle est la norme pour la mutation FLT3 TKD et la PCR avec séquençage pour FLT3 ITS/LM. La PCR quantitative en temps réel est la méthode de référence pour PRVI, un nouveau marqueur diagnostique à l'étude dans la Polycytémie vraie.

La principale cause de résultats non interprétables est la qualité médiocre des échantillons et ceci, tant pour la PCR, que la RT-PCR et le FISH. La proportion de ces tests est le plus souvent estimée de 5 à 10%. Les tests ARN sont plus sensibles que les tests ADN. Les conséquences sont surtout d'ordre financier lorsqu'un deuxième échantillon doit être prélevé. Des résultats faussement positifs et faussement négatifs sont, selon les experts, généralement associés à un impact négatif sur la santé du patient et à un surcoût médical.

En héματο-oncologie, les 5 années à venir apporteront peu de changements dans la technologie des tests ou leurs volumes. L'introduction de tests à puces pour la détection de translocations et une utilisation croissante d'amorces propres au patient pour le suivi de MRD sont des innovations attendues.

Messages clés

- Les méthodes “maison” représentent encore 79% des méthodes de tests rapportées.
- D’après l’enquête, seules 33% des méthodes “maison” ont été validées.
- Un élément frappant est la grande diversité des méthodes “maison” pour un seul et même test sans qu’une équivalence clinique pertinente soit formellement documentée entre les méthodes utilisées.
- La proportion de tests PCR faussement positifs n’est pas toujours connue avec exactitude et est donc difficile à distinguer de la contamination.
- La sensibilité de la PCR en temps réel est telle qu’une valeur seuil de pertinence clinique n’a pas encore été déterminée pour certains microorganismes.
- La précision diagnostique des tests RT-PCR en hémato-oncologie est insuffisamment documentée. Ces tests paraissent cependant importants pour le suivi thérapeutique des maladies résiduelles minimales.
- La base scientifique (l’évidence clinique) est relativement peu développée au sein des CDM. Ceci constitue un obstacle important dans la prise de décisions scientifiquement fondées.

2.3 Aspects en rapport avec la qualité

2.3.1 Contrôle de qualité lors de tests moléculaires

Le Comité National des CDM avait également comme tâche la mise en place des contrôles de qualité tant internes qu’externes pour les tests CDM et les tests moléculaires déjà repris à la nomenclature. Cette dernière tâche est en contradiction avec le rôle légal de l’ISP. L’Institut Scientifique de Santé Publique (ISP) n’a pas organisé de contrôle de qualité externe spécifique pour les 5 tests moléculaires repris dans la nomenclature. Les laboratoires avaient toutefois l’opportunité d’utiliser une technique moléculaire lors des contrôles de qualité existants pour *M. tuberculosis* et HCV qualitatif. Actuellement, il n’existe pas de réglementation spécifique pour la gestion de la qualité au sein des CDM pas plus que pour les CGH. Néanmoins, il existe de très nombreuses directives internationales pour les aspects de qualité dans le diagnostic moléculaire (section 6.3).

Les laboratoires ont tendance à s’accréditer selon les normes ISO 15189 pour les tests DIV et donc aussi pour le diagnostic moléculaire. Cette norme récente est plus spécifique pour les laboratoires cliniques que l’ancienne norme ISO 17025. Trois des 18 CDM sont déjà accrédités pour la majorité des tests moléculaires. Les contrôles de qualité CDM exécutés actuellement sont susceptibles d’amélioration à divers égards et ne constituent pas vraiment un contrôle de qualité externe. Les principes à la base du contrôle de qualité externe mis en œuvre par certains CDM étaient parfois excellents mais parfois aussi minimalistes. Ceci n’a bien sûr pas empêché certains CDM de satisfaire aux exigences de qualité les plus élevées. Les pouvoirs publics n’ont toutefois pas reçu de garanties de qualité concluantes ni documentées pour les 18 CDM.

Un aperçu des programmes de qualité internationaux existants pour le diagnostic moléculaire a été annexé (annexe 6). Si l’on se fonde sur les questionnaires méthodologiques et les coûts intégrés (tableau 12), la participation des CDM à des programmes de contrôle de qualité internationaux fut de toute façon très limitée.

2.3.2 Satisfaction des clients des CDM

Au total, 6 hôpitaux ont été visités et des questions furent posées à des prescripteurs de nombreux tests de diagnostic moléculaire (interniste infectiologue, gastro-entérologue, neurologue, pédiatre, hémato-oncologue) et auprès des chefs de service de laboratoire locaux.

Certains cliniciens et les médecins des laboratoires locaux sont encore insuffisamment informés de l'éventail de tests CDM/CGH existants et ne sont pas au courant des méthodes utilisées, de la fiabilité et des règles d'interprétation. Des informations sont surtout obtenues par des contacts informels à l'occasion de séminaires, mais une information mise à jour ne peut pas toujours être trouvée sur le site internet des CDM (par exemple quel CDM/CGH dispose d'une large expérience pour quel test). Un grand intérêt semble exister pour toutes les formations et tous les séminaires en diagnostic moléculaire, mais l'offre émanant des CDM/CGH est ressentie comme plutôt limitée, y compris en ce qui concerne les lieux de formation. L'offre de service ne répond pas toujours non plus aux attentes, par exemple le délai entre le prélèvement et le protocole de réponse est parfois trop long pour la détection d'HSV en cas d'encéphalite. Certains cliniciens et médecins de laboratoire locaux voudraient donc être impliqués plus étroitement dans l'élaboration de l'offre en tests existants au sein des CDM/CGH. Toutefois, les CDM encadrent les laboratoires locaux lors du démarrage du diagnostic moléculaire et diffusent donc aussi leurs méthodes "maison". Les laboratoires locaux ne sont souvent pas informés de la possibilité de prendre part aux contrôles de qualité CDM. Ces laboratoires ressentent parfois les CDM/CGH comme non transparents et même concurrents vis-à-vis d'eux. Il existe donc théoriquement un risque de différence d'accessibilité pour des tests coûteux entre les hôpitaux.

Le choix du CDM s'effectue encore souvent par l'entremise du médecin demandeur, ce qui entraîne des envois à plusieurs CDM par hôpital. L'implication, le rôle de coordination et l'influence du laboratoire local diffèrent d'un hôpital à l'autre. Les tests CDM/CGH ne figurent pas sur les formulaires de demande locaux. Parfois, des formulaires de demande spécifiques sont utilisés. En hémato-oncologie, c'est le clinicien qui dans de nombreux cas se charge de l'envoi d'échantillons, parfois simultanément vers les CDM et les CGH, ce qui peut entraîner une redondance des tests. Pour une matière aussi complexe que le diagnostic en hémato-oncologie, il est préférable qu'un seul coordinateur au sein du laboratoire assure l'échelonnement des tests faits dans la maison et des tests réalisés à l'extérieur, la répartition des envois et l'assemblage des résultats. Ceci est déjà le cas avec succès dans quelques hôpitaux. Pour obtenir l'agrégation d'un programme de soins oncologiques, un hôpital devrait inclure les procédures de diagnostic moléculaire dans le manuel d'oncologie. Généralement, il faut habituellement rappeler le CDM et le CGH pour connaître les résultats du test, puisque le protocole écrit se fait souvent longtemps attendre. En outre, les cliniciens espèrent une présentation normalisée des protocoles, par exemple le dosage quantitatif de BCR-ABL.

Les tests CDM qui sont demandés le plus souvent (et moins souvent) :

- HCV qualitatif et quantitatif, génotypage HCV, HBV, HSV, Entérovirus, VZV, CMV, Toxoplasmose, *M. tuberculosis*, (EBV, Polyoma, Mycoplasma, Borrelia)
- Réarrangement Ig et TCR, BCR-ABL, t(14;18) et t(11;14)
- HER2

Les tests moléculaires qui sont exécutés (ou prévus) par les laboratoires locaux :

- *C. trachomatis*, *N. gonorrhoeae*, HCV qualitatif, (HSV, entérovirus, MRSA, *B. pertussis*, *S. agalactiae*)
- HLA-B27, FV Leiden, FII, MTHFR, Hémochromatose, (BCR-ABL)
- (HER2)

Messages clés

- Une partie des laboratoires a demandé l'accréditation ISO 15189 pour les tests DIV et donc aussi pour le diagnostic moléculaire.
- L'auto-évaluation effectuée par les CDM ne peut pas être considérée comme un contrôle de qualité externe et n'a pas apporté d'améliorations évidentes.
- La participation des CDM à des programmes internationaux de contrôle de qualité a été très limitée.
- Certains cliniciens et les médecins biologistes sont encore insuffisamment informés de l'offre en tests CDM/CGH.
- La sélection des CDM s'effectue encore souvent par le médecin demandeur ce qui entraîne des envois à plusieurs CDM par hôpital.
- L'implication, le rôle de coordination et l'influence du laboratoire local diffèrent d'un hôpital à l'autre.

2.4 Volume et coût des tests CDM

Nous nous sommes basés sur les rapports CDM, de même que sur les factures et les frais de personnel introduits auprès de l'INAMI pour les 40 premiers mois (1^{er} octobre 2000 – 31 janvier 2004). Les rapports CDM couvraient deux périodes de 8 mois, suivies de 2 périodes de 12 mois. Les données de facturation de la seconde période de 12 mois ont été obtenues chez 16 des 18 CDM et utilisées pour les calculs approfondis.

Le nombre total de tests rapportés sur une base annuelle en microbiologie et hématologie-oncologie-pathologie tend à croître d'année en année. Pour la période la plus récente du 1^{er} février 2004 au 31 janvier 2005, on recense un total déclaré de 117 139 tests de microbiologie et de 29 611 tests en hématologie-oncologie, ce qui représente une augmentation de respectivement 27% et 24% par rapport à l'année précédente. Néanmoins l'INAMI n'a pas encouru de risque financier puisque le budget total attribué aux CDM était cadencé.

Sur la base des factures d'achat de *Taq* polymérase (annexe 5), une bonne estimation du nombre total de réactions PCR par CDM est possible pour la période allant du 1^{er} février 2003 au 31 janvier 2004. Ces chiffres sont toutefois parfois un multiple important du nombre de tests PCR "maison" rapportés (tableau 12), ce qui laisse supposer qu'outre l'optimisation, la validation et l'exécution de test CDM, ces réactifs ont aussi été utilisés dans un certain nombre de centres pour l'exécution d'un nombre non négligeable de tests non CDM.

Il est par ailleurs évident que plus le volume de tests est important, plus faible est le temps consacré par le personnel (techniciens, licencié/Dr. Sc et secrétariat), ce qui réduit le coût par test (table 14 et figure 2).

Nous avons calculé le coût moyen par test PCR "maison" réalisé en double. Ce coût moyen inclut donc aussi bien les tests exécutés en séries par les CDM que les tests moins fréquents mais urgents qui ne peuvent attendre l'exécution de la prochaine série.

Pour des tests PCR « maison » exécutés en double, les coûts suivants ont été obtenus:

- Consommables : 9,82 € (test ARN) et 6,81 € (test ADN)
- Frais de personnel : 10,86 € à 50,22 € par CMD, moyenne 21,64 €

- Amortissement et entretien (basés sur un Taqman 7700 à 96 puits) : 2,98 €

Ceci nous amène à un coût total d'environ 33 € allant de 22 € pour le centre ayant les coûts les plus faibles en personnel à 60 € pour certains petits centres. Le coût moyen de tests quantitatifs comme la RT-PCR en héματο-oncologie où la mesure en double de l'expression d'un gène de contrôle est nécessaire pour permettre une normalisation du résultat s'élève à 66 €. La grande variation entre CDM peut être attribuée aux coûts de personnel. La méthode de financement des CDM n'incitait pas à limiter les frais de personnel. Il convient d'ajouter que l'utilisation de contrôles positifs et négatifs ou de calibrateurs pour le dosage augmente le coût par test de 8%. S'y ajoutent les coûts d'optimisation et de validation du test, de même que les frais de licence à payer éventuellement (non encore mentionnés par les CDM). Il convient également de remarquer que les tests « maison » ne sont pas toujours exécutés en double, et que la courbe d'étalonnage n'est pas nécessairement établie à chaque série. Les coûts se réduisent en cas de recours à des techniques PCR multiplex (amplification simultanée dans une réaction).

Pour les tests CDM généralement exécutés (de manière simple et non en double) avec une trousse DIV, les coûts de réactifs proprement dits s'établissent comme suit (les frais de 8% pour les contrôles sont déjà inclus à ce niveau) :

- HBV quantitatif, COBAS Amplicor HBV Monitor RUO, Roche : 58.11 €
- HCV qualitatif, Amplicor HCV AMP GEN-2 C, Roche : 20.34 €
- HCV quantitatif, Amplicor HCV Monitor, Roche : 85.10 €
- HCV génotypage, LINEPRB HCV GENO, Bayer : 70.72 €
- HPV, Hybrid Capture II, Digene : 14.28 €
- HER-2/neu, HER-2/neu, Ventana : 87.31 €

Lorsqu'on utilise une trousse de diagnostic, les frais de personnel par test devraient normalement être inférieurs à ce qu'ils sont pour les tests « maison » et peuvent être estimés à une seule réaction PCR « maison » au maximum (moyenne 10,86 €). Les frais d'amortissement et d'entretien peuvent être ici estimés à quelque 3,00 € (l'appareil COBAS Amplicor a 48 puits).

Messages clés

- Le nombre de tests de biologie moléculaire croît de plus de 20% par an.
- Le coût total d'une exécution en double d'un test PCR « maison » est aujourd'hui d'environ 33€ et est moins élevé dans les centres qui réalisent un volume important de tests.
- La grande variation entre CDM s'explique par les frais de personnel.
- L'INAMI n'a pas encouru de risque financier grâce à une enveloppe budgétaire fermée.

2.5 Discussion

L'Assurance Maladie a, grâce à l'expérience CDM, rendu le diagnostic moléculaire relativement accessible et ce pour un coût sans surprise. La mission des CDM, comme décrite à l'article 24 de l'AR du septembre 1998, était prometteuse et ambitieuse. L'extension imprévue du nombre de CDM impliqués et l'absence d'un contrôle par les pouvoirs publics de l'exécution de toutes les dispositions dudit AR expliquent que certaines attentes initiales n'aient pas été rencontrées. Ceci ressort également de l'enquête auprès des utilisateurs de tests. Même s'il existe un site central internet pour tous les CDM donnant des informations, la communication de l'offre en tests disponibles et leur indication est encore insuffisante voire même confuse pour le clinicien. L'absence de règles contraignantes lors de la prescription d'un test débouche parfois sur l'exécution de tests qui ne sont pas toujours adaptés à la demande ou sur une redondance lorsque plusieurs laboratoires (CDM et CGH) travaillent en parallèle sans communication. Les utilisateurs des résultats souhaitent également une entente sur la normalisation des méthodes et les unités de mesure utilisées, mais les CDM n'ont encore rien proposé. La communication écrite des résultats des tests devrait être plus rapide selon les prescripteurs. Les CDM n'ont aussi que partiellement répondu au grand besoin de formation en diagnostic moléculaire que ressentent les biologistes cliniques et les anatomo-pathologistes.

L'utilisation fréquente de méthodes moléculaires "maison" s'accompagne d'une validation minimale de la technique utilisée. Ceci ne favorise pas une validation clinique robuste.

L'évaluation de la valeur diagnostique des techniques moléculaires, y compris des trousse de diagnostics in vitro (DIV) n'est pas diligentée systématiquement ou au moins documentée. La liste des tests disponibles et leurs indications sont bien revues annuellement (des tests étaient ajoutés mais aucun ne fut supprimé) et des propositions de nomenclature ont été élaborées pour un grand nombre de tests moléculaires. Les CDM ont mis sur pied des contrôles de qualité entre eux et diffusé les résultats, mais la participation à des programmes internationaux de contrôle de qualité est restée limitée. Les CDM n'ont organisé aucun contrôle de qualité pour les rares tests moléculaires qui figurent actuellement à la nomenclature. A ce sujet, il convient de remarquer que l'ISP aussi est légalement habilité pour l'organisation du contrôle de qualité des tests moléculaires.

Le nombre croissant de CDM impliqués (un centre pour près de 500 000 habitants) n'a pas permis d'atteindre une masse critique individuelle sur le plan scientifique, de mettre en place une structure solide et de développer des résultats tangibles pour répondre à toutes les attentes. Cette expérience doit apporter des enseignements pour le futur. Pour l'INAMI, il peut être indiqué de faire appel, si nécessaire, à une expertise scientifique extérieure et à d'autres instances indépendantes avec le soutien, par exemple, du KCE.

L'auto-évaluation et l'évaluation de la qualité n'ont plus de sens dès lors qu'il existe un conflit d'intérêt majeur pour tous les membres du collège. L'auto-évaluation de la qualité a montré des lacunes évidentes sans que les mesures appropriées soient prises. Le choix d'un financement par un budget fermé et le paiement sur base des factures introduites offrent toutefois en soi une garantie pour l'Assurance Maladie. Par ailleurs, il ressort de ce rapport que ceci ne donne pas l'assurance d'une utilisation efficace de ces moyens dans l'ensemble des 18 CDM. Certaines factures posent question et le prix de revient par test varie considérablement entre les centres. De même, le nombre final de centres autorisés à participer à l'expérience et leur taille interpellent. Il existe sans nul doute des arguments de nature juridique qui justifient l'augmentation du nombre de centres. Au début de cette année, une décision de justice a mis fin brutalement à la convention. Sous l'angle de la méthodologie scientifique et de l'objectif d'évaluer une technologie de pointe, cette expérience ne peut donc pas être considérée comme un succès total pour l'assurance-maladie.

Messages clés

- L'Assurance Maladie a, grâce à l'expérience CDM, rendu le diagnostic moléculaire relativement accessible et ce pour un coût sans surprise.
- Pour l'assurance-maladie, l'expérience CDM ne peut pas être considérée comme un succès total.

3. Comparaison avec l'étranger

3.1 Trousses de diagnostic *in vitro* et tests "maison"

La directive européenne 98/79/EC exige que le producteur suive, via un système de qualité, le développement, la production et la vente des trousse DIV. Le dossier d'un petit nombre de tests dits à "hauts risques" doit être approuvé par un "Notified Body" désigné par les pouvoirs publics avant que la trousse de diagnostic puisse être commercialisée. La plupart des trousse de diagnostic moléculaire ressortissent toutefois au règlement d'auto-certification par lequel le producteur s'attribue lui-même le label CE DIV.

Une liste non exhaustive de plus de 200 trousse DIV portant le label CE pour la réalisation des tests CDM est présentée à l'annexe 2. Elle est basée sur la documentation reçue du fabricant. Une minorité seulement de ces trousse est utilisée par les CDM.

Contrairement à ce qui passe dans l'UE, la réglementation de la FDA exige une évaluation préalable de toute trousse DIV avant de pouvoir mettre celle-ci sur le marché américain. Les caractéristiques tant analytiques que diagnostiques et cliniques du test sont examinées. La description de l'indication comme "aide au diagnostic" est bien parfois imprécise. La liste des trousse de diagnostic moléculaire *in vitro* approuvés par la FDA aux EU (annexe 2) est encore fort courte et comprend, à côté du Facteur V Leiden, des trousse pour les tests CDM comme l'identification du MRSA, la détection de *M. tuberculosis* et d'HPV, la recherche qualitative des CMV et HCV, le dosage d'HCV, la mise en évidence du statut HER-2 (FISH), la présence d'aneuploïdie dans le cancer de la vessie (FISH).

Les tests moléculaires "maison" (« home-brew ») sont encore utilisés fréquemment un peu partout dans le monde, avec une validation minimale de la technique utilisée. Ceci ne favorise pas une validation clinique robuste. Sans validation, le test ne peut être considéré comme fiable dans la pratique clinique puisqu'il expose le patient à des résultats faussement positifs ou faussement négatifs avec de sérieuses conséquences possibles. Seul un nombre restreint de pays, dont l'Australie, disposent d'une réglementation spécifique pour la validation analytique et diagnostique de tests "maison". Aux Etats-Unis, les Analyte Specific Reagents, ou composants pour tests "maison" tombent également sous le coup d'une réglementation spécifique de la FDA qui considère les aspects de bonne pratique lors de la production.

Les trousse et réactifs commercialisés sous l'appellation RUO (pour Research Use Only) ne doivent pas satisfaire à la directive UE ni offrir de garantie de reproductibilité lors de la production. Aux Etats-Unis, l'utilisation de produits RUO à des fins de diagnostic n'est pas autorisée, de même que leur utilisation dans des tests "maison". Ce dernier point n'est pas clair en Europe.

3.2 Financement des tests moléculaires à l'étranger

Des données concernant le financement des tests moléculaires étudiés ont été colligées pour la France, l'Allemagne, le Royaume-Uni, les Pays-Bas, la Suisse, les Etats-Unis et l'Australie (tableaux 21 et 22). Des données concernant le volume de tests ont été uniquement obtenues pour la France et cela pour les seuls soins ambulatoires. Aux Etats-Unis, l'approbation de la trousse DIV par la FDA est une des conditions au remboursement du test. En l'absence de rapports HTA aux EU, la décision de remboursement peut être basée sur l'attitude d'autres assureurs.

La liste des tests moléculaires remboursés dans les pays étudiés n'est pas toujours similaire et suggère l'utilisation de différents critères pour l'inscription au remboursement. En microbiologie, il existe généralement un code de nomenclature distinct par microorganisme détecté. Un code générique existe en outre en Allemagne, aux Pays-Bas et en Australie pour la détection de l'acide nucléique par hybridation ou amplification. En hématologie-oncologie, les codes sont plutôt génériques tant pour l'amplification que pour les techniques cytogénétiques. En Australie, le remboursement n'est accordé que pour la détection de translocations par PCR en cas de leucémie aiguë et pour la leucémie

myéloïde chronique. En Suisse, une liste limitative de translocations est autorisée en PCR. En France, il n'existe aucun code pour les tests PCR d'hémato-oncologie exécutés chez les patients extra-muros. Le montant facturé par test, en ce compris la contribution personnelle du patient, varie considérablement d'un pays à l'autre. Seuls l'Allemagne et la France ont des montants comparables.

Une politique de licence stricte en ce qui concerne la propriété intellectuelle est d'application aux Etats-Unis et dans certains pays de l'UE. Le coût supplémentaire par test peut ainsi aller jusqu'à 20 USD.

Messages clés

- Plus de 200 trousses DIV sont commercialisées dans l'UE. C'est beaucoup plus qu'aux EU où chaque trousse doit être approuvée par la FDA avant sa commercialisation.
- En microbiologie, il existe habituellement un remboursement (code) distinct par microorganisme détecté. En hématologie-oncologie, les codes sont plutôt génériques tant pour les techniques cytogénétiques que pour l'amplification.

4. Proposition de solution basée sur une approche scientifique

4.1 Introduction

Une distinction doit être faite entre les tests d'utilité clinique prouvée et les tests pour lesquels il manque encore de l'évidence scientifique avant de les introduire dans la routine. Pour effectuer cette évaluation le KCE propose d'utiliser une méthode d'évaluation standardisée ("framework").

Seuls les tests moléculaires avec une précision analytique et diagnostique suffisante peuvent entrer en ligne de compte pour une utilisation en routine dans les soins aux patients. Les tests moléculaires qui sont encore en phase d'étude pour leurs propriétés analytiques et/ou diagnostiques/cliniques n'en font pas partie et forment une catégorie distincte.

Une validation de la technique est nécessaire avant la validation clinique. Sans validation, le test ne peut être considéré comme fiable dans la pratique clinique puisqu'il expose le patient à des résultats faussement positif ou faussement négatifs avec de sérieuses conséquences possibles. L'appréciation de l'efficacité diagnostique (« efficacy ») d'un test obtenu sous des conditions expérimentales utilise une échelle à six niveaux, comme détaillé ci-dessous. De plus il y a d'autres aspects à considérer pour arriver à une implémentation optimale et à un test efficient (« effectiveness ») dans la pratique clinique quotidienne. Ces aspects sont discutés pour les tests dont l'efficacité diagnostique est suffisante.

Pour les tests de microbiologie à faible volume, une autre solution doit être trouvée. L'approche diagnostique en hémato-oncologie est plus complexe que celle qui prévaut pour une analyse infectieuse et a exigé une mise en œuvre spécifique, ne fût-ce également que par la coexistence dans certains cas de laboratoires CGH et CDM.

Un certain nombre de données signalées pour les tests exécutés au sein des CDM ont été rassemblées dans le tableau 4a et le tableau 4b.

4.2 Modèle d'évaluation

4.2.1 Niveaux d'efficacité d'un test

Les tests diagnostiques peuvent être utilisés à des fins différentes pour diminuer l'incertitude concernant la présence ou non d'une infection, pour surveiller l'évolution d'une infection, pour supporter des décisions en relation avec un traitement, etc. Par là, les tests diagnostiques ont un effet éventuel sur le traitement, le résultat et le bien-être général du patient. Des tests qui sont insuffisamment précis et fiables peuvent entraîner des effets négatifs chez le patient. L'utilisation d'un test diagnostique n'est donc jamais neutre et une évaluation prudente du test avant son introduction dans la pratique clinique quotidienne s'indique.

Pour l'évaluation de l'efficacité d'un test, nous distinguons différents niveaux qui sont classés de manière hiérarchique en se basant sur le modèle de Fryback et Thornbury. Si un test obtient un score médiocre à un niveau inférieur, il est peu probable qu'il donnera un bon score à un niveau supérieur. Au sens strict du terme, il n'est pas nécessaire qu'un test soit efficace à chaque niveau avant de pouvoir être utilisé dans la pratique quotidienne, mais l'utilisation des niveaux fait que l'on connaît clairement le bénéfice qui peut être apporté par le test et celui à propos duquel une incertitude existe encore.

- Niveau 1 : l'efficacité technique
- Niveau 2 : l'efficacité diagnostique
- Niveau 3 : le bénéfice diagnostique
- Niveau 4 : l'effet sur la gestion ultérieure du patient
- Niveau 5 : l'effet sur le résultat chez le patient (« outcome »)

- *Niveau 6 : le rapport coût-efficacité*

Le **premier** niveau est l'efficacité technique, en d'autres termes, le test est-il à même de fournir des informations utiles dans des "conditions de laboratoire" ? Des mesures du résultat sont la sensibilité analytique, la reproductibilité, la dépendance de l'opérateur, etc.

Le **second** niveau est l'efficacité diagnostique : le test est-il en mesure de détecter l'infection chez les patients ou de l'exclure ? Les mesures de résultat sont la sensibilité, la spécificité, les valeurs prédictives, les rapports de cote, les fonctions discriminantes (ROC).

Le **troisième** niveau concerne le bénéfice diagnostique. Le test est-il en mesure de transformer la probabilité initiale en une probabilité a posteriori pertinente sur le plan clinique ? Sur base des rapports de cote, il est possible de calculer cette transformation mais le bénéfice diagnostique est-il également pertinent sur le plan clinique ? L'évaluation subjective du médecin concernant le risque de maladie avant et après la connaissance du résultat du test peut être utilisée à cette fin.

Le **quatrième** niveau est un effet sur la gestion ultérieure du patient. Le résultat peut résider dans le nombre de procédures invasives évitées, dans le nombre de nouveaux traitements initiés à l'occasion du résultat du test, etc.

Ce quatrième niveau fonctionne à vrai dire comme un intermédiaire pour le **cinquième** niveau, à savoir l'effet sur le résultat chez le patient. A ce niveau, les avantages du test, comme l'amélioration du pronostic, sont mis en balance vis-à-vis des désavantages du test, comme la charge pour le patient. Un essai clinique avec tirage aléatoire est le plus apte, sur le plan méthodologique, à fournir une réponse à ce niveau, mais il ne sera pas toujours possible d'exécuter ce type d'essai. Il existe également des diagnostics d'infections qui ne débouchent pas sur un résultat tangible. Dans ce cas, des mesures de la qualité de vie peuvent être utilisées pour évaluer l'effet du test.

Le **dernier** niveau (*niveau 6*) a trait au rapport coût-efficacité : le prix à payer est-il acceptable ? Les études de coût-efficacité calculent un coût par unité de résultat, comme le nombre d'années de vie gagnées. Les informations obtenues à partir des niveaux précédents peuvent être utilisées comme intrants, par exemple le nombre d'opérations évitées.

4.2.2 Autres caractéristiques importantes lors de la mise en place de tests

Outre le schéma proposé pour l'évaluation de l'utilité clinique, d'autres caractéristiques des tests doivent être également considérées pour une mise en œuvre rationnelle des tests et l'obtention de l'efficacité dans la routine. C'est ainsi que l'accréditation ISO garantit en principe l'équivalence du test en routine et lors des études qui ont montré l'efficacité diagnostique. Cette équivalence est documentée par la validation de la méthode et les contrôles de qualité internes et externes. On veillera également à exécuter les tests et à communiquer les résultats aussi rapidement que lors des études qui ont documenté l'utilité clinique.

Les variables suivantes doivent certainement être prises en considération lors d'un choix d'organisation et de financement et sont également reprises dans le schéma de recommandation.

- 1. *Disponibilité du test en trousse DIV ou test "maison"*
- 2. *Validation du test, en ce compris robustesse et contrôle de qualité*
- 3. *Prise en compte de l'utilité clinique et du risque, ainsi que des conditions nécessaires comme le temps de réponse maximal*
- 4. *Coûts et impact budgétaire*
- 5. *Conséquences d'une décentralisation sur les variables énoncées ci-avant sur*

- *les soins aux patients (qualité du test, temps nécessaire à l'obtention du résultat, interprétation du test, possibilité d'exécuter des tests complémentaires si nécessaire, communication du résultat)*
 - *le coût par test*
- *6. Risque de prescription ou d'exécution inappropriée du test (règles de diagnostic)*

4.2.3 Tests moléculaires et critères de remboursement de médicaments

Une situation particulière se pose lors de l'introduction d'un nouveau médicament pour lequel la sélection des patients ou le suivi de la sécurité ou de l'efficacité dépendent d'un DIV moléculaire ou de tout autre nouveau DIV. C'est le cas pour les tests moléculaires suivants rendus obligatoires pour bénéficier d'un remboursement du traitement par l'INAMI.

- Déterminations qualitative et quantitative de HCV-ARN, et génotypage. Ces tests sont décisifs pour évaluer les chances de succès ainsi que la dose et la durée du traitement de l'hépatite C chronique, de même que la surveillance de l'effet thérapeutique de l'interféron pégylé (PEGINTRON, PEGASYS) en combinaison avec la ribavirine.
- Détermination quantitative de HBV-ADN pour la sélection des patients et la surveillance du traitement de l'hépatite B chronique par lamuvidine (ZEFFIX).
- Test HER2 (FISH) dans l'identification du cancer du sein métastaté avec surexpression de HER2/neu pour bénéficier d'un traitement par trastuzumab (HERCEPTIN). Le test HER2 FISH doit être exécuté dans le cadre d'un CDM reconnu. L'adaptation de cette obligation s'impose donc.
- La détection et la quantification BCR-ABL sont nécessaires pour la sélection des patients et la surveillance du traitement de la leucémie myéloïde chronique par l'imatinib (GLIVEC).

A cet égard, la connaissance du nombre de tests révélés positifs pourrait s'avérer utile à l'INAMI pour anticiper la consommation des médicaments en question. Le nombre de tests HER2 positifs (408 en 2002, 819 en 2003, 689 en 2004) et BCR-ABL positifs (280 en 2002, 275 en 2003, 211 en 2004) au moment du diagnostic permet d'estimer le nombre de patients susceptibles de bénéficier prochainement d'un traitement par trastuzumab et imatinib. Si le nombre de patients diagnostiqués diminue quelque peu en 2004 par rapport à 2003, il est bien au dessus du nombre attendu lors de l'autorisation de remboursement délivrée par l'INAMI pour l'HERCEPTIN (170 par an) et pour le GLIVEC (120 par an).

A l'avenir, les traitements génétiquement ciblés exigeront des tests moléculaires parfois complexes. Ces tests seront exécutés pendant le développement clinique dans un nombre limité de laboratoires centraux mais doivent trouver leur place dans la pratique normale au moment de l'introduction du médicament.

Lors de l'évaluation d'un nouveau médicament par les instances compétentes (notamment l'INAMI), il faut donc veiller à une coordination indispensable entre la cellule du médicament et la cellule des actes techniques. Une intervention doit être prévue en cas d'utilité avérée du test moléculaire.

Dans un certain nombre de cas, le test diagnostique n'a pas été repris explicitement dans les critères de remboursement du médicament, mais le test est toutefois essentiel pour le choix thérapeutique lors du diagnostic ou pendant le suivi, comme énoncé lors des entretiens avec les prescripteurs de tests. C'est le cas pour des translocations spécifiques en cas de diagnostic de leucémie aiguë qui peuvent concourir au choix du traitement. De même, la persistance d'une maladie résiduelle minimale documentée par PCR après un mois de traitement d'une leucémie lymphoïde aiguë influence profondément le traitement ultérieur.

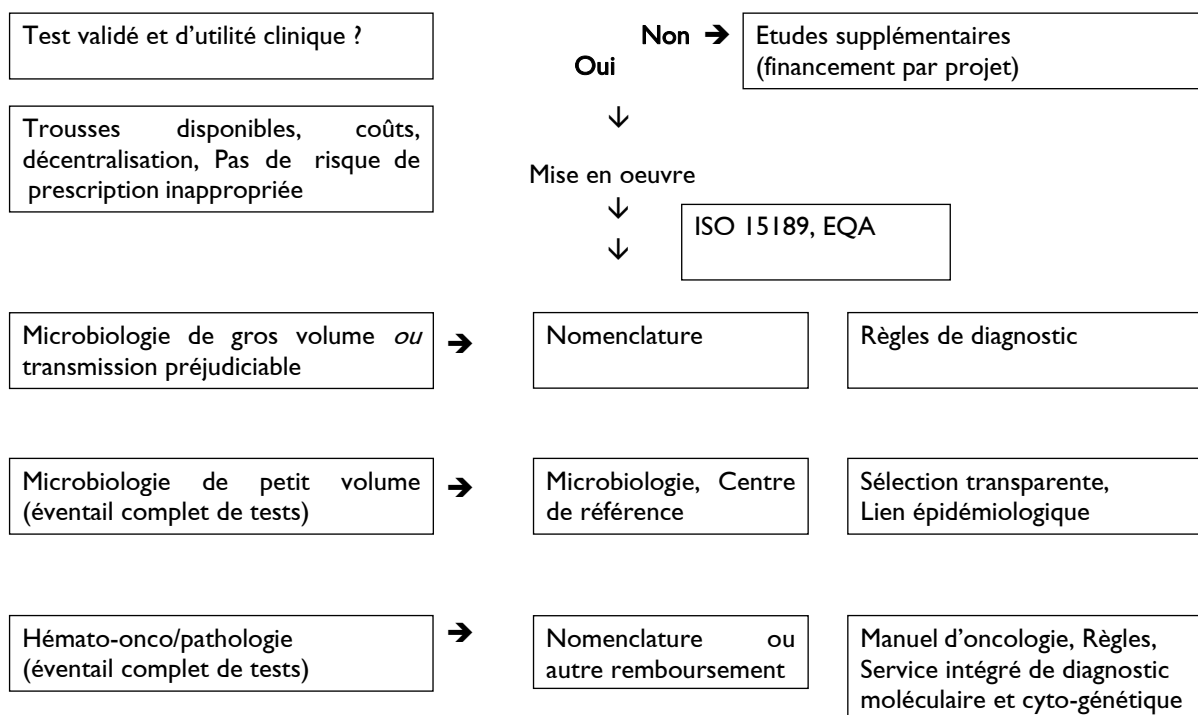
Messages clés

- Pour l'évaluation d'un test et son implémentation rationnelle, le KCE propose d'utiliser un protocole standardisé ("framework") qui, à côté d'une évaluation de l'efficacité clinique, recherche aussi l'efficience du test en routine.
- L'évaluation de l'efficacité d'un test se fonde sur 6 niveaux hiérarchiques différents depuis l'efficacité sur le plan technique (1) jusqu'à un test qui soit coût-efficace (6).
- Lors de l'évaluation d'un nouveau médicament par les instances compétentes (notamment l'INAMI), il faut toujours veiller à ce que les tests diagnostiques nécessaires soient évalués parallèlement et qu'une intervention soit prévue en cas d'utilité avérée.

4.3 Recommandations d'organisation

Comme mentionné plus haut, il convient d'opérer une distinction entre les tests d'utilité clinique documentée et ceux pour lesquels une analyse complémentaire s'impose. Il est en soi possible bien que peu réaliste d'exiger de chaque test le plus haut niveau d'efficacité clinique avant de l'implémenter. Dès le niveau 3 et certainement au niveau 4, l'utilité clinique prêterait peu à discussion. Pour les tests de niveau inférieur et à coup sûr pour les tests qui ne dépassent pas le niveau 1, on ne peut pas encore parler d'intérêt clinique et ces tests sont en phase expérimentale. De plus, les recommandations particulières d'implémentation des tests doivent faire la différence entre les tests de microbiologie de volume important ou modeste, et ceux d'hémato-oncologie.

Nous proposons le schéma suivant pour l'évaluation des tests diagnostiques :



4.3.1 Tests moléculaires encore en phase d'étude

Les tests pour lesquels l'utilité clinique n'a pas encore été documentée peuvent uniquement être exécutés dans le cadre de protocoles d'étude. La sélection et le financement des études doivent s'effectuer sur la base de la valeur scientifique et clinique du protocole d'étude. Les évaluations pilotes réalisées ont montré que l'évaluation d'un nouveau DIV moléculaire doit se faire progressivement puisqu'une précision analytique suffisante est une condition nécessaire avant de pouvoir étudier la valeur diagnostique ou clinique au début ou en cours du traitement et, le cas échéant, pouvoir mener ensuite une analyse de coût-efficacité. La FDA exige aussi dans la plupart des cas que des études soient conduites dans plusieurs centres de manière à évaluer également la robustesse analytique de la trousse de diagnostic entre les mains des utilisateurs futurs. La validation clinique doit toujours être abordée dans un contexte clinique et requiert donc la collaboration des médecins traitants. L'exécution d'études cliniques exige de plus une expertise particulière. Contrairement à la situation qui prévaut pour l'étude des médicaments, des études prospectives à grande échelle avec les trousse DIV ne sont que rarement supportées par l'industrie. Ces études ne sont généralement pas requises pour la commercialisation des produits DIV en dehors des Etats-Unis, ceci contrairement aux médicaments. En outre, l'investissement à consentir dans une étude à grande échelle par l'industrie est souvent disproportionné par rapport aux bénéfices escomptés, ne serait-ce que parce que la technologie DIV vieillit rapidement. La mise à disposition de fonds de recherche par les pouvoirs publics peut ici s'avérer utile, certainement si l'impact budgétaire attendu est important.

Le financement doit être transparent et basé sur des résultats à atteindre clairs, y compris le rapport qui doit montrer l'utilité clinique indiscutable du test dans une indication précise. Comme pour les médicaments, ces rapports d'étude seraient idéalement destinés aux pouvoirs publics aux fins d'autorisation et d'assurer le financement lors de l'utilisation en routine.

A ce jour, le fondement scientifique pour une utilité clinique est absent pour de nombreux tests moléculaires récents, ce qui pose problème étant donné les conséquences médicales et économiques possibles, et certaines en cas d'application généralisée. Les pouvoirs publics doivent investir dans l'expertise nécessaire pour une évaluation scientifique et économique de l'information disponible lors de l'implémentation de chaque nouveau test diagnostique. L'INAMI pourrait créer une cellule d'évaluation des tests moléculaires (et d'autres moyens de diagnostic plus récents) de manière à évaluer l'efficacité diagnostique des tests proposés. Ceci pourrait se faire en utilisant l'expertise existante, notamment pour le HTA, au sein du KCE. L'ISP doit prêter son concours au contrôle de qualité des laboratoires. Si cette évaluation est négative, l'utilisation du test pour cette indication ne doit pas être autorisée.

Il serait préférable que la décision d'attribuer une utilité clinique ou pas à une trousse DIV (et d'afficher la mention CE) ne soit plus laissée au fabricant, comme c'est le cas aujourd'hui avec le régime d'autocertification dans l'UE. Le concept d'un "dossier génétique" qui existe déjà au Royaume-Uni pour les tests génétiques et sera peut-être élargi sous l'égide de l'EMA, peut être étendu aux autres trousse DIV. Ceci peut se faire en collaboration avec des organisations internationales, y compris la FDA et la CLSI (anciennement NCCLS). La banque de données avec toutes les trousse DIV CE mise sur pied selon la directive DIV sera vraisemblablement rapidement opérationnelle.

4.3.2 Tests d'utilité clinique documentée

Tests moléculaires de microbiologie de volume moyen à élevé

Les tests moléculaires de microbiologie d'utilité clinique documentée et dont le volume est par exemple supérieur à 1000-2000 tests par an, peuvent être inclus dans la nomenclature de biologie clinique. Les exigences de qualité requièrent une accréditation ISO obligatoire et la participation à des contrôles de qualité externes (cfr infra). Un rapport d'activité pourrait être utile pour pouvoir mieux suivre l'évolution et l'utilité de certains tests. L'évaluation pilote des tests moléculaires dans l'hépatite C montre qu'une estimation du volume des tests est possible lorsque des directives claires existent.

Tests moléculaires de microbiologie à faible volume.

Ces tests d'utilité clinique documentée et dont le volume est inférieur à 1000-2000 tests par an, sont, pour des raisons de qualité et de coût, exécutés de préférence dans un ou plusieurs laboratoires. Ceux-ci pourraient être moins nombreux qu'actuellement.

La sélection et le financement de ces laboratoires de référence en microbiologie doivent s'effectuer de façon transparente (par ex. par convention ou par adjudication publique) et comprendre les exigences de service suivantes :

- qualité (accréditation ISO obligatoire et participation à des contrôles de qualité internationaux),
- présence d'expertise technique et clinique (case-load, publications scientifiques en cliniques dans le domaine),
- emploi d'une méthode identique ou équivalente à la méthode qui a montré son utilité clinique en cas d'existence de plusieurs centres de référence,
- délai de réponse à ne pas dépasser (assorti d'une permanence pendant le week-end si nécessaire),
- protocole écrit en temps utile,
- information claire et correcte via le site internet pour des tests peu fréquents et accessibilité téléphonique,
- rédaction d'un rapport d'activité (comme actuellement pour les CDM) et d'un rapport d'audit interne.

S'il s'agit de tests rares qui sont plutôt exécutés par paquet, il semble logique que l'exécution des tests moléculaires et non moléculaires ait lieu dans le même laboratoire (p.ex. éventail de tests pour la pneumonie atypique, la méningo-encéphalite virale). Dans certains cas, ce laboratoire pourrait également jouer le rôle de laboratoire de référence, comme décrit dans la proposition émanant de l'ISP, pour autant que la recherche épidémiologique et les activités de routine soient clairement séparées. Pour des raisons de transparence, les travaux de recherche sur l'efficacité diagnostique, qui auraient lieu dans ces mêmes centres, doivent être financés de manière distincte. Ce financement se fera sur base de protocoles d'étude qui doivent souvent associer des cliniciens.

Tests moléculaires/cytogénétiques en hémato-oncologie/pathologie

La démarche diagnostique dans le laboratoire d'hémato-oncologie, y compris la cytogénétique et le diagnostic moléculaire, est complexe et s'effectue par étapes. Les tests moléculaires et cytogénétiques sont parfois complémentaires, parfois concurrents. Idéalement, un seul coordinateur assurera l'échantillonnage du prélèvement, la demande des tests réalisés dans l'hôpital et à l'extérieur, le suivi ainsi que l'assemblage des résultats de laboratoire. Une démarche pratique et transparente est d'avoir un schéma détaillé des étapes diagnostiques, qui est rattaché au manuel oncologique rédigé en accord avec tous les cliniciens et laboratoires concernés. Le collège d'oncologie pourrait, en collaboration avec l'INAMI, rédiger des directives nationales qui détaillent les normes de protocole et le contrôle des procédures du manuel. Le développement de directives nationales pour les

demandes d'analyses est fortement recommandé pour favoriser l'échange d'informations et l'usage judicieux des tests. Comme également recommandé dans un rapport HTA (NICE, 2003), l'exécution de la cytogénétique et du diagnostic moléculaire pour un problème diagnostique se fera de préférence dans un seul laboratoire. Ce laboratoire peut conclure une convention de service révisable annuellement avec le département d'oncologie et le laboratoire local. Cette convention mentionnera explicitement le délai de communication des résultats et du protocole complet. Un nouveau financement remplacera dès lors la facturation des prestations pour affections génétiques acquises qui se faisait improprement au moyen de codes de l'article 33, puisque ces codes de nomenclature furent créés spécifiquement pour les affections génétiques congénitales.

La nombre des laboratoires moléculaires/cytogénétiques peut être fixé sur base de critères de sélection ou non. Les exigences posées pour l'offre d'une gamme complète de tests cytogénétiques et moléculaires en hématologie-oncologie, l'accréditation obligatoire pour ces tests, la mise en place d'une convention de service avec les hôpitaux concernés et le respect de cette convention constitueront un filtre suffisant pour que les hôpitaux aient encore le choix. Pour le financement des laboratoires de diagnostic moléculaire/cytogénétique en hématologie-oncologie, plusieurs modèles sont imaginables :

- l'utilisation d'une nomenclature générique,
- idem mais avec utilisation d'une nomenclature spécifique (un code par test),
- idem mais avec utilisation d'une nomenclature générique selon l'utilisation (diagnostic *versus* suivi),
- l'adjudication publique pour laquelle le laboratoire soumet lui-même un volume et un prix,
- un budget fixe réparti entre les laboratoires selon le volume de tests, sur la base de factures (convention, comme le modèle CDM).

L'option d'une nomenclature générique selon l'utilisation permet le décompte des tests exécutés pour le diagnostic et des tests de suivi. Les règles habituelles pour l'exécution de tests dans un laboratoire extérieur peuvent être applicables. Le fait que le budget ne soit pas fermé dans cette proposition, contrairement au financement CDM, rend d'autant plus nécessaire la transparence et le respect des schémas diagnostiques basés sur les données scientifiques présentées dans les manuels d'oncologie.

La redondance de tests au moment du diagnostic (comme actuellement dans certains CDM et CGH) doit être évitée et lors du suivi, un nombre maximal de tests par an doit être stipulé. Chaque test présenté au remboursement doit être évalué sur base de son utilité par rapport au modèle proposé (voir ci-dessus) et mériter sa place dans les schémas diagnostiques.

On veillera de plus à ne pas introduire de distorsion de remboursement selon le choix de la méthode utilisée (caryotypage, FISH, PCR).

Comme ce domaine se caractérise par un éventail de méthodes "maison" souvent non validées, les recommandations susdites concernant la qualité doivent s'appliquer sans exception (accréditation obligatoire selon l'ISO 15189 pour la majorité des tests et participation à des circuits de qualité externes internationaux). Le nombre restreint de patients et la rareté de tests positifs plaident également pour une centralisation de ces tests.

Les tests moléculaires pour l'hématologie-oncologie sont exécutés pour un groupe spécifique limité de patients. Cette donnée permet d'envisager une forme alternative de financement (via les centres d'oncologie) fondée sur l'enregistrement rendu obligatoire des cancers et les résumés cliniques minimaux. Un tel financement accorde aux centres une grande liberté pour intégrer rapidement des tests et des méthodes de pointe (comme le micro-array), ce qui n'est pas toujours possible dans le cadre d'une nomenclature rigide. Une étude de faisabilité doit être exécutée avant de pouvoir recommander cette proposition.

Les tests moléculaires/cytogénétiques en anatomo-pathologie (pas en hématologie) peuvent également être financés par le biais des propositions élaborées pour l'hématologie.

oncologie. Pour la pathologie, un financement alternatif des tests HER2 FISH devrait également être possible sur base du nombre de cancers du sein rapportés.

4.3.3 Recommandations générales

Recommandations pour la qualité et le service des laboratoires

Si l'on veut offrir des soins de qualité, il convient de cibler l'utilisation des tests diagnostiques en s'appuyant sur les données scientifiques et cliniques. C'est pourquoi la garantie de qualité du test n'est pas un élément négligeable.

Indépendamment du modèle choisi pour l'organisation et le financement, il existe une tendance évidente en direction de l'accréditation obligatoire des tests de biologie clinique selon l'ISO 17025 ou mieux ISO 15189 (BELAC, avant BELTEST), et ceci également pour les tests moléculaires.

L'accréditation ISO obligatoire pour tous les tests solutionne immédiatement le problème des nombreuses méthodes « maison » non validées. Pour des tests peu fréquents non encore validés, par exemple en l'absence d'échantillons bien documentés, cette absence doit être également mentionnée dans le rapport. Les protocoles finaux des tests et les résultats intermédiaires doivent être également transmis au demandeur par écrit et sans retard. La participation aux contrôles de qualité nationaux ou internationaux sous le contrôle de l'ISP devrait aussi devenir la règle.

La validation de la mise en œuvre locale de méthodes qui font appel à des trousseaux DIV peut être plus efficace si les producteurs mettent systématiquement les données concernant la validation analytique et diagnostique à la disposition des laboratoires (comme ceci est également prévu par la directive DIV). Dans le contexte de l'accréditation, ceci devrait permettre d'éviter la répétition partielle ou complète d'un certain nombre de validations. Une concertation avec l'organisation des producteurs semble ici indiquée pour réaliser cet objectif, par exemple en utilisant un site internet centralisé. Les producteurs de trousseaux CE doivent être informés lorsque les trousseaux ne sont pas satisfaisants tandis que les "effets indésirables" doivent être signalés aux autorités compétentes comme légalement prescrit.

L'exécution des tests ne doit pas seulement offrir des garanties de qualité ; le prescripteur doit aussi disposer des connaissances nécessaires concernant les caractéristiques des tests ainsi que les conséquences possibles pour le traitement et le pronostic. Lors de l'introduction d'un nouveau test, la question doit donc être posée de savoir si des restrictions sont également indiquées dans ce cas, comme par exemple la demande d'HCV quantitatif ou de génotypage par le généraliste.

Les prescripteurs de tests moléculaires et les laboratoires locaux doivent pouvoir fournir une réponse rapide et correcte à des questions comme celles-ci : où le test est-il exécuté, quel type d'échantillon est nécessaire, quelle est la fiabilité du test sur le plan analytique et quelle est sa valeur diagnostique, dans quel délai puis-je obtenir le résultat et à quoi dois-je également faire attention lors de l'interprétation. Un site internet tenu à jour semble être une solution avec la disponibilité des experts nécessaires pour répondre aux questions éventuelles. Un protocole écrit et normalisé (unités) disponible rapidement évitera les oublis lors de l'interprétation. D'autre part, l'exécutant doit disposer des informations cliniques pertinentes et doit, si nécessaire, pouvoir contacter facilement le demandeur.

Les tests moléculaires doivent, comme les autres méthodes diagnostiques in vitro, figurer au programme de la formation et du recyclage des exécutants concernés et des prescripteurs de tests.

Recommandations complémentaires pour l'Assurance Maladie

Indépendamment de l'option retenue pour le financement et l'organisation, il peut être utile de disposer de chiffres annuels fiables concernant le nombre de patients testés et le nombre de tests exécutés pour chacun des tests diagnostiques. De cette manière, on peut, si nécessaire, intervenir de manière pertinente à partir de l'évolution du nombre de tests

exécutés. A cet égard, le modèle CDM est plus transparent que le codage selon l'article 24 et que la nomenclature CGH générique selon l'article 33 pour connaître le nombre de tests spécifiques exécutés et le nombre de résultats positifs.

Une consultation de routine d'une banque de données centrale avant qu'un test soit demandé (et remboursé), accessible via la combinaison des codes du médecin et du patient, pourrait éviter la répétition inutile des tests. Les risques et les avantages d'une telle banque de données devraient être analysés. Actuellement, et pour autant qu'il existe un code de nomenclature, ces données sont transmises aux mutuelles, après l'exécution du test diagnostique.

L'évolution vers un diagnostic moléculaire plus robuste et accessible est en marche. Comme pour les techniques DIV utilisant des anticorps monoclonaux, toute évolution requiert un certain nombre d'ajustements de trajectoire avant de rendre possible l'exécution des tests en routine. Il est important de donner à cette évolution toutes ses chances, en veillant à ce que les fondements de chaque test soient solides. Étant donné l'évolution constante observée en technologie, il faut également revoir régulièrement l'indemnisation des tests moléculaires, comme leurs indications, ainsi que les conséquences éventuelles sur la forme d'organisation recommandée (centre de référence ou labo de routine).

Cette révision de l'indemnisation, adaptée aux techniques PCR meilleur marché, semble aussi nécessaire pour les tests moléculaires dans d'autres domaines. Par exemple, le test PCR Facteur V Leiden dans les CGH est encore remboursé à environ 300 € par test.

Messages clés

- Les tests dont l'utilité clinique n'a pas encore été documentée ne peuvent être exécutés que dans le contexte de protocoles d'étude.
- Les tests moléculaires en *microbiologie* d'utilité clinique documentée et de volume important peuvent être repris dans la nomenclature de la biologie clinique.
- Les tests moléculaires en *microbiologie* d'utilité clinique documentée et de volume faible se font dans des laboratoires de référence pour la microbiologie. Le nombre de centres doit être limité pour des raisons d'expertise, de coût et de qualité. La sélection doit être faite de façon transparente. Le service, mais aussi la vitesse d'exécution, doivent être garantis.
- Le laboratoire moléculaire/cytogénétique et les représentants du programme de soins en oncologie doivent élaborer des schémas diagnostiques à suivre pour les problèmes les plus courants en hémato-oncologie.
- Pour le financement des tests moléculaires en *hémato-oncologie* il existe plusieurs options, y compris la nomenclature.
- Les exigences posées à l'offre d'une gamme complète de tests cytogénétiques et moléculaires pour l'hémato-oncologie, l'accréditation obligatoire pour ces tests et l'établissement, ainsi que le respect de la convention de service avec les hôpitaux concernés constitueront en soi un filtre suffisant pour que la sélection puisse avoir lieu librement et être ouverte à d'autres laboratoires que les CGH.
- L'imputation de tests en hémato-oncologie et oncologie par le biais de la nomenclature réservée aux CGH doit cesser.
- L'accréditation ISO obligatoire de tous les tests moléculaires fournit d'emblée une réponse au problème des nombreuses méthodes « maison » non validées.
- Il faut revoir régulièrement l'indemnisation des tests moléculaires, de même que les indications.
- La duplication de tests et une double imputation ne sont pas acceptables.

5. Evaluations pilotes et applicabilité du modèle

Une évaluation HTA exhaustive de chaque test CDM individuel (au total, nous avons compté 94 tests) était impossible dans le cadre de ce projet. C'est pourquoi un certain nombre de tests ont été sélectionnés pour une étude plus détaillée de l'utilité clinique. Ces études pilotes avaient aussi pour but de formaliser et d'affiner le modèle d'évaluation proposé plus haut. Pour les autres tests CDM on a recherché des HTA et des revues systématiques.

5.1 Evaluations pilotes

Un nombre limité de tests ont été sélectionnés pour une étude plus détaillée de l'utilité clinique et de la situation des tests locaux. La sélection consistait en des tests de volume important ou faible, des tests de microbiologie ainsi que des tests d'héματο-oncologie.

Comme exemple de tests normalisés à grand volume, on a opté pour une évaluation pilote des tests moléculaires dans le cadre du traitement de l'hépatite C chronique. Comme test microbiologique moins normalisé, on a opté pour l'évaluation d'un entérovirus dans le cadre de la méningite. Pour l'héματο-oncologie, on a opté pour l'élaboration du rôle de t (14; 18) PCR dans le lymphome folliculaire. A la lumière d'une enquête de l'ISP auprès des laboratoires de biologie clinique en 2003, il est apparu que Facteur V Leiden était le test génétique qui était proposé par le plus grand nombre de laboratoires; ce test a également été sélectionné pour une évaluation pilote. Chacune de ces études pilotes a été commentée par un groupe d'experts externes, y compris des spécialistes cliniques du domaine.

Nous avons à chaque fois opéré une distinction entre les propriétés analytiques du test, étudiées dans des conditions de laboratoire, et les propriétés diagnostiques pour lesquelles l'examen du patient est nécessaire. Enfin, nous avons aussi examiné l'implication clinique du test. Pour ce faire, la bibliographie a été étudiée systématiquement à chaque fois dans au moins deux banques de données différentes, avec l'aide d'une stratégie de recherche exhaustive. Les articles obtenus ont été inclus ensuite sur base de la qualité de l'étude estimée selon une échelle validée.

5.1.1 Hépatite C

La prévalence de l'hépatite C en Belgique est estimée à 1%. Sa transmission s'opère principalement par le sang infecté et la consommation intraveineuse de drogues. Sur l'ensemble des patients contaminés par l'hépatite C, 85% environ développera une infection chronique. Parmi ceux-ci, environ 20% fait une cirrhose du foie tandis qu'un nombre peu élevé développera un carcinome hépatocellulaire. Le traitement standard pour l'hépatite C chronique est l'interféron pégylé, combiné à la ribavirine.

Pour l'hépatite C, différents tests moléculaires existent, à savoir le génotypage, la détermination quantitative et la détermination qualitative de l'ARN. Ceux-ci sont utilisés pour évaluer les chances de succès au départ du traitement et pour déterminer la réponse intermédiaire et définitive après la fin du traitement.

La qualité générale des études qui ont été identifiées a été de moyenne à médiocre. La précision analytique des tests était bonne. Le seuil de détection des tests qualitatifs est inférieur à celui des tests quantitatifs, à savoir 50-100 UI/ml, certaines déterminations présentant une limite encore inférieure. Les tests quantitatifs ont une linéarité suffisante pour différentes concentrations virales, cependant la correspondance entre les différents tests disponibles est insuffisante. Le suivi chez un même patient doit donc s'effectuer avec le même test. Le génotypage est précis, encore que certains patients ne puissent être typés.

Les études diagnostiques et cliniques montrent que les patients infectés par un génotype 2 ou 3 présentent une meilleure réponse au traitement. Les patients qui ont une charge virale plus élevée, mesurée par tests quantitatifs, ont une probabilité plus faible de réponse. Un test qualitatif négatif 6 mois après l'arrêt du traitement définit la réponse à celui-ci. Chez les patients avec génotype 1, l'absence d'une diminution supérieure ou égale à 2 log

10 de la concentration en HCV-ARN lors d'un test quantitatif, ou la persistance de l'HCV-ARN lors d'un test qualitatif a une valeur prédictive négative très élevée pour la réponse finale au traitement. En d'autres termes, les patients sans diminution de $2 \log_{10}$ ou dont l'ARN est encore détectable après 12 semaines de traitement ont une très faible chance de présenter une réponse et il est donc préférable de mettre fin au traitement. L'utilisation de tests moléculaires pendant le traitement de patients avec génotype 1 est coût-efficace. Une même stratégie diagnostique chez les patients avec génotype 2 ou 3 entraîne toutefois des coûts supplémentaires sans gain thérapeutique, étant donné l'excellente réponse au traitement pour ces génotypes.

Les tests moléculaires pour l'hépatite C sont suffisamment précis sur les plans analytique et diagnostique et ont un impact clinique évident.

5.1.2 Entérovirus

Les entérovirus sont la cause de plus de 90% des cas de méningites aseptiques pour lesquelles un agent responsable peut être identifié. L'évolution naturelle est favorable mais en l'absence de diagnostic différentiel avec la méningite bactérienne, ces infections entraînent une hospitalisation et un traitement empirique. Les tests moléculaires devraient permettre une identification rapide de l'entérovirus et donc exclure plus rapidement une méningite bactérienne.

La précision des tests analytiques n'était pas bonne. La sensibilité variait entre 61% et 91%, et la spécificité entre 86% et 98%. De même, lors des études diagnostiques, on notait une variation importante des résultats rapportés avec une sensibilité comprise entre 85 et 100% et une spécificité comprise entre 80% et 100%. La pléocytose du liquide rachidien semble influencer les caractéristiques du test. Des intervalles de confiance ne sont indiqués nulle part ; l'absence d'un test de référence rend encore plus incertain les résultats des études. Vu l'indication pour les tests moléculaires ici, à savoir l'exclusion d'une autre cause par l'identification d'un entérovirus, la faible spécificité constitue le problème principal. Pour une spécificité de 80%, il y a 20% de faux positifs et donc un nombre de patients, variable selon la prévalence, aura à tort un diagnostic de méningite à entérovirus et n'aura pas de traitement approprié.

Quelques études ont examiné l'impact clinique des tests moléculaires pour l'entérovirus. C'est ainsi que l'on a observé une durée d'hospitalisation plus courte pour les patients avec test positif que pour les patients avec test négatif. Les effets négatifs éventuels des tests n'ont été analysés nulle part.

Les tests moléculaires pour l'entérovirus sont insuffisamment précis sur les plans analytique et diagnostique.

5.1.3 PCR pour t(14;18) dans le lymphome folliculaire

Le lymphome folliculaire (LF) est, en terme de fréquence, la seconde forme de lymphome non-Hodgkinien. Son incidence en Belgique est estimée à 400 cas par an. La translocation t(14; 18) est observée dans la toute grande majorité des cas de LF et est nettement moins fréquente pour d'autres lymphomes. La détection de t(14; 18) lors de la mise au point est utile dans 5% des cas, lorsque la morphologie et l'immuno-histochimie ne sont pas univoques.

Pour la détection de t(14; 18), la sensibilité diagnostique de la technique FISH (plus chère) est nettement plus élevée que la sensibilité PCR. Ceci provient de la grande diversité en points de rupture chromosomiques pour t(14; 18). L'utilisation d'une PCR quantitative t(14; 18) pour le suivi du traitement du LF n'est donc possible que dans la moitié des cas. L'utilité clinique de cette surveillance est encore à l'étude.

La détection de t(14; 18) peut être utile lorsqu'il s'agit de poser le diagnostic de lymphome folliculaire. La technique FISH semble toutefois ici supérieure à la PCR sur le plan diagnostique.

5.1.4 Factor V Leiden

Le Facteur V Leiden est la cause la plus fréquente de thrombophilie associée à un risque trois à sept fois plus élevé de thrombose veineuse profonde.

On a retenu un nombre limité d'études de qualité moyenne à bonne, tant pour la précision analytique que pour la précision diagnostique. Les études analytiques rapportent toutes une concordance de 100% entre les tests et les méthodes de référence. De même, les études diagnostiques trouvent une concordance supérieure à 98%. Des mesures de précision ou de reproductibilité n'ont pas été rapportées.

L'impact clinique de tests pour cette mutation est moins évident. Après un épisode de thrombose veineuse profonde, le traitement de patients avec mutation n'est pas différent de celui de patients ayant présenté une thrombose veineuse profonde idiopathique. Le dépistage chez la femme qui souhaite une contraception orale ou une substitution hormonale n'est pas conseillé. Les sujets avec antécédents personnels ou familiaux évoquant une mutation homozygote ou la présence de 2 facteurs de thrombophilie peuvent toutefois entrer en ligne de compte pour le test mais l'impact thérapeutique n'est pas établi.

Les tests moléculaires pour la mutation du Facteur V Leiden sont sans doute suffisamment précis sur les plans analytique et diagnostique. L'impact clinique est insuffisamment documenté.

5.2 Rapports HTA et revues systématiques des tests CDM

Pour les tests CDM à propos desquels aucune évaluation pilote n'a été exécutée, on a recherché des rapports HTA et des revues systématiques. Des rapports HTA de haute qualité et des revues systématiques donnent une bonne synthèse des données scientifiques disponibles.

Une première recherche bibliographique a permis d'identifier des revues systématiques pour *Mycobacterium tuberculosis* et HPV. Les revues de littérature trouvées pour *Borrelia burgdorferi* et Herpes simples virus doivent être interprétées avec prudence étant donné que tous les critères d'une revue systématique n'étaient pas remplis. Deux rapports HTA établis en 2003 par le MSAC australien supportent le remboursement des tests PCR pour le diagnostic et le suivi de patients avec leucémie aiguë promyélocytaire ou myéloïde.

Une très petite minorité seulement des tests CDM étudiés a fait l'objet de rapports HTA ou de revues systématiques. En l'absence de ceux-ci, les évaluations de diagnostic moléculaire devront faire appel à des études originales.

Les évaluations pilotes, les rares HTA et revues systématiques disponibles, avec leur niveau d'efficacité diagnostique, sont résumées ci-dessous pour les tests CDM et de manière plus détaillée dans le tableau 20.

Test	Indication	Niveau d'efficacité diagnostique	Référence
HCV qualitatif, quantitatif et génotypage	Sélection et suivi du traitement basé sur le peg-interféron	Niveau 6: coût-efficace	Evaluation pilote
<i>Mycobacterium tuberculosis</i>	Echantillon positif	Niveau 6: coût-efficace si les tests sont centralisés	Dowdy, 2003 ¹
<i>Borrelia burgdorferi</i>	Maladie de Lyme	Niveau 2 : sensibilité diagnostique modérée, pas pour le diagnostic primaire	Dumler, 2001 ²
PCR Herpes simplex virus	Méningo-encéphalite	Niveau 1: étude supplémentaire nécessaire	Linde, 1997 ³
PCR Entérovirus	Méningite	Niveau 1: précision analytique insuffisante	Evaluation-pilote
PCR t(8;21) AML1-ETO et inv(16) CBFB-MYH11	Leucémie myéloïde aiguë	Niveau 6: bon rapport coût-efficacité	MSAC, 2003 ⁴
PCR t(15;17) PML-RARA	Leucémie promyélocytaire aiguë	Niveau 6: bon rapport coût-efficacité	MSAC, 2003 ⁵
PCR t(14;18) BCL2-IgH	Lymphome folliculaire	Niveau 2: sensibilité diagnostique inférieure mais meilleur marché que FISH	Evaluation pilote

Ce rapport ne propose aucune recommandation pour l'emploi du test HPV dans le dépistage du cancer du col de l'utérus. Ce sujet fait l'objet d'un projet KCE dont les résultats sont attendus en 2006.

Messages clés

- Très peu de tests CDM étudiés ont déjà fait l'objet de rapports HTA ou de revues systématiques.
- Les tests moléculaires pour l'hépatite C ont une précision analytique et diagnostique suffisante, un impact clinique clair et sont coût-efficaces.
- Les tests moléculaires pour l'entérovirus sont insuffisamment précis sur les plans analytique et diagnostique.
- La détection de t(14 ; 18) peut être utile dans le diagnostic du lymphome folliculaire. La technique FISH reste toutefois supérieure à la PCR sur le plan diagnostique.
- Les tests moléculaires pour la mutation Facteur V Leiden sont probablement suffisamment précis sur les plans analytique et diagnostique. L'impact clinique est insuffisamment documenté.

Abstract

Introduction

Depuis la mise au point de la réaction en chaîne à la polymérase (PCR) en 1983, l'usage et le nombre de tests moléculaires ont connu une croissance considérable.

Pour certaines maladies infectieuses (hépatite C, tuberculose) et pour certaines formes de cancer (leucémies, lymphomes, cancers du sein) les tests moléculaires sont devenus essentiels pour l'initiation et le suivi du traitement. Les tests peuvent être exécutés au moyen de méthodes "maison" ou de trousse de diagnostic in vitro (DIV).

L'Assurance Maladie (INAMI) a opté, lors de l'introduction en 1998 du diagnostic moléculaire dans les soins de santé belges, pour la reconnaissance d'un certain nombre de Centres de Diagnostic Moléculaire (CDM). Par là, les autorités visaient à la fois un objectif scientifique (l'évaluation d'une nouvelle technologie et une garantie de qualité pour les tests), un objectif clinique (la mise en place des tests moléculaires et le développement de recommandations pour les prescripteurs) et un objectif financier (un nombre limité de centres et un carcan budgétaire pour l'Assurance Maladie). En exécution d'une décision de justice de 1998, le nombre de centres a crû de 10 à 18. Le Conseil d'Etat, dans un arrêt rendu au début de 2005, a annulé les bases légales de la convention entre l'INAMI et les CDM.

Etude de l'expérience CDM

L'expérience CDM a introduit un éventail de 94 tests moléculaires en échange pour l'INAMI, d'une enveloppe annuelle fixe de 6,53 millions d'euros. Le volume des tests moléculaires a crû d'année en année aussi bien en microbiologie (117 139 tests en 2004) qu'en hémato-oncologie (29 611 tests en 2004). La nouvelle technologie de PCR en temps réel a permis de réduire à 33 euros en moyenne le coût d'un test PCR exécuté en double. Le coût était inférieur dans les CDM importants.

Les hôpitaux sans CDM ont une opinion nuancée de l'expérience CDM et mettent en avant la nécessité d'une communication plus efficiente et de l'exécution plus rapide de certains tests.

Sur certains plans dont celui de la qualité des tests effectués au sein des CDM, les autorités n'ont pas obtenu de garanties absolues. La majorité des nombreuses méthodes PCR "maison" (développées dans le centre) ne sont pas validées. La participation aux programmes internationaux de contrôle de qualité existants est restée très limitée. Au sein des CDM, il y a eu peu d'activités documentées de normalisation des tests et d'évaluation de l'efficacité diagnostique en clinique ("efficacy"). Ceci constitue une entrave importante à la prise de décisions stratégiques fondées sur une base scientifique. Les objectifs de l'expérience CDM, comme mentionné plus haut, n'ont donc été que partiellement atteints.

Comparaison avec l'étranger

Les fabricants de trousse DIV ont fourni les informations concernant leurs produits. La nomenclature existante pour les tests moléculaires fut obtenue auprès des institutions d'assurance maladie de chaque pays étudié. Plus de 200 trousse DIV pour tests moléculaires portent la mention déposée CE. C'est bien plus que le nombre de trousse pour tests moléculaires commercialisées aux E.U. où la FDA doit évaluer au préalable l'efficacité analytique et diagnostique de chaque trousse. Cette reconnaissance par la FDA aux E.U. est un pré-requis à tout remboursement.

Très peu de pays ont une législation adaptée aux tests "maison" et à l'évaluation de leur qualité. Dans les pays étudiés, la nomenclature concerne les soins ambulatoires et est

plutôt spécifique en microbiologie et générique (selon la méthode) pour les tests moléculaires et cytogénétiques en hémato-oncologie.

Recommandation pour une solution future spécifiquement fondée

Un modèle d'évaluation pour les (nouveaux) tests moléculaires est proposé. Ce modèle se fonde sur une échelle à 6 niveaux depuis une efficacité purement technique (1) jusqu'à une efficacité qui soit coût-efficace (6) ("efficacy"). A partir des niveaux 3-4, on peut parler d'utilité clinique. Une assurance maladie efficace se doit d'investir dans l'expertise nécessaire à ce type d'évaluation.

Le modèle considère d'autre part certaines conditions supplémentaires à remplir pour un usage efficient en routine ("effectiveness"), comme la prescription judicieuse, la qualité du test (accréditation ISO et participation aux contrôles de qualité externes pour tous les tests) et le service offert (exécution prompte et protocole standardisé).

Le Centre Fédéral d'Expertise conseille, pour les tests qui doivent encore être documentés, de ne les exécuter et financer que par le canal d'études où les médecins en charge du traitement peuvent être impliqués. Les tests d'utilité clinique documentée peuvent être introduits dans la pratique clinique journalière et honorés à un coût raisonnable par test. Les tests de volume important en microbiologie peuvent être admis à la nomenclature.

Les tests moins fréquents en microbiologie sont, pour des raisons d'expertise et de qualité, exécutés dans un, voire, quelques centres de référence. Les tests moléculaires en hémato-oncologie prennent place de préférence dans le centre qui effectue les tests cytogénétiques puisque ces tests complexes requièrent une sélection progressive, le choix entre des méthodes complémentaires et une interprétation globale.

Le modèle d'évaluation proposé a été appliqué concrètement à quelques tests moléculaires: détection, dosage et génotypage de l'ARN viral de l'hépatite C (utilité clinique et coût-efficacité); détection de l'entérovirus dans la méningite par PCR (précision analytique insuffisante); translocation t(14;18) dans le lymphome folliculaire par PCR (valeur diagnostique supérieure du FISH vis-à-vis de la PCR); détection du facteur V Leiden dans la thrombophilie par PCR (impact clinique insuffisamment documenté).

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Glossary

ACMG	American College of Medical Genetics	www.acmg.net
AF	Amniotic fluid	
ALL	Acute lymphoblastic leucemia	
AML	Acute myeloid leukemia	
AMP	Association for Molecular Pathology	www.ampweb.org
AP	Anatomo-pathology	
APR-DRG	All Patient Refined-Diagnosis Related Groups	
ARL	AIDS Reference Laboratory	
ASM	American Society of Microbiology	www.asm.org
ASR	Analyte specific reagent	
BAL	Brochoalveolar lavage	
BCSH	British Committee for Standards in Haematology	www.bcsghguidelines.com
bDNA	Branched DNA	
BL/BLL	Burkitt/Burkitt-like lymphoma	
BM	Bone marrow	
CAP	Community acquired pneumonia	
CAP	College of American Pathologists	www.cap.org
CDC	Centers for Disease Control and Prevention	www.cdc.gov
CISH	Chromogenic in situ hybridization	
CLIA	Clinical Laboratory Improvement Act	
CLL	Chronic lymphocytic leukemia	
CLSI	Clinical and Laboratory Standards Institute	www.clsi.org
CMD	Centre for Molecular Diagnosis	http://webhost.ua.ac.be/cmd/index.htm
CMG	Centre for Medical Genetics	
CMGS	Clinical Molecular Genetics Society	www.cmgs.org
CML	Chronic myelogenous leukemia	
CMV	Cytomegalovirus	
CSF	Cerebrospinal fluid	
DG	Directorate general	
DLBCL	Diffuse large B cell lymphoma	
DNA	Deoxyribonucleic Acid	
DORA	Directory of rare analytes	
EAC	Europe Against Cancer	
EBV	Epstein-Barr Virus	
EIA	Enzyme immuno-assay	
EM	Electron microscopy	

EMA	European Medicines Agency	www.emea.eu.int
EMQN	European Molecular Genetics Quality Network	www.EMQN.org
EQA	External quality assurance	
EU	European Union	
EVR	Early virologic response	
FDA	Food and Drug Administration	www.FDA.org
FISH	Fluorescence In Situ Hybridisation	
FL	Follicular lymphoma	
GFCH	Groupe Français de Cytogénétique Hématologique	
GMP	Good manufacturing practice	
HBV	Hepatitis B virus	
HCV	Hepatitis C virus	
HIV	Human immunodeficiency virus	
HO	Hemato-oncology	
HPV	Human papilloma virus	
HSV	Herpes simplex virus	
HUS	Hemolytic uremic syndrome	
IC	Immunocompromised	
ICU	Intensive care unit	
Ig	Immunoglobulin	
IgH	Heavy chain of immunoglobulin	
IHC	Immunohistochemistry	
INAMI	Institut national d'assurance maladie invalidité	http://inami.fgov.be/
IPH	Institute for Public Health	www.iph.fgov.be
IQC	Internal quality control	
ITG	Instituut voor Tropische Geneeskunde	
IUO	Investigational use only	
IVD	In vitro diagnostic	
IVDMDD	In Vitro Diagnostic Medical Devices Directive	
LCR	Ligase chain reaction	
LPD	Lymphoproliferative disorder	
MB	Microbiology	
MCL	Mantle cell lymphoma	
MDS	Myelodysplastic syndrome	
MM	Multiple myeloma	
MRD	Minimal residual disease	
mRNA	Messenger RNA	
MRSA	Methicillin resistant Staphylococcus aureus	
MSAC	Medical Services Advisory Committee	
MTHFR	Methylenetetrahydrofolate reductase	

MZL	Marginal zone lymphoma	
NASBA	Nucleic-acid-sequence-based amplification	www.msac.gov.au
NAT	Nucleic acid test	
NCCLS	Now CLSI	www.clsi.org
NCCN	National Comprehensive Cancer Network	www.nccn.org
NHS	National Health Service	www.nhs.uk
NIH	National Institutes of Health	www.nih.gov
NP	Nasopharynx	
NPA	Nasopharynx aspirate	
PBL	Peripheral blood lymphocytes	
PBMC	Peripheral blood mononuclear cells	
PCR	Polymerase chain reaction	
PMA	Pre-market approval	
QA	Quality assurance	
RCT	Randomised controlled trial	
RD	Royal decree	
RIZIV	Rijksinstituut voor ziekte- en invaliditeitsverzekering	www.riziv.fgov.be
RNA	Ribonucleic Acid	
ROC	Receiver operating characteristic	
RT-PCR	Reverse transcriptase PCR	
RUO	Research use only	
SF	Synovial fluid	
SOP	Standard operating procedure	
SVR	Sustained virologic response	
TAT	Turnaround time	
TCR	T cell receptor	
TMA	Transcription mediated amplification	
VAT	Value added tax	
VRE	Vancomycin resistant Enterococci	
VTEC	Verocytotoxin-producing E.coli	
VZV	Varicella zoster virus	
WHO	World Health Organisation	

I. PROJECT DEFINITION AND RESEARCH QUESTIONS

Molecular diagnostics have seen multiple technological innovations over the last two decades, which have increased the potential impact for the clinical routine. Only recently the technology has emerged allowing the long awaited move of molecular diagnostics to fully automated random access instruments performing sample preparation to data analysis in a minimum amount of time. This evolution will drastically change the molecular laboratory organisation and workflow and could increase the value of molecular diagnostics in the clinic.

The 2004-2005 KCE Health Technology Assessment project on molecular diagnostics concerns the molecular diagnostic testing in Belgium, as being performed at the Belgian Centres for Molecular Diagnosis (CMDs). This project report includes both an assessment of the characteristics of individual tests as well as an evaluation of the organisation, financing, and quality assurance aspects.

For a long time clinical routine testing of DNA and RNA was limited to a few Belgian Centres for Medical Genetics (CMG). The increasing number of clinical research applications of nucleic acid based tests after the invention of PCR has led to the creation of Centres for Molecular Diagnosis (CMDs, Royal Decree of September 24, 1998, published October 22, 1998, <http://webhost.ua.ac.be/cmd/index.html>). The aim of the CMD structure was to network the expertise in molecular diagnostics available in microbiology, hemato-oncology, and pathology departments. Furthermore, each CMD was to form an association with one of the eight Centres for Medical Genetics (CMG). In addition to the National CMD Committee, separate Working Groups were created for microbiology, hemato-oncology and pathology. The most recent list of tests performed at the CMDs includes 94 tests.

According to the Royal Decree of September 24, 1998, the CMDs were to

- inform the hospitals on the molecular tests offered and the indications for testing; the list of tests offered at the CMDs was to be updated every year
- provide routine molecular testing
- guide the specialist formation of laboratory physicians and pathologists with respect to molecular methods
- continuously evaluate the molecular techniques, including IVD kits

In addition, the CMD National Committee was to

- implement and optimize the internal and external quality assurance including participation to international EQA programs
- organize the quality control of the molecular tests included or being included in the nomenclature
- make proposals for the introduction of molecular tests into the nomenclature
- provide guidance with respect to test indications and test interpretation, in the context of an evaluation of the diagnostic value of the tests

Candidate CMDs had to prove expertise in molecular diagnostics and have the appropriate laboratory infrastructure to avoid sample contamination. Given the experimental nature of the CMD structure, funding contracts were for a two year period. The contracts have been renewed thereafter. The number of appointed CMDs was 10* in 1999. This number has increased the first years to 18 CMDs and has remained stable since July 3, 2000.

List of CMDs:

- AZ Sint-Jan, Brugge*
- OCMW Ziekenhuizen Antwerpen (now ZNA), Antwerp*
- UCL Cliniques Universitaires St Luc, Brussels*
- CHU Brugmann, Brussels*
- UZA, Antwerp*
- UZ Gent, Gent*
- UZ Leuven, Leuven*
- CHU de Liège, Liège*
- AZ VUB, Brussels*
- Hôpital Erasme, Brussels*
- Association de diagnostic moléculaire, Loverval
- Virga Jesse OCMW, Hasselt
- O.L. Vrouwziekenhuis, Aalst
- Institut Jules Bordet, Brussels
- H. Hartziekenhuis, Roeselare
- Cliniques Universitaires UCL, Mont-Godinne
- CHR de la Citadelle, Liège
- Centre Hospitalier de Jolimont-Lobbès

Many but not all CMDs are based at a university hospital. The overall budget for the CMDs has remained fixed at 6,54 Mio Euro per year. This fixed overall yearly budget is divided between the CMDs, driven by costs for personnel, invoiced consumables and investments. The most recent agreements for funding of the CMDs were to end January 31, 2006. The Council of State rejected on January 27, 2005 the legal basis of the CMDs, and thus also their further financing. Over the last years 5 molecular microbiology tests have been introduced in the RIZIV/INAMI nomenclature article 24, applicable to any licensed laboratory. This concerns detection of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, HCV qualitative, *Mycobacterium tuberculosis* and *Mycobacterium avium* intracellulare (Royal Decrees of April 29, 1999 and July 16, 2001). The volume of testing, the number of laboratories performing the tests and the costs directly associated with the tests are given in table I. Of note, the majority of tests for *N. gonorrhoeae* and *C. trachomatis* in 2003 were performed by non-CMD laboratories.

Table I. Volume, number of laboratories, and costs directly associated with the molecular tests currently covered by the clinical biology nomenclature.

		<i>N. gonorr.</i>	<i>M. tuberc.</i>	<i>M. avium</i>	HCV qual.	<i>C. tracho.</i>
Ambulatory	N tests 2003	15092	284	17	4915	28689
	N tests 2004*	16464	290	28	5314	31868
	N of labs 2003**	19	9	0	30	46
	Costs 2003***	41 545 €	3 892 €	233 €	27 032 €	78 987 €
Hospitalized	N tests 2003	603	704	29	1273	1152
	N tests 2004*	864	854	42	1236	1648
	N of labs 2003**	7	18	0	13	14
	Costs 2003***	1 659 €	9 658 €	397 €	6 999 €	3 167 €

* The number of tests for 2004 was extrapolated based on the data for the first 6 months of 2004

** Only laboratories performing more than 10 tests per year

*** Costs directly paid by RIZIV/INAMI to laboratory (rule: 25% of overall cost).

It should be noted that in addition to cytogenetic testing, some of the CMGs also perform a broad range of molecular tests for hemato-oncology and pathology. CMGs receive reimbursement for the hemato-oncology tests using a generic nomenclature (RIZIV/INAMI article 33), in fact created only for human genetic testing (Royal Decree July 22, 1988). No differentiation is made between simple and more complex tests based on DNA hybridization. In contrast to the funding of regular laboratories, article 33 tests are financed entirely on a test volume basis. The total cost for tests reimbursed to the centres amounted 30,8 Mio Euro in 2003. The costs for tests based on DNA hybridization amounted to 15,7 Mio Euro in 2003 and 8,5 Mio Euro for the first half of 2004. The current nomenclature nor the activity reports of the CMGs provide the volume and cost details of specific tests performed.

Out of the scope of this project are molecular diagnostic tests performed at the AIDS Reference Laboratories (ARLs), tests performed for blood supply screening, tissue typing, epidemiological surveillance, research, industrial or forensic purposes. The broad field of human genetic testing has mainly been left out of scope. HPV screening is the subject of a separate KCE project.

The project tries to answer the following research questions.

- Which are the molecular diagnostic tests in use and what are their characteristics ?
- Do the tests fulfil the diagnostic requirements for appropriate clinical use? (analytical and diagnostic accuracy, clinical utility, utility for society)
- Does the current implementation meet the health care service needs in Belgium?
- How can the implementation further be optimized with respect to organisation, financing and quality?

2. PEOPLE AND METHODS

The project was conducted by the KCE project team in accordance with the KCE procedures. Test quality aspects were covered by the Clinical Biology Department of the Scientific Institute of Public Health (IPH). The characteristics of the CMD tests were documented with the help of the experts in the CMD Working Groups, the existing CMD reports and as well as a basic literature search. An estimate of testing cost was based on invoices reported by the CMDs. Documentation on IVD kits for the molecular tests was obtained from the manufacturers. The clinical need for molecular testing and the feedback on the service provided by the CMDs to the requesting physicians was documented at 6 non-CMD hospitals. A more detailed pilot assessment of the clinical utility and the local situation was performed for a small number of tests: hepatitis C tests, enterovirus, PCR t(14;18) in follicular lymphoma, and Factor V Leiden (the only non-CMD genetic test evaluated in the context of this project). Each pilot assessment underwent external review by a group of experts, including clinical experts. International information on test organisation, financing and test quality aspects were obtained from the relevant institutions abroad. A number of available EU documents on the related field of genetic testing served also as a reference. Finally, the methods used and progress made for this project, were assessed at regular intervals by an external Process Steering Group (Klankbordgroep). This report was also validated by this group of experts.

2.1. METHODS FOR EVALUATING DIAGNOSTIC TESTS

A diagnostic test can be evaluated at several levels. Six possible levels have been described by Pearl⁶, starting from a more technical/analytical level to the test impact on patient outcome and society. These levels are discussed in detail in the context of a framework for the evaluation of molecular tests (section 5.5). It is important for the reader to distinguish the analytical validity (also named technical or analytical accuracy and including analytical sensitivity and specificity) from the clinical validity (also named diagnostic accuracy or clinical accuracy and including diagnostic sensitivity and specificity, ACMG Standards and Guidelines, www.ACMG.net). In addition clinical validity should be distinguished from clinical utility. The following definitions can be used⁷.

Analytical validity refers to how well a test performs in the laboratory - that is, how well the test measures the property or characteristic it is intended to measure. In other words, does the test do what its makers claim it does? If so, it must produce the same results repeatedly and in different laboratories (given the same set of procedures).

Clinical validity refers to the accuracy with which a test predicts the presence or absence of a clinical condition or predisposition. Initially, the test has to be conducted on individuals who are known to have the condition (as well as those who do not) to determine its success rate.

Clinical utility refers to the usefulness of the test and the value of information to the person being tested. If a test has utility, it means that the results - positive or negative - provide information that is of value to the person being tested because he or she can use that information to seek an effective treatment or preventive strategy. Even if no interventions are available to treat or prevent disease, there may be benefits associated with knowledge of a result.

A phased process of assessment has been suggested for diagnostics^{8,9}.

- Phase I: Establishing the normal range
- Phase II: Establishing sensitivity and specificity and other measure of diagnostic accuracy
- Phase III: Randomised trials to determine whether patients benefit from the testing.
- Phase IV: Large continuous surveillance studies to identify consequences of testing in clinical practice.

Similar to pharmaceutical development, the clinical studies of a diagnostic test have also been grouped into exploratory type of studies, followed by challenge studies, and then by large scale prospective confirmatory studies¹⁰. For most of the molecular diagnostic tests considered in this report, and for most IVDs in general, no large prospective phase III studies have been reported.

In contrast with treatment trials often sponsored or co-sponsored by the pharmaceutical industry, the IVD industry is more reluctant to sponsor expensive and large scale prospective trials, certainly in regions of the world where no regulatory requirements impose such practice, or when the potential return does not justify the investment. The clinical validation of markers predictive of treatment response is best achieved using specific trial designs. For cancer treatment trials two classes of clinical trial designs have been proposed: a 'marker by treatment interaction' design and 'marker-based strategy' design¹¹. A quality grading system for diagnostic clinical studies has also been published^{12,13}. Specific methods are available for the examination of heterogeneity in systematic reviews of diagnostic test accuracy.¹⁴ In-house methods are still frequently used for many molecular diagnostic tests. The level of test robustness and test validation are important if laboratories want to implement such methods¹⁵. Also if a test kit procedure is modified the modification needs to be fully validated by the user as is the case for all in-house methods.

2.2. METHODS USED IN THIS PROJECT

The methodology followed for this project can be summarized into 5 steps as follows. Documents were preferably to be prepared in electronic format and in English language.

Step I: Define the tests under evaluation.

The tests listed at the CMD web site (<http://webhost.ua.ac.be/cmd/index.html>), was used as a starting point and fine tuned with the three CMD working groups. This resulted in a list of 94 molecular diagnostic tests (38 in microbiology, 34 in hemato-oncology and 22 in pathology) detailed in **table 2**. Some of the tests showed some overlap between specialties such as HPV and EBV (overlap microbiology with pathology), and some of the lymphoma tests (overlap hemato-oncology with pathology). Cytogenetic analyses for hemato-oncology are traditionally performed at centres for medical genetics. The introduction of the interphase FISH technique has lowered the entry barrier for other laboratories. Some of the microbiology testing performed at some of the CMDs is part of their reference centre activity. A recent proposal¹⁶ by the IPH for assuring the quality and financing of this type of activity should thus be considered together with this report.

Table 2. List of molecular diagnostic tests performed at the CMDs

Microbiology tests	Hemato-oncology tests	Pathology tests
<i>Bartonella henselae</i> , <i>Bartonella quintana</i>	Ig rearrangements in NHL	HPV (see also microbiology)
<i>Bordetella pertussis</i> ^{17, 18, 19}	Ig rearrangements in AML/ALL	EBV (see also microbiology)
<i>Borrelia burgdorferi</i> ²⁰	t(2;5) NPM-ALK	B cell monoclonality in lymphoma (see Ig rearrangements under hemato)
<i>Chlamydia pneumoniae</i> ²¹	TCR rearrangements in NHL	T cell monoclonality in lymphoma (see TCR rearrangements hemato)
<i>Corynebacterium diphtheriae</i>	TCR rearrangements in AML/ALL	t(14;18) in follicular lymphoma (see under hemato)
<i>Escherichia coli</i> (VTEC) ²²	IgVH sequencing ²³	t(1;14) in mantle cell and anaplastic lymphoma
Enterococci (Vancomycin resistant) ^{24, 19}	Patient-specific PCR	t(11;14) in mantle cell lymphoma (see under hemato)
<i>Helicobacter pylori</i> (macrolide resistance)	t(1;14) SIL-TAL	t(8;14), t(8;22), t(2;8) in Burkitt lymphoma
<i>Legionella pneumophila</i> ²⁵	t(1;19) E2A-PBX	t(2;5) in anaplastic lymphoma (see under hemato)
<i>Mycoplasma pneumoniae</i> ²⁶	t(12;21) TEL-AML1	inv(2) in anaplastic lymphoma
<i>Mycobacterium tuberculosis</i> (direct and culture) ^{27, 28}	MLL 11q23 translocation t(4;11) AF4-ALL1 in ALL and AML ²⁹	t(11;18) in MALT lymphoma
<i>M. tuberculosis</i> (resistance genes) ³⁰	t(8;21) AML1-ETO ⁴	Neu/HER2 in breast carcinoma
Staphylococci (resistance genes) ^{31, 19, 32}	t(15;17) PML-RAR ^{5, 33}	m-RNA neuroendocrine products – nesidioblastosis
Identification of bacteria difficult to identify	inv16 MYH11-CBCF ⁴	m-RNA neuroendocrine products - graft
Molecular typing of nosocomial pathogens	MLL 11q23 translocation t(9;11) MLL-AF9 in AML	m-RNA receptors - nesidioblastosis
Cytomegalovirus (CMV) qualitative ³⁴	FLT3	m-RNA somatostatin receptor
Cytomegalovirus (CMV) quantitative ^{35, 36, 37, 38}	WT1	Aneuploidy in Transitional Cell Carcinoma
Epstein-Barr virus (EBV) qualitative ³⁹	MLL translocations in AML	LOH 1p-19q
Epstein-Barr virus (EBV) quantitative	t(11;14) JH-BCL1 qualitative	EGFR gene amplification/mutation
Hepatitis B virus DNA quantitative	t(14;18) JH-BCL2 qualitative	t(X;18) in synoviosarcoma
Hepatitis B virus DNA qualitative	t(11;14) JH-BCL1 quantitative	t(11;22) in Ewing sarcoma
Hepatitis C virus (HCV) qualitative	t(14;18) JH-BCL2 quantitative	t(X;13) in alveolar rhabdomyosarcoma

Hepatitis C virus (HCV) quantitative	t(8;14) JH-MYC and variants	
Hepatitis C virus (HCV) genotyping	cyclin-D1 overexpression	
Human Papillomavirus (HPV)	trisomy 12	
Enterovirus	t(9;22) BCR-ABL in CML diagnosis ³³	
Herpes simplex virus ^{40, 41, 34, 42}	t(9;22) BCR-ABL in ALL diagnosis ⁴³	
Human herpesvirus type 8 (HHV8)	t(9;22) BCR-ABL in CML follow up ^{44, 45}	
Parvovirus B19 ^{46, 47}	t(9;22) BCR-ABL in ALL follow up	
Polyomaviruses JC and BK	HUMARA	
Rubella virus	PRV1 ⁴⁸	
Varicella Zoster Virus (VZV) ³⁴	Chimerism	
Toxoplasma <i>gondii</i>	t(6;9)	
Aspergillus ⁴⁹	t(11;18)	
Candida		
Identification fungi		
Pneumocystis <i>jiroveci</i> (<i>carinii</i>)		

Step 2: Define and collect the key variables for each test

First the key test variables were defined. Key variables are test variables considered of relevance for an optimal implementation. Then those variables are collected for each test using the available sources (CMD experts, requesting clinicians, industry, literature, ..) and summarized in a tabular format.

Volumes and INAMI/RIZIV reimbursed cost per test for tests included under specific nomenclature were obtained from INAMI/RIZIV. The CMD activity reports (activity tables for February 1, 2003 to January 31, 2004 are given in appendix I) provide an excellent overview of the number of specific tests performed and the proportion of positive tests. The volume of a specific test reported by the CMDs may not be fully representative for Belgium as some haematological and other oncology tests are also performed at the CMGs.

The level of detail of the reports for the CMD QA rounds (<http://webhost.ua.ac.be/cmd/index.html>) varies by report, and the results demonstrate the need for continued QA efforts in this area. The reported CMD personnel costs and invoices for reagents and instrumentation, in theory allow for the calculation of a cost per test.

The clinical need for molecular testing and the feedback on the service provided by the CMDs to the requesting physicians was documented using a structured interview with the main requesting clinicians in 6 non-CMD hospitals. The hospitals were visited by a KCE expert to conduct the interviews. Also the interface role of the local laboratory was documented this way.

In accordance with Article 12 of the In Vitro Diagnostic Medical Devices Directive 98/79/EC a European database, accessible to the Competent Authorities has been created to hold relevant data relating to registration of manufacturers and their IVDs. Currently this database is however not yet operational. A list of manufacturers of IVD kits was therefore obtained from the Belgian Association of IVD Manufacturers (FIDIAG/Pharma.be). However, as some IVD manufacturers are not a member of FIDIAG, other sources of information were also explored, including the responses to the CMD method questionnaires, a basic literature search, and a visit to the

MEDICA trade show. The head of regulatory of each manufacturer was then contacted by Dr. Jean-Claude Libeer, IPH, Competent Authority for Belgium, and was requested to provide the list of marketed molecular diagnostics and their test performance data. In most cases, the product insert was received, containing most of the requested information. Tables with the kits marketed in the EU for the CMD tests as well as the molecular diagnostics approved or cleared by the FDA are given in appendix 2. In case of doubt on the regulatory status of the products, the company received an extract from the database with their products for verification and completion. This table is a best efforts result without any claim of completeness or accuracy.

A method questionnaire (sample form in appendix 3) and an expert questionnaire (sample form in appendix 4) were used to document the tests in use at the CMDs. The method questionnaire was e-mailed to all CMDs, with the request to complete this short questionnaire for each of the molecular methods in use. It covered the test method used (in-house or kit), the time to result and the participation of the lab to external quality assessment schemes. Each CMD was also requested to provide a copy of the Standard Operating Procedure (SOP) for performing the test, as well as the summary pages of the test method validation report, if available. As laboratory accreditation efforts for molecular diagnostics have only started over the last years, this item was not yet collected.

For each test, the expert appointed by the CMD working group completed an expert questionnaire with the following set of questions concerning the existing test methods, their performance characteristics, clinical utility, and the expected 5 year evolution. Each completed questionnaire was circulated for review to all CMDs before being considered final. The items collected can be summarized as follows.

Test Method / Indication and Analytical Performance Characteristics

- The diagnostic test, the target population and the test indication.
- A comparison with alternative techniques or test methods.
- The biological material needed for the test method.
- Data on between-lab reproducibility of the test method.
- Diagnostic accuracy data. In absence of a 'gold standard': what does this test add in comparison with other tests?

Aspects of Clinical Utility and Cost-benefit

- Proportion of positive tests and clinical decisions/actions that follow a positive test result (or typing result).
- Proportions of routine tests which are non-interpretable, false positive or false negative, and the impact on patient health and health care costs. For typing assays: what is the proportion of incorrect typing results? For quantitative tests: what is the proportion of incorrect measures?
- Number of samples expected to get tested in Belgium per year?
- Critical appraisal of the clinical utility of the used test method/indication in comparison with existing method or no testing, whatever is appropriate.
- Cost per test performed.
- Critical appraisal of the cost-effect of the used test method/indication.
- Impact of fully reimbursing this test via the 'nomenclature'?

Expected 5 year evolution

- Any major change in indication, number of tests performed, or in test method (kit, automation, ...) within the next 5 years?

In addition to the CMD/CMG structure a small number of clinical routine molecular tests are also performed in laboratories outside of the CMD/CMG structure, as assessed in 2003 by the IPH using a questionnaire mailed to all Belgian routine labs, including the CMDs. The report of this survey (data kindly provided by Dr K Vernelen, IPH) shows molecular diagnostics were in use at 45 out of the 203 labs participating to this survey. The tests most frequently offered were

HCV qualitative (n=20) and quantitative (n=17), *Chlamydia trachomatis* (n=17) and *Mycobacterium tuberculosis* complex (n=15). Of these tests, only HCV quantitative was not included in the nomenclature of 2003 and it is assumed this test is performed exclusively at CMDs. A small number of genetic tests was also offered, the most frequently offered genetic test being Factor V Leiden (n=8).

Step 3: Pilot assessments

A detailed evaluation of all molecular tests being performed at the CMDs was not possible within the scope of this project and ready-to-use guidelines are scarce in this area. Only a few tests were selected for a more detailed assessment (pilot assessment). The methods used for such a pilot assessment include a systematic review of the literature, followed if feasible and appropriate by a calculation of the number of tests expected in Belgium and the associated costs. Both the literature review and financial impact data were reviewed by a panel of experts, including clinical experts in the field of interest. The overall goal of the pilot assessments was to define a framework for the evaluation of molecular diagnostics.

The aim was to select tests which could be considered representative for a group of tests. Two test cases were selected first by the Project Steering Group. As representative for high volume and well-documented microbiology tests, the molecular diagnostics in hepatitis C were selected (HCV RNA qualitative and quantitative, HCV genotyping). As a representative of a lower volume, less standardized microbiology test, enterovirus detection in meningitis was selected.

Two pilot assessments were added later. As a representative of a rather high volume molecular test in hemato-oncology PCR for t(14;18) in follicular lymphoma was selected. Finally, also factor V Leiden was included as a pilot assessment as this test is a frequently performed genetic test outside of the centres for medical genetics.

Step 4: Comparison with other countries

A comparison in terms of organisation, financing and quality has been performed versus other countries for the tests under study. A closer look to the related field of genetic testing was also found of interest as some further steps have already been taken for this field at EU level.

Financial data are reported by the CMDs in their yearly activity report. Invoices for reagents and personnel were reviewed and used to estimate a cost per test. Reimbursement fees for molecular tests were obtained from agencies in the neighbour countries.

Comparative information and guidelines on quality measures appropriate for in vitro diagnostics in general and molecular diagnostics in particular were obtained from the Institute for Public Health (IPH). A collaboration agreement with the IPH was signed for this purpose.

Step 5: Recommendations

The testing needs and the associated costs are estimated, based on the collected information. Recommendations are provided with respect to organisation and financing as well as quality. For quality aspects the expert advice by the IPH was incorporated into the recommendations.

3. INTRODUCTION TO MOLECULAR DIAGNOSTICS

3.1. EVOLUTION OF TECHNIQUES AND USE IN MICROBIOLOGY

PCR, from bench to bedside

The first nucleic acid assay for detection of *Mycobacterium tuberculosis* in culture was published in 1987⁵⁰ and was based on DNA hybridization. After the invention of the Polymerase Chain Reaction (PCR) technique in 1983 by Kary Banks Mullis at Cetus corporation, the first PCR based microbiology kits (HIV-I and *Chlamydia trachomatis*) were introduced in 1992⁵¹. It took until 1995 to see the first commercially available semi-automated amplification-detection systems as well as the first standardized PCR quantification kits for HIV-I and HCV. Amplification techniques can be subdivided into target-amplification techniques (PCR, ligase chain reaction or LCR, transcription mediated amplification or TMA, nucleic-acid-sequence-based amplification or NASBA,...) and signal-amplification techniques (branched DNA or bDNA,...). PCR is still the most commonly used amplification technique. Multiplex PCR enables the simultaneous detection of several target sequences by incorporation of multiple sets of primers. To increase sensitivity and specificity, a double amplification step can be done with appropriately designed “nested” primers. Amplification may be made less specific using degenerate primers to detect divergent genomes by randomising portions of the primer sets. Finally, RNA (rather than DNA) can be detected by converting RNA into a complementary DNA copy, and then amplifying (so-called reverse transcriptase PCR, or RT-PCR), enabling evaluation of RNA viruses or gene expression.

The success of PCR and the advantage of heightened sensitivity have been offset by demanding or exacting assay conditions. Because “all-inclusive,” automated clinical instruments for molecular microbiology are not yet available, laboratories currently run assays in semi-manual formats. This requires careful assessment of the functional reliability of the instruments and equipment employed. These include procedures for DNA and RNA extraction, temperature control, and prevention of molecular contamination of reactions by amplified products from previous reactions leading to false-positive results. To avoid problems, fastidious laboratory practices must be employed, eg use of separate rooms for pre- and post-amplification work, and enzymatic inactivation of carry-over DNA. In addition, quality assurance (QA) measures and experimental controls must be carefully designed and executed. The quantification of nucleic acid further adds to the test complexity. Furthermore, many qualitative and quantitative molecular tests are still performed using in-house developed (“home-brew”) PCR methods, which are often not validated. The competence of staff at performing laboratory tasks is also considered a critical factor to control contamination.

Real-time PCR methods

A significant advancement in PCR technology has been the introduction of quantitative real-time PCR, in which amplification and detection of amplified products are coupled in a single reaction vessel⁵². Real-time PCR monitors the fluorescence emitted during the reaction as an indicator of amplicon production during each PCR cycle (ie, in real time) as opposed to the endpoint detection. Formats used for real-time PCR are still evolving (<http://www.gene-quantification.de>) and include non-specific intercalating (DNA minor groove binding) dyes such as SYBR green and sequence specific fluorescent probes such as TaqMan probes and molecular beacons.

SYBR green, when unbound in solution emits only minimal fluorescence. After the primer binds, SYBR green intercalates within the DNA, emitting a significant amount of fluorescence if excited. The SYBR green method is a relatively low-cost but non-specific amplification since mis-priming or primer-dimer artefact will also generate signal.

Hydrolysis probes (eg Applied Biosystems ABI TaqMan Probes) are oligonucleotides that contain a reporter fluorescent dye, and a quenching dye at the other end. When irradiated, the fluorescence emitted by the excited fluorescent dye is captured by the nearby quenching dye molecule. TaqMan probes are designed to anneal to an internal region of a PCR product. When the polymerase replicates a template on which a TaqMan probe is bound, its 5' exonuclease activity cleaves the probe. This ends the activity of quencher and the reporter dye fluorescence is now detectable and increases in each cycle proportional to the rate of probe cleavage.

LC™ Hybridization Probes (HybProbes, Roche's real-time online LightCycler™ PCR System) use two sequence specific, fluorescently labelled oligonucleotide probes, called Hybridization Probes. After reaching the annealing temperature, PCR primers and HybProbes hybridise to their specific target regions. The donor dye now comes into close proximity of the acceptor dye. Energy emitted from the donor dye excites the acceptor dye, which now emits red light of 640 or 705 nm.

Molecular beacon probes also contain a fluorescent reporter and a quencher molecule at their end. Molecular beacons form a closed hairpin structure at ambient temperature bringing the quencher close to the reporter molecule thus preventing fluorescence from occurring. By raising the temperature to 95 degrees Celsius both the double stranded DNA and the molecular beacon are denatured. The temperature is then quickly lowered causing the DNA primers and the molecular beacon to anneal to the target sequence, increasing the distance between fluorescent molecule and its quencher. Fluorescence in this stage becomes detectable. Raising the temperature to initiate replication will dissociate the molecular beacon probe which will return to its closed configuration, no longer able to emit reporter fluorescence.

Real-time amplification reactions are characterized by fluorescence appearance during the exponential phase of amplification when none of the reagents is limiting and, therefore, allowing a more precise real-time quantification. This eliminates the need for laborious post-amplification processing (ie, gel electrophoresis) conventionally needed for amplicon detection. Note: post-amplification steps may however still be needed in specific cases (see below). Also the chances of carryover contamination are reduced. Being based on fluorescence detection, this technique allows analysis of minimal amounts of template with high sensitivity. Development of automated instrumentation with quantitative capacity insures reproducibility. Many instruments allow for the use of multiple formats. Other advantages over conventional PCR are speed, simplicity, an increase in dynamic range of detection and the requirement of less RNA than conventional assays. Speed is also increased because of the short amplification cycles used.

A search for all articles published in the Journal of Clinical Microbiology from 2000 through 2003 which evaluated real-time PCR as a test method for pathogen detection and/or identification of genes or mutations associated with antimicrobial resistance in pathogens revealed a total of 109 articles. Among these articles, 84 described assays with the LightCycler instrument (Roche Diagnostics Corporation, Indianapolis, Ind.); 21 described assays with the ABI PRISM 7000, 7700, or 7900H instrument (Applied Biosystems, Foster City, Calif.); 2 described assays with the SmartCycler instrument (Cepheid, Sunnyvale, Calif.); and 2 described assays with the iCycler instrument (Bio-Rad Laboratories, Hercules, Calif.). The availability of nucleic acid-based technology, such as real-time PCR, along with conventional staining and culture methods and immunoassays, can provide laboratories of many sizes with a comprehensive and responsible approach to the detection of both commonly encountered and emerging or re-emerging pathogens¹⁹. However, because of the many possible pitfalls, some consider quantitative real-time RT-PCR still a research tool for the time being⁵³.

Generally two strategies can be performed in real-time RT-PCR. The levels of expressed genes may be measured by absolute or relative quantitative real-time RT-PCR. Absolute quantification relates the PCR signal to input copy number using a calibration curve, while relative quantification measures the relative change in mRNA expression levels. Relative quantification is easier to perform than absolute quantification because a calibration curve is not necessary. It is based on the expression levels of a target gene versus a housekeeping gene (reference or control gene) and in theory is adequate for most purposes to investigate physiological changes in gene expression levels.

All real-time PCR probe/beacon based chemistries allow simultaneous detection of multiple DNA species (multiplexing) by designing each probe/beacon with a spectrally unique fluor/quench pair or make use of a difference in melting temperature between genetic variants. DNA microarrays may also be used for the sometimes complex spectral analysis of such reaction. Normally SYBR green is used in singleplex reactions, however when coupled with melting point analysis, it can be used for multiplex reactions. One-step real-time RT-PCR performs reverse transcription and PCR in a single buffer system and in one tube. In two-step RT-PCR, these two steps are performed separately in different tubes. For multiplex real-time RT-PCR, one-step PCR remains a technical challenge.

Random access instruments for automated DNA/RNA extraction are being linked to thermocyclers capable of running separate real-time PCR reactions in parallel. They may provide results in just 30 minutes and will change the current batch-oriented workflow in the molecular lab. Such instruments will probably first be used for indications benefiting from a round the clock service, and could trigger the introduction of DNA amplification techniques into most routine laboratories, or even as a point of care test.

Use in microbiology

The microbiological diagnosis relies on identification of a microbial pathogen in clinical specimens, or the immunological response of the host. Molecular methods using DNA probes, nucleic acid hybridization, and amplification reactions are promising substitutes for the conventional methods of diagnosis, such as isolation of pathogens in culture, serology, antigen detection and microscopic visualization. These new molecular techniques often save time, can obviate the need for in vitro culture, have excellent specificity and, in some cases, offer enhanced sensitivity⁵⁴. Based on an extensive literature review⁵⁵, the process of introducing a molecular assay into the clinical microbiology laboratory can be broken down into 4 major components: (1) initial phase of assay development, (2) polymerase chain reaction assay verification in which analytic sensitivity and specificity is determined, (3) assay validation to determine clinical sensitivity and specificity, and (4) interpretation of results and ongoing quality assurance activities.

In case of viral infections, NATs have already today replaced most of the virus isolation work, and also bacterial culture systems have been replaced by PCR in selected cases. This evolution is not without risk as was illustrated by the 'outbreak' of pertussis in New York State, caused by false positive PCR reactions¹⁷. This event highlights the importance of appropriate clinical laboratory quality assurance programs, of the limitations of the PCR test, and of interpreting laboratory results in the context of clinical disease. PCR is of use for the detection of organisms with fastidious growth requirements, or those refractory to in vitro culture, e.g., mycobacteria (culture can take weeks), *Legionella* (culture takes a few days), mycoplasma (difficult to culture), *Borrelia burgdorferi* (very difficult to culture), human immunodeficiency virus (HIV), and other sexually transmitted diseases such as *Chlamydia trachomatis*. The sensitivity of the NAT also depends on the nucleic acid extraction method used, as shown for HSV real-time PCR⁵⁶.

Quantification of viral load is already well established for HIV-I, HCV and HBV, where it has proven useful in assessing disease severity or monitoring treatment efficacy. For EBV and CMV³⁸ the interpretation of quantitative assessments is still under study, as is the clinical advantage of quantitative CMV DNA over pp65 antigenemia^{57,58}. A short test turnaround time is not so much needed for chronic viral infections eg chronic hepatitis B and C, but this variable could be critical eg in cases of viral meningo-encephalitis^{59,42,60} or in monitoring immunosuppressed patients⁶¹. HCV genotyping is now performed routinely to guide interferon-based treatment, while genotyping of HBV may also prove of use in treatment selection.

Bacterial culture, a relatively inexpensive technique, so far has not been replaced by molecular methods. In absence of a rapid and reliable method for pathogen identification a conservative management approach is adopted in the acute care setting using empiric intravenous antibiotic therapy. Using primers for conserved regions, eg of the 16S rRNA gene, PCR can theoretically establish the presence of any bacteria in an otherwise sterile clinical specimen⁶², after a comprehensive validation⁵⁹. As an example, a UK government sponsored RCT has been announced to evaluate the role of molecular diagnosis of central venous catheter associated infections⁶³. The same concept of broad spectrum pathogen detection could theoretically also be used for viruses or fungi. Significant technical difficulties in automation, multiplexing, and avoiding false positives are to be overcome. Such panel tests may be of clinical value eg for meningo-encephalitis, pneumonia, or sepsis. Perhaps even more technically challenging is the detection of microbial resistance using PCR based tests. This has proven feasible and reliable for detection of methicillin resistant *Staphylococcus aureus* (MRSA)³² and for rifampicin resistance in *M tuberculosis*. PCR followed by sequencing is now the standard method used to test for mutations conferring HIV-I drug resistance.

Surveillance programs for MRSA and VRE (vancomycin resistant Enterococci) may benefit from rapid, sensitive and easy-to-perform tests like the LightCycler⁶⁴ and Smart Cycler⁶⁵ nucleic acid-based tests. The new instruments allowing routine around-the-clock service and without the

need for highly trained personnel could also prove useful for the rapid detection of *B. pertussis*, *M. tuberculosis* testing, HSV, enterovirus or Group B streptococcus.

3.2. USE IN HAEMATO-ONCOLOGY AND ONCOLOGY

Molecular and cytogenetic techniques

Molecular techniques are of increasing practical importance in the analysis of haematological and other malignancies, for diagnostic purposes, in order to evaluate prognosis, to monitor minimal residual disease and to select specific treatment²³. Chromosomal abnormalities include Ig/TCR rearrangement errors and translocations. Chromosomal translocations may be detected directly (genomically) or by the associated fusion RNA transcript.

At the genomic level the chromosomal breakpoints are typically in introns and may occur anywhere within a several-kilobase region. These regions are often too large to be spanned by conventional amplification protocols, making molecular detection of the chimeric gene impractical. PCR can be used for the detection of the t(11;14) bcl-1/IgH rearrangement in about half of patients with mantle cell lymphoma and for the detection of t(14;18) bcl-2/IgH rearrangement, most frequently associated with follicular lymphoma (see also pilot assessment).

RT-PCR circumvents the problem of intron breakpoint variability for detection of many other translocations. The structural requirements for oncogenesis lead to a focusing of the points of fusions within the chimeric mRNAs. This leads to predictable patterns of fusion mRNAs generated from the derivative chromosomes and relatively small, easily amplified fusion sequences. In addition to patient-to-patient variability in fusion point, alternative splicing patterns are to be considered in designing RNA amplification-based molecular diagnostic tests. The most common technique employed is RT-PCR. In the design of such a test the choice of primers is critical. Multiplex PCR in a single tube can be used to screen for a number of common leukaemia-associated translocations³³. Note that validation of the individual components is not a substitute for validation of the multiplex format.

A quantitative analysis of the fusion transcript can be of greater utility than qualitative results, eg increasing levels of BCR-ABL fusion transcript in CML or ALL can reflect increased transcriptional activity or increasing tumor burden and has been shown to predict clinical relapse⁴⁵. The source and handling of the RNA can be critical for this application⁴⁴. Also critical for the analysis of residual disease is the test validation, including the validation of the selected normalisation strategy to control for experimental error introduced during the multistage process required to extract and process the RNA⁶⁶. Validation includes the precision of the assay over the full range of clinically relevant quantitation, using multiple, independent RNA sources.

In addition, hybridisation probe assays have evolved. These assays use chemiluminescence or fluorescence (eg FISH) for detection. An increasing number of largely complementary techniques targeting specific chromosomal abnormalities have thus become available (Southern blotting, PCR amplification of DNA or RNA, and FISH). It is necessary to evaluate their relative roles and to assess their contribution with respect to classical techniques, including morphology, immunophenotype and karyotype analysis. Prudent clinical use of molecular laboratory methods requires a thorough understanding of the sensitivity and technical artifacts associated with these methods, extreme care in assay performance (eg to avoid carry-over of amplification products), and the ability to prudently weigh the results, together with clinical findings and histology, to arrive at a diagnosis.

The standard method for genome-wide screening is still the cytogenetic banding technique (karyotyping). This test provides a low-power screening method for detecting dislocated or missing chunks of chromosomes, in contrast to the tests targeting specific chromosomal abnormalities. Many of the dangerous rearrangements as seen in leukaemia and lymphoma can be detected using standard cytogenetics, but not all. For example, the t(12;21), associated with a favourable prognosis, is detected in about 25% of childhood ALL cases using molecular techniques but is frequently missed using karyotyping. Karyotypic analysis requires the in vitro induction of metaphases to obtain individual chromosomes for identification after banding, which is a slow and labor-intensive process. A cost-effectiveness comparison of karyotyping with PCR for acute leukemias at diagnosis demonstrated that sequential strategies are more cost-effective than simultaneous use of both techniques for minimising the risk of false negatives⁶⁷.

Especially the use of FISH assays for molecular evaluation of malignant lymphomas and leukemias has increased remarkably over the past years, and has blurred the lines between classical cytogenetics and molecular pathology. FISH allows the study of chromosome exchanges and gene rearrangements, amplifications, and deletions at the single-cell level. FISH can be done on blood, bone marrow, tissue touch preparations (interphase or metaphase cells attached to glass microscope slides), body fluids, and even paraffin-embedded fixed tissue. FISH overcomes one of the biggest problems with routine cytogenetic analysis of many lymphoma and chronic leukemia samples (i.e. the need for metaphases in tumors with only a small number of dividing cells), as FISH can be done with either metaphase or interphase preparations. Interphase FISH may thus be possible in case metaphase induction is not possible, a situation which is not infrequent²³. FISH assays are also particularly useful in detection of chromosomal translocations that are not amenable to PCR detection due to widely distributed breakpoints, because FISH probes are much larger than the probes and primers used in PCR analysis. FISH assays may also detect some genetic abnormalities that are karyotypically silent⁶⁸.

Genomic probes for the genetic abnormalities of many leukemias, lymphomas, and even myeloproliferative and myelodysplastic disorders are now readily available from commercial sources. Most FISH assays are based on the ability of single stranded DNA to bind (hybridize) to complementary DNA. Some RNA FISH assays are also available. There are several different strategies for the design of FISH assays. Single fusion-dual color FISH assays for translocations utilize 2 probe hybridization targets located on 1 side of each of the 2 genetic breakpoints. Dual fusion-dual color FISH assays for translocation utilize large probes that span 2 breakpoints on the different chromosomes. FISH using dual color-break apart probes is very useful in the evaluation of genes known to have multiple translocation partners; the differently colored probes hybridize to targets on opposite sides of the breakpoint in the known gene. This split-signal FISH approach has three main advantages over the classical fusion-signal FISH approach⁶⁹. First, the detection of a chromosome aberration is independent of the involved partner gene. Second, split-signal FISH allows the identification of the partner gene or chromosome region if metaphase spreads are present, and finally it reduces false-positivity. Multicolor FISH using 3 to 4 differently colored probes can be done in selected cases to determine the overlap of different genetic abnormalities in different cell populations. FISH with centromeric probes is useful for detection of changes in chromosome number (i.e., monosomy, diploidy, trisomy).

It should be remembered that FISH assays are useful mainly around the time of initial diagnosis, in the early phases of treatment or at relapse, when there is a relatively high level of abnormal cells⁴⁵. FISH is not useful for detection of low level minimal residual disease (MRD) following therapy, as the sensitivity of even the best dual fusion-dual color FISH assay is only approximately 1 positive cell in 100 normal cells, not sufficient for detection of MRD.

Another FISH-based technique, comparative genomic hybridization (CGH), can identify chromosome losses and gains in tumor cells without prior knowledge of the chromosomal loci involved. The capacity to hybridize simultaneously 24 or more DNA probes in the FISH-based karyotyping of chromosomes has resulted in several novel techniques, such as multiplex FISH (MFISH), spectral karyotyping (SKY), combined binary ratio labeling (COBRA), and color-changing karyotyping. Such complementary tests for genome-wide screening of chromosomal abnormalities allow for the simultaneous visualization of all chromosomes of a metaphase in a single hybridization step.

Tests for genome-wide screening of chromosomal abnormalities remain extremely important in the initial diagnosis and follow-up of patients with hematopoietic malignancies. Focusing only on tests that target specific genetic abnormalities, like FISH and PCR, can result in the failure to detect the additional important cytogenetic abnormalities that may be present initially or that may occur following therapy. For example, the need for intermittent cytogenetic analysis is very clear in chronic myelogenous leukemia (CML) patients⁴⁵. A number of these CML patients have developed clonal karyotypic abnormalities in Philadelphia chromosome-negative cells while on therapy with imatinib mesylate; these abnormalities would not have been detected by FISH or PCR analyses for BCR-ABL.

Analysis of large cohorts of data using learning algorithms based on artificial intelligence approaches can allow the discrimination between two groups of samples ("supervised" learning, such as distinguishing the presence or absence of cancer), or to identify data clusters within a population set that may represent novel disease entities ("unsupervised" learning). Such analysis of "fingerprints" can be based either on gene expression profiling using DNA array data or based

on proteomic patterns based on surface-enhanced laser desorption ionization time-of-flight (SELDI-TOF) analysis. Multiple reports have been published on the clinical utility of such microarrays for lymphomas and leukemias^{70,29}, or other malignancies⁷¹. However, standardization of the software used to analyse the data and the lack of appropriate controls remains an issue. Such microarrays interrogate not only the tumor cells but also the microenvironment. Slowly progressive follicular lymphoma (FL) could thus be discriminated from more rapidly progressive FL based on a specific expression profile of the surrounding (non-tumor) cells. A specific T cell signature corresponded with improved survival whereas a macrophage/dendritic cell profile did not⁷⁰. No microarrays are however available already for routine clinical use. Such transcriptome analysis on tumor specimens may also delineate a set of “genes of interest”, that can be monitored by RT-PCR, alleviating the need of nowadays more expressive micro-array approaches.

As an example, the techniques most commonly used in B-cell lymphomas are given in the table below, copied from Brazier et al, 2003⁷².

Table 3. Non-random chromosomal abnormalities in B-cell lymphomas

Lymphoma Subtype	Nonrandom Chromosomal Alterations	Genes Involved	Assay Used for Diagnosis / Prognosis†
CLL/SLL	Del 13q14	Unknown	FISH
	Trisomy 12	Unknown	FISH
	Del 11q22-23	ATM	FISH
	Del 17p1	TP53	FISH, karyotyping
LPL	t(9;14)(p13;q32)	PAX5/IgH	FISH, karyotyping
MZL	t(11;18)(q21;q21)	API2/MALT1	FISH, PCR
	t(1;14)(p22;q32)	BCL10/IgH	FISH, PCR
	t(14;18)(q32;q21)	IgH/MALT1	FISH
	Trisomy 3	?BCL6	FISH
FL	t(14;18)(q32;q21)	IgH/BCL2	PCR, FISH
MCL	t(11;14)(q13;q32)	Cyclin D1/IgH	FISH, PCR
DLBCL	3q27	BCL6	FISH
	t(14;18)(q32;q21)	IgH/BCL2	FISH, PCR
BL / BLL	t(8;14)(q24;q32)	c-MYC/IgH	FISH for c-MYC
	t(2;8)(p11;q24)	Igk/c-MYC	FISH for c-MYC
	t(8;22)(q24;q11)	c-MYC/IgI	FISH for c-MYC
Abbreviations: CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; LPL, lymphoplasmacytic lymphoma; MZL, marginal zone lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; DLBCL, diffuse large B-cell lymphoma; BL/BLL, Burkitt/Burkitt-like lymphoma.			
†The assays listed are the most commonly used today for clinical testing today, but many of these abnormalities can also be detected by other techniques.			

Immunoglobulin and T-cell receptor genes

Identification of a monoclonal population may assist significantly in arriving at the diagnosis of leukaemia or lymphoma, or in detecting its recurrence at levels below those discernible using other techniques⁷³.

Historically gene rearrangement studies were performed using Southern blotting techniques (DNA is transferred to nitrocellulose or nylon support followed by probe hybridization). The major problem with Southern Blot analysis of Ig/TCR gene rearrangements is the need for a sufficient amount of material and the relatively long time taken to obtain results (one or two weeks). Compared with Southern-blot, amplification-based procedures require less material, may be successfully performed in formalin-fixed, paraffin-based material, and may be accomplished over a time of one to two days. Therefore, several more rapid and cheaper PCR based techniques are used at present. Following PCR amplification, products are separated using flat gel electrophoresis or capillary electrophoresis, and visualized. Although these techniques yield a relatively high number of false negative results, they are very useful as an initial screening tool⁷⁴.

Southern blot techniques are now reserved for cases in which amplification-based assays are unsuccessful. Indeed, amplification-based assays may not identify all possible rearrangements that can be recognized by Southern blotting techniques, or the sensitivity for detecting small monoclonal populations may be lower or higher, depending on the particular rearrangement. It is advisable that Southern blot be available to the laboratory as a supplemental method⁷⁵.

Because the ability of the assays to detect a monoclonal population depends in part upon the fraction of the cell population that demonstrates the rearrangement, the sensitivity of gene rearrangement assays may sometimes be improved by microdissection and amplification of a morphologically suspicious cell population. The presence of a monoclonal gene rearrangement does not necessarily reflect the presence of a lymphoid neoplasm. Transient clonal proliferations can occur, particularly in immunocompromised patients and patients with certain viral infections. Also clonal T-cell receptor gene rearrangements have been described even in normal thymus. Lineage infidelity can occur: B-cell neoplasms demonstrating rearranged T-cell receptor genes or myeloid neoplasms demonstrating T-cell receptor gene rearrangements.

Laboratories should make available information regarding the sensitivity and the specificity of their assays, eg the fraction of possible gene rearrangements that can be identified, an indication of the fraction of the relevant neoplasms that are identified, and a statement of the percentage of cells that belong to a monoclonal population visualized in the assay⁷⁵.

CLL patients with a high proportion (>2%) of IgV_H somatic mutations survive longer^{76,23}. However, this prognostic variable is based on a technically difficult test requiring sequencing. Possibly, easier to detect surrogate markers as ZAP-70 expression may replace sequencing in routine diagnostics.

Tests for minimal residual disease

Although many patients with haematological malignancies achieve a complete clinical remission and even a complete pathologic remission by standard morphologic and immunologic criteria, a relatively high proportion of them will ultimately relapse. The source of this relapse is clearly from a persistent malignant cellular population that is present at a low level. This reservoir of neoplastic cells, detected only by sensitive molecular methods, is commonly referred to as minimal residual disease (MRD)^{77,78,79,80,81}. If achieving a molecular remission is confirmed to be an important goal following therapy for most haematological malignancies, as seems likely, MRD testing will become a much larger component of testing in molecular diagnostics laboratories.

Ideally, techniques used for MRD detection should have a sensitivity level in the 10⁻⁵ to 10⁻⁶ range, be applicable to almost all patients with the disease, provide some quantification of the target, and be rapid, inexpensive, readily standardized, and disease-specific. Also of critical importance for the clinical utility of tests for MRD detection is good interlaboratory reproducibility and standardization of reporting. In reality, most commonly used molecular analyses for MRD detection do not meet many of these criteria. A particular problem for clinicians is the lack of standardization of testing techniques and primers between laboratories, which essentially mandates follow-up testing for MRD be performed in the laboratory that did the previous testing to allow comparison of results. With frequent shifts in patient locations, sending follow-up specimens to the same laboratory may be impossible. The Europe Against Cancer (EAC) network has tried to standardize methods for sample preparation and processing, the use of primers and control genes^{79,82}.

Only a few commonly used techniques are sensitive enough for detection of MRD in leukemias and lymphomas. Nested PCR and quantitative real-time PCR can be used for disease-associated translocations or rearrangements³³. If the malignant clone does not carry a good translocation target for PCR analysis, patient-specific gene rearrangements may be targeted, using either nested or quantitative real-time PCR. Nested PCR analyses can detect up to 1 malignant cell in 10⁶ normal cells. Quantitative real-time PCR assays, with a sensitivity of 1 in 10⁴-10⁵, are almost as sensitive as the nested PCR. The major disadvantage of standard real-time PCR testing in a number of settings is the inability to compare the size of any detected rearrangements to that of the original malignant clone without additional testing. A substantive number of studies of MRD detection have been performed in only a few hematopoietic malignancies, specifically chronic myelogenous leukemia, follicular lymphoma, and childhood acute lymphoblastic leukemia.

Patient/clone-specific IgH PCR technology for MRD monitoring

This method is used in childhood pre-B-ALL studies of MRD and takes advantage of the fingerprint-like sequences of the junctional regions of rearranged IgH genes, which differ in length and composition for each lymphocyte clone. To obtain these sequences, standard IgH PCR

analysis is performed at diagnosis and/or relapse, followed by sequencing of junctional regions of the clonal IgH rearrangements. The different IgH rearrangements are then used for design of patient-specific oligonucleotide primers that are subsequently used in real-time PCR assays to follow the patient. Patient-specific IgH primers increase PCR sensitivity up to 1000-fold compared to standard consensus primers for IgV_H gene rearrangements, and could even prove of help for detection of CNS involvement⁸³. This same type of patient/clone-specific IgH PCR technology could be used for MRD detection in B-cell lymphoma or multiple myeloma, in which either no translocation-associated molecular event is available for MRD testing or the recurrent translocations occur in too low a proportion to be clinically useful.

Chimerism

Analysis of chimerism after allogeneic hematopoietic cell transplantation is important for assessing engraftment and the early detection of graft failure⁸⁴. Novel transplant procedures, for example dose-reduced conditioning protocols, rely on chimerism analysis to guide intervention, i.e. the reduction of immunosuppression or infusion of donor lymphocytes. XY-FISH analysis of sex chromosomes after transplantation from a sex-mismatched donor or analysis of polymorphic DNA sequences, i.e. short tandem repeats (STR) or variable number of tandem repeats (VNTR), are used in the assessment of chimerism.

Additional applications in oncology

As more target specific tumor treatments become available there is an increasing need for markers, including molecular markers, to detect the appropriate treatment candidates as well as to monitor treatment response. This increasing demand for personalized medicine and the integration of molecular diagnostics with therapeutics are driving the market for molecular diagnostics.⁸⁵ Although the medical literature is replete with reports of putative prognostic or predictive markers for cancer, few new diagnostics have been incorporated into routine clinical practice. A methodological approach to the development of such markers has been proposed⁸⁶, including the use of specific trials designs¹¹.

The two best known examples are the introduction of Trastuzumab (HERCEPTIN) monoclonal antibody therapy in HER2-driven metastatic breast cancer, where HER2 FISH tests are now used for treatment selection⁸⁷, and the targetting of the BCR-ABL kinase domain by imatinib mesylate (GLIVEC) in CML patients⁴⁵. Sequencing for mutations in the tyrosine kinase domain of EGFR gene, or FISH test for determining the EGFR copy number may help identify responders to tyrosine kinase inhibitors (gefitinib, erlotinib) in small cell lung carcinoma⁸⁸. Similarly, molecular markers may be of use at diagnosis and for monitoring of bevacizumab (AVASTIN) anti-VEGF antibody treatment, anti-CDK treatments and anti-NF-kappa-beta treatment (eg Bortezomib). Quantification of mRNA may allow detection of residual breast cancer cells in the circulation and molecular markers of microsatellite instability may be of use in the diagnosis of the Hereditary Non-polyposis Colorectal Cancer Syndrome and in the prognosis of colorectal cancer⁸⁹.

3.3. IN VITRO DIAGNOSTIC KITS OR IN-HOUSE TESTS

In-house tests

In-house methods (or “home-brew”) are still used for many molecular tests. The two main reasons given in a 2003 Australian survey are a lack of IVD kits with good performance characteristics and cost (<http://www.tga.gov.au/docs/pdf/ivdsurv.pdf>). Since 2004 validation is also required for in-house IVD tests in Australia¹⁵, and if a non-validated test is used the requesting physician should at least be made aware of this. In the US in-house tests must meet Clinical Laboratory Improvement Amendment standards but are exempt from FDA regulation. However, in-house tests may be developed using reagents prepared in-house, or using commercially manufactured analyte-specific reagents (ASRs), which must meet certain FDA criteria of good manufacturing practices (GMP). In the EU it remains unclear whether individual reagents must comply with the IVD Directive 98/79/EC.

The variety of in-house molecular methods in use for many molecular tests, makes it virtually impossible to define the clinical utility of such tests, as the performance characteristics for most of the individual in-house methods have not been established and may show great variation when studied. Even when international efforts have tried to optimize and standardize in-house methods eg in hemato-oncology⁷⁴, only part of the recommended primers and probes are often used because of cost reasons. Costs for in-house tests will likely increase significantly if test validation would be required and/or intellectual property licenses would have to be paid.

Regulatory context and list of kits marketed in the EU

All companies producing and distributing in-vitro-diagnostics in the European Union (EU) have to comply with the In Vitro Diagnostic Medical Devices Directive 98/79/EC (IVDMDD) issued by the EU. The purpose of the IVDMDD is to establish and to guarantee a uniform quality standard of medical diagnostics within the EU. The IVDMDD requires manufacturers (or their representatives) placing in vitro diagnostic medical devices on the Community market to provide certain information to a Competent Authority in a Member State where they have a registered place of business. One basic requirement of the IVDMDD is the establishment of a quality management system within the manufacturing company to monitor product development, production and sales (overlaps with the ASR regulation in the US). The Competent Authority appoints notified bodies to implement the requirements of the directive with regard to certification of manufacturers' CE marking and quality systems. Notified Bodies are typically test laboratories and quality systems houses that audit quality systems of medical device companies and test their products for compliance with applicable standards.

The IVDMDD also requires a Technical Documentation which has to be presented for each diagnostic product. This information includes test performance data (IVDMDD Annex I, part A, section 3). The IVDMDD has four classifications of product: List A and List B devices (as referred to in Annex II of the Directive), self-test devices and all other devices roughly in descending order of risk. Minimum criteria for test performance have been defined and IVD kit dossiers pre-market approval by a Notified Body is required only for a limited number of IVDs (listed under Annex II of the Directive). Thus most IVDs are CE labelled by the manufacturer and fall under the self-certification rule. Unfortunately, some IVD products marketed are still labelled in an ambiguous way eg “In vitro diagnostic in non EU countries. In EU countries which follow the provision of the IVD directive 98/79/EC this kit is available for research use only (RUO).” On the other hand, kits without clear clinical utility can still be labelled by the manufacturer as CE IVD. In the EU, the decision on the clinical utility of a diagnostic test is thus still left to the individual manufacturer. The kits marketed in the EU for performing the molecular tests under evaluation are listed in appendix 2 of this report. For nearly all tests under evaluation CE-labelled IVD kits are now available.

Regulatory context and list of kits marketed in the US

In the US, commercial molecular diagnostic kits or reagents for routine clinical use are regulated by the FDA⁹⁰. A distinction is made between IVDs and ASRs. An IVD, in vitro diagnostic, is cleared or approved by the FDA for one or more specific intended uses with established analytical and clinical performance characteristics. FDA approval refers to products that are approved for marketing under the PMA (pre-market approval) process for new devices. FDA clearance refers to devices that are cleared for marketing under a 510 (K) review. Generally the PMA process is more stringent, targeting truly novel products. Devices that are conceptually similar to those already on the market, or that represent improvements over existing products, can elect FDA clearance under a 510(K).

ASRs, analyte specific reagents, are products for use in “home-brew” testing, which have manufacturer assurances of GMP. ASRs must be labeled in accordance with 21 CFR § 809.10(e). Advertising and promotional materials are regulated by 21 CFR § 809.30(D). The laboratory that develops an in-house test using the ASRs shall inform the ordering person of the test result by appending to the test report this statement according to 21 CFR § 809.30(e): “This test was developed and its performance characteristics determined by (laboratory name). It has not been cleared or approved by the U.S. FDA.” ASRs do not have any intended use claims; the laboratory is responsible for establishing intended use and cutoffs. In the US, RUO (research use only) tests are not intended for use as building blocks for laboratory-developed assays and cannot be used for clinical laboratory testing.

Surprisingly few molecular tests have been cleared or approved by the FDA. The tables in appendix 2 are current through November 2, 2004 and have been copied from the Association for Molecular Pathology web site (www.ampweb.org).

3.4. GUIDELINES

The Clinical and Laboratory Standards Institute (formerly NCCLS, www.nccls.org) has developed a number of guidelines on molecular diagnostics. These guidelines are kept up to date on a regular basis and provide testing and QA guidance for the following topics. Further guidelines on quality aspects are also given under section 7.1.

- Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline⁹¹.
- Quantitative Molecular Methods for Infectious Diseases; Approved Guideline⁹².
- Nucleic Acid Amplification Assays for Molecular Hematopathology; Approved Guideline⁷⁵.
- Immunoglobulin and T-Cell Receptor Gene Rearrangement Assays; Approved Guideline – Second Edition⁷³.
- Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline⁹³.
- Fluorescence In Situ Hybridization (FISH) Methods for Medical Genetics; Approved Guideline⁹⁴.
- Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline⁹⁵.
- Genotyping for Infectious Diseases: Identification and Characterization; Proposed Guideline⁹⁶.
- Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Proposed Guideline⁹⁷.
- Proficiency Testing for Molecular Methods; Proposed Guideline⁹⁸.

Guidelines on the diagnosis and treatment of specific disorders in the area of hemato-oncology have been published by professional bodies such as the British Committee for Standards in Haematology (BCSH)⁹⁹. These include guidelines on the provision of facilities for the care of patients with haematological malignancies (including leukemia and lymphoma and severe bone marrow failure)¹⁰⁰. These guidelines describe four levels of care. For example, centers

performing autologous or allogeneic transplants, including stem cell transplantations, should have access on site (or immediately available) to cytogenetics and molecular biology services.

The National Institute for Clinical Excellence (NICE), UK, recommends that clinical services for patients with haematological cancers should be delivered by multi-disciplinary haemato-oncology teams which serve populations of 500000 or more¹⁰¹. The key recommendations also include the following. “In order to reduce errors, every diagnosis of possible haematological malignancy should be reviewed by specialists in diagnosis of haematological malignancy. Results of tests should be integrated and interpreted by experts who work with local haemato-oncology multi-disciplinary teams and provide a specialised service at network level. This is most easily achieved by locating all specialist haemato-pathology diagnostic services in a single laboratory.” Richards and Jack¹⁰² describe the practical development in Leeds, UK, of an integrated laboratory for diagnosis of tumours of the haematopoietic system performing flow cytometry, histopathology, molecular diagnostics and cytogenetics in a systematic and co-ordinated way. This organisation meets the requirement for the results of laboratory investigations and diagnosis of all cases of haematological malignancy to be reviewed by experts and specialist haematopathologists. The role of the IT system for an effective service is also highlighted.

UK guidelines on FISH scoring¹⁰³ mention interphase FISH studies are often carried out because of the absence of sufficient metaphases. If metaphases are present, they can add significant information to the analysis, eg for the interpretation of unusual signal patterns. Even experienced cytogeneticists need a proper training programme when starting FISH analyses. In most haematological disorders it is preferable to use FISH at diagnosis as an adjunct to, and not in place of, a conventional cytogenetic study. Where no fresh material is available (eg lymphoma), FISH may be the only possibility. FISH studies are reported as suitable for assessing initial response to treatment, but are not sensitive enough for detecting MRD. The choice of FISH or molecular tests is left to the laboratories and their users. The guidelines thus only apply in case FISH is selected.

The Groupe Français de Cytogénétique Hématologique (GFCH) has recently published recommendations with respect to the use of cytogenetic testing in specific haematological malignancies¹⁰⁴. Karyotyping is positioned as first line test, while FISH has its place according to specific guidelines. The correct interpretation of interphase FISH often requires access to the metaphase FISH data. The use of interphase FISH as stand alone test is therefore considered inappropriate because of possible errors in interpretation. This guidance document does not mention the use of tests for follow-up.

The National Comprehensive Cancer Network, Inc (<http://www.nccn.org>) lists in its oncology practice guidelines the use of cytogenetics/FISH as well as molecular genetic analyses but no flow chart of diagnostic tests is given.

4. LOCAL SITUATION

4.1. ANALYSIS OF THE CMD TEST METHOD QUESTIONNAIRES.

All 18 CMDs returned completed questionnaires (sample method questionnaire given in appendix 3) for the tests they were performing. As the tests offered may slightly change over time the offerings obtained using the questionnaire may differ from the tests listed on the CMD activity report covering mainly the 2003 situation. Comparing the responses obtained with the CMD activity reports we conclude the survey covers the vast majority of the tests actually performed at the respective CMDs. The method questionnaires were entered into an Excel spreadsheet which was mailed to the CMDs for verification, before the start of the data analysis.

A total of 594 method questionnaires were entered (268 for microbiology, 309 for hemato-oncology and 17 for pathology). Pathology tests which overlapped with microbiology (HPV, EBV) or hemato-oncology (lymphoma tests) were entered under microbiology or hemato-oncology, respectively. The complete databases for microbiology, hemato-oncology and pathology are listed in appendix 3, together with the derived pivot tables on test methods by centre, turn around time (TAT), test interpretation and external quality assurance (EQA) participation. Microbiology tests offered by at least 10 out of the 18 CMDs are *Chlamydia pneumoniae*, CMV qualitative, enterovirus, HCV qualitative, quantitative and genotyping, HSV, HPV, *Legionella pneumophila*, nosocomial pathogen typing, *Mycobacterium tuberculosis*, *Mycoplasma pneumoniae*, Staphylococci resistance, and VZV. Hemato-oncology tests offered by at least 10 CMDs are Ig and TCR rearrangements, t(8;21), t(9;22) qualitative and quantitative, t(12;21), t(14;18) qualitative, t(15;17). For pathology (excluding overlap tests) the most frequent test is HER2, being reported by 7 CMDs. For some tests two different or complementary test methods are offered for the same test, eg RT-PCR for detection of the fusion transcript and FISH for the detection of the translocation itself.

The median value for the average test turnaround time for microbiology tests is 3 days (mean 4 days), with over 7 days on average for HCV quantitative, HPV and typing of nosocomial infectious agents. The lowest average TATs reported were 1.8 days for *B. pertussis*, 2.0 days for Polyoma virusses and *E. coli* (Verotoxin producing), 2.2 days for Enterococci (resistance genes, VRE) and HSV, 2.3 days for *Pneumocystis jirovecii* (*carinii*) and 2.4 days for *Legionella pneumophila* and *M. tuberculosis* (direct). For hemato-oncology the average TAT was 8 days with no test having a TAT under 5 days on average. The reported average TAT for the HER2 test in pathology was 6.1 days. Test interpretation is provided in over 80% of the methods by the laboratory, for all three area's. The use of dialogue with the requesting physician to discuss test interpretation varied mainly by CMD, more then by test.

Despite the availability of CE labelled kits on the market for most tests (see list of kits in appendix 2), the majority of tests are still performed at the CMDs based on in-house methods or sometimes also based on modifications of kits (overall 79% of the reported methods). Only 28% of the methods reported for microbiology and 13% of the methods used in hemato-oncology are based on commercial kits, most carrying the IVD CE label. For microbiology, testing kits are used mainly for HCV, HPV, and mycobacterium testing, all characterized by a rather large test volume. Interestingly, the pathology-specific methods reported are performed mainly using commercial kits, eg for HER2 FISH. Overall 33% of the in-house methods had been validated. For microbiology, 39% of the in-house amplification methods and 20% of the commercial kit based methods were reportedly validated (in 23%, respectively 14% of methods, a validation report summary was also provided). For most validated test methods a SOP for test execution was provided, however a SOP was made available only for 60% of the non-validated methods.

CMDs have organized and reported on the compulsory QA rounds between CMDs as part of their mission (see CMD QA reports, <http://webhost.ua.ac.be/cmd/index.html>). Participation to international EQA programs over the last years was reported for 93 out of 268 microbiology test methods. At least 5 centres reported EQA participation for CMV (qualitative and quantitative), enterovirus, HBV quantitative, HCV qualitative and quantitative, HSV, *Mycobacterium* and VZV. These initiatives were mainly restricted to 11 out of the 18 centres. For hemato-oncology such participation to EQA was recorded for 46 out of 309 test methods. At least 5 centres reported EQA participation for t(15;17) and t(9;22). Overall, EQA participation for hemato-oncology was

restricted mainly to 5 centres, 3 of these are non-university centres. For the pathology-specific tests no international EQA participation was reported.

Discussion

The increasing number of clinical research applications of nucleic acid based tests after the invention of PCR has stimulated the creation of CMDs. The expertise at the CMDs has been developed together with the research and often the in-house tests were developed in the context of a thesis work at the centre. As people moved, the methods often moved along. Also, published methods were reproduced or modified from the literature. Test validation is clearly not yet the rule for in-house tests. As the first labs start to pursue test accreditation, the proportion of tests validated will probably soon increase. For applications with a commercially attractive volume, mainly in the infectious disease area, specific kits have been marketed. For many applications the available CE kits are not performing well enough according to some members of the microbiology working group, necessitating continued use of in-house methods. Another reason to choose for an in-house method may be cost, provided the volume is sufficiently large to absorb the cost of research and development, and validation (if done). These two reasons are also the two main reasons cited for the use of in-house IVD tests in an Australian survey (<http://www.tga.gov.au/docs/pdf/ivdsurv.pdf>). The development of fully automated extraction procedures and multiplex real-time PCR applications goes beyond the development capacity available in most of the centres and can only be afforded by industry. This evolution may cause a shift towards the use of kits for more applications.

For hemato-oncology a number of European initiatives have tried to standardize PCR and RT-PCR assays (eg BIOMED Concerted Actions^{74,78} and Europe Against Cancer programs⁷⁹), but the complete sets of recommended primers are not always used at the CMDs for cost reasons. Another important variable in the low volume hemato-oncology tests is the number of tests per run, with the cost per test easily dropping to half if a sufficiently high number of tests can be performed in the same run. This is an argument for more centralized testing.

Tests offered at CMG and CMD overlap, and the introduction of interphase FISH at some CMDs has added to the complexity. Most often the CMG is well aware of the tests being performed at CMD level but sometimes redundant testing has been seen in case the requesting physician ships samples in parallel to different labs. Furthermore often two or more (sometimes complementary) techniques are available: eg interphase FISH for detection of a specific translocation and RT-PCR for the detection or quantification of its fusion transcript. In addition to the price difference of the two techniques, there is the potential risk for preferential use of a specific technique and/or testing environment because different financing is used for CMG and CMD. CMG hemato-oncology tests are now being reimbursed using a generic nomenclature (art 33) in fact developed only for human genetic testing. For the CMDs the fixed overall yearly budget is divided according to the CMDs, driven by costs for personnel, invoiced consumables and investments.

4.2. CHARACTERISTICS OF INDIVIDUAL TESTS

The completed expert questionnaires were considered together with the information available from other sources eg CMD nomenclature proposal 2001, comparative data from abroad, pilot assessments. Overall, completed questionnaires were received from the molecular laboratory experts for all 94 CMD tests. In most cases the questionnaire was completed by a molecular laboratory expert only. In some cases the questionnaire was completed by a laboratory expert and a clinical expert. Most completed questionnaires contained references to the literature. For the 37 microbiology test reports received all but 4 contained references to the literature. Data on between-center reproducibility are available for kits approved or cleared by FDA but are scarce for other tests (outside of EQA programs). In 5 out of 37 microbiology test the between-center reproducibility of the test method had been assessed independently from quality assurance rounds.

Microbiology

For tests being used in completely different settings (antenatal or not) separate forms were completed. The clinical validity or diagnostic accuracy data (diagnostic sensitivity and specificity) were reported versus a reported gold standard. This could be viral or bacterial culture, a nucleic acid based test mentioned as gold standard technique, a well documented clinical diagnosis, or a combination of variables. The gold standard or the diagnostic accuracy data vary not only with the micro-organism but also with other factors.

- The test indication
 - Detection of CMV prenatally (gold standard is demonstration of human CMV excretion in the first 2 weeks of life)
 - or monitoring of immunocompromised patients (gold standard is CMV disease or monitoring viremia using shell vial assay or culture or pp65 antigenemia).
 - Parvovirus in a prenatal setting (PCR is best standard, more sensitive than serology)
 - or aplastic anemia and other indications (Parvovirus detected using qualitative PCR may not be specific enough, clinical cut-off may be needed)
 - HSV in encephalitis (PCR on CSF as gold standard)
 - or other indications and samples (dermal, ocular, genital, biopsies) where viral culture is still gold standard.
- The tissue type
 - False positive NAT results for aspergillus are an issue for respiratory samples, but not for blood samples.
 - MRSA PCR has a somewhat lower accuracy when performed directly on a nasal swab compared with blood culture.

For some micro-organisms the NATs are reported as gold standard

- DNA sequencing was reported as reference technique for the identification of HCV genotype, nosocomial pathogens, bacteria difficult to identify, and cultured fungi.
- Human herpesvirus type 8 has been identified in 1994 on the basis of DNA sequences in Kaposi's sarcoma lesions. In this case molecular detection has been the standard from the start.
- NAT is now the gold standard test for HCV, HBV, EBV, HSV (encephalitis)

For some micro-organisms the analytical sensitivity of NATs (eg real-time PCR) may be so high that a clinical cut-off value or follow-up pattern may need to be established, requiring further study.

- CMV disease in immunocompromised: NATs are more sensitive than antigen pp65 (especially in leukopenia), may result in earlier detection of disease (quantitative PCR needs cut-off), and allow for sample storage before analysis.
- HBV: NATs have no alternative for follow-up, added value of increased analytical sensitivity of real-time PCR over bDNA assay still unclear for patient follow-up
- Parvovirus in serum (indication different from prenatal, also role of genotypes 2 and 3): also detected in asymptomatic patients
- Polyoma virus in urine: criteria for monitoring and treatment impact under evaluation
- Pneumocystis in respiratory samples: cut-off may help discriminate colonization from disease

Sometimes the gold standard result requires further patient follow-up.

- CMV PCR in amniotic fluid has a sensitivity of 80-90% for the clinical disorder, defined as human CMV excretion in urine or saliva in the first 2 weeks of life.

- *Toxoplasma gondii* in amniotic fluid has a sensitivity of 60-81% for disease defined using serology at one year of age.
- *Aspergillus* PCR has a sensitivity of 25-84% for a diagnosis which is often made postmortem.
- NATs are earlier than pathology findings as for EBV, or much earlier as for HPV.

In other situations the reported diagnostic sensitivity of the NAT is low versus a gold standard which includes a clinical diagnosis.

- VTEC PCR has a diagnostic sensitivity of 61% for HUS
- *Borrelia burgdorferi* PCR in synovial fluid has a diagnostic sensitivity of 50-96% in case of Lyme disease, PCR may help resolve cases with unclear serology
- *Toxoplasma* PCR in CSF has a diagnostic sensitivity of 65% for cerebral toxoplasmosis.

The gold standard is shifting to NATs for some micro-organism from a gold standard which was based on culture. NATs are often more sensitive, and faster, or may be more specific, or easier to perform. It remains difficult to assess the specificity of the NAT if culture was a gold standard with a low diagnostic sensitivity.

- *Bartonella*: bacteriological culture as gold standard but very difficult
- *B. pertussis*: bacteriological culture as gold standard but PCR more sensitive and faster
- *L. pneumophila*: gold standard consists of clinic plus culture or serology or antigen; NATs are more sensitive and are also possible after the start of antibiotic therapy
- *M. pneumoniae*: expanded gold standard of three independent techniques; PCR is more sensitive, specific and faster than culture and serology
- *C. pneumoniae*: expanded gold standard of three independent techniques; PCR is more sensitive, specific and faster than culture, EIA and serology. Remark: the values reported for diagnostic accuracy referred to a comparison of different NATs, and not versus the gold standard.
- *M. tuberculosis*: culture is gold standard but NATs are more specific, and faster than culture
- *M. tuberculosis* resistance: culture plus drug susceptibility testing as gold standard but NATs can resolve grey zone phenotype data
- Enterovirus: gold standard of clinic plus CSF cytology or viral culture
- HSV: viral culture as gold standard already replaced by PCR for encephalitis indication
- VZV: PCR is becoming the gold standard, is more sensitive and faster than culture (CSF and ocular samples)
- *Toxoplasma* prenatal: NAT more sensitive than mice culture of amniotic fluid
- Cerebral toxoplasmosis: gold standard is a combination of culture, clinic, CT exam and response to therapy, PCR is single most sensitive test
- Rubella prenatal: gold standard is viral culture, PCR is more sensitive and faster, and sample shipment conditions are less critical
- CMV prenatal: NAT more sensitive than viral culture or specific IgM
- VTEC: no gold standard technique, PCR is more sensitive than phenotype
- VRE: no gold standard technique, PCR is standard for detection of resistance genes, more sensitive than phenotype
- MRSA: PCR already sometimes used as gold standard, faster than phenotype
- *Candida*: gold standard is clinic plus culture, NATs are more sensitive and faster
- Polyomavirus: no gold standard, PCR is faster than culture and more sensitive than EM

The reported proportion of microbiology tests performed routinely which cannot be interpreted

- 0% (n=8)
- = or < 1% (n=11)
- = or < 3% (n=4)
- = or < 6% (n=6)
- = or < 10% (n=1)
- Low (n=3)
- Not available (n=4)

Consequences for non-interpretable microbiology tests were reported for 29 tests. In 12 out of 29 tests this has no negative impact. In about half of the tests (n=14) this is associated with additional health care costs and in a quarter with a possible negative impact on patient health (n=7).

Reported proportion of false positive test results among the test-positives in routine testing (or incorrect typing result or incorrect quantitative measure).

- 0% (n=10)
- = or < 1% (n=5)
- = or < 3% (n=3)
- = or < 6% (n=4)
- = or < 10% (n=2)
- Low (n=2)
- Not available (n=11)

Possible consequences of such results were reported for 35 tests. False positives or incorrect typing results are associated in over half of the tests (n=20) with a negative impact on patient health. For 19 tests such results create additional health care costs. Both types of consequences were reported for 16 tests. For 12 tests false positives or incorrect results have no consequences.

The proportion of false negative test results was reported for 15 tests:

- 0% (n=3)
- = or < 1% (n=1)
- = or < 3% (n=2)
- = or < 10% (n=3)
- = or < 20% (n=2)
- 20-75% (n=4)

Consequences of false negative results were reported for 31 tests and consist mainly of additional health care costs (n=20), and/or a negative impact on patient health (n=15). Both consequences were reported for 12 tests and no consequences were reported for 8 tests.

Expected changes in indications/number of tests or test methods over the next 5 years were reported for 37 tests. For most tests (n=27) method changes are expected consisting mainly of automation of extraction, real-time PCR, and kits. An expected increase in test volume, or additional indications were reported for *B. pertussis*, *L. pneumophila*, *M. tuberculosis*, MRSA, VZV, and *Toxoplasma gondii*, or a transient volume increase in HCV tests.

Hemato-oncology tests

For hemato-oncology tests the contribution of individual tests to the diagnosis is more complex. CMD experts mention it should be the molecular haematologist to decide on the test to perform after accurate integration of available clinico-biological data.

Overall, few data on diagnostic accuracy are available for RT-PCR tests in hemato-oncology.

The reference diagnosis is obtained according to WHO criteria¹⁰⁵. For some diagnoses the presence of a specific translocation is part of the WHO criteria or has been proposed.

- The WHO definition of CML: a myeloproliferative disorder with t(9;22) or BCR/ABL
- Translocation t(11;14) with resulting cyclin-D1 expression is hallmark of MCL (WHO)
- c-myc rearrangements, including t(8;14), t(2;8), t(8;22), have been proposed as a criterium for Burkitt lymphoma to the WHO classification advisory committee

Most often however the diagnostic sensitivity of the translocation is below 50% for a given diagnosis, but the detection of the translocation may be performed for several reasons.

- It has a prognostic impact, eg BCR-ABL in ALL
- It may serve as a molecular marker for MRD during follow-up
- It may give prognostic information and some additional evidence for the diagnosis, eg Trisomy 12 in B-CLL (diagnostic sensitivity 12-50%)

Note that the numbers for diagnostic sensitivity of PCR or RT-PCR are thus dependent on the reference used, eg RT-PCR for inv(16)/CBFB-MYH11 is present in 10% of AML and in >99% of inv(16)+AML (based on cytogenetics).

At diagnosis, the gold standard technique to detect most translocations is cytogenetics, which may have to rely on interphase FISH in case no metaphases can be induced. This is the case for up to 50% of the B-CLL cases. PCR has an advantage over cytogenetics that it can be performed also if only small amounts of material are available. Furthermore it costs much less. According to the experts, for many translocations no studies comparing the performance of RT-PCR to the gold standard (cytogenetics) have been reported, eg for t(1;14), t(1;19), t(12;21), MLL translocations, t(11;18). No diagnostic accuracy data are thus available for those RT-PCR tests. The t(11;14) PCR and t(14;18) PCR can only detect about half of cytogenetic (FISH) positive cases due to scattering of the genomic breakpoints. Instead of PCR for t(11;14), the resulting cyclin-D1 expression is a more sensitive marker and hallmark of MCL (WHO). For Burkitt lymphoma, the scattering of breakpoints in 8q24 makes detection using PCR even not feasible for routine diagnosis. Immunohistochemistry is also sometimes being used to detect translocations. For detection of t(2;5) immunohistochemistry for detection of the ALK protein is used more widely than FISH or RT-PCR.

During MRD follow-up, RT-PCR is often considered the gold standard itself, and no diagnostic accuracy data are given. Sometimes FISH is also used (but it does not have the high analytical sensitivity of PCR). However, no studies have been performed comparing FISH with RT-PCR of the transcript during patient MRD follow-up, eg for t(8;21) or t(15;17). Important to mention is that any changes of lab during follow-up (eg for BCR-ABL) should be avoided. Care must also be taken with highly sensitive PCR assays for t(14;18) or RT-PCR assays for transcripts of t(2;5), t(8;21) or t(15;17) as they may test positive in some healthy individuals. Checking the amplicon size versus the original tumor may thus sometimes be needed. The clinical utility of MRD follow-up using PCR for t(11;14) is controversial or under trial for PCR t(14;18).

PCR plus restriction enzyme digest or sequencing is the gold standard for FLT3 exon 20 TKD mutation (D835) analysis and PCR plus sequence for FLT3 ITD/LM. Real-time quantitative PCR is the reference method for PRV1, a new diagnostic test under evaluation for polycythemia vera.

Bad quality of the sample DNA or RNA is the main reason why RT-PCR, PCR or even FISH tests cannot be interpreted. The proportion of tests that cannot be interpreted varies from <1% for PCR t(14;18) in hematology to 5-12% of samples tested in pathology (bad quality of the samples received or paraffin embedding of the biopsy). For RNA based tests the proportion of bad quality samples is around 5-10%. Also for FISH the proportion of non-interpretable tests is 5-10% due to poor quality of the sample (fixation conditions) or the section (overlapping nuclei).

Non-interpretable tests mainly have an impact on cost if a second sample needs to be collected. False positive and false negative results are most often associated both with a negative impact on patient health and additional costs.

The experts expect over the next 5 years only few changes in the test technology or volume, with the exception of the introduction of chip based tests for the detection of translocations, and a further increase in the use of patient specific primers for MRD follow-up.

The following items have been listed in **table 4a** for individual microbiology and pathology tests performed at the CMDs. A slightly modified table is available for tests in hemato-oncology (**table 4b**).

- Name of the test.
- Sample type and indication as mentioned in the nomenclature proposal 2001, and adapted as appropriate by the CMD expert.
- The type of test impact: initial diagnosis, prognosis, treatment selection or monitoring, patient isolation, outbreak control.
- The technique used at the CMDs for amplification/detection: in-house only, in-house mainly, kits only, kits mainly.
- The availability of kits. FDA=FDA approved/cleared test exists, also with CE label. CE= kit with CE label exists but no FDA approved/cleared kit. RUO=Only RUO product available. No: for all other situations.
- The average test turnaround time (TAT): average of the average TAT reported by the CMDs for this test.
- CMD tests in 2003 based on CMD activity report for period 1 February 2003 – 31 January 2004. Please note that for hemato-oncology a significant part of the molecular testing is also being performed at the CMGs (no exact numbers available per test).
- CMD positive tests in 2003 based on CMD activity report for period 1 February 2003 – 31 January 2004.
- Number of CMDs performing this test, based on the received method questionnaires.
- Number of CMDs participating to non-CMD international QA proficiency testing program, entry = NA if no such program available.
- Countries with a specific reimbursement code for the test, Au=Australia, Fr=France, Ge=Germany, UK=United Kingdom, Sw=Switzerland. Note that in Australia and in Germany there are also generic codes for PCR detection of genomic mutations or micro-organisms.
- Expert comments on comparative test, financing, or observed differences for volume estimates, eg if volume CMD differs from estimates in 2001 nomenclature proposal or expert estimate.

Table 4a. Characteristics of individual microbiology and pathology tests, as reported by the CMD experts.

Nr	Test	Sample type and indication	Type of Impact	Techn. CMD	IVD Kits	TAT day avg.	CMD tests 2003	CMD pos. tests 2003	CMD ctrs (#)	EQA ctrs (#)	Spec Reimb Countr	Expert comments on comparative test, financing, or observed differences for volume estimates
1	<i>Bartonella henselae</i> , <i>Bartonella quintana</i>	Non-fixed lymph node aspirate or biopsy to confirm Cat Scratch Disease; non-fixed tissue biopsy to confirm bacillary angiomatosis. Test performed together with histopathology.	Treatment selection, prognosis	In-house	No	3	121	28	3	NA	Sw	Culture is difficult, role serology unclear in angiomatosis
2	<i>Bordetella pertussis</i>	Exclusively using NPA or NP smear, in case of clinical suspicion of pertussis (see criteria) or for contact tracing	Treatment selection, outbreak control	In-house	CE	1,8	747	75	5	0	Sw	More sensitive than culture
3	<i>Borrelia burgdorferi</i>	CSF to confirm (plus intrathecal antibodies) or monitor neuroborreliosis; SF (main sample type) to confirm or monitor Lyme arthritis; skin biopsy for atypical erythema migrans; urine testing only together with one of above	Treatment selection	In-house mainly	CE	2,6	627	12	4	0	Sw	Serology often sufficient, culture ok for skin lesions only
4	<i>Chlamydia pneumoniae</i>	Respiratory sample (NPA, sputum, throat swab, BAL,...). CAP (on chest X-ray, and sputum stain or culture negative for pneumococcus), or prolonged cough (>2 weeks).	Treatment selection (few data)	In-house mainly	CE	2,6	1255	18	11	0	Fr, Ge, Sw	No alternative, but also NAT not-standardized
5	<i>Corynebacterium diphtheriae</i>	Isolate presumptively <i>C. diphtheriae</i>	Correct diagnosis	In-house	No	NA	6	0	1	1	Sw	No alternative, not for nomenclature
6	<i>Escherichia coli</i> (VTEC)	Fecal specimen or culture, in case of HUS, bloody diarrhea or diarrhea outbreak (3 cases in 1 week in one community)	Patient isolation, outbreak control	In-house	CE	2	190	48	1	0	Sw	NAT probably most performing. 5000 tests (2001 proposal), 500 tests if strict HUS criterium
7	Enterococci (Vancomycin resistant, VRE)	Isolate 1. atypical phenotype, confirm glycopeptide resistance in case of clinical or nosocomial infection, admission at ward at risk; 2. confirm VanA, VanB phenotype; 3. identify <i>E. casseliflavus</i> or <i>E. gallinarum</i>	Correct diagnosis, treatment selection	In-house	No	2,2	146	61	5	0		Second line test, fast. If reimbursed, should be restricted using diagnostic rules
8	<i>Helicobacter pylori</i> (macrolide resistance)	Gastric biopsy or mucus, persistent infection after macrolide containing treatment or to confirm unclear antibiogram (or non growing isolates)	Epidemiol. (Treatment selection)	In-house	No	3	94	67	1	NA		Second line test. Not for nomenclature
9	<i>Legionella pneumophila</i>	BAL or sputum. 1. adult patient with CAP or nosocomial pneumonia (on chest X-ray) and sputum neg. for Gram-stain (or other rapid test) and any of the following: a: IC, b: outbreak, c: requiring ICU care d: CAP after travel abroad.	Treatment selection, outbreak control.	In-house mainly	CE	2,4	852	66	11	4		5000 tests in 2001 proposal. Urinary Ag test is ok for routine, PCR in theory more sensitive. Restrict to urinary Ag negatives?
10	<i>Mycoplasma pneumoniae</i>	Respiratory sample (NPA, sputum, throat swab, BAL,...): 1. CAP (on chest X-ray, and sputum stain or culture negative for pneumococcus); 2. prolonged cough (>2 weeks). CSF: meningitis/encephalitis with CSF neg. for bacterial culture, HSV and enterovirus	Treatment selection	In-house mainly	CE	2,2	1652	28	12	0	Sw	Higher sens than culture and serology. 6000 tests in 2001 proposal

Nr	Test	Sample type and indication	Type of Impact	Techn. CMD	IVD Kits	TAT day avg.	CMD tests 2003	CMD pos. tests 2003	CMD ctrs (#)	EQA ctrs (#)	Spec Reimb Countr	Expert comments on comparative test, financing, or observed differences for volume estimates
11	Mycobacterium tuberculosis	New indications. Sputum: smear neg. AND respiratory signs for > 3 weeks AND no diagnosis using classical techniques AND pulmonary lesions on chest X-ray or CT. CSF with high protein. Lymph node, biopsy, exsudate: no diagnosis using classical tests (aerobic and anaerobic cultures) or suggestive histology. Culture: identification M. tuberculosis on solid medium or non-tb mycobacteria (liquid or solid culture).	Treatment selection, patient isolation	Kits mainly	FDA	2,5	4795	732	13	6	Fr, Ge, Sw	1000 of 7500 (estim.) tests already reimbursed (direct or culture-based detection of nucleic acid in smear pos. tb) , volume control needed for smear neg.
12	Mycobacterium tuberculosis (resistance genes)	Culture M. tuberculosis: confirmation of RMP-R (or doubtful) or in patients strongly suspected of primary or secondary RMP-resistance. Clinical samples of TB patients strongly suspected of primary or secondary resistance AND smear or nucleic acid pos. Max 1x/6M/patient.	Treatment selection, patient isolation	Kits mainly	CE	3,3	119	9	5	NA		Second line test. Diagnostic rules to be respected
13	Staphylococci (resistance genes, MRSA)	1. Staphylococcal culture: atypical phenotype; 2. confirmation mupirocin resistance of isolate; 3. new indication under study: direct MRSA detection in blood, culture, endotracheal aspirate, nasal and skin swab.	Treatment selection, patient isolation	In-house	FDA	2,5	2081	1019	13	0		Second line test (screening if new indication). 8000 in 2001 proposal, >50000 tests if new indications (direct screening test)
14	Identification of bacteria difficult to identify	Pure culture: if exact identification of species causing endocarditis, meningitis, osteomyelitis... is clinically relevant	Treatment selection	In-house	No	4,4	2036	NA	8	1		Superior to non-molecular techniques
15	Molecular typing of nosocomial pathogens	Pure culture. 1. outbreak investigation by hospital infection control team; 2. evaluation of measures: monitoring spread of multi-resistant bacteria, 3. differentiate between relapse and infection with another strain.	Infection control, treatment selection	In-house	CE	10,1	1496	NA	11	2		PFGE is gold standard, serotyping ok for Salmonella. 1600 sets of testing, coordinate with infection control unit
16	Cytomegalovirus (CMV) qualitative	Blood, PBMCs, serum, plasma, BAL, CSF, ocular fluid, biopsy: diagnosis IC patients. Blood: monitoring CMV neg acceptor, pos donor of organ or blood. AF: 1. suspected primary infection, eg based on serology; 2. pregnancy with ultrasonographic abnormalities	Treatment selection	In-house	FDA	3,3	10014	1542	12	8	Au, Fr, Sw	More sensitive than pp65 in leukopenia; culture is slower, IgM sometimes of use. Diagnostic rules and limitation of requesting physicians, 1000-2000 tests antenatal
17	Cytomegalovirus (CMV) quantitative	Blood, PBMCs, PBL, plasma: monitoring IC patients up to twice weekly (CMV pos donor and/or acceptor)	Treatment selection (research)	In-house	CE	3,5	8533	1838	8	6	Sw	Cut-off not established, pp65 Ag is not sensitive in leukopenia. Limited patient population, 35000 tests (incl pp65, now reimbursed at B1400), shift towards PCR
18	Epstein-Barr virus (EBV) qualitative	CSF: 1. encephalitis in IC or after exclusion more prevalent causes; 2. cerebral lymphoma (HIV). Blood: primary infection post liver or BM transplant (max 10x post transplant). Tissue biopsy (ISH): EBV related lymphoproliferation or tumor	Correct diagnosis	In-house	CE	3,6	1294	316	7	3	Sw	ISH for RNA transcripts is reference test
19	Epstein-Barr virus (EBV) quantitative	Blood: diagnosis and monitoring of infection in post pediatric liver or BM transplant, leukemia treatment, EBV related lymphoma	Treatment selection	In-house	CE	4,1	1888	855	5	4		More reliable than serology. Integrate into transplant or cancer treatment cost
20	Hepatitis B virus (HBV) qualitative	Serum, plasma: when serology or infection is unclear. Max 1x/year/patient.	Correct diagnosis	In-house	CE	3,4	457	285	4	2	Fr, UK, Sw	No alternative in these patients. No reimbursement needed (low volume)

Nr	Test	Sample type and indication	Type of Impact	Techn. CMD	IVD Kits	TAT day avg.	CMD tests 2003	CMD pos. tests 2003	CMD ctrs (#)	EQA ctrs (#)	Spec Reimb Countr	Expert comments on comparative test, financing, or observed differences for volume estimates
21	Hepatitis B virus (HBV) quantitative	Serum, plasma: start and monitoring of treatment. Max 3x/year/patient treated.	Treatment selection, treatment effect	In-house mainly	CE	9	3192	1810	9	6	Ge, UK, Sw	No alternative. Integrate into treatment cost
22	Hepatitis C virus (HCV) qualitative	Serum, plasma, (liver biopsy): confirm active infection (serology unclear, or neg in IC). Max 2x/year/patient treated. Max 1x/patient untreated.	Confirm diagnosis, treatment effect	Kits mainly	FDA	4,8	7368	3359	12	6	Au, Fr, Ge, UK, Sw	Needed to detect clearance in serology pos. Integrate into treatment cost, limit test to anti-HCV sero pos
23	Hepatitis C virus (HCV) quantitative	Serum, plasma: at treatment start and at 12 weeks in genotype 1 infection	Treatment effect, prognosis	Kits mainly	FDA	9,4	3211	NA	13	5	Au, Fr, Ge, UK, Sw	Ag test may become alternative. Savings made in terms of treatment cost
24	Hepatitis C virus (HCV) genotyping	Serum, plasma: if treatment is considered. Max 1x/patient.	Treatment selection, prognosis	Kits mainly	CE	8,3	2627	NA	12	4	Au, Fr, Ge, UK	
25	Human Papillomavirus (HPV)	Cell brush, liquid cytology: cervical ASCUS/AGUS in women > 30y, LSIL, residual HPV: max 2x in 6 months after intervention.	Treatment selection, treatment effect	Kits mainly	FDA	9,5	24213	11381	16	1	Au, Fr, Ge, UK, Sw	More sensitive, less specific than histology. 28000 tests (2001 proposal), 40000 tests (expert)
26	Enterovirus	CSF: presumed viral meningitis or meningoencephalitis. Blood, pericardial fluid, myocardial biopsy: acute pericarditis/myocarditis. Fetal blood, AF: antenatal diagnosis of fetal death or in case of specific echographic findings	Treatment selection (if early results)	In-house	CE	3,1	2924	373	12	9	Sw	More sensitive and potentially faster than culture. Limitation because of sample type, max. 5000 tests (2001 proposal and expert)
27	Herpes simplex virus	CSF: meningitis, encephalitis, myelitis, neonatal herpes. Ocular fluid: keratitis, uveitis, retinitis. Non-fixed biopsy: IC with oesophageal or intestinal lesions.	Treatment selection, treatment effect if quantitative	In-house	CE	2,2	3315	361	13	8	Au, Sw	CSF PCR is superior to brain biopsy culture. Suggestion for reduction in test volume: no CSF PCR if normal leucocyte and protein levels
28	Human herpesvirus type 8 (HHV8)	Plasma, serum, PBMC, biopsy: suspected Kaposi's sarcoma, disease of Castleman, primary effusion lymphoma.	Treatment selection	In-house	CE	3	16	3	2	NA		No alternative
29	Parvovirus B19	AF, fetal blood or tissue: specific echographic abnormality or fetal death or symptomatic infection during pregnancy. SF: unexplained arthropathy. Blood or bone marrow: aplastic crisis, red cell aplasia or unexplained pancytopenia in IC.	Pregnancy monitoring, treatment selection	In-house mainly	CE	3,2	297	26	3	1	Fr, Sw	Used together with maternal IgM. 1000-2000 tests (2001 proposal)
30	Polyomaviruses JC and BK	CSF: progressive multifocal encephalopathy. Urine: hemorrhagic cystitis post BMT or leukemia treatment, TIN post kidney transplant.	Treatment selection (research)	In-house	CE	2	2043	935	3	NA	Sw	Culture, EM, serology of little value. 350 tests (2001 proposal) is too low, much follow-up testing
31	Rubella virus	AF: suspected primary rubella infection in first 16 weeks of pregnancy (seroconversion or unclear serology).	Pregnancy interrupt.	In-house	CE	2,5	14	0	2	NA	Fr, Sw	More sensitive than culture, IgM may be false pos. Reference labs only (low volume)

Nr	Test	Sample type and indication	Type of Impact	Techn. CMD	IVD Kits	TAT day avg.	CMD tests 2003	CMD pos. tests 2003	CMD ctrs (#)	EQA ctrs (#)	Spec Reimb Countr	Expert comments on comparative test, financing, or observed differences for volume estimates
32	Varicella Zoster Virus (VZV)	CSF: encephalitis, meningitis, myelitis. Ocular fluid: keratitis, uveitis, retinitis. Vesicular fluid: atypical varicella, zoster. BAL: atypical pneumonia in IC. AF: varicella during pregnancy.	Treatment selection	In-house	CE	2,5	1349	209	10	5	Au, Fr, Sw	More sensitive than culture, specificity higher than serology. 1500 tests without atypical zoster, 3000 tests if also approved for atypical varicella/zoster, volume control for latter indication: hospital practice only?
33	<i>Toxoplasma gondii</i>	CSF or brain biopsy: serological, clinical and radiological indications for cerebral toxoplasmosis in IC or neonatal. AF: congenital toxoplasmosis (seroconversion, serology unclear, specific congenital complications). Anterior chamber fluid: chorioretinitis.	Treatment selection, pregnancy interrupt.	In-house	CE	2,5	1276	52	8	0	Fr	More sensitive than culture, faster TAT. 1000-2000 tests expected for prenatal diagnosis
34	<i>Aspergillus</i>	BAL, biopsy, CSF, serum: IC with fever under broad spectrum antibiotics, pulmonary or cerebral lesion on CT lesion, cough, cerebral disease.	Treatment selection	In-house	No	3,5	793	50	2	NA		Complements galacto-mannan test (hemato indication). Increasing to 1000-1500 tests (100-200 in proposal 2001); criteria: Asciglu et al.; communication with physician is essential
35	<i>Candida</i>	Blood, serum: fever despite antimicrobial treatment in selected ICU or IC (transplant, neutropenia) patients.	Treatment selection	In-house	No	NA	470	114	NA	NA		Faster and more sensitive than culture
36	<i>Pneumocystis jiroveci (carinii)</i>	BAL, sputum, blood: unexplained lung infiltration in IC patient (AIDS, transplant, neutropenic...) and BAL microscopic exam for <i>P. carinii</i> neg or unclear.	Treatment selection	In-house	No	2,3	571	77	4	NA		Cytology is ok (PCR too sensitive?). Not for nomenclature (low volume); restrict to centres treating at risk patients
37	Identification cultured fungi	Culture: same as for phenotypic identification, currently only used if phenotype unclear.	Treatment selection	In-house	No	NA	257	NA	NA	NA		Phenotypic identification at same cost but slower.
38	Neu/HER2	Tissue section: metastatic breast carcinoma eligible for Herceptin therapy	Treatment selection	Kits (FISH, CISH)	FDA	6,1	1928	819	7	0		More reproducible and specific than IHC. 400 new treatments HERCEPTIN per year expected.
39	Aneuploidy TCC (bladder cancer)	Urine, bladder washing: follow-up treatment of transitional cell carcinoma of the bladder, neg. cystoscopy plus cytology equivocal.	Correct diagnosis, prognosis	Kits (FISH)	FDA	7	1000 estim	100 estim	1	0		More sensitive than cytology
40	LOH 1p-19q	Tissue section: prognostic, aid in diagnosis in complex cases of glioma.	Correct diagnosis, prognosis	Kits (FISH)	CE	7	50 estim	25 estim	1	0		No alternative
41	EGFR amplification/ mutation gene	Tissue section: grade III and IV astrocytoma.	Correct diagnosis, prognosis	Kits (FISH, CISH)	CE	5,5	200 estim	50 estim	2	0		IHC not reliable

Table 4b. Characteristics of individual hemato-oncology tests, as reported by the CMD experts.

Test	Sample type and indication	Type of Impact	Techn. CMD	IVD Kits	TAT day avg.	CMD tests 2003 C+M	CMD pos. tests 2003 C+M	CMD ctrs (#)	EQA ctrs (#)	Spec Reimb Countr	Expert comments on comparator, financing, or observed differences for volume estimates
PCR (BIOMED2) for VH-JH IgH / DH-JH IgH / Kappa and Lambda gene rearrangement.	DNA from blood, BM, LN, tissue block. (Suspected) nonconclusive B-cell or uncertain lineage LPD. LPD in IC. Classification/staging LPD. Discrimination relapse from second malignancy.	Correct diagnosis. Treatment selection.	In-house	CE probe/ RUO PCR	6	4864 +1424	2065 +572	12	0	Sw	Southern blot was reference but slower, larger biopsy needed, no paraffin tissue. BIOMED-2 PCR detects >98% of clonal B cell LPD.
PCR/ PAGE (BIOMED 2) for TCR rearrangement in NHL	DNA from tissue, skin, lymph node, BM, (suspected) T cell LPD, or organ involvement, especially skin lesions and lymphadenopathy. Follow up always after 3 months (to exclude false pos). max 4x/year or in stem cell collections, best using allele specific primers.	Correct diagnosis. Treatment selection.	In-house	RUO	7	1847 +460	429 +127	12	1	Sw	Southern blot needs more sample and >5% malign. cells. Southern blot mainly replaced by PCR. PCR best in duplo. PCR has 20-30% false neg (paraffin tissue, primer set incomplete) and 5-20% false pos (in non malignancy, depends on primers). Sequencing often used for confirmation or for patient specific primers.
PCR/ PAGE (BIOMED 2) for TCR rearrangement in AML/ALL	DNA from blood, BM, biopsy, tissue. Following conventional diagnosis of acute leukemia (precursor B-ALL, T-ALL, AML). If no other marker, MRD detection best using allele specific primers.	Correct diagnosis. Treatment selection. MRD detection.	In-house	RUO	9	incl. in above	incl. in above	10	1	Sw	False pos or false neg no issue here. Sequencing needed to produce allele specific primer test. 400 new acute leukemia's per year in Belgium.
Patient specific (RQ)-ASO PCR	DNA from BM or blood. MRD in ALL, AML and MM. Possible only after Ig/TCR rearrangement test. Technique possible in >85% of ALL and >60% of MM.	Treatment selection eg BMT if pos day 35 in pediatric ALL. MM: unclear.	In-house		NA	NA	NA	NA	NA		400 new acute leukemia per year. No data for MM. More testing expected in future.
IgVH sequencing	DNA from blood or BM. Hypermutation detection in typical CLL.	Poor prognosis. Treatment selection.	In-house		21	NA	NA	6	0		600 CLL per year in Belgium. Test is becoming more widespread.
RQ/RT-PCR (EAC) for t(1;14) BCL10-IgH	RNA from blood or BM, follow-up of t(1;14) cytogenetic positive T-ALL (MALT lymphoma).	MRD detection	In-house		10	256 +28	5 +3	4	0		FISH / cytogenetics at diagnosis. No study comparing sens of RQ/RT-PCR with FISH. 4-8 new pos per year. PCR standardisation: EAC, Gabert et al. Leukemia 2003.
RQ/RT-PCR (EAC) for t(1;19) E2A-PBX1	RNA from blood or BM, follow-up of childhood pre-B ALL, present in 25% of cases, only if t(1;19) cytogenetic positive	MRD detection (bad prognosis in adults)	In-house	CE	8	305 +23	1 +0	10	1	Sw	FISH / cytogenetics at diagnosis. No study comparing sens of RQ/RT-PCR with FISH. 20 new pos per year.
RQ-PCR (EAC) for t(12;21) TEL-AML1	RNA from blood or BM, follow-up of childhood pre-B ALL, present in 25% of cases, only if t(12;21) FISH positive?	MRD detection (good prognosis in children)	In-house mainly	CE	9	307 +50	16 +3	10	1	Sw	Typically missed by karyotyping, but detected using FISH. No study comparing sens of RQ/RT-PCR with FISH. 80 new pos per year (pediatric and adult).

Test	Sample type and indication	Type of Impact	Techn. CMD	IVD Kits	TAT day avg.	CMD tests 2003 C+M	CMD pos. tests 2003 C+M	CMD ctrs (#)	EQA ctrs (#)	Spec Reimb Countr	Expert comments on comparator, financing, or observed differences for volume estimates
RQ/RT-PCR (EAC) for MLL 11q23 translocation t(4;11) AF4-ALLI in ALL and AML	RNA from blood or BM. Subtype ALL (5% of ALL, >50% of infant ALL) and AML (<1%, M5 AML). Role for follow-up still unclear.	Poor prognosis. MRD detection.	In-house mainly	CE	11	195 +19	8 +0	9	2	Sw	PCR difficult for MLL. FISH / cytogenetics and southern blot as alternative at diagnosis.. At diagnosis: 700 for ALL (12 pos in 400) + AML (13 pos in 300).
RQ/RT-PCR for MLL 11q23 translocation t(9;11) MLL-AF9 in AML	RNA from blood or BM. Subtype AML (freq 2-5%, 25% of M5a in children). Role for follow-up still unclear.	Poor prognosis, MRD target	In-house mainly	RUO	12	208 +17	0 +0	7	1		PCR difficult for MLL. FISH and southern blot alternative at diagnosis, not sensitive enough for follow-up. At diagnosis: 300 tests in AML (10 pos). No positives found!!
RQ/RT-PCR (EAC) for t(8;21) AML1-ETO	RNA from blood or BM, subtyping of CBF AML when WHO M2 AML/ t(8;21)/AML1-ETO+ is suspected, identify target for follow-up, present in 10% of AML, in 40% of M2 AML, follow-up of treatment	Good prognosis, MRD detection	In-house mainly	CE	9	525 +138	15 +38	10	4	Au, Sw	Standard is karyotyping and FISH. RQ-PCR for follow-up. 10-15 new pos per year.
RQ/RT-PCR (EAC) for t(15;17) PML-RARA bcr 1, 2 and 3 fusion gene transcripts	RNA from blood or BM. Subtyping when WHO M3 AML/t(15;17)+/PML-RARA+ or variant is suspected; follow-up of treatment using RQ-PCR	Treatment selection, MRD detection	In-house mainly	CE	9	747 +177	15 +23	10	5	Au, Sw	FISH or PCR to complement karyotype at diagnosis. 1 new pos per year. FISH recommended for follow-up until neg by expert, also for local cost reason
RQ/RT-PCR for inv(16) CBFB-MYH11	RNA from blood or BM. Subtype all AML (M4eo, 10% of AML), follow-up up to 4x/year if pos	Good prognosis, MRD detection	In-house mainly	CE	9	647 +122	20 +52	9	3	Au, Sw	PCR detects also cryptic translocations. FISH as alternative at diagnosis, not sensitive enough for follow-up.
RFLP PCR for FLT3 exon 20 TKD mutation (D835)	RNA from blood or BM. Subtype adult and pediatric AML (5-10%) and pediatric ALL (1-3%), MDS with blast excess? (2-5%). At diagnosis and relapse, no role for MRD detection.	None today, awaiting FLT3 inhibitors	RFLP PCR	RUO	11	NA	NA	7	2		Complements cytogenetics in AML diagnosis. Confirm with sequencing. Class II tyrosin kinase inhibitors expected in AML
RFLP PCR for FLT3 exons 14/15 internal tandem duplication (ITD) or length mutation	RNA from blood or BM. Subtype adult (25%) and pediatric (12%) AML, pediatric ALL (25%), MDS with blast excess (3%), CMML. At diagnosis and relapse, MRD detection role unclear.	None today, awaiting FLT3 inhibitors	RFLP PCR	RUO	11	NA	NA	7	2	Sw	Complements cytogenetics in AML diagnosis. Confirm with sequencing. Class II tyrosin kinase inhibitors expected in AML
RQ/RT-PCR for WTI overexpression in malignant blasts	RNA from blood or BM. Subtype AL (30% pos), MDS, CML. Diagnosis and follow-up in rare cases of BCR-ABL neg CML or MDS.	Sometimes only available marker for follow up	In-house		16	NA	NA	4	0		Potential tool for monitoring 30% of AL, if other markers lacking. 400 tests 30% pos. Method standardisation lacking. Potentially 400+800 AL, 250+1000 MDS tests at diagnosis and foillow-up.
PCR for t(11;14) BCL1-IgH qualitative	DNA from blood, BM, lymph node, tissue. 1. B-NHL CD5+ or unclear phenotype. 2. Test for secondary organ involvement. Present in 95% of MCL.	Treatment selection. MRD detection.	In-house mainly	RUO	6	1032 +308	91 +31	8	0	Sw	Nested or real-time. BIOMED standard. FISH also possible. 1200 NHL/year, 5% MCL. PCR in-house. No FISH needed if PCR positive.

Test	Sample type and indication	Type of Impact	Techn. CMD	IVD Kits	TAT day avg.	CMD tests 2003 C+M	CMD pos. tests 2003 C+M	CMD ctrs (#)	EQA ctrs (#)	Spec Reimb COUNTRY	Expert comments on comparator, financing, or observed differences for volume estimates
PCR or FISH for t(11;14) BCL1-IgH in pathology	DNA from tissue. (Suspected) MCL (histology, immunophenotype) or other B-cell LPD associated with t(11;14) such as MM (20% is pos), hairy cell leukemia and prolymphocytic leukemia. Of help when IHC cyclin D1 unclear.	Diagnosis confirmed. Treatment selection. MRD detection. Prognosis (MM).	In-house mainly	RUO	6	incl. in above	incl. in above	8	0	Sw	PCR only 50% sensitive but best for MRD. Higher sensitivity with FISH. Cyclin D1 over-expression (IHC, not RT-PCR) as alternative. Shift towards FISH instead of PCR.
RQ-PCR for t(11;14) BCL1-IgH quantitat	Blood or BM. BCL1-IgH pos lymphoma (MCL, atypical B-CLL, sporadic other LPD or MM): detection tumor load and response, MRD, safety stem cell collection. Significance of remission unclear.	Poor prognosis, MRD detection.	In-house	RUO	6	incl. in above	incl. in above	2	0	Sw	FISH less suitable for MRD. RQ-PCR cyclin-D1 over-expression also indicates tumor load. 40 pos cases/year, or 200 samples/year. Follow-up short because MCL is aggressive disease.
PCR for t(14;18) BCL2-IgH qualitative	DNA from blood, BM, lymph node, tissue. 1. B-NHL and B-CLL. Complements morphology/ immunophenotype. 2. Test for secondary organ involvement. Present in 85% of FL and in 30% of DLBCL.	Treatment selection. MRD detection.	In-house mainly	RUO	6	1448 +432	255 +89	11	0	Sw	Small sample sufficient for PCR. BIOMED standard. Normals may test pos for highly sensitive test.
PCR for t(14;18) BCL2-IgH in pathology	DNA from biopsy, tissue. (Suspected) FL (histology, immunophenotype).	Correct diagnosis.	In-house mainly	RUO	6	incl. in above	incl. in above	11	0	Sw	Requested only by hematologist or pathologist.
RQ-PCR for t(14;18) quantification	Blood or BM. BCL2-IgH pos lymphoma (FL, DLBCL): detection tumor load and response, MRD, safety stem cell collection. RQ-PCR may be pos in healthy.	MRD under trial. Treatment selection at relapse.	In-house		6	incl. in above	incl. in above	5	0	Sw	RQ-PCR superior to competitive nested PCR. FISH less suitable for MRD.
FISH for 8q24: t(8;14), t(8;22), t(2;8) C-MYC	DNA from blood, BM, tissue section. BL/BLL (C-MYC rearr. is hallmark), high grade lymphoma. Transformation of FL.	Correct diagnosis. Treatment selection.	Kits mainly	RUO	14	482 +151	9 +0	3	0		FISH segregation assay best. Long distance PCR difficult. Southern blot requires much material. 100 tests/year. Important test because immunophenotype often not clear.
RQ-PCR for cyclin-D1 overexpression	Differential diagnosis MCL. t(11;14) with resulting cyclin-D1 expression is hallmark for MCL. MRD if no other marker.	Diagnosis, prognosis, treatment selection.	In-house mainly		9	281 +20	65 +9	9	0		IHC variable. Genomic BCL1-IgH via PCR (in 40%) or FISH (in most). Cytogenetics too slow. 1200 NHL/year, 5% MCL
FISH for trisomy 12	BM, blood, tissue. Diagnosis (differential diagnosis atypical lymphoproliferative disorders, pos in 50% of B-CLL), relapse or transformation of trisomy neg B-CLL, MRD in B-CLL if no other genetic marker	Diagnosis Poor prognosis.	Kits and in-house	FDA	10	210 +12	29 +4	3	0		Karyotype in B-CLL slow or in up to 50% impossible. FISH panel +12, del13, del17, del11 often used. 1500 samples/year diagnosis, 500 pos follow up. Sum 2000/year.
RQ-PCR (EAC) for t(9;22) BCR-ABL transcripts b2a2, b3a2 and e1a2 in CML diagnosis	MPD/MDS with hematological suspicion of CML or CML variants. t(9;22) or BCR-ABL is hallmark of CML (WHO)	Treatment selection	In-house mainly	CE	7	1757	275	12	5		RNA integrity needed for RQ-PCR, viability for karyotype, cell integrity for FISH

Test	Sample type and indication	Type of Impact	Techn. CMD	IVD Kits	TAT day avg.	CMD tests 2003 C+M	CMD pos. tests 2003 C+M	CMD ctrs (#)	EQA ctrs (#)	Spec Reimb Countr	Expert comments on comparator, financing, or observed differences for volume estimates
(RQ)-PCR t(9;22) BCR-ABL transcripts b2a2, b3a2 and e1a2 in ALL diagnosis	BM or blood. Precursor B-ALL (precursor T-ALL?).	Prognosis.	In-house	CE	6	incl. in above	incl. in above	12	5		
RQ-PCR in t(9;22) in CML follow-up	BM or blood. Autologous or allogeneic stem cell transplant or other CML treatment. 4-6 times per year depending on indication.	MRD detection	In-house mainly	CE	6	1085	642	12	5		RQ-PCR is superior to single or multiplex PCR, FISH, cytogenetics. Change of lab to be avoided (quantitative assay). 120 GLIVEC treatments per year (expected).
RQ-PCR in t(9;22) in ALL follow-up	BM or blood. After chemotherapy (4x) or stem cell transplantation (6x in first year). Consider relapse as new diagnosis. Year 2: 2x, year 3: 1x.	MRD detection	In-house	CE	6	incl. in above	incl. in above	12	5		RQ-PCR is superior to single or multiplex PCR, FISH, cytogenetics Change of lab to be avoided (quantitative assay).
PCR for HUMARA (human androgen receptor locus on X-chromosome)	BM or blood. Testing for clonal hematopoiesis in rare cases of MPD and MDS with diagnosis unclear, in females <65. Proposed new indication: clonality in essential thrombocytosis.	Correct diagnosis.	In-house		14	11 +0	3 +0	1	0		Difficult test.
RQ/RT-PCR for PRVI	Blood only. Suspected polycythaemia vera.	Correct diagnosis.	In-house		8	29 (HI 2004)	13 (HI 2004)	6	0		Too early to know clinical utility, could potentially substitute other tests (red cell mass, epo assay, cytogenetics)
Short Tandem Repeat (DNA fingerprinting) for chimerism	DNA from blood or BM. All related and unrelated allogeneic hematopoietic stem cell transplant. Test donor and patient first before transplant. Estimate en monitor engraftment succes max 6x/year.	Treatment selection	Kits mainly		8	405 +331	295 +203	6	2		ABO bloodgroup (if differs), sex chromosome via FISH or karyotype (if sex mismatch). No quantitation. 1200-1800/year: 300 transplants x 4-6/year, requested by centers performing HSCT.
RQ/RT-PCR for t(6;9) DEK-CAN	RNA from blood or BM. AML with t(6;9) on cytogenetics as seen in AML with maturation and increased basophilia (1% of AML is pos), but also in MDS.	MRD detection.	In-house mainly		7	NA	NA	5	0		310 new cases of AML in Flandres (Belgium 500?), thus 5 new cases/year.
RQ/RT-PCR for t(11;18) API2-MLT	RNA from biopsy, tissue, BM, (blood). Diagnosed or suspected (by pathology) (extranodal) marginal zone lymphoma. Up to 30-50% pos. Not quantitative.	Diagnosis confirmed. Treatment selection.	In-house mainly		5	NA	NA	5	0		Cytogenetics, conventional FISH requires living cells or mitosis in culture. 1200 new NHL in Flandres (2000 in Belgium?), of which 5-10% MALT GI/pulmonary.
RQ/RT-PCR for t(2;5) NMP-ALK, inv(2)	RNA from biopsy. Anaplastic large cell lymphoma of T cell or null cell type (ALCL is 3% of the lymphoma's). CD30+ LPD of skin. Rare large B cell LPD with unusual morphology/phenotype.	Diagnosis confirmed. Treatment selection.	In-house mainly	RUO	10	27 +1	4 +0	6	0		RQ/RT-PCR complements IHC. Quality RNA often not ok, plus RT-PCR low specificity. FISH takes time. IHC as reference test. 46 new cases per year.

4.3. QUALITY ASPECTS

4.3.1. Laboratory Quality Management

Molecular diagnosis is considered as a discipline of clinical biology (RD 3-12-1999, article 1, 2). “Klinische biologie: de verstrekkingen in de domeinen biochemie, hematologie, de microbiologie alsmede van de op deze domeinen betrekking hebbende moleculaire biologische toepassingen en immunologische toepassingen, ongeacht of daarbij gebruik gemaakt wordt van koude of radio-isotopische markers. Biologie clinique: les prestations couvrant les domaines de biochimie, d’hématologie, de microbiologie ainsi que les applications en biologie moléculaire et les applications immunologiques se rapportant à ces domaines, qu’elles fassent appel ou non à des marqueurs froids ou radioisotopiques. ». Currently only 5 parameters are included in article 24 of the nomenclature of clinical biology (ambulatory-hospitalization).

- 550911-550922: *Neisseria gonorrhea*
- 550933-550944: *Mycobacterium tuberculosis*
- 550211-550222: *Mycobacterium avium*
- 550233-550244: Hepatitis C qualitative
- 550255-550266: *Chlamydia trachomatis*

The volume of testing, the number of laboratories performing the tests and the costs directly associated with the tests have been listed in Table I above. For these parameters, all quality management requirements outlined in the license decree of 3-12-1999 are applicable. In January 2005, the Commission of Clinical Biology introduced a proposal for the inclusion of Factor V Leiden by molecular amplification in article 24. The following table summarizes the current quality requirements for parameters in the nomenclature in the clinical biology laboratory and those performed in the CMD's.

Table 5. Quality requirements, comparison between Clinical Biology Laboratory and CMD.

	Clinical Biology Laboratory	CMD
Legislation	RD 3-12-1999	RD 24-9-1998
Available know-how	+	+ and scientific expertise
Adequate infrastructure	+	+
Qualification of technologists	+	-
Training of technologists	+	-
Mandatory IQC	+	-
Mandatory participation in EQA	+	Free participation
Use of validated methods	+	-
Evaluation of technology and diagnostic kits	-	+
Quality system	+	-
SOP's	+	-

There are currently no quality requirements for pathology laboratories and for CMG laboratories. On the other hand, for two particular types of reference laboratories (Aids Reference Laboratories, ARLs, and the Tropical Institute, ITG) specific requirements are imposed and also formal BELTEST accreditation according to ISO

17025, in accordance with the RD of 8-10-1996 (ARLs) and the RD of 28-1-1998 (ITG). The steering committee of the CMD's (RD 24-9-1998, article 2§5) is in charge of the elaboration of quality requirements but there are no strict quality criteria required for the proper functioning of CMD's.

EQA within CMDs

For the quality evaluation of the CMDs (as required in article 2.4 of the RD of 24-09-1998) two rounds of interlaboratory comparisons were organized in the different working groups and the reports are available on the CMDs website <http://webhost.ua.ac.be/cmd/tests/tests.html>. Within the CMDs two cycles of interlaboratory comparisons for molecular microbiology were organized in 2001 and 2003. The organized programs were of different quality varying from excellent to poor regarding to EQA concepts used. The following remarks can be made.

- Not all CMDs have participated; which quality can be expected from those who refused participation?
- Response time sometimes unacceptable (up to 68 days after mailing of samples; one participant even lost his samples!)
- No participation of other laboratories outside Belgian CMDs (except for Bordetella pertussis)
- Some materials were well characterised (reference strains, EQA strains); in some surveys so called "reference material" was provided by the scheme organizer and declared "as such" as gold standard. In this last case there is a risk that the results are only harmonised between the participants without any guarantee on the trueness of results.
- For those schemes where one or more laboratories failed, corrective actions were taken; however efficiency of these actions was not checked. So was there no improvement assessed after the two surveys for Legionella pneumophila.
- For Enterovirus no consensus was obtained for some samples between the two reference laboratories (testing in order to assess sample quality and homogeneity). It is not appropriate to include these samples in a survey knowing the information from the pre-test.
- No accreditation for the organisation of EQA has been obtained by the CMD scheme organisers.

Within the CMD's two cycles of interlaboratory comparisons for hemato-oncology were organized in 2002 and 2003. As there are only few data available on the organisation of such surveys, the organized surveys were more or less experimental. Especially pre-analytical phase conditions must further be tested. Comparability of results was in general very good. Response time is here also sometimes too long (up to 153 days after mailing for one participant!). However this situation is rather exceptional and is much better as compared to the molecular microbiology surveys. Two rounds were organized (2002 and 2004) for the Her-2/neu FISH pathology test. All involved centers have participated. Samples were validated before sending out. No data are available on the response time for the first round; for the second round all results were available within one month.

Sources of information:

- Website CMD's: <http://webhost.ua.ac.be/cmd/tests/tests.html> for reports for microbiology and hemato-oncology.
- CMD documents "First HER-2/ FISH quality control program" and "External Quality Control Program for HER2/neu amplification in breast carcinoma".

EQA within clinical laboratories

According to the license decree (RD 3-12-1999, article 29§1), the laboratory must participate in the national external quality evaluation programs organized by the Institute of Public Health (IPH), which has been accredited for this activity. This mission of the IPH is clearly defined in article 31. The needed financial funding is regulated according to the RD of 10-6-2001. The EQA surveys for tests in the nomenclature under article 3, 18 and 24 are financed by an advance rebate of 0.2% on the annual total RIZIV/INAMI budget of clinical biology. The National Committee of the CMD in article 3, 1 of the RD 24-9-1998, is also given a mission to organize EQA schemes for all users of molecular biology tests. No financial regulation is foreseen. No initiatives were undertaken to organize EQA for other laboratories. Availability of EQA surveys for those parameters of molecular biology in article 24 of the nomenclature:

- *Neisseria gonorrhea*: not included in the current EQA programs of the IPH.
- *Mycobacterium tuberculosis*: a yearly scheme is organized
- *Mycobacterium avium*: not started due to the limited number of involved laboratories.
- Hepatitis C qualitative: included in the existing EQA schemes
- *Chlamydia trachomatis*: no national EQA scheme is organized due to the difficulties to find appropriate and stable sample material.

4.3.2. Feedback by requesting clinicians on the CMD services.

A survey was conducted at 6 regional hospitals (without CMD or CMG) focussing on the medical need for molecular tests and the quality of the services offered by the CMDs. The six hospitals were

- AZ St Augustinus – Wilrijk
- AZ Groeninge – Kortrijk
- AZ St-Lucas – Gent
- CH St-Vincent, St-Elisabeth, Clin St-Joseph - Rocourt / Montegnee
- CH de Charleroi – Charleroi
- CH Peltzer – La Tourelle – Verviers

For each hospital, the chief physician was contacted first, and then the head of the laboratory (or the CMD coordinator). The physicians requesting most of the molecular tests were identified and interviews were arranged. The main users were specialists in internal medicine (infectious diseases, gastroenterology), neurology, pediatrics and hemato-oncology. The findings have been ordered by specific role assigned to the CMDs by the Royal Decree of September 24, 1998.

CMD Role: Inform hospitals of test offering

- Clinicians and local lab physicians often do not know the offering by CMD/CMG, and their expertise, the test indications, shipment conditions, method limitations, test interpretation rules.
- Information is often obtained informally eg at seminars.
- Information is difficult to find when needed (web site should be up to date and could also mention test volume, method, TAT, interpretation).
- Service offered does not always meet the need (eg TAT HSV).
- Clinicians and non-CMD labs would like to get more involved in the selection of the test offering (some are well positioned, being involved eg in oncology clinical trials).

- CMD/CMGs are sometimes perceived as non-transparent or even competitive.
- Risk that lab service provided at CMD/CMG hospital differs from other hospitals, eg when new expensive tests are being introduced such as Ig VH hypermutation test in CLL.

The CMD tests most frequently requested are:

- HCV qualitative and quantitative, HCV genotyping, HBV qualitative, HSV, Enterovirus, VZV, CMV, Toxoplasma, *M. tuberculosis*, (EBV, Polyoma, Mycoplasma, Borrelia)
- Hemato-oncology: Ig and TCR rearrangements, BCR-ABL, t(14;18) and t(11;14)
- HER2

Tests performed by local labs having started or starting molecular testing:

- *C. trachomatis*, *N. gonorrhoeae*, HCV qualitative, CSF HSV, (enterovirus, MRSA, *B. pertussis*, *Streptococcus agalactiae*)
- HLA-B27, FVLeiden, FII, MTHFR, Hemochromatosis, (BCR-ABL, IgVH sequencing for hypermutation status)
- (HER2)

CMD Role: Perform routine molecular testing

- The CMD is often selected by the clinician, this may result in many CMDs/CMGs offering services to a single hospital.
- The CMD/CMG tests are often not listed on the local lab request forms, this may lead to a less standardized way of communication. Some CMDs provide clear request forms.
- The involvement of the local lab in CMD/CMG logistics varies by hospital and subspecialty.
- For hemato-oncology, the logistics towards the CMD/CMG are still often taken care of by the hemato-oncologist. Non specific test requests may result in redundant testing in CMD and CMG.
- In some hospitals this issue has successfully been solved by having subsampling and logistics performed by a single coordinator for the local clinical biology/pathology lab.
- Test selection/addition by receiving lab is often the rule at CMD/CMG (sometimes also for microbiology eg HCV genotyping).
- Many CMD/CMGs need to be phoned to obtain results.
- There is not always backup expertise available at the CMD lab.
- There is an unacceptable delay for printed results at some CMD/CMGs.
- There is no standardisation between CMDs for reporting of key tests eg BCR-ABL quantification.

CMD Role: Guide specialist formation

- There seems to be an imbalance between the demand and the offering of information. There is massive attendance of local laboratories for molecular test topics but little efforts to spread expertise at CMD/CMG.
- Often, little attention is paid to molecular techniques during the clinical biology specialist training.

- Local labs starting molecular testing received training by a CMD to use their in-house method.

CMD Role: Evaluate diagnostic value and propose nomenclature

- No CMD reports available on diagnostic value and test interpretation.
- Test indications on CMD web site not always up to date or not always respected.

CMD Role: Implement internal and external QA of CMD tests and nomenclature tests

- No internal QA guidelines available on CMD website.
- Local labs often do not know the possibility to participate to CMD QA rounds.

4.4. ORGANISATION AND FINANCING

Materials and methods

During the first 40 months, the 18 CMDs submitted 4 annual reports to the RIZIV-INAMI. The centres had to report the investments done during the 4 previous years, the maintenance contracts, the small equipments and repair costs paid during the covered year, the number and qualification of the people employed at the centres, the costs for consumables, the volume for each of the 94 authorized tests, the number of patients tested and the number of positive tests. The 2 first reports covered each a 8-month period because of an initial delay in the number of centres, and agreed by the RIZIV-INAMI.

The activity reports (and their annexes) produced every year by 16 CMDs constitute the main source of data. The financial information (invoices or listing of goods purchased) received from the centres has not been audited in the context of this project, as this was not planned and not needed for the project aims. The financial information was used mainly to estimate a cost per test. The findings of this project were presented to the CMDs and the feedback received has been incorporated before finalisation of this document.

At start of the investigation, the INAMI-RIZIV had received a complete set of invoices for the period Feb 03 to Jan 04 from the following centres: Brugge, Sart Tilman, Brugman, UZ Gent, UZ Antwerpen, Virga Jesse, HH Roeselare, IJ Bordet, Jolimont, OLV Aalst. Upon individual request of the INAMI-RIZIV, the following material was added: a complete set of invoices for ULB, Lovreval, Mont-Godinne; an incomplete set of invoices with a complete listing for UCL and Citadelle.

From ZN Antwerpen, we received a concise listing of the expenses charged with a description of the goods purchased but without any invoices. From KU Leuven, we received an abridged list of goods without any invoices. Finally, VUB had sent in due time a spreadsheet file with all the expenses charged to the RIZIV-INAMI. KCE had prematurely agreed with the format before checking the detailed content, which at a later stage could not be used for the intended purpose. So 17 out of the 18 centres transmitted to the RIZIV-INAMI a detailed list of the goods bought during the period Feb 03 to Jan 04 and 15 out of the 18 centres submitted copies of the invoices charged to the RIZIV-INAMI.

Evaluation of the mean cost of molecular diagnostic assays at the CMDs.

For the two first 8-month periods, depreciation and labour costs were reduced to 8/12 of the costs submitted by the 18 centres. Other expenses were fully accounted. For the two last periods, all costs submitted were fully accounted as stated in the annual reports of the centres for the RIZIV-INAMI.

The annual depreciation rate for the investments of a value of >2 479 € was 25% per year of the acquisition costs with a 21% VAT included whatever the country of origin of the equipment. The annual slice of 25% was further reduced if the equipment was used simultaneously by the CMD and another laboratory according to their respective use. The depreciation was split between the Microbiology (MB), Hemato-oncology (HO) or Pathology (AP) departments. Small equipment ($\leq 2\,479$ €) was 100% deductible during the year of acquisition as well as maintenance & repair of equipment. Consumables were charged according to the department that has consumed the goods. The costs of consumables were all the varied variable costs. In one centre, the variable costs and the cost of small equipment had been inverted for the last period and a correction was done accordingly.

Labour costs were detailed in

- Management costs: clinical biologists & pathologists
- Operators: MSc (Licentiaat), PhDs & technicians
- Secretary

In a further analysis, the expenses were grouped in 4 classes:

- Fixed costs: annual depreciation, small equipment, maintenance and repairs
- Management costs
- Technico-scientific and secretary costs
- Consumables

The number of determinations performed during the exercise (8- or 12-month period) for each of the allowed tests was entered according to the figures submitted by the centers in their annual report to the RIZIV-INAMI.

Costing of individual tests

Invoices collection and analysis

Every individual invoice received from the INAMI-RIZIV was split in as many lines as the number of articles bought. The complete description of the good was retrieved from the product list found on the website of the manufacturer. Every line of every invoice (available) for molecular diagnostic reagents was entered in a MSExcel spreadsheet as described hereunder:

Centre	Depart.	Invoice date	Manufacturer, short description & number of items	ref. number	Invoice number
17	AP	1/01/03	PathService B, 1*480 PAP smear material	(GYN-0480-E)	# 03 001 531
18	AP	1/02/03	Digene UK, 1*96 tests HC II HPV DNA Kit, HC2	(5196-1230)	# 01 274

Price of goods: $\text{Round}[\text{Round}(((n*u*(1-d))+t);2)*1.21;2]$

where:

n = number of packs,

u = unit price in EURO,

d = discount,

t = transport cost,

2 = rounding the value up to 2 digits,

1.21 = price VAT included if bought in Belgium, otherwise, the local VAT rate applies since CMDs may not deduct VAT.

2 = rounding the value up to 2 digits

Administrative & transportation costs, dry ice, safety costs are either split between the similar reagents billed on the same invoice or added to the single reagent costs. Not all invoices were enrolled since a minority of centres never complied with the obligation to submit their invoices to the INAMI-RIZIV. Finally, $\pm$ 3 000 invoices and 6 000 lines were entered. For the calculation of costs, when same goods were bought several times in a year by the same centre, we kept the latest invoice available. The cost of a same item bought by several centres was then compared and the second lowest price in case of regular discounts was selected.

For goods produced and bought outside Belgium, we have added the VAT at the local rate since the centre may not recover the VAT on goods. However many centres accepted that the seller does not charge any local VAT on the goods bought. It is then the responsibility of the buyer to pay the VAT (at the Belgian rate, what is always higher than the local one). Only a minority of centres had traces of VAT effectively paid in their books. The majority added 21% to these invoices, sometimes twice, without producing any convincing document. We did not check if the taxes were really paid.

For most of the products used specifically for molecular testing, we identify - from the reference number of the product - the number of tests achievable according to the manufacturer. This gives us the gross number of tests and controls really performed.

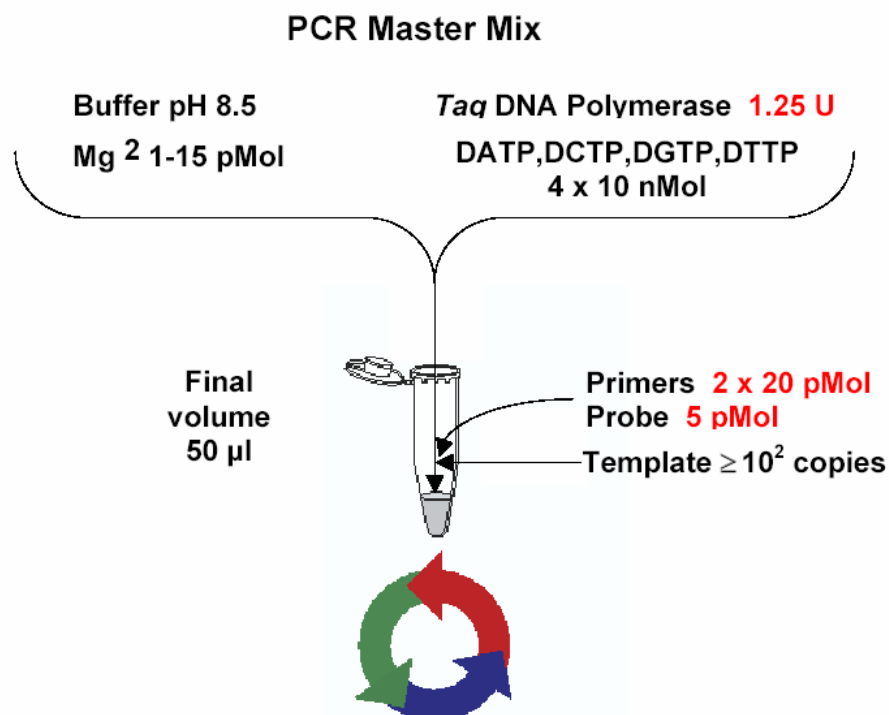
Calculation of the cost of consumables used for the individual tests

In-house methods use RNA/DNA extraction and DNA/cDNA amplification. Extraction and purification of the RNA/DNA are mainly preceded by a preliminary lysis of cells or pathogen agents with proteinase K (200 μ g/sample) followed by a purification of the genetic material on a silica gel mini column. This step is tricky and relatively expensive. The separation columns are delivered with proteinase if needed, buffers and collection tubes. The purified RNA/DNA is then amplified with the Taq DNA polymerase, 2 primers (forward & reverse) and a (fluorescent) probe in a Mg^{++} rich buffer. Each PCR amplification is performed in a maximal volume of 50 μ l where the following reagents are present:

- 1.25 units of Taq DNA polymerase in a universal master mix
- 10-20 pMol of a specific forward primer
- 10-20 pMol of a specific reverse primer
- 5 pMol of a specific (fluorescent) probe
- DNA to be amplified

For RNA, a cDNA copy is first obtained with the help of a specific reverse primer and a reverse transcriptase, then the cDNA is amplified; the two steps are often conducted sequentially in the same vial (one- or two-step reaction). The figure 1 below depicts the reagents mix and the respective quantities needed to perform the PCR reaction. The composition of the PCR master mix is quite constant whatever the kind of DNA that is amplified. The length of the test-specific primers varies between 15 and 30 nucleotide bases. The number of cycles depends of the initial DNA content available. For quantitative PCR (real-time PCR), 4 to 5 calibrated standards are often simultaneously amplified and a calibration curve is built. The amplicon is either directly read in case of the use of a fluorescent probe (real-time PCR) or further isolated by a polyacrylamid or agarose gel electrophoresis.

Figure 1. PCR Reaction



For in-house tests, the quantities needed were calculated either from the SOP supplied by some of the CMDs or from the reference paper. Consumables like polypropylene tubes & vials, pipettips, filtertips (tips with a physical barrier to prevent cross-contaminations by aerosol of particles), and gloves were also accounted. The depreciation rate for the main equipment was calculated per PCR.

Results

Evolution in volume of tests declared

The table 6 shows the number of tests performed in microbiology and hemato-oncology from October 2000 till January 2005. The number of tests increased by more than 25 % during the last period.

Table 6: Number of tests declared yearly in hemato-oncology and microbiology

	10/00–05/01 8 months	06/01–01/02 8 months	02/02–01/03 12 months	02/03–01/04 12 months	02/04–01/05 12 months
Hemato-oncology	10 948	12 445	23 235	23 897	29 611
Microbiology	50 144	46 381	80 024	92 339	117 139
TOTAL	61 092	58 826	103 259	116 236	146 750

Investments in heavy equipments supported by the RIZIV-INAMI

Total depreciation for the 4 periods was 3 643 203 €. Investment for equipment was reported for Real Time PCR analyzers (n=53), Cyclers (n=55), Hybrid Capture II (n=7), laminar air flow cabinets (n=29), - 80 °C Freezers (n=14), centrifuges (n=19), CO₂ incubators (n=3), spectrophotometers (n=7), Palm Robots (n=2), Cytovision (n=2),

microscopes (n=10), microdissection microscopes (n=3), microtomes (n=6), and other equipments (n=16).

Table 7 describes the number of assays reported from October 2000 to end January 2004, the reported costs ('really spent') split in fixed costs (depreciation, maintenance), personnel costs, variable costs (consumables, small equipment, repairs) and the amount of money paid-out by the RIZIV.

The centres with the lowest volumes reported high fixed costs and high management costs.

Fourteen centres received between €1.3 and €1.8 millions for a number of tests varying from 11 606 to 38 964. The RIZIV grants did not cover the expenses reported and the centre with the highest volume of tests suffered also the deepest financial loss.

The table 8 shows the previous costs standardized per test. The centres reported a cost per test varying from €59 to €175 for a reimbursement of €39 to €178. The reasons for a lower reimbursement lie to the system of a ceiled reimbursement by centre whatever the number of tests performed. The more tests a center reported, the less that center received by test.

Eleven of the centres had 4 to 6 employees at work (at bench and secretary) (table 9).

Table 7: Grants to CMDs from 1st October 2000 to 31st January 2004

Centre	# Assays reported	Fixed costs	Management	Labour	Variable costs	Really spent	Paid by RIZIV
1	4 134	173 575 €	80 817 €	188 410 €	213 115 €	655 917	695 249 €
2	5 263	96 697 €	207 329 €	414 882 €	203 028 €	921 937	934 510 €
3	8 035	183 763 €	307 388 €	567 384 €	267 474 €	1 326 009 €	1 185 938 €
4	11 606	95 212 €	267 725 €	535 863 €	450 942 €	1 349 742 €	1 332 280 €
5	12 528	106 199 €	264 420 €	754 178 €	488 917 €	1 613 715 €	1 329 712 €
6	12 746	252 204 €	446 209 €	613 642 €	908 903 €	2 220 958 €	1 637 105 €
7	14 579	271 047 €	315 071 €	550 119 €	482 055 €	1 618 292 €	1 309 796 €
8	16 407	150 923 €	132 822 €	970 665 €	477 181 €	1 731 592 €	1 302 732 €
9	17 330	291 522 €	411 505 €	702 077 €	696 217 €	2 101 321 €	1 477 424 €
10	18 672	199 590 €	213 189 €	655 473 €	579 849 €	1 648 100 €	1 399 728 €
11	18 916	203 466 €	268 965 €	786 199 €	811 610 €	2 070 240 €	1 494 681 €
12	19 560	289 768 €	287 559 €	748 229 €	890 484 €	2 216 041 €	1 576 773 €
13	19 836	405 483 €	366 882 €	1 165 723 €	918 348 €	2 856 436 €	1 613 599 €
14	21 034	228 607 €	330 526 €	534 459 €	512 999 €	1 606 591 €	1 306 297 €
15	23 713	277 614 €	338 786 €	1 265 660 €	1 126 185 €	3 008 245 €	1 788 759 €
16	26 310	203 379 €	406 542 €	724 840 €	685 120 €	2 019 881 €	1 479 308 €
17	38 964	146 542 €	315 502 €	1 122 958 €	727 539 €	2 312 542 €	1 521 773 €
18	49 780	203 254 €	330 526 €	2 004 742 €	1 717 039 €	4 255 561 €	2 124 589 €
All	339 413	4 086 704 €	5 291 765 €	14 305 505 €	11 849 145 €	35 533 119 €	25 510 253 €

Really spent = sum of the expenses stated by the centres in their annual reports;

Paid by the RIZIV = Amount paid-out by the RIZIV for the 4 periods

Table 8: Mean costs per CMD test from 1st October 2000 to 31st January 2004

Centre	# Assays reported	Fixed costs	Management	Labour	Variable costs	Really spent/test	Paid/test by RIZIV
1	4 134	41.99 €	19.55 €	45.58 €	51.55 €	158.66 €	168.18 €
2	5 263	18.37 €	39.39 €	78.83 €	38.58 €	175.17 €	177.56 €
3	8 035	22.87 €	38.26 €	70.61 €	33.29 €	165.03 €	147.60 €
4	11 606	8.20 €	23.07 €	46.17 €	38.85 €	116.30 €	114.79 €
5	12 528	8.48 €	21.11 €	60.20 €	39.03 €	128.81 €	106.14 €
6	12 746	19.79 €	35.01 €	48.14 €	71.31 €	174.25 €	128.44 €
7	14 579	18.59 €	21.61 €	37.73 €	33.07 €	111.00 €	89.84 €
8	16 407	9.20 €	8.10 €	59.16 €	29.08 €	105.54 €	79.40 €
9	17 330	16.82 €	23.75 €	40.1 €	40.17 €	121.25 €	85.25 €
10	18 672	10.69 €	11.42 €	35.10 €	31.05 €	88.27 €	74.96 €
11	18 916	10.76 €	14.22 €	41.56 €	42.91 €	109.44 €	79.02 €
12	19 560	14.81 €	14.70 €	38.25 €	45.53 €	113.29 €	80.61 €
13	19 836	20.44 €	18.50 €	58.77 €	46.30 €	144.00 €	81.35 €
14	21 034	10.87 €	15.71 €	25.41 €	24.39 €	76.38 €	62.10 €
15	23 713	11.71 €	14.29 €	53.37 €	47.49 €	126.86 €	75.43 €
16	26 310	7.73 €	15.45 €	27.55 €	26.04 €	76.77 €	56.23 €
17	38 964	3.76 €	8.10 €	28.82 €	18.67 €	59.35 €	39.06 €
18	49 780	4.08 €	6.64 €	40.27 €	34.49 €	85.49 €	42.68 €
All	339 413	12.04 €	15.59 €	42.15 €	34.91 €	104.69 €	75.16 €

Table 9. Full-time equivalents employed at the CMDs by CMD (1 Feb 2003 – 31 Jan 2004)

CMD	MScs & PhDs	Technologists	Secretaries
1	1.70	7.10	0.00
2	1.60	3.73	0.10
3	2.08	3.05	0.65
4 MB/HO	0.10	4.00	0.25
5	1.70	7.30	0.20
6	4.50	3.00	0.20
7	1.30	4.90	1.20
8	1.00	2.75	0.30
9	0.30	5.00	0.60
10	3.60	9.43	1.70
11	4.26	2.45	0.20
12	2.00	3.21	0.40
13	1.00	3.50	0.50
14	3.00	2.65	0.35
15	0.00	1.00	0.00
16 MB/HO	0.00	3.20	0.50
17	1.15	3.38	0.37
18	1.00	2.50	0.75
AP	0.00	2.00	0.20
All CMDs	30.29	74.15	8.47

Deviations observed

Several important deviations with regard to the initial agreement were observed. Some of them are most probably clerical errors.

Depreciation :

- Inadequate allocation of depreciations according to their use
- Year to year variation in the acquisition value of the same equipment
- No mention of the acquisition date
- Extra financial costs charged
- Equipment not located in the CMD (but invoiced): one CMD claimed in the last 3 years for the depreciation of a GeneAmp 9600 (Feb 2000) and a Taqman 7700 (Feb 2000) both devices delivered at the Vesale Hospital (Montignies-le-Tilleul)!
- Two Bioanalysers Agilent 2100 were paid by the INAMI-RIZIV, one in Liège in Aug 2002, the second in Brussels in 2002 but never used for CMDs tests: We were only able to find a single invoice for reagents used with the device in Liège. For the equipment located in Brussels, no further invoices from the company that produces that equipment were charged afterwards!

Small equipment :

- Inadequate allocation of equipments according to their use
- Heavy equipment sliced under the threshold of 2 479 €.
- Not allowed expenses (office, computer, printers,...)
- Expenses nested elsewhere (usually within the reagents)
- Discrepancy with regards to the accounted value

Maintenance contracts & repairs:

- Inadequate allocation of the contracts according to their use
- Date of contract starts before the considered period
- Repairs nested elsewhere (usually within the reagents)
- Discrepancy with regard to the accounted value

Reagents :

- No copy of the invoice for the reagents bought (mandatory)
- Invoices out of the time period considered
- Renting costs of heavy equipment
- Cost of testing *Mycobacteria tuberculosis* (n = 5), *Chlamydia trachomatis* or *Neisseria gonorrhea* (n = 5), HIV-1 (n = 1), already reimbursed through the nomenclature system, haemochromatosis (n = 2), alpha 1-antitrypsin deficit (n = 1) and Factor V Leiden (n = 1), inherited diseases under the responsibility of the CMGs and forensic medicine (n = 2).
- Computers and office furnitures, scientific books, translations
- Training, membership and registration to scientific meetings
- Transportation costs for employees or for the collection of samples
- Catering for meetings
- Financial agreement with other non CMD laboratory
- No mention of the VAT identification number on some intracommunautaire invoices for many centres
- VAT not paid on goods bought outside Belgium according to the hospital books (1 centre has also 4 VAT registration numbers)
- Belgian VAT billed twice

Coexistence of two reimbursement systems for *M tuberculosis* and HCV-qualitative assays

Two tests, *M tuberculosis* and Hepatitis C virus qualitative, could be reimbursed through either the nomenclature system or within the CMD system. Several centres used both systems (table 10).

It is difficult to assess, for some CMDs, if a same test (HCV-qual or *M tuberculosis*) was accounted or charged twice within the same laboratory: once through the nomenclature system and once through the fixed budget of CMDs.

We also found evidences of invoices of kits for the determination of *M tuberculosis* and Hepatitis C virus qualitative in the reports of 5 CMDs although they did not declare any of these tests in their activity reports. Similarly, 8 CMDs included the invoices of kits for the determination of *C trachomatis* and *N gonorrhea* in their financial report, tests which are reimbursed through the nomenclature system.

Table 10. Reimbursement of molecular diagnostic tests (test volume) through the nomenclature system from 1st Feb 2003 to 31st Jan 2004

Centre No.	Nomencl. HCV- Qual	Nomencl. M. <i>tuberculosis</i>	Nomencl. C. <i>trachomatis</i>	Nomencl. N. <i>gonorrhea</i>	Total nomenclature	CMD HCV- Qual	CMD M. <i>tuberculosis</i>
1	0	6	205	138	349	563	605
2	117	25	0	0	142	76	164
3	72	149	52 ⁺	0	273	102	237
4	0	0	5	5	10	540	1 244
5	168	0	0	0	101	58	0
6	0	102	6 ⁺	0	108	284	205
7	2	14	77	0	93		0
8	118	0	0	0	118		34
9	81	8	455	0	544	423	198
10	432	58	154 ⁺	152 ⁺	796	1 375	300
11	0	0	10 ⁺	0	10	1 018	453
12	25	0	38	0	63		
13	235	38	1 019 ⁺	0	1 292	12	668
14	345	79	577 ⁺	312 ⁺	1 313	503	520
15	108	37	1	0	146	831	118
16	858	29	724 ⁺	0	1 611	32	49
17	1 444	116	5 540	5 205	12 305	0	0
18	1 359	65	219 ⁺	0	1 643	1 551	0
CMDs	5 297	726	9 082	5 812	20 917	7 368	4 795
Not CMDs	891	262	20 759	9 883	31 795	NA	NA
Total	6 188	988	29 841	15 695	52 712		

The figures for the nomenclature tests reimbursed in 2003 are based on RIZIV-INAMI data from Jan to Dec 2003 and for the CMDs from Feb 2003 to Jan 2004.

For HPV determinations, it was difficult to reconcile the number of tests notified and the number of tests expected from the kits invoiced (table 11).

Table I I. Variability between the number of HPV tests stated and the number of tests bought from 1st February 2003 to 31st January 2004

Centre	Quantities ordered and invoiced	HPV tests reported	HPV tests purchased
1	7*96 tests HC II HPV DNA Assay Kit, HC2 (5196-1230)	301	672
2	No invoices	0	0
3	14*96 tests HC II HPV DNA Assay Kit, HC2 (5196-1230)	1 506	1 344
4	4*96 tests HC II HPV DNA Assay Kit, HC2 (5196-1230)	532	384
5	8*96 tests HC II HPV DNA Assay Kit, HC2 (5196-1230)	0	768
6	34*96 tests HC II HPV DNA Assay Kit, HC2 (5196-1230)	820	3 264
7	54*96 tests HC II HPV DNA Assay Kit, HC2 (5196-1230)	3 471	5 184
8	14*96 tests HC II HPV DNA Assay Kit, HC2 (5196-1230)	984	1 344
9	17*96 tests HC II HPV DNA Assay Kit, HC2 (5196-1230)	860	1 632
10	9*96 tests HC II HPV DNA Assay Kit, HC2 (5196-1230)	875	864
11	52*96 tests HC II HPV DNA Assay Kit, HC2 (5196-1230)	2 678	4 992
12	No detailed invoices	1 859	± 2 500
13	20*96 tests HC II HPV DNA Assay Kit, HC2 (5196-1230)	1 168	1 920
14	9*96 tests HC II HPV DNA Assay Kit, HC2 (5196-1230)	1 395	864
15	NB: 1.750 HPV determinations and no expenses charged!	1 750	0
16	No invoices	1 166	0
17	8*96 tests HC II HPV DNA Assay Kit, HC2 (5196-1230)	2 282	768
18	No invoices	1 566	0
		24 213	± 26 500

HPV tests notified = number of tests presented in the last annual report

Expenses reported by the CMDs for participation to international External Quality Assurance programs

We have derived the effective participation in External Quality Assurance programs from the collected invoices for every CMD (table 12). Very few CMDs participated effectively to an EQA program during the period Feb 2003 to Jan 2004.

Table 12: Costs reported for participation to international EQA programs from 1st February 2003 to 31st January 2004

Centre no. 1	No expenses											
Centre no. 2		CMV									MTB	
Centre no. 3	No expenses											
Centre no. 4				EV								
Centre no. 5	No expenses											
Centre no. 6	No expenses											
Centre no. 7	No expenses											
Centre no. 8	No expenses											
Centre no. 9					HBV	HCV		HIV			MTB	CT
Centre no. 10	No expenses											
Centre no. 11	No expenses											
Centre no. 12	No expenses											
Centre no. 13	No expenses											
Centre no. 14	No expenses											CT*
Centre no. 15		CMV	EBV	EV	HBV	HCV	HCV-genot.	HIV	HSV	VZV		
Centre no. 16	No expenses											
Centre no. 17		CMV	EBV	EV	HBV	HCV		HIV	HSV	VZV		
Centre no. 18	No expenses											

Note that CT (*Chlamydia trachomatis*) is not a CMD test

CT*: No invoice produced, order form only

Overall testing activity of the centres.

Aside of the officially declared activity, a sensitive, yet theoretical yardstick for the true activity of the CMDs is the consumption of a unique reagent, the *Taq* DNA polymerase, the cornerstone of all PCR reactions. The table 13 shows the quantities of *Taq* DNA polymerase purchased and charged to the INAMI-RIZIV during the period 31 Jan 2003 – 1 Feb 2004 by the 16 centres with in-house tests and a detailed listing of the consumables bought. The pathology departments of 2 centres which worked in close association and submitted their invoices together make the centre AP. The detailed invoices can be found in appendix 5. The number of units were converted in number of tests achievable, to say 1.25 unit of enzyme per 50 µl assay (1 000 units = 800 tests) although some centres used 0.625 unit of enzyme per 25 µl assay (according to their SOPs), especially for enterovirus, HSV, VZV, CMV qualitative and quantitative, EBV qualitative and quantitative, HBV quantitative, HCV qualitative and parvovirus assays. The sum of *Taq* DNA polymerase bought does not take into account the direct use of *Taq* polymerase included in the commercially available kits used by the centres.

These quantities give a clearly different picture of the respective activity of the CMDs. Table 13 also shows the distribution of tests assayed with kits and with in-house methods. The ratio of the number of PCR reactions versus the number of in-house tests should normally not largely exceed 2.0 if all the assays are quantitative (that means 4 extra assays per calibration curve) and done in duplicate. The observed values varied between 2.3 and 14.8. This indicates that many centres performed far more assays than officially declared either for research or other purposes.

Table 13. Repartition of the tests by method and by centre

CMD	Total tests ¹	Kits ²	In-house ³	PCRs ⁴	Ratio ⁵
1	5 172	1 083	4 089	10 320	2.5
2	8 808	1 652	7 156	23 300	3.3
3	4 459	2 133	2 326	8 000	3.4
4	8 378	2 639	5 739	21 400	3.7
5	7 062	1 168	5 894	22 000	3.7
6*	1 682	77	1 605	7 200	4.5
7	19 373	5 619	13 754	65 800	4.8
8	6 801	2 119	4 682	25 300	5.4
9*	7 341	2 745	4 596	28 000	6.1
10	5 022	1 166	3 856	27 412	7.1
11	5 533	3 471	2 062	15 600	7.6
12	3 317	1 506	1 811	14 450	8.0
13	5 865	2 639	3 226	26 856	8.3
14	4 004	0	4 004	36 450	9.1
15	5 218	1 987	3 231	33 200	10.3
16	8 219	4 366	3 853	57 000	14.8
16 CMDs	106 254	34 370	71 884	422 288	5.9
AP	2 571	2 270	301	3 900	13.0
17	5 619	1 859	3 760	75 100	20.0
Jolimont	1 792	1 792	0	0	

¹Total tests: Number of tests reported in the annual report;

²Kits: Number of tests performed with commercial kits (reported number of tests for which kits were purchased at centre). Aside of the tests included in the tasks of the CMDs, many centres charged reagents and kits for other tests that are not foreseen like *M. tuberculosis* (55 09 33/44), *C. trachomatis* (55 02 55/66), *N. gonorrhea* (55 09 11/22), HCV qualitative (55 02 33/44), HIV-1; these tests were done on Cobas Amplicor devices with commercial kits from Roche.

³In-house: Assays developed in-house; a minority of centres determined also Factor V Leiden, Hereditary haemochromatosis and Polycythemia Vera, tests at that time not included in the lists of CMD tests.

⁴PCRs: Number of reactions based on the quantities of *Taq* DNA Polymerase bought

⁵Ratio: Number of PCR reactions vs number of in-house tests

* These 2 centres performed mainly 25 µl assays

Cost of personnel directly involved in PCR assays

We have then allocated the human resources in time as well as in € in function of that “molecular biology” activity (table 14)

Table 14: Mean time charged for personnel per PCR (50 µl in single) by category and centre

Centre	PCRs	Scientists	Technologists	Secretaries	Total
1	10 320	14 min.	33 min.	1 min.	47 min.
2	23 300	1 min.	19 min.	2 min.	23 min.
3	8 000	11 min.	31 min.	3 min.	46 min.
4	21 400	7 min.	31 min.	1 min.	39 min.
5	22 000	7 min.	29 min.	0 min.	36 min.
6	7 200	13 min.	31 min.	9 min.	53 min.
7	65 800	5 min.	13 min.	2 min.	20 min.
8	25 300	0 min.	11 min.	2 min.	13 min.
9	28 000	14 min.	10 min.	1 min.	25 min.
10	26 612	14 min.	8 min.	1 min.	23 min.
11	15 600	6 min.	20 min.	3 min.	29 min.
12	14 450	7 min.	21 min.	2 min.	31 min.
13	26 856	0 min.	13 min.	1 min.	15 min.
14	36 450	7 min.	7 min.	1 min.	15 min.
15	33 200	5 min.	9 min.	1 min.	15 min.
16	57 000	2 min.	8 min.	2 min.	12 min.
16 CMDs	421 488	6 min.	15 min.	2 min.	22 min.
AP	3 900	0 min.	46 min.	5 min.	51 min.
Jolimont	No in-house				

The figures and assumptions used to compute the costs in personnel are presented in table 15. Briefly, the wages agreed at start for the several types of personnel were inflated according the “health index” progression from Oct 2000, the first month of the first period; index = 106,04) to June 2005, the last available value (=116,29). A full-time worker was supposed to work 1 500 hours a year. For the sake of simplicity, all the assays were supposed to be performed in single; if now all of these were done in duplicate, the costs will double.

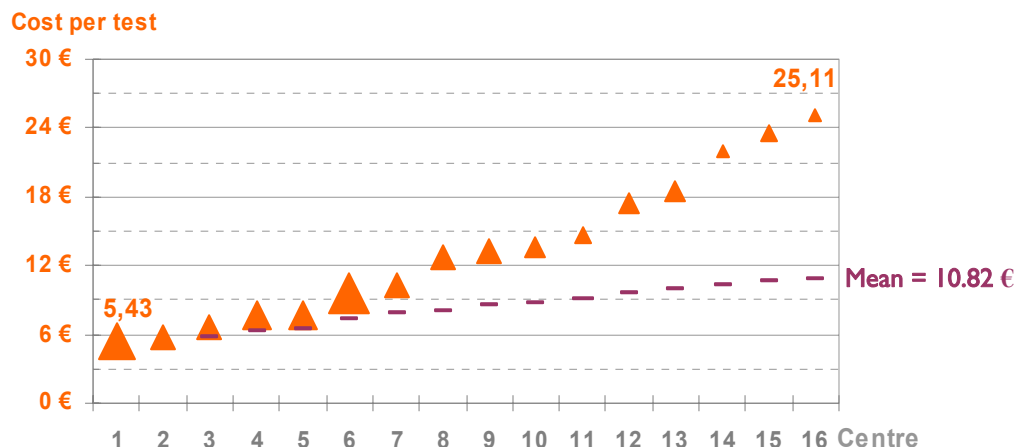
Table 15. Human resources engaged in the PCRs of the 16 CMDs laboratories

Full-time scientists	28.21
Full-time technologists	68.10
Full-time secretaries	7.62
Working days per year (assumption)	200
Working hours per day (assumption)	7.5
Percentage of tests in duplicate (base case)	0.00%
Hours of scientists	42 315
Hours of technologists	102 150
Hours of secretaries	11 430
Index Oct 2000	1.0604
Index June 2005	1.1629
(Yearly inflation rate	2.00%)
Years since the reference year (1 st report)	4.67
Mean wage of scientists at start and during the 4 periods	49 579 €
Mean wage of technologists at start and during the 4 periods	37 184 €
Mean wage of secretaries at start and during the 4 periods	29 747 €
Mean wage of scientists today	54 379 €
Mean wage of technologists today	40 784 €
Mean wage of secretaries today	32 627 €

The results are presented in figure 2. The triangles show the 16 active CMDs ranked by ascending cost per test. The surface of each triangle is proportional to the number of tests performed by the centre. The dashes represent the weighted average cost per test from the least expensive centre till the most expensive one.

The cost of personnel per PCR varied from 5.43 € to 25.11 €. The median and the mean personnel costs for the 421 488 in-house tests (50 µl final volume) were respectively 9.66 € and 10.82 €.

Figure 2. Cost of personnel per reaction (PCR in single)



Legend: Triangles show the mean cost of personnel per PCR for each of the 16 centres; Dashes indicate the weighted average cost per PCR, starting with the lowest cost on the left of the chart

Cost of consumables used for the individual tests when kits are used.

- HBV quantitative with the kit COBAS Amplicor HBV Monitor RUO (I 118 331) from Roche in 9 centres: 58.11 € per test
- HCV qualitative with the kit Amplicor HCV AMP GEN-2 C (I 111 132) from Roche in 9 centres: 20.34 € per test
- HCV quantitative with the kit Amplicor HCV Monitor (I 118 404) from Roche in 10 centres: 85.10 € per test
- HCV genotyping with the kit LINEPRB HCV GENO from Bayer in 11 centres: 70.72 € per test
- HPV with the kit Hybrid Capture II (5196-1230) from Digene in 14 centres: 14.28 € per test
- HER-2/neu with the kit HER-2/neu (F-ISH-CPT200) from Ventana in 8 centres: 87.31 € per test

These costs per test include 8 controls (positive/negative) per 100 samples

Cost of consumables for in-house tests.

Two examples are given. HCV qualitative (table 16) is an example of an RNA test and HBV qualitative (table 17) is an example of a DNA test. In both examples duplicate testing is included in the calculation.

Table 16. Cost of consumables for an in-house real-time HCV Qualitative RT-PCR (20 µl in duplicate)

Purification	Consumable	Invoice	1st test	2d test
Silica column	1*250 QIAamp Viral RNA Mini Kit (QI 52 906) Spin Columns, Carrier RNA, Collection Tubes (2 ml), RNase-free Buffers	787,50 €	3,7485 €	
Elution	1*1 L Ethanol 99 - 100% PA UCB 1115	30,39 €	0,0517 €	
Reverse Transcriptase				
Buffer	1*1 ml M-MLV RI Buffer (18 057 018)	15,17 €	0,0607 €	0,0607 €
Protector	1*2 gr DTT (0 197 777)	44,87 €	0,0001 €	0,0001 €
Nucleotides	1*4,000 tests (DATP,DCTP,DGTP,DTTP) 40 µMOLES (UI240)	208,25 €	0,1239 €	0,1239 €
Protector	1* 10,000 units recomb RNasin® Ribonuclease Inhibitor (N2 515)	195,00 €	0,4641 €	0,4641 €
R primer 14pMol	1*HCV 1 0.20 µMol (4 353 424)	0,89 €	0,0001 €	0,0001 €
RT 20 units	1* MLV-REVERSE TRANSCRIPTASE 40,000 U (28 025 013)	250,97 €	0,1255 €	0,1255 €
Amplification				
Taq polymerase	1*2000 tests TaqMan® Universal PCR Master Mix (4 305 719)	3.879,26 €	1,9396 €	1,9396 €
F primer 7.5 pMol	1*HCV 1 0.20 µMol (4 353 424)	0,89 €	0,0000 €	0,0000 €
R primer 7.5 pMol	1*HCV 2 0.20 µMol (4 353 425)	0,89 €	0,0000 €	0,0000 €
Probe 1.66 pMol	1*HCV S 0.20 µMol TAQFT (4 353 426)	252,50 €	0,0001 €	0,0001 €
Ballast*	1*(2 x 25ml Nuclease-Free Water) (PI 193)	34,81 €	0,0139 €	0,0139 €
Consumables				
Reaction well	1*(20*96) ABI PRISM 96-Well Optical Reaction Plate with Barcode (4 306 737)	134,31 €	0,0700 €	0,0700 €
Caps	1*(300*8) ABI PRISM Optical Caps (4 323 032)	103,46 €	0,0431 €	0,0431 €
Filtertips	1*(10*96) Filtertips 1-100 µl (ART2065E)	79,86 €	0,0832 €	0,0832 €
Filtertips	1*(10*96) Filter tips 1-200 µl (MBP2069)	59,85 €	0,0742 €	
Filtertips	1*(8*100) Filtertips 100-1000 µl (MBP2079E)	59,85 €	0,0890 €	
Gloves**	1*100 safeskin purple nitril gloves M (SSK52 002M)	16,49 €	0,0330 €	0,0330 €
TOTAL			9,8192 €	

Ballast*: a volume of 20 µl nuclease-free water is charged per test

Gloves**: a pair of gloves for every 10 samples is charged

Table 17. Cost of consumables for an in-house HBV Qualitative PCR (50 µl in duplicate)

Purification	Consumable	Invoice	1st test	2d test
Silica column	1*250 QIA amp DNA Blood Mini Kit (QI 51 106) Mini Spin Columns, Protease, Reagents, Buffers, Collection Tubes (2 ml)	580.50 €	2.7632 €	
Elution	1*6 L Ethanol 99 - 100% PA UCB 1115	182.32 €	0.0407 €	
Amplification				
Taq polymerase	1*800 tests HotStarTaq PCR Master Mix 1000 U (QI 203 445)	0.7280 €	0.8663 €	0.8663 €
F primer 25 pMol	1*DNA oligo purif. Sc. 200 nMol	2.13 €	0.0003 €	0.0003 €
R primer 25 pMol	1*DNA oligo purif. Sc. 200 nMol	2.13 €	0.0003 €	0.0003 €
Ballast*	1*(2 x 25ml Nuclease-Free Water) (PI 193)	34.81 €	0.0278 €	0.0278 €
Electrophoresis				
250 ml gel 2%	1*500 gr Pronarose D-I LEO (S103a)	200.00 €	0.1983 €	0.1983 €
1000 ml buffer	1*4 l Tris-Borate-EDTA Buffer 10x Concentrate (T4415)	131.53 €	0.1713 €	0.1713 €
Marker 100 bp	1*250 µl (50 lanes) 100 bp dna ladder (G2101)	60.00 €	0.1190 €	0.1190 €
Consumables				
Reaction well	1*1000 thin wall PCR tubes 200 µl with cap (I79 401)	54.00 €	0.0643 €	0.0643 €
Filtertips	1*(10*96) Filtertips 1-100 µl (ART2065E)	79.86 €	0.0832 €	0.0832 €
Filtertips	1*(10*96) Filter tips 1-200 µl (MBP2069)	59.85 €	0.0742 €	
Filtertips	1*(8*100) Filtertips 100-1000 µl (MBP2079E)	59.85 €	0.0890 €	
Gloves**	1*100 safeskin purple nitril gloves M (SSK52 002M)	16.49 €	0.0330 €	
TOTAL			6,1447 €	

Ballast*: a volume of 40 µl nuclease-free water is charged per test

Gloves**: a pair of gloves for every 10 samples is charged

Electrophoresis reagents are used for 12 lanes

Depreciation and maintenance costs

Cost of depreciation for the devices used is based on the acquisition cost of the Taqman 7700, the main used equipment for PCR during the previous years. New equipment like the Taqman 7900 or the LightCycler allow smaller reactions volume and consequently shorter amplification cycles (reduced heating time).

Main parameters

Cost of the device :	107 000 €
Maximum number of assays per plate :	96
Duration for 1 amplification :	150 minutes
Max. # of amplification runs per day :	3 runs
Working days per year :	200 days
Maximal number of assays achievable theoretically :	57 600

The depreciation rate for the Taqman 7700, was calculated per reaction (table 17). A constant depreciation cost of 1.49 € per test is obtained (table 18) when the use of the device varies from 2 years in intensive conditions (36 000 tests per year) to 5 years in smaller centres (14 400 tests per year).

Table 18. Cost of the depreciation of the Taqman 7700 for different occupancy rates.

Occupancy rate	PCRs per year	Use (in years)	Total assays performed	Cost per PCR
62,50%	36 000	2	72 000	1.49 €
41,67%	24 000	3	72 000	1.49 €
31,25%	18 000	4	72 000	1.49 €
25,00%	14 400	5	72 000	1.49 €

Maintenance costs

Maintenance costs were very low since the Taqman 7700 has very few mechanical parts: basically, it has a heater/cooler element and a fluorimeter with selected filters. The main repairs were the regular replacement of the 21 v / 150 w halogen bulb (from 1 to 5 bulbs/year depending of the activity at 160 each (# 4 309 224) and the cathode electrode at 213 (# 5 914) by the personnel of the centre.

Conclusions

The fixed budget of the CMDs for the 4 periods allowed the 18 CMDs not only to buy specific equipment for molecular testing but also to build a very well equipped laboratory, sometimes starting from scratch. We saw investments in laminar flow cabinets, freezers, centrifuges, microscopes, water baths, seats, computers and library. The financing can thus be considered generous. However, the appropriateness of the expenses made with regard to the mission of the CMDs was not checked by the RIZIV/INAMI.

The financing by CMD was almost completely independent of the number of test realized. In 63 out of 72 occasions (4 x 18) the maximum fixed amount per year (for personnel and investments) was received by the CMD. This way of splitting the budget did not encourage smaller centers to reduce their personnel and investment costs.

Although the CMD experiment can be considered expensive, it has permitted us to calculate precisely the production cost per molecular test in the CMDs.

The overall average cost of an in-house DNA or RNA PCR test run in duplicate, amounts to about 33 €. The main cost driver is the personnel cost which varies heavily by CMD. Overall cost of an in-house PCR test in duplicate thus varies between 22 € and 60 €. For tests performed using a kit no duplicate testing is the standard. In addition to the cost of the IVD kit, some personnel (varies from 5 € to 25 €) and depreciation costs (about 3 €) have to be added.

The future allocation of resources should now take into account these real production costs in fixing the reimbursement for those tests, which are supported by clinical evidence.

With respect to hemato-oncology tests at diagnosis, the number of positive test results is extremely low, often 1 or 2% (see CMD activity report, appendix I). The overall testing cost per positive result is thus several thousands of Euro's. As some CMD's only test a limited number of patients per year, the chances of finding a positive results is also very low. For reasons of test validation and continued quality assurance, centralisation of such testing may be appropriate.

5. PILOT ASSESSMENTS AND FRAMEWORK FOR TEST EVALUATION

The purpose of the pilot assessments was a more detailed evaluation of a small number of representative tests, in order to derive one or a few general frameworks or models for evaluating specific molecular diagnostics for their test performance, clinical utility, and health economic aspects. Organisational, financing and QA aspects, and international comparative data, may also be considered in the evaluation. Two test cases were selected first by the Project Steering Group. As representative for high volume and well-documented microbiology tests, the molecular diagnostics in hepatitis C were selected (HCV RNA qualitative and quantitative, HCV genotyping). As a representative of a lower volume, less standardized microbiology test, enterovirus detection in meningitis was selected. Two pilot assessments were added later. As a representative of a rather high volume molecular test in hemato-oncology PCR for t(14;18) in follicular lymphoma was selected. Finally, also factor V Leiden was included as a pilot assessment as this test is the most frequently performed genetic test outside of the centres for medical genetics. Underneath, a summary is provided for each of the pilot assessments. Separate KCE reports are available for each of the pilot assessments.

5.1. HEPATITIS C

Hepatitis C, formerly defined as non-A, non-B hepatitis, is caused by the hepatitis C virus (HCV), a single-stranded RNA virus. It has been estimated that currently 170 million people worldwide are chronically infected with HCV, corresponding to a global prevalence of approximately 3%. In Belgium the prevalence is estimated at 1%. Transmission is mainly associated with infected blood products or intravenous drug abuse. Following initial HCV infection, it is estimated that up to 85% of patients will have a chronic hepatitis C infection. Up to 20% of those chronically infected will develop cirrhosis over a period of decades and a small number of these patients will develop hepatocellular carcinoma. Whereas genotype 1 is the most prevalent genotype in patients with chronic hepatitis C, new infections are now often associated with intravenous drug abuse and caused frequently by genotype 3 virus, which is more amenable to interferon-based treatment. This report summarizes the existing evidence on the clinical utility of molecular tests in patients with hepatitis C, by searching the literature in Medline, Embase, DARE, Medion and INAHTA. Studies were assessed on quality and data were extracted in a predefined fashion.

Molecular tests evaluated

Three molecular tests are considered: HCV genotyping, and qualitative and quantitative HCV-RNA tests. The tests are used to support treatment decisions and to assess the response to treatment. Interferon-alpha-2a or 2b (IFN), and more recently its pegylated version (PEG-IFN) combined with oral ribavirin (RBV), is the current standard treatment for patients with moderate to severe chronic hepatitis C.

Sustained virologic response to treatment (SVR) is defined as plasma HCV-RNA levels below the detection limit of a qualitative HCV-RNA assay 6 months after the end of treatment. Obtaining SVR is the main goal of IFN-based treatment, as this is assumed to be associated with long term patient benefit. While the SVR after 48 weeks of PEG-IFN plus RBV in patients with HCV genotype 1 infection is about 50%, genotype 2/3 patients achieve SVR rates of > 75% after 24 weeks of treatment. Higher levels of HCV RNA before the start of treatment have been shown to decrease the chances of achieving a SVR, in any genotype. An early virologic response (EVR) is defined as a drop in plasma HCV-RNA levels of at least 2 log₁₀ (quantitative assay) or HCV-RNA no longer detectable (qualitative assay) 12 weeks after the start of treatment.

In general, the analytical accuracy of the molecular tests is good. Qualitative tests have a lower limit of detection of 50-100 IU/ml. This is lower than the detection limit of the current generation of quantitative HCV-RNA assays. The quantitative tests have

sufficient linearity across different viral load levels and reasonable within and between-run variability. The agreement between different assays is not sufficient, therefore the same assay should be used in monitoring one patient. Genotyping is accurate, although a proportion of patients will not be typable with any molecular test.

In clinical studies, qualitative tests have proven to be useful in assessing an early viral response during treatment, although the prognostic value of the test changes as the lower limit of detection changes. Genotype 1 patients who do not show an EVR have virtually no chance in achieving a SVR, and can discontinue treatment early (12 week stopping rule). Compliance with the 12 week stopping rule in genotype 1 patients increases the cost-effectiveness of IFN-based treatment in chronic hepatitis C. Testing of EVR in genotype 2 or 3 HCV patients reduces the efficiency of treatment, as it adds to costs without generating substantial additional benefits. Similarly genotype 1 patients with a 2 log drop in HCV-RNA but with HCV-RNA still detectable at week 12, may be tested with a qualitative HCV-RNA test at week 24 and discontinue treatment if still positive (24 week stopping rule). A serological assay quantifying HCV core Ag could be an alternative assay for the assessment of EVR, while its limit of detection is higher than for a qualitative HCV-RNA test. The new generation real-time RT-PCR assays combine the high sensitivity of qualitative assays with a broad range of quantitative measurement.

Local situation and health economic aspects

The RIZIV-INAMI reimbursement of the PEG-IFN / RBV 1000mg daily combination treatment for 48 weeks in genotype 1/4/5/6 patients amounts to 19 564 €. The reimbursement of the PEG-IFN / RBV 800mg daily combination treatment for 24 weeks in genotype 2/3 patients amounts to 8 978 €. For the estimation of the numbers of HCV molecular tests the model presented in table 19 was used (makes use of the 24 weeks stopping rule).

Table 19: HCV molecular tests before, during and after PEG-IFN plus RBV

Genotype ; treatment duration	Wk 0	Wk 12	Wk 24	Wk 48	Wk 72	Wk 0-72
Genotypes 1, 4, 5, 6; 12-48 weeks						
HCV Genotyping	+					1
HCV-RNA qualitative test	+		(+ ⁺)	+ ⁻	+ ⁻	4
HCV-RNA quantitative test	+	+				2
Genotypes 2, 3; 24 weeks						
HCV Genotyping	+					1
HCV-RNA qualitative test	+		+	+ ⁻		3
HCV-RNA quantitative test	-					0

+⁻ conditional qualitative test performed only if previous result was negative

(+⁺) conditional qualitative test performed only if the HCV-RNA decreased by 2 log₁₀ but was still positive

During the year 2004, 3114 HCV genotype tests were performed in 14 CMDs. The most prevalent types were genotype 1 (60.4%), genotype 3 (18.5%), genotype 4 (12.3%) and genotype 2 (6.1%). Based on the reimbursement requests submitted to the RIZIV-INAMI by insured patients, about 60% of those patients genotyped decided to start (PEG)IFN plus RBV. It is assumed 60% of patients start treatment within each genotype stratum. Sensitivity analyses have also been performed using other assumptions. It can be calculated that for every patient genotyped, an average of 1.21 quantitative tests and 2.23 qualitative HCV-RNA assays will be performed. The number of quantitative HCV-RNA tests needed is thus 3758 and the number of qualitative HCV-RNA tests is 6938. The number of patients with SVR is 1137.

Similar to the situation in France, the number of genotyping and quantitative HCV-RNA tests performed in Belgium increased by 30% from 2002 to 2003 and by another 20% from 2003 to 2004. Based on the number of genotype tests in 2003 (2627), the number

of expected quantitative assays in the year 2003 was 3170, which corresponds almost perfectly with the observed figure of 3211. This is not the case for the number of HCV qualitative assays performed at the CMDs in the same year (7368). Furthermore 6188 tests were reimbursed that year using the nomenclature. There is thus a strong excess of tests with regard to the figure calculated in the HCV treatment context. These extra tests may have been used for the diagnosis of active chronic HCV infection and for the detection of acute infection after accidental exposure. The reported calculations of the cost per test start from as low as 8 Euro for a HCV-RNA quantification using real-time PCR. Reimbursement fees for HCV molecular tests vary considerably between countries but are remarkably similar in France and Germany: 40.90€ to 54.00€ for HCV qualitative, 81.00€ to 89.50€ for HCV quantitative and 102.30€ to 108.00€ for HCV genotyping. A downward revision of the tariffs is under consideration in France.

In conclusion, the HCV molecular tests evaluated are of clinical utility in the context of guiding IFN-based treatment. The existing testing guidelines, together with the number of treatments started, allow for a good estimation of the testing volumes and cost expected in the context of IFN-based treatment. For 2004, the RIZIV-INAMI costs for hepatitis C therapy can thus be estimated at about 28 Million €, and the associated molecular tests at about 1 Million € (for nearly 14 000 tests at an estimated cost of 75€ on average), while 1 137 patients of the 1 868 patients treated can be expected to achieve a SVR. For each patient with a SVR, there is thus an overall payer cost of about 25 000 € for therapy and of nearly 1 000 € for approximately 12 molecular tests.

5.2. ENTEROVIRUS

Background

Enteroviruses cause a myriad of symptoms, involving almost every organ system. More importantly, they are responsible for more than 90% of cases of aseptic meningitis for which an etiologic agent can be identified. Although the natural course is usually benign, the differential diagnosis with bacterial meningitis leads to hospitalisation and empirical treatment until diagnosis has been established.

Enteroviruses are mainly transmitted by the faecal-oral route. Due to prolonged shedding of virus from permissive sites, such as the pharynx or stool, the identification of Enterovirus from these sites does not establish causality adequately, in contrast to identification from non-permissive sites, such as the central nervous system, vascular system and urinary tract. The use of molecular tests in patients with suspected meningitis could lead to a fast and accurate identification of Enterovirus, and thus exclude bacterial meningitis.

Methods

We have summarised the evidence on molecular tests for Enterovirus, both for analytical accuracy, clinical accuracy and clinical impact of testing. We searched the literature for HTA reports, systematic reviews and original diagnostic research in several databases. Studies were selected on the basis of predefined inclusion and exclusion criteria. Included studies were subsequently assessed for quality. Poor quality studies were excluded from the review. Data were extracted on study design, population included and test characteristics.

We were not able to identify any HTA reports or systematic reviews that met our criteria. In total, we included 16 original studies, of which 6 were on the analytical accuracy, 7 on the clinical accuracy and 3 on the clinical impact of the tests.

Results

The analytical accuracy was reported poorly in general. Moreover, results were heterogeneous with sensitivity ranging from 61%-91% and specificity ranging from 86%-

98%. The overall quality of the clinical accuracy studies was equally poor as the analytical studies. In addition, results were difficult to compare because of differences in case definition and reference test. Confidence intervals were not reported. Sensitivity ranges from 85% to 100%; specificity from 80% to 100%. As a true 'golden' standard does not exist for Enterovirus meningitis, these estimates are uncertain. CSF pleocytosis influences the test characteristics.

In theory, a positive PCR test could lead to important clinical consequences, such as immediate discharge or refraining from further antibiotic treatment. A significant difference on a number of outcomes, such as length of stay, between patients with a positive and with a negative PCR test result was found by several authors. In two studies, a relevant part of the study population was excluded from the analyses, thus embellishing the results and reducing the applicability in clinical practice. Possible adverse consequences of the use of these molecular tests were not addressed.

Conclusion

In conclusion, both the analytical and clinical accuracy of the Enterovirus PCR tests are not sufficient at this moment to be introduced in clinical routine practice. Although a positive clinical impact of introducing such tests could be assumed on theoretical grounds and has been partly analysed in some studies, the uncertainty of the accuracy of these tests is too large.

5.3. PCR FOR T(14;18) IN FOLLICULAR LYMPHOMA

Introduction

Follicular lymphoma (FL) is the second most common form of non-Hodgkin lymphoma. The incidence of FL in Belgium is estimated at 400 cases per year. The patients are mainly elderly and the median survival is 8 to 10 years. A frequent chromosomal aberration of FL is the translocation t(14;18)(q32;q21) (Bcl-2/IgH), involving the immunoglobulin heavy chain (IgH) gene on chromosome 14q32 and the Bcl-2 gene on chromosome 18q21. This translocation results in the juxtaposition of the antiapoptotic Bcl-2 gene and the IgH heavy chain locus on chromosome 14, leading to upregulation of Bcl-2 protein expression in most cases of FL, and an inhibition of cell death.

Initially, the biological material available for diagnosing FL is most frequently a lymph node, but can also consist of bone marrow. The lymph node tissue is best shipped fresh and not fixed. Coordination of the different tests involved in the local pathology and hematology lab, and at external laboratories such as CMDs and CMGs is best handled by a single coordinator, according to a diagnostic scheme outlined in the local oncology handbook. Diagnosis of follicular lymphoma can be based on morphology and immunohistochemistry in over 95% of the cases of FL. In the remaining cases tests for monoclonality or for t(14;18) may help the pathologist to define malignancy and the diagnosis of FL. However, testing for t(14;18) is routinely performed anyhow in most cases of FL as part of an integrated diagnostic work-up. Other tests pathologists perform for FL include IHC on frozen tissue slides or flow cytometry. If immediately available, IHC on frozen tissue slides may help select samples for karyotyping.

Possible testing algorithms

Three possible ways to test for t(14;18) are given. The local situation may define the most appropriate scenario. The most comprehensive approach to t(14;18) testing in FL starts with karyotyping. Metaphase induction needs to start immediately after receipt of a fresh sample, consisting of lymph node tissue in most cases of FL. Induction of metaphases after 24-48h of culture (48-72h for CLL) is successful in 70% of cases overall, but will be somewhat lower for lymph node tissue in FL. Karyotyping is the preferred option in case of a diagnostic challenge, or if the initial diagnosis is unclear, as additional cytogenetic abnormalities are also visualized. This approach also avoids the

need to perform another biopsy in such cases. If karyotyping is not possible or if results are unclear interphase FISH (Vysis) is used to detect t(14;18). This particular FISH assay is relatively easy to interpret, and will generate only unclear results (which could benefit from backup karyotype information, if available) in about 10% of t(14;18) FISH tests. An issue which still needs to be resolved is the marketing of the product, which is currently still for research use only, excluding clinical use.

In theory centers for medical genetics can provide results for karyotyping within one week, in writing, provided the current backlog in terms of technician and secretary work is tackled first. In case the sample provided falls within the 5% category of diagnostic challenges, already today priority is given to such karyotyping work upon simple request. As an alternative approach interphase FISH can be used as the first line test for detecting t(14;18) in FL at diagnosis. The use of interphase FISH as stand alone test is thus not considered inappropriate in this situation (differs from the guidance published by the Groupe Français de Cytogénétique Hématologique). In case the diagnosis is not conclusive based on this approach another biopsy may be needed.

A third approach consists of the use of (less costly) PCR as a first line test at diagnosis, followed by (more expensive) interphase FISH if negative. Also using this approach another biopsy may be needed if the diagnosis remains inconclusive. In order to have a reasonable clinical/diagnostic sensitivity the PCR test is recommended

- to cover as many well documented breakpoints as possible (thus minimally MBR and mcr breakpoints), diagnostic sensitivity of such PCR is 45-70% versus 88-100% for FISH
- not to show a too high analytical sensitivity (in order to avoid picking up the t(14;18) translocations present in a few cells in a significant fraction of the population)
- to characterize the amplicon eg on gel as a quality check, especially relevant for t(14;18) PCR
- to be validated (eg based on BIOMED-2 efforts, same comment as for FISH: RUO kits cannot be used for routine diagnosis, in-house method requires full validation)

The only indication mentioned at the expert meeting for quantitative t(14;18) PCR was for the safety of peripheral stem cell collections. For this purpose it may be appropriate to perform t(14;18) PCR already at diagnosis. There is currently no role for quantitative PCR at diagnosis as a prognostic variable in clinical routine. Quantification of tumour load at diagnosis and during follow-up or the detection of minimal residual disease using molecular methods should be limited to research protocols. The clinicians do not see a role for t(14;18) PCR monitoring in the routine setting, awaiting a better molecular understanding of the disease (and associated tests) and more targeted treatment options. It was recommended to store away biological material or the extracted RNA and DNA (lymph node, bone marrow, blood) for later use. This storage is associated with significant costs and should preferably be conducted under research protocols.

5.4. FACTOR V LEIDEN

Background

Factor V Leiden mutation is the most frequent cause of heritable thrombophilia, associated with a three to sevenfold risk of deep venous thrombosis. It is not as yet clear whether testing for factor V Leiden improves patient outcome. Before widespread testing or screening is introduced, evidence will need to prove that carriers benefit from being diagnosed with this mutation.

In this review, we present data on the analytical and clinical performance of molecular tests for the factor V Leiden, together with the possible clinical consequences of testing.

Methods

We searched Medline, Embase, INAHTA, Medion and several other databases. The articles were selected on the basis of pre-specified inclusion and exclusion criteria, and assessed for quality. Low quality studies were excluded.

Results

We found a limited number of studies of good or fair quality on the analytical characteristics of the various molecular assays. The included studies all reported a 100% concordance with the reference method.

In addition, the overall quality of clinical studies was low, leading to a large proportion of excluded studies. All included studies reported a concordance of >98.7%, with very little samples producing equivocal or invalid results. Measures of precision or reproducibility were not reported.

As the modified APC resistance test has sensitivity and specificity approaching that of molecular tests, this test should be performed first, only verifying positive test results with a molecular test.

Factor V Leiden is an established risk factor for the occurrence and recurrence of VTE. Screening women before starting oral contraceptives, antenatally and relatives of patients is not recommended. Management of patients with a first episode of VTE with the factor V Leiden mutation is not different from patients suffering from an idiopathic VTE. Patients with a personal and/or family history suggestive of co-inheritance of two thrombophilic conditions or homozygosity of the factor V Leiden mutation could be considered for testing, although evidence on the optimal treatment in these patients is equally lacking.

Conclusion

The factor V Leiden mutation tests seem to have sufficient analytical and diagnostic efficacy, although evidence is scarce and of low quality. The clinical impact of testing is unclear, as the identification of the mutation does not offer any advantages on treatment. Only patients with a family or personal history suggestive of homozygosity or co-inheritance of another thrombophilia could be considered for testing.

5.5. FRAMEWORK FOR MOLECULAR TEST EVALUATION

5.5.1. Introduction

Molecular tests, as all diagnostic tests, are used for various purposes. The purpose can be to increase certainty on the presence or absence of a disease by using the discriminative power of the test; to monitor the clinical course when a disease is left untreated or during and after treatment; to support clinical management, for example determining presence, localisation, and shape of a lesion for treatment decisions; or assess prognosis as the starting point for clinical follow up and informing patients¹⁰⁶. As a consequence, diagnostic tests have a potential effect on management, patient outcome and patient well being.

Tests which do not have the potential to produce any of these effects are obsolete and should not be performed at all. In addition, tests that are not sufficiently reliable may cause adverse effects in patients, by leading to inappropriate treatment decisions, causing unnecessary concern or contrarily, unjustified reassurance. The use of diagnostic tests is therefore never neutral and should be considered with proper care, as is the case for therapeutic interventions. Moreover, health care purchasers are demanding an accounting of value received for their money spent.

In this paper, we present a framework by which the utility of tests can be evaluated, before introducing them into clinical practice. The aim of the evaluation is to present clear and explicit information by which decision makers or physicians can decide whether the test is useful and to what extent it will help them in treating individual patients.

5.5.2. Information gathering

In order to provide data for further assessment, efforts must be made to gather the appropriate information. This information can be found in the literature and in unpublished data provided by for example manufacturers.

As outlined in section 5.5.3, we propose a hierarchy of diagnostic efficacy. It follows that in order to decide on any effect on patient outcome or societal value, diagnostic accuracy studies do not suffice. Additional data on clinical impact and cost-effectiveness of the test must be sought, for which other research designs will be more useful.

However, if not all the available evidence are presented, conclusions drawn from the evidence risk being prone to selection bias. This occurs when important studies were either missed in the search, or a purposive sample of studies has been chosen to support one's own opinion, ignoring any contradictory results. Therefore, we propose the following strategy of information gathering:

Evidence syntheses

In order to prepare good quality evidence syntheses, the literature has already been searched, appraised and synthesized. It is crucial, however, to critically appraise the quality of the evidence synthesis itself, as not all reviews or reports are of equal high quality.

Good quality Health Technology Assessment (HTA) reports should be searched for first, as they provide information on the various levels of diagnostic efficacy, as outlined later in this paper. Additional aspects that are important for the implementation of the technology are possibly considered as well, such as organisational, financial and ethical issues. If the HTA reports are outdated, they can be supplemented by newer material.

The second source of evidence synthesis, are systematic reviews. Again, in case of a good quality systematic review, the effort of searching and appraising evidence does not have to be duplicated and the efficiency of the search process is increased. A systematic review however, has a narrow focus and will therefore not provide an answer to all the questions regarding the implementation of the technology.

Original studies

If HTA reports or systematic reviews do not exist, are of inferior quality or are outdated, original research should be searched. Published literature must be searched in at least the two largest databases, being Medline and Embase. Additional databases can be searched as well, for example Medion, the Cochrane Library, DARE, or the IFCC database. Published material can be complemented by unpublished study results. A clear description of the selection and quality assessment process should be provided, conform the QUOROM statement¹⁰⁷.

In order to complete the hierarchy of diagnostic efficacy as outlined later in the manuscript, original studies should not be restricted to diagnostic accuracy studies.

Other designs will answer the other research questions, such as the effect of the diagnostic technology on therapeutic management and patient outcome.

Evidence tables

The data that were found in the various evidence sources should be summarized in evidence tables. This increases the transparency of the review and provides the necessary details for readers and decision makers. These tables should include essential data on the methodology of the studies as well as on their results.

5.5.3. Hierarchy of diagnostic efficacy

Fryback and Thornbury have described a hierarchy of diagnostic efficacy, which is used as the basis of this paper¹⁰⁸. Efficacy is defined as the probability of benefit from a medical technology to individuals in a defined population under ideal conditions of use¹⁰⁹. In other words: can the diagnostic test work? This is not the same as effectiveness, which assesses the test's ability to work in the real world: does it work in clinical practice? Finally, in efficiency the test's financial implications are considered: is it worth it?¹¹⁰ The model presented here mainly assesses the test's efficacy, although cost-effectiveness considers its efficiency.

The model is characterized by a change in perceived goals. It is hierarchical: on one extreme are endpoints describing only the technical performance of the test, on the other extreme are endpoints pertaining to the value of the diagnostic technology to society. If a test performs poorly at one level, it is unlikely to perform well at a higher level. The reverse, however, is not true: increases in the technical performance of a test will not necessarily guarantee improvement at a higher level, for example effect on patient outcome.

A diagnostic test does not necessarily have to have demonstrated effectiveness at each level before it can be used in clinical practice⁶, but using this approach the possible gain and remaining uncertainty on the test's efficacy is clearly presented.

Level 1: technical efficacy

The technical efficacy of a test refers to the ability to produce usable information.

The *test's feasibility* and *operator dependence* refer to in what circumstances and by whom the test can be performed.

The *analytical sensitivity* is the ability to detect small quantities of the measured component. This should be distinguished from the diagnostic sensitivity, the ability of a test to detect disease.

Biochemical tests may be subject to *interference* from other substances. The possibility of interference by relevant clinical substances must have been explored.

The precision or *reproducibility* of results is the ability to obtain the same test results on repeated testing or observations. It is influenced by analytical variability and observer interpretation. Analytical variability consists of inaccuracy and imprecision. Inaccuracy implies systematic error, such as calibration error. Imprecision implies random error. Agreement between two continuous test methods can be expressed in a regression analysis or Bland & Altman plots¹¹¹. A correlation coefficient does not provide information on agreement. The agreement between two observers (interobserver) or the same observer on different occasions (intraobserver) can be expressed with a kappa statistic.

It is often assumed that the technical efficacy does no longer need to be evaluated once a test is being used in clinical practice. However, in our review on molecular tests for the detection of enterovirus, the technical efficacy of the tests was insufficient to recommend its use in clinical practice, despite the fact that the test is currently used in patients with suspected meningitis.

Level 2: diagnostic accuracy

This level refers to the test's ability to detect or exclude disease in patients compared with a criterion standard or reference test. Test characteristics are sensitivity, specificity, predictive values, likelihood ratios and ROC curves.

Sensitivity and specificity are the most widely used outcome measures, but are sensible to spectrum bias. Spectrum bias may occur when the study population has a different clinical spectrum (more advanced cases, for instance) than the population in whom the test is to be applied^{112, 113}. If sensitivity is determined in seriously diseased subjects and specificity in clearly healthy subjects, both will be grossly overestimated relative to

practical situations where diseased and healthy subjects cannot be clinically distinguished in advance^{114, 106}. This design has been called 'inappropriate case-control design' in the pilot assessments.

Predictive values, with the positive predictive value being the proportion of patients with a positive test result that actually has the disease and the negative predictive value the proportion of patients with a negative test result that does not have the disease, are dependent on disease prevalence in the study sample. For example, in a situation where disease prevalence is very low, say 1%, the negative predictive value of the test will be easily over 95% as already 99% of the population do not have the disease. Prevalence and the setting in which patients were recruited should be noted to reflect on this.

The *likelihood ratios* show how a test result alters the pre-test probability into a post-test probability, using Bayesian reasoning. The pre-test probability depends on the prevalence of the target condition and the results of previous tests, for example history, clinical examination, imaging or laboratory tests.

Another outcome measure which is sometimes used, is the *number needed to diagnose*, analogous to the number needed to treat in intervention studies. However, using this measure it is assumed that diagnostic testing is always done to rule in a target condition, to diagnose the target condition, while in clinical practice tests are also used to rule out a target condition.

Finally, test accuracy can be illustrated using an *ROC curve*. The ROC curve graphs test sensitivity versus 1-specificity for various cut-off points. The area under the curve provides a summary measure of the test performance. It also allows us to compare two different tests by testing the two areas under the curve or by testing partial areas under the curve in which the test is most useful.

Clearly, the first level of diagnostic efficacy, technical efficacy, contributes to the diagnostic accuracy. But it also becomes apparent that there may be a point beyond which improvement in technical performance no longer improves diagnostic accuracy. Assuming therefore that diagnostic accuracy can be estimated on the basis of technical accuracy studies is not correct.

Level 3: diagnostic thinking

This level of diagnostic efficacy is concerned with assessment of the effect of test information on diagnostic reasoning and disease categorization. Studies on diagnostic thinking serve as a proxy for estimating the effect of a test on patient care. Patients' outcome can not be influenced by the diagnostic technology unless the physician is led to do something different than would have been done without the test information.

Using the *likelihood ratio* and calculating the post-test probability, this change in diagnostic thinking can be computed. However, the pre-test probability of a disease is not always available in clinical practice and depends not only on setting, but also on patient characteristics and other selection processes, such as referral and the results or previous tests. Clinicians who wish to apply the Bayesian properties of diagnostic tests require accurate estimates of the pre-test probability of target disorders in their area and setting. These estimates can come from five sources — personal experience, population prevalence figures, practice databases, the publication that described the test or one of a growing number of primary studies of pre-test probability in different settings⁸.

An alternative are studies that empirically test the *change in the physician's subjective assessment* on the probability of disease. In these studies, physicians are asked to estimate the probability of disease before knowing the test result, and estimating it again after the test result has been disclosed. Efficacious tests are those that significantly increase or lower pre-test probabilities assumed by the physician or computed by likelihood ratios using Bayesian reasoning.

One major difficulty with this level of diagnostic efficacy is that it is not always known what post-test probability of disease should be used as a threshold. Which probability of disease is low enough to exclude disease, which is high enough to treat the patient?

These thresholds will differ according to the target condition and the treatments that are available¹¹⁵.

Level 4: therapeutic impact

The most efficacious tests at this level are those that lead to the institution of a new management strategy. Studies can assess this empirically by comparing the intended management before the test result is known with that after the test result has been disclosed. In what *proportion of patients did the information change the intended management?* in some cases, management changes are considered not only in the patient himself, but also in other persons, for example prophylactic measures in case of an infectious outbreak. These prospective case-series, however, can be subject to bias such as selection bias. The lack of a concurrent control group may lead to confounding, as there is no information on those patients not enrolled in the study and therefore not receiving the new technology. These considerations underscore the need for randomized controlled trials. But, in the absence of RCT's they do play an important role as an intermediate.

Level 5: patient outcome

The ultimate goal of health care is to improve patient outcome. For diagnostic tests that are expensive, dangerous or widely used, knowledge about patient outcome efficacy seems particularly important. It is at this level that expected harm, such as burden, pain, risk, can be weighed directly against its expected benefit, such as improving life expectancy, quality of life, avoiding other test procedures, etcetera.

The *randomized controlled trial* is the study design the least prone to bias to estimate these harm and benefit. However, it is not always feasible to perform an RCT for ethical, financial or other reasons. In those cases, case-series collected before and after the introduction of a new test technology or case-control studies may provide some of the answers.

A methodological difficulty with this level is that the independent contribution of test technology to patient outcomes may be small in the context of all the other influences and therefore very large sample sizes may be required. But, in spite of these difficulties, RCT's on diagnostic tests are feasible. Various designs are possible, according to the specific research question¹¹⁶.

Some tests, however, will never be able to prove a change in 'objective' patient outcomes such as mortality or morbidity, simply because there is no treatment available at this moment that has an impact on these outcomes. This is the case in for example dementia or Amyotrophic Lateral Sclerosis (ALS). A diagnostic test will therefore never produce a difference in mortality, but may improve *quality of life measures* by giving the patient (and the carer) an affirmative diagnosis and providing an explanation for the signs and symptoms the patient experiences.

Level 6: cost-effectiveness analysis

This level goes beyond the individual risks and benefits, but assesses whether the cost for use of a given test is acceptable for society. Is the price for the positive effect on patient outcome worthwhile? Resources can not be allocated twice; money spent on one technology can not be spent on another.

Cost-effectiveness studies compute a cost per unit of output. Any of the measures of the previous levels can be used as input, for example cost per surgery avoided, cost per appropriately treated patient, cost per life year gained or cost per quality adjusted life year gained. Final outcomes, such as life years gained or QALYs gained, are preferred over intermediate outcomes in economic evaluations, as they allow comparisons across a broader range of health interventions, e.g. diagnostic and therapeutic interventions. Because data on these outcomes and costs of the diagnostic and subsequent therapeutic paths are not routinely available from observations, modelling becomes inevitable to examine the cost-effectiveness of diagnostic tests. The validity of the model input parameters is crucial for the credibility of the model. The values of all input variables

must be based on solid evidence obtained from literature or observations. Sensitivity analyses can illustrate the robustness of the conclusions, by demonstrating the sensitivity of the results to changes in the values of remaining uncertain input parameters.

5.5.4. Implementation characteristics

The characteristics of both the test itself as the condition for which it is being used, have an impact on the way the test is implemented into clinical practice.

The *prevalence of the target condition/indication* refers to the volume of tests that can be expected. The prevalence of the target condition itself is not sufficient, as a rare but important target condition can have several, more frequent, differential diagnoses. The prevalence of the test indication is more appropriate to estimate the volume of tests needed: what is the indication for which the test will be used, and what is the frequency of this indication? For example, in testing for the factor V Leiden mutation, it is not sufficient to know that the general prevalence of the mutation is 5%, as the test might be considered in patients experiencing a first episode of venous thrombo-embolism. The relevant question is how many patients have a VTE every year, therefore how frequent is the indication to test for factor V Leiden mutation?

With what *speed* is the test result required in order to have an impact on patient management? Acute conditions that should be treated promptly need faster test results than chronic conditions for which a delay in treatment of days or even weeks is not an issue. This will have implications for the organisation of the test implementation.

Finally, the test can be used for *outbreak surveillance or scientific monitoring*, without immediate impact on patient management.

5.5.5. Effectiveness

As we already discussed in the introduction, effectiveness refers to the use of the test in clinical practice. How should the performance of the test be done and organized in clinical practice to obtain similar results as in efficacy studies, which of course reflect ideal conditions and not daily practice conditions.

In molecular testing, the test method used should be *validated*, especially when an in-house method is being used. In addition, the data on the validation of commercial kits should be made publicly available, in order for the user to assess the methodological quality of the validation.

It is important to assess whether the test method that is used is in fact *equivalent* to the methods used in analytical and clinical studies. Only this way will the results of those studies be transferable to real life.

In addition, when several centres use the same or equivalent methods, *harmonizing* the test methods has the advantage that interpretation by clinicians is facilitated and duplication of tests can be avoided.

Other parameters should be guarded when applying a test in clinical practice, for example test *turn around time*. If studies have found that a test has a positive effect on patient treatment under the condition that the result is available within 24 hours, this will only hold if the test result can be delivered equally fast in clinical practice.

Finally, diagnostic test will only prove effective if they are performed in the indications in which they have proven to be efficacious. Performing the test in other settings, on other patients, at a different point in the diagnostic strategy can affect test characteristics and make the conclusion of efficacy studies no longer valid.

5.5.6. Conclusion

With this hierarchical model, the diagnostic efficacy of a test can be presented transparently and systematically. It is important to bear in mind that a lower level has to be achieved in order to perform at a higher level.

A diagnostic test can be introduced into practice without fulfilling all levels. In fact, for some tests it may be impossible to gain information on all levels, for ethical or organisational reasons. However, the limitations of the knowledge on the test's efficacy should be emphasized and proper use of the test should be guarded, as the effect on patient outcome is uncertain. Further research on the test's efficacy in clinical trials should be promoted until the highest level possible has been achieved. The assessment of a test's efficacy is therefore a continuous process, with conclusions that need to be updated to the publication of new evidence.

As molecular tests claim superior test characteristics than the tests currently used, in terms of for example higher sensitivity and faster test results, the tests should be able to achieve at least a level 4 diagnostic efficacy before introduction in routine practice, as this evaluates the impact on patient management. A faster test result does not necessarily mean that patient management will be influenced, so this will need to be examined. Higher levels of diagnostic efficacy are recommended because extremely sensitive tests may detect clinically irrelevant quantities and benefit from treatment is highly uncertain in these patients. This can only be analysed in good quality trials.

5.6. THE FRAMEWORK APPLIED TO THE CMD TESTS

Within the time restraints of the project, it was impossible to evaluate every test as we did with the pilot assessments and as described in the framework. However, as some guidance on the value of these tests was needed, a very rapid review was performed for all tests, considering only good quality evidence synthesis, being HTA reports and systematic reviews.

Methods

We searched several databases for HTA reports or systematic reviews: INAHTA, HTA database, DARE database, NHS EED database and Medline (Clinical Queries). Search terms used were the names of the microbiological agent or the genetic translocation. Secondly we searched the databases using more generic terms, such as pneumonia, meningitis, genetic translocation, genetic rearrangement or sequence analysis.

HTA reports were defined as a synthesis of all available evidence with a transparent and systematic literature search and quality appraisal, assessing the value of the technology by comparing it to the currently used diagnostic test and estimating its cost-effectiveness. Systematic reviews were defined as an evidence synthesis with a transparent and systematic literature search and quality appraisal of a specific diagnostic technology, possibly with a meta-analysis.

Narrative reviews were excluded, as well as primary studies. Molecular tests for which a more extensive pilot assessment had been done (HCV, enterovirus, and t(14;18)) were excluded from this review.

Systematic reviews were identified for the following tests:

- *Borrelia burgdorferi*²
- *Mycobacterium tuberculosis*^{1, 117}
- Human papilloma virus¹¹⁸⁻¹²³
- Herpes simplex virus³

The reviews by Dumler² and Linde³ should be treated with caution, though, as they did not fulfil all criteria of a systematic review.

In addition to the MSAC HTA report on Hepatitis C viral load testing¹²⁴, only two Health Technology Assessment Reports on the concerned molecular diagnostic tests were identified, developed by MSAC Australia (<http://www7.health.gov.au/msac/reports.htm>). All three HTAs support public funding of the procedure.

- Polymerase chain reaction in the diagnosis and monitoring of patients with PML-RARalpha and PLZF-RARalpha gene rearrangement in acute promyelocytic leukaemia – March 2003⁵.
- Polymerase chain reaction in the diagnosis and monitoring of patients with AML1-ETO and CBFbeta-MYH11 gene rearrangement in acute myeloid leukaemia – August 2003⁴.

Results are summarized in table 20. The table was subsequently completed with the other variables only for those tests for which at least level 4 evidence was found in support of the use of the test.

Table 20. Molecular tests used in the CMDs, with their indication and levels of diagnostic efficacy

Test	Indications considered	Level of diagnostic efficacy	Commercial/ in-house; interpretation	Maximum time request-result
<i>Bartonella henselae</i> , <i>Bartonella quintana</i>	Cat Scratch Disease	No evidence found	In-house	
<i>Bordetella pertussis</i>	Pertussis	No evidence found	In-house	
<i>Borrelia burgdorferi</i>	Neuroborreliosis Lyme arthritis Erythema migrans	<u>Level 2</u> ² : fair sensitivity for skin (68%) and synovial fluid assays (73%); not suitable for primary diagnosis	In-house mainly	
<i>Chlamydia pneumoniae</i>	Community acquired pneumonia or prolonged cough.	No evidence found	In-house mainly	
<i>Corynebacterium diphtheriae</i>	Diphtheria	No evidence found	In-house	
<i>Escherichia coli</i> (VTEC)	Haemolytic uremic syndrome, bloody diarrhea or diarrhea outbreak	No evidence found	In-house	
Enterococci (Vancomycin resistant, VRE)	glycopeptide resistance; VanA, VanB phenotype; identify <i>E. casseliflavus</i> or <i>E. gallinarum</i>	No evidence found	In-house	
<i>Helicobacter pylori</i> (macrolide resistance)	Persistent infection after macrolide treatment or to confirm unclear antibiogram (or non growing isolates)	No evidence found	In-house	
<i>Legionella pneumophila</i>	Pneumonia + Gram-stain sputum negative + IC, outbreak or after travel abroad.	No evidence found	In-house mainly	

Test	Indications considered	Level of diagnostic efficacy	Commercial/ in-house; interpretation	Maximum time request-result
<i>Mycoplasma pneumoniae</i>	Community acquired pneumonia; prolonged cough; Meningitis/encephalitis	No evidence found	In-house mainly	
<i>Mycobacterium tuberculosis</i>	Smear neg patients; CSF with high protein; Lymph node, biopsy, exsudate; Identification M. tuberculosis on solid medium or non-tb mycobacteria	<u>Level 2</u> ¹⁷ for tuberculous meningitis: commercial tests sensitivity 56% (46-66%); specificity 98% (97-99); no summary accuracy results of in-house methods due to wide variability Potential role in confirming tuberculous meningitis; due to low sensitivity not able to rule out tuberculous meningitis <u>Level 6</u> ¹ routine testing of smear positive specimens: not cost-effective; Smear-negative testing may be useful, although cost-effectiveness remains an obstacle	Kits mainly	Preferably within one week
<i>Mycobacterium tuberculosis</i> (resistance genes)	RMP-resistance.	No evidence found	Kits mainly	
Staphylococci (resistance genes, MRSA)	Staphylococcal atypical phenotype; Mupirocin resistance; Direct MRSA detection	No evidence found	In-house	
Identification of bacteria difficult to identify	Endocarditis, meningitis, osteomyelitis	No evidence found	In-house	
Molecular typing of nosocomial pathogens	Outbreak investigation; spread of multi-resistant bacteria; differentiate relapse from new infection	No evidence found	In-house	

Test	Indications considered	Level of diagnostic efficacy	Commercial/ in-house; interpretation	Maximum time request-result
Cytomegalovirus (CMV) qualitative	IC patients; CMV neg acceptor- pos donor; Primary infection in pregnancy; Foetal abnormalities	No evidence found	In-house	
Cytomegalovirus (CMV) quantitative	Monitoring IC patients (CMV pos donor and/or acceptor)	No evidence found	In-house	
Epstein-Barr virus (EBV) qualitative	Encephalitis in IC; Cerebral lymphoma (HIV); Primary infection post-liver or BM transplant; Lymph proliferation or tumour	No evidence found	In-house	
Epstein-Barr virus (EBV) quantitative	post pediatric liver or BM transplant, leukemia treatment, Lymphoma	No evidence found	In-house	
Hepatitis B virus (HBV) qualitative	Serum, plasma: when serology or infection is unclear. Max 1x/year/patient.	No evidence found	In-house	
Hepatitis B virus (HBV) quantitative	Start and monitoring of treatment.	No evidence found	In-house mainly	
Hepatitis C virus (HCV) qualitative	Confirm active infection before, during and after treatment	Pilot assessment: level 6	Kits mainly	Not urgent due to chronic nature of infection

Test	Indications considered	Level of diagnostic efficacy	Commercial/ in-house; interpretation	Maximum time request-result
Hepatitis C virus (HCV) quantitative	At treatment start and at 12 weeks in genotype 1 infection	Pilot assessment: level 6	Kits mainly	Not urgent; at week 12 preferably within one week to avoid unnecessary treatment
Hepatitis C virus (HCV) genotyping	Before treatment	Pilot assessment: level 6	Kits mainly	Not urgent
Human Papillomavirus (HPV)	Cervical ASCUS/AGUS in women > 30y, LSIL, residual HPV	<u>Level 2</u> : HPV testing instead of cervical smear is not justified ^{119, 121} ; as an adjunctive test to PAP smears for targeted high risk groups, HPV DNA testing increases sensitivity. However, there is no evidence to suggest a change in patient management ¹¹⁹ ; HPV testing, alone or with cytology, is more sensitive but less specific than PAP smears ^{118, 120} . Women after treatment of CIN 3 might benefit from HPV testing as the negative predictive value is high ^{122, 123} .	Kits mainly	Not urgent
Enterovirus	Meningitis/encephalitis Pericarditis/myocarditis Antenatal diagnosis of fetal death or specific echographic findings	<u>Pilot assessment: level 1</u> : insufficient technical efficacy.	In-house	
Herpes simplex virus	Meningitis, encephalitis, myelitis, neonatal herpes; keratitis, uveitis, retinitis; IC with oesophageal or intestinal lesions.	<u>Level 1</u> ³ : further studies were considered to be needed at the time of this review (1997) before the value of the tests could be determined	In-house	
Human herpesvirus type 8 (HHV8)	Kaposi's sarcoma, disease of Castleman, primary effusion lymphoma.	No evidence found	In-house	

Test	Indications considered	Level of diagnostic efficacy	Commercial/ in-house; interpretation	Maximum time request-result
Parvovirus B19	Echographic abnormality, foetal death or symptomatic infection during pregnancy; Arthropathy; Aplastic crisis, red cell aplasia or unexplained pancytopenia in IC.	No evidence found	In-house mainly	
Polyomaviruses JC and BK	Progressive multifocal encephalopathy. Hemorrhagic cystitis post BMT or leukemia treatment, TIN post kidney transplant.	No evidence found	In-house	
Rubella virus	Primary rubella infection in first 16 weeks of pregnancy	No evidence found	In-house	
Varicella Zoster Virus (VZV)	Encephalitis, meningitis, myelitis; Keratitis, uveitis, retinitis; Atypical varicella zoster; Atypical pneumonia IC; Varicella during pregnancy.	No evidence found	In-house	
<i>Toxoplasma gondii</i>	Cerebral toxoplasmosis in IC or neonatal; Congenital toxoplasmosis; Chorioretinitis.	No evidence found	In-house	
<i>Aspergillus</i>	IC with fever under broad spectrum antibiotics, pulmonary or cerebral lesion on CT lesion, cough, cerebral disease.	No evidence found	In-house	
<i>Candida</i>	fever despite antimicrobial treatment in selected ICU or IC	No evidence found	In-house	

Test	Indications considered	Level of diagnostic efficacy	Commercial/ in-house; interpretation	Maximum time request-result
Pneumocystis <i>jiroveci</i> (<i>carinii</i>)	Unexplained lung infiltration in IC patient and BAL microscopic exam for P. carinii neg or unclear.	No evidence found	In-house	
Identification cultured fungi	phenotypic identification if phenotype unclear.	No evidence found	In-house	
Neu/HER2	Metastatic breast carcinoma eligible for Herceptin therapy	No evidence found	Kits (FISH, CISH)	
Aneuploidy TCC (bladder cancer)	Urine, bladder washing: follow-up treatment of transitional cell carcinoma of the bladder, neg. cystoscopy plus cytology equivocal.	No evidence found	Kits (FISH)	
LOH 1p-19q	Tissue section: prognostic, aid in diagnosis in complex cases of glioma.	No evidence found	Kits (FISH)	
EGFR gene amplification/ mutation	Tissue section: grade III and IV astrocytoma.	No evidence found	Kits (FISH, CISH)	
VH-JH IgH / DH-JH IgH / Kappa and Lambda gene rearrangement.	Not conclusive B-cell or uncertain lineage LPD. LPD in IC. Classification/staging LPD. Discrimination relapse from second malignancy.	No evidence found	In-house	
TCR rearrangement in NHL	T cell LPD, or organ involvement Follow up after 3 months	No evidence found	In-house	
TCR rearrangement in AML/ALL	Following conventional diagnosis of acute leukemia	No evidence found	In-house	

Test	Indications considered	Level of diagnostic efficacy	Commercial/ in-house; interpretation	Maximum time request-result
Patient specific (RQ)- ASO PCR	Minimal residual disease in ALL, AML and MM, after Ig/TCR rearrangement test	No evidence found	In-house	
IgVH sequencing	Hypermutation detection in typical CLL.	No evidence found	In-house	
t(1;14) BCL10-IgH	Follow-up of t(1;14) cytogenetic positive T-ALL (MALT lymphoma).	No evidence found	In-house	
t(1;19) E2A-PBX1	Follow-up of childhood pre-B ALL, if t(1;19) cytogenetic positive	No evidence found	In-house	
t(12;21) TEL-AML1	Follow-up of childhood pre-B ALL, if t(12;21) FISH positive	No evidence found	In-house mainly	
MLL 11q23 translocation t(4;11) AF4-ALL1 in ALL and AML	Subtype ALL and AML Role for follow-up?	No evidence found	In-house mainly	
MLL 11q23 translocation t(9;11) MLL-AF9 in AML	Subtype AML Role for follow-up?	No evidence found	In-house mainly	
t(8;21) AML1-ETO	Subtyping of CBF AML when WHO M2 AML/ t(8;21)/AML1-ETO+ is suspected, identify target for follow-up, follow-up of treatment	<u>Level 6⁴</u> : on the basis of the safety, effectiveness and cost- effectiveness, the use and public funding of this test is recommended	In-house mainly	Not urgent
t(15;17) PML-RARA bcr 1, 2 and 3 fusion gene transcripts	Subtyping when WHO M3 AML/t(15;17)+/PML-RARA+ or variant is suspected; follow-up of treatment	<u>Level 6⁵</u> : on the basis of the safety, effectiveness and cost- effectiveness, the use and public funding of this test is recommended	In-house mainly	Not urgent
inv(16) CBFB-MYH11	Subtype all AML follow-up	No evidence found	In-house mainly	

Test	Indications considered	Level of diagnostic efficacy	Commercial/ in-house; interpretation	Maximum time request-result
FLT3 exon 20 TKD mutation (D835)	Subtype adult and pediatric AML and pediatric ALL, MDS with blast excess? Diagnosis and relapse	No evidence found	RFLP PCR	
FLT3 exons 14/15 internal tandem duplication (ITD) or length mutation	Subtype adult and pediatric AML, pediatric ALL, MDS with blast excess, CMML. Diagnosis and relapse	No evidence found	RFLP PCR	
WT1 overexpression in malignant blasts	Subtype AL, MDS, CML. Diagnosis and follow-up in rare cases of BCR-ABL neg CML or MDS.	No evidence found	In-house	
PCR for t(11;14) BCL1-IgH qualitative	B-NHL CD5+ or unclear phenotype Test for secondary organ involvement.	No evidence found	In-house mainly	
for t(11;14) BCL1-IgH in pathology	(Suspected) MCL or other B-cell LPD associated with t(11;14) such as MM, hairy cell leukemia and prolymphocytic leukemia. Of help when IHC cyclin D1 unclear.	No evidence found	In-house mainly	
t(11;14) BCL1-IgH quantitat	BCL1-IgH pos lymphoma: detection tumor load and response, MRD, safety stem cell collection.	No evidence found	In-house	
t(14;18) BCL2-IgH qualitative	B-NHL and B-CLL. Complements morphology/ immunophenotype Test for secondary organ involvement.	No evidence found	In-house mainly	
t(14;18) BCL2-IgH in pathology	(Suspected) FL	No evidence found	In-house mainly	

Test	Indications considered	Level of diagnostic efficacy	Commercial/ in-house; interpretation	Maximum time request-result
t(14;18) quantification	BCL2-IgH pos lymphoma detection tumor load and response, MRD, safety stem cell collection	No evidence found	In-house	
FISH for 8q24: t(8;14), t(8;22), t(2;8) C-MYC	BL/BLL, high grade lymphoma. Transformation of FL.	No evidence found	Kits mainly	
cyclin-D1 overexpression	Differential diagnosis MCL. MRD if no other marker.	No evidence found	In-house mainly	
FISH for trisomy 12	Diagnosis, relapse or transformation of trisomy neg B-CLL, MRD in B-CLL if no other genetic marker	No evidence found	Kits and in- house	
t(9;22) BCR-ABL transcripts b2a2, b3a2 and e1a2 in CML diagnosis	MPD/MDS with hematological suspicion of CML or CML variants. t(9;22) or BCR- ABL is hallmark of CML (WHO)	No evidence found	In-house mainly	
t(9;22) BCR-ABL transcripts b2a2, b3a2 and e1a2 in ALL diagnosis	Precursor B-ALL	No evidence found	In-house	
t(9;22) in CML follow- up	Autologous or allogeneic stem cell transplant or other CML treatment.	No evidence found	In-house mainly	
t(9;22) in ALL follow-up	After chemotherapy or stem cell transplantation	No evidence found	In-house	
Human androgen receptor locus on X- chromosome	Clonal hematopoiesis in rare cases of MPD and MDS with diagnosis unclear, in females <65. Clonality in essential thrombocytosis.	No evidence found	In-house	

Test	Indications considered	Level of diagnostic efficacy	Commercial/ in-house; interpretation	Maximum time request-result
PRV1	Polycythaemia vera.	No evidence found	In-house	
Short Tandem Repeat (DNA fingerprinting) for chimerism	Allogeneic hematopoietic stem cell transplant. Test donor and patient before transplant. Estimate and monitor engraftment success	No evidence found	Kits mainly	
t(6;9) DEK-CAN	AML with t(6;9) on cytogenetics as seen in AML with maturation and increased basophilia, and in MDS.	No evidence found	In-house mainly	
t(11;18) API2-MLT	Marginal zone lymphoma.	No evidence found	In-house mainly	
t(2;5) NMP-ALK, inv(2)	Anaplastic large cell lymphoma of T cell or null cell type CD30+ LPD of skin. Rare large B cell LPD with unusual morphology/phenotype.	No evidence found	In-house mainly	

6. COMPARISON WITH OTHER COUNTRIES AND GENETIC TESTING

A comparison in terms of organisation, financing and quality (see chapter on quality) has been performed versus other countries for the tests under study. A closer look to the related field of genetic testing may also be of interest as some steps have been taken for this field at EU level.

6.1. THE GENETIC TESTING SITUATION

Introduction

Within the overall field of molecular diagnostics, genetic testing has a special place. Specific familial, ethical and social consequences can be associated with such tests, indicating the need for counselling. These sensitive issues, the repeated demonstration of quality issues associated with the test performance, and the evolution towards large scale pharmacogenetic testing have raised the interest of the EU and US authorities.

Genetic tests have increased in number of different tests and also in number of tests performed. Pharmacogenetic testing is still limited now and not yet penetrated into routine diagnostic service. An estimated yearly growth of 20% for this type of testing has however been predicted for the US. At the NIH sponsored GeneTests directory (www.geneclinics.org) laboratories and genetic tests for over 700 disorders are listed. In the EU at least 735000 tests were performed in 2002 with a mean cost of 573 Euro per test¹²⁵. These are estimates based on 715 laboratories performing genetic tests in 21 countries, and a further 936 clinical chemistry/haematology centers estimated to also offer genetic tests. A reliable database of labs is needed. The most frequent tests in EU are for hemochromatosis, factor V Leiden, factor II, cystic fibrosis, and fragile X mental retardation. Testing using relatively easy techniques (kits) for frequent conditions with well known mutations is done in numerous centers. For other tests the production of a kit format may prove impossible because of patent licenses needed or because of the complexity of the test. It may also prove not cost-effective for rare disorders.

Most CE labelled IVD kits for genetic testing are currently self-certified by the manufacturer. This is an indication for quality production, however it should not be considered an indication of clinical utility. There remains thus a need for assessment of clinical utility. Instead of leaving it to each member state, a review board linked to EMEA has recently been set up to assess the clinical utility of each test (like is done now in the UK with "gene dossier").

Quality

External quality assessment schemes (in the EU and the US) have repeatedly pointed to shortcomings, even after the introduction of IVD kits, eg for cystic fibrosis. Based on these findings, efforts are underway in the EU and the US to ensure the safe and effective use of genetic tests. By its very nature, the integration of genetics into clinical and public health practice is international in scope, also because testing for rare genetic conditions may necessitate shipment of samples across national boundaries. An overview of genetic testing laboratory databases and quality assurance efforts in the US and in Europe has been published¹²⁶. Issues of quality can be grouped into issues of laboratory practice or issues of patient management. Laboratory practice issues include the definition of genetic testing, lab certification/accreditation, QA system to make sure the quality-control job is done effectively, personnel standards, quality control, external quality assessment or proficiency testing, analytical and clinical validity of the test, record retention, report requirements, and advice regarding follow-up testing. Patient management issues include informed consent, counselling, use of residual samples, privacy/confidentiality, access to services and education. These issues are only in part addressed by the guidance available from international governmental/professional groups (Council of Europe, OECD, ESHG, EMQN, HGSA and WHO) or country based

organizations in the US, Australia, Austria, France, Canada, Germany, the Netherlands or the UK.

In the US, the minimum standards for clinical lab practice of CLIA apply also for genetic testing labs. Testing kits need market approval by the FDA. Most genetic tests are developed in-house for the laboratory's own uses and are thus not subject to FDA reviews. However, components of these tests are subject to the Analyte Specific Reagents Rule, which subjects reagent manufacturers to certain general controls, such as good manufacturing practices. In many countries testing for acquired mutations is not included under the category of genetic testing. Guidelines for Clinical Genetics Laboratories and evaluation of genetic tests have been issued by the American College of Medical Genetics (www.acmg.net). An approved guideline for Molecular Diagnostic Methods for Genetic Diseases is also available from NCCLS (www.nccls.org).

Little or no legal requirements on quality aspects specific for genetic diagnostic laboratories exist in the different member states of the EU. Obtaining an accreditation or certification is entirely voluntary. This is in contrast with clinical chemistry labs where the requirement for quality manuals is being implemented. These and other issues are raised in a document "Towards quality assurance and harmonisation of genetic testing services in the EU", which has been prepared for DG JRC. This report was based on a European wide survey and a more detailed survey in Spain¹²⁵. In addition, 25 Recommendations on the ethical, legal and social implications of genetic testing have been published by DG Research¹²⁷. These are the result of the activities of the STRATA Expert Group¹²⁸. This report concludes 'genetic exceptionalism' is inappropriate, but stresses the need for increased attention to quality and confidentiality for all medical data with high information content. The European Commission has organized informal meetings with EU member state experts and officials on genetic testing, including quality aspects, in an effort to appreciate the need for EU legal action in this domain. In the US, a number of meetings on the evolution of genetic testing took place in 2003 and 2004, organized by the National Institutes of Health Secretary's Advisory Committee on Genetics, Health and Society (www4.od.nih.gov/oba/sacghs.htm). The topics also included cost-effectiveness determinants for genetic tests, and criteria for coverage by the payers. Of note, in addition to HTAs, coverage of a test by other payers was also mentioned as a possible factor. A draft report on the coverage and reimbursement of genetic tests and services is available for public comment¹²⁹. Also in the US continued attention is being given to improve the quality of genetic testing as illustrated by the CDC funding no 04137 (2004): "Improving the Quality of Genetic Testing and Assuring Its Appropriate Integration into Clinical and Public Health Practice".

Organisation and financing

Especially for rare disorders, testing is best performed in a European wide network of reference labs. This testing situation points to the need for standard arrangements for data handling (informed consent, privacy and language used for accompanying information and results), sample handling and documentation, assuring pre- and post test counselling where needed, and harmonized test reimbursement). Currently reimbursement systems for genetic tests vary greatly across Europe. As is the case in Belgium, a single level reimbursement scheme is used in the Netherlands and in France. In Germany components of the testing method are each reimbursed separately. Reimbursement could be used to reinforce QA, as it is being developed in the UK. For a test to be refunded by NHS, a steering group will include it in the list of tests that should be offered by approved laboratories in the UK Genetic Testing Network.

Intellectual property protection is a condition for research and development. However, the complexity of the patent situation may not only impact the individual lab (eg restriction on licences to test for BRCA1 and BRCA2) but may also limit the industry in the development of kits, or limit the development and distribution of certified reference materials by the relevant authorities. To date almost no certified reference materials are available for genetic testing. This material is needed for verifying analytical accuracy. Public support is needed for this activity.

The European Molecular Genetics Quality Network (www.EMQN.org) and the European Concerted Action on Cystic Fibrosis provide EQA schemes for 10 hereditary disorders and cystic fibrosis respectively. Participation to the local or European external quality assessment schemes is voluntary. Participation both to local (regional mutations) and European wide EQA schemes would seem appropriate for genetic testing labs. Ideally, genetic tests should be performed only at public or private laboratories, which are fully accredited (Beltest, ISO 15189) and have the necessary patent licenses, pass local/European/US proficiency schemes, and always use up to date methods. Those labs can theoretically cover most of Europe, if assuring an acceptable turn around time and appropriate reporting.

There are two possible scenarios for the relationship between genetic testing and counselling services, one of closer integration and one of progressive dislocation of the two elements. As it is likely that more and more samples will be sent away to be tested in centralized facilities, the laboratory activities can be considered separate from the counselling activities. However, also near-patient testing performed by the primary practitioner may become a reality for some tests, and depend on the level of genetic counselling training the physician has received. In general, genetic counselling could conditionally be spread downwards to the secondary and primary healthcare levels. Counselling centers should ideally be as close as possible to the patients or clients. However, they need to guarantee the highest standards of genetic counselling and medical genetic knowledge. Common agreed international guidelines are needed. One way to guarantee counselling is that laboratories only take samples referred by institutions that provide genetic counselling or are linked to those that do.

6.2. ORGANISATION AND FINANCING

Data about financing of the molecular tests could be documented for France¹³⁰, Germany¹³¹, UK (NHS), the Netherlands, Switzerland¹³² and Australia¹³³ (tables 21 & 22). Data on the test volume could only be obtained for France and only for ambulatory care. Also for the other countries no exhaustive research has been performed for hospitalized patients. For microbiology there is often a specific reimbursement code for each agent detected. In addition, for the detection of any other micro-organism using nucleic acid hybridisation or amplification there is a generic reimbursement code in Australia and in Germany. There are striking differences between countries in the list of reimbursed tests. The amounts reimbursed per test are rather similar in France and Germany. For cytogenetic and molecular tests in hemato-oncology the reimbursement codes are generally generic. For the reimbursement of PCR detection of translocations or their fusion transcripts a list of specific translocations has been published in Switzerland. In Australia, gene rearrangement tests are only reimbursed for acute forms of leukemia or in case of chronic myeloid leukaemia. In France, no specific nomenclature is available for ambulatory molecular hemato-oncology PCR tests.

Table 21. Laboratory reimbursement rate at 100% (includes any patient contribution) for selected microbiology molecular tests in several countries

Parameter	Belgium [#]	France	Germany	Netherlands	UK (NHS)*	Switzerl [†] *	US*	Australia*
<i>N gonorrhea</i>	2.80		(16.40)	(23.50-73.20)		22.73-51.95	12.88-38.81	(17.28)
<i>C trachomatis</i>	2.80	16.20	16.40	(23.50-73.20)		51.95	12.88-38.81	17.28
<i>C pneumoniae</i>		27.00	16.40	(23.50-73.20)		110.39	12.88-38.81	17.28
<i>M pneumoniae</i>			(16.40)	(23.50-73.20)		110.39	12.88-38.81	(17.28)
<i>M tuberculosis</i>	13.99	40.50	61.40	(23.50-73.20)		97.40	12.88-38.81	(17.28)
<i>L pneumophila</i>			(16.40)	(23.50-73.20)			12.88-38.81	(17.28)
HBV qualitative		40.50	(16.40)	(35.20-73.20)	32.03	110.39	12.88-38.81	(17.28)
HBV quantitative			89.50	(177.00)	55.32	162.34	31.38-47.37	(17.28)
HCV qualitative	5.60	54.00	40.90	95.40	82.98	129.87	12.88-38.81	55.58
HCV quantitative		81.00	89.50	(177.00)	165.96	178.57	31.38-47.37	108.67
HCV genotyping		108.00	102.30	(177.00)	132.48		86.31-284.68	123.49
HCV geno + quant					215.46			
CMV qualitative		162.00	(16.40)	(23.50-73.20)		32.47-110.39	12.88-38.81	17.28
CMV quantitative		162.00	(16.40)	(177.00)		162.34	31.38-47.37	(17.28)
EBV qualitative			(16.40)	(23.50-73.20)		32.47-110.39	(12.88-38.81)	(17.28)
EBV quantitative			(16.40)	(177.00)			(31.38-47.37)	(17.28)
Herpes simplex			(16.40)	(23.50-73.20)		32.47-110.39	12.88-38.81	17.28
Enterovirus						32.47-129.87	(12.88-38.81)	
Varicella Zoster Virus		162.00	(16.40)	(23.50-73.20)		110.39	(12.88-38.81)	17.28
<i>Toxoplasma gondii</i>		162.00	(16.40)	(23.50-73.20)			(12.88-38.81)	(17.28)
Rubella virus		121.50	(16.40)	(23.50-73.20)		129.87	(12.88-38.81)	(17.28)
Parvovirus		162.00	(16.40)	(23.50-73.20)		110.39	(12.88-38.81)	(17.28)

Fees in brackets refer to the use of a generic code; range of fees for qualitative tests covers lowest fee for hybridization to highest fee for amplification test (across reimbursement systems for the US or automated versus manual method in The Netherlands)

[#]Cost per test, directly paid to lab (25% rule)

*2005 prices, exchange rates from the Eur. Central Bank May 13th

Table 22. Laboratory reimbursement rate at 100% (includes any patient contribution) for selected hemato-oncology molecular tests in several countries.

Parameter	France*	Germany**	Netherlands**	Switzerland**#	Australia#
IgH rearrangement	135-270	32-62.20	134.38	32.47-162.34	140.74
TCR rearrangement	135-270	32-62.20	134.38	32.47-162.34	140.74
DNA t(14,18) BCL2-IgH in follicular lymphoma	135-270	32-62.20	134.38	32.47-162.34	
t(2,8)-, t(8,14)-, t(8,22) in Burkitt-lymphoma	135-270	32-62.20	134.38	162.34	
t(9,22) BCR-ABL in CML	135-270	32-62.20	134.38	32.47-162.34	140.74
t(4;11) MLL-AF4, t(9;22) BCR-ABL, t(12;21) TEL-AML1 in precursor B-ALL	135-270	32-62.20	134.38	32.47-162.34	140.74
t(15;17) PML-RARα in AML-M3	135-270	32-62.20	134.38	32.47-162.34	140.74
t(1;19) E2A-PBX1 in pre-B-ALL	135-270	32-62.20	134.38	32.47-162.34	140.74
t(8;21) AML1-ETO in AML	135-270	32-62.20	134.38	32.47-162.34	140.74
del(1)/SIL-TAL1 in T-ALL	135-270	32-62.20	134.38	162.34	140.74
t(2;5) in anaplastic large cell NHL	135-270	32-62.20	134.38	162.34	
t(11;14) BCL1-IgH in mantle cell NHL	135-270	32-62.20	134.38	32.47-162.34	
11q23 MLL rearrangement in acute leukemia	135-270	32-62.20	134.38	32.47-162.34	140.74
inv(16) CBF-beta-MYH11 in AML-M4 Eo	135-270	32-62.20	134.38	32.47-162.34	140.74
FLT3 ITD in acute leukemia	135-270	32-62.20	134.38	32.47-162.34	140.74

*Metaphase DNA hybridization tests, maximum two (no fees for PCR tests)

**Fee varies. Germany: 32 Euro for PCR-based detection, 39.90 Euro for hybridisation-based detection and 64.20 Euro for post-karyotyping hybridisation or FISH. The Netherlands: 73 to 134 Euro depending on the specific code used. Switzerland: 32.47 € for PCR to 162.34 € for post-karyotyping FISH

prices, exchange rates from the Eur. Central Bank May 13th

7. QUALITY

7.1. QUALITY GUIDELINES AND EQA

A review of available control materials, proficiency programs, standard reference materials, and written guidelines for molecular assays for infectious diseases has been published¹³⁴. It remains a challenge for the clinical laboratory scientist to incorporate the programs and services in the context of good laboratory practice and current laboratory regulation.

Classes of examination procedures

For molecular diagnosis a distinction can be made between following categories of test procedures.

1. In vitro medical device (bearing the CE label).

For those kits the manufacturer must satisfy to the requirements of EU directive 98/79 and the national transpositions. For Belgium: RD of 14-11-2001. Since 7-12-2003 only CE labeled IVD kits can be put on the European market. It is up to the manufacturer to set up performance criteria (both analytical and clinical). Only for those IVD's belonging to Annex II list A, IVD's must comply with the Common Technical Specifications as defined by the EU Commission. (<http://www.pei.de/ivd/cts.htm>). All IVD kits have been validated by the manufacturer and data must be available for the users on all aspects of analytical and clinical validation. (Essential requirements in Annex I of directive 98/79).

2. Devices for performance evaluation.

These devices are also regulated in the IVD directive. If a device is in performance evaluation phase it can be made available to institutions or laboratories to be subject to one or more evaluation studies intended to gather information on performance evaluation parameters in field conditions (multi-center studies) which would be used for its conformity assessment. These devices still have no intended medical purpose. When a medical purpose has been established based on sufficient and broadly agreed upon scientific, diagnostic and clinical evidence, then the product must comply with the requirements of the directive before the manufacturer can place it on the market with an intended IVD use. According to the IVD directive, these products must be clearly labeled as such to distinguish them from products that fall outside the scope of the IVD directive. In contrast to the US where FDA decides on the status of approval of IVD's including scientific, diagnostic and clinical evidence in Europe this decision belongs to the responsibility of the manufacturer in the gray zone between IVD and RUO.

3. Modification from an existing IVD by the user.

4. RUO (Research Use Only); ASR (Analyte Specific Reagent); IUO (Investigational Use Only).

These devices are not regulated by the IVD directive. Some RUO products are industrial prepared kits but are not validated from an analytical point of view. There is no clinical validation performed by the manufacturer. Other RUO products are raw materials and must be considered as raw materials that are incorporated in "home-brew" kits. There is a strict interpretation given for RUO kits. An RUO product cannot have intended medical purpose or objective. (Meddev. 2.14/2 rev 1: http://europa.eu.int/comm/enterprise/medical_devices/meddev/index.htm).

5. "Home-brew" kits (in-house method).

The IVD directive 98/79 makes a distinction between devices manufactured and used in the same premises of their manufacture and others. In the first case, directive 98/79 is not applied. A discussion on the use of "home-brew" kits only for in-house patients was initiated by the Medicines and Healthcare products Regulatory Agency of the UK. This discussion is now considered closed. There are no restrictions for the use of "home-

brew” kits (they may also be used for referral patient samples). More information is available at <http://devices.mhra.gov.uk>.

According to the legislation for clinical laboratories (RD 3-12-1998), the IVD directive EC 98/79 and the ISO accreditation standard 15189, responsibilities of involved parties can be summarized as follows (table 23):

Table 23. Responsibilities of involved parties for the validation of IVDs

Type of test	Minimum specifications	Analytical validation	Clinical validation	User Responsibilities
IVD not belonging to annex II	Defined by the manufacturer	Performed by the manufacturer	Performed by the manufacturer	Implementation validation
IVD annex II list A	CTS	Performed by the manufacturer	Performed by the manufacturer	Implementation validation
IVD annex II list B	Defined by the manufacturer	Performed by the manufacturer	Performed by the manufacturer	Implementation validation
RUO, ASR, IUO	/	To be done by the user	To be done by the user	Full validation
Partial modified IVD's	/	Performed by the manufacturer (partially)	Performed by the manufacturer (partially)	Implementation validation + changes made
“Home-brew”	/	To be done by the user	To be done by the user	Full validation

EQA in European countries

One can distinguish in Europe three types of EQA approaches.

- In most countries the participation to EQA schemes is voluntary
- In some countries the participation in EQA schemes is mandatory in order to be recognized or licensed (Belgium, Luxembourg, France, Switzerland)
- Only few countries link EQA results to reimbursement. This is the case in Germany.

Molecular diagnostic tests are not often included in the panel of offered EQA programmes and if offered they do not belong to the mandatory panels. Table 24 summarises the situation in different European Countries

Table 24. Mandatory participation to EQA programs by country

Country	Mandatory EQA schemes	Mandatory EQA for molecular diagnostics
Belgium	Yes	HCV, M <i>tuberculosis</i>
France	Yes	Only genetic profile
Great Britain	No	No
The Netherlands	No	No
Denmark	No	No
Sweden	No	No
Finland	No	No
Norway	No	No
Germany	Yes	No
Switzerland	Yes	Some tests (see QUALAB)*
Germany	Yes	No

*<http://www.famh.ch/QUALAB.htm>

Literature search

A literature search was performed on quality management aspects related to molecular biology. The CMD laboratories must be seen also as referral laboratories; consequently recommendations and guidelines for referral laboratories are also applicable.

Guidelines and standards for quality management

- clinical laboratories in general¹³⁵
- specific aspects¹³⁶
- checklist for the accreditation of molecular biology tests^{137, 138}

Information for patient preparation, sampling, transport

- in general¹³⁵
- specific aspects^{92, 91, 138, 139}

Acceptance and rejection criteria

- in general⁷⁵
- specific aspects^{92, 91, 140, 138}

Validation of tests

- in house testing¹³⁶
- specific aspects^{92, 91, 141, 138}

Infrastructural requirements

- in general⁷⁵
- specific aspects^{92, 91, 138, 139}

Competence of staff^{75, 138}

Guidelines for internal QC in molecular biology^{92, 140, 138, 139}

Guidelines for EQC

- for the users^{91, 138}

- for PT scheme organizers⁹⁸
- alternative procedure¹⁴²

Description of references

Nucleic Acid Amplification Assays for Molecular Hematopathology; Approved Guideline. NCCLS vol 23 number 17: MM5-A; 2003⁷⁵

- This guideline addresses the performance and application of assays for gene rearrangement and translocations by both polymerase chain reaction (PCR) and reverse-transcriptase polymerase chain reaction (RT-PCR) techniques and includes information on specimen collection, sample preparation, test reporting, test validation, and quality assurance.

Quantitative Molecular Methods for Infectious Diseases; Approved Guideline. NCCLS Vol 23 number 28: MM6-A; 2003⁹²

- This document provides guidance for the development and use of quantitative molecular methods, such as nucleic acid probes and nucleic acid amplification techniques of the target sequences specific to particular microorganisms.
- It also presents recommendations for quality assurance, proficiency testing, and interpretation of results

Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline. NCCLS Vol 25 number 22: MM3-A; 1995⁹¹

- This document contains guidelines for the use of nucleic acid probes and nucleic acid amplification techniques of the target sequences specific to particular microorganisms; quality assurance; limitations; proficiency testing; and interpretation of results

Assessment of Laboratory Tests When Proficiency Testing is Not Available; Approved Guideline. NCCLS Vol 22 number 26: GP29-A; 2002¹⁴²

- This document is intended for use by laboratories as alternative assessment procedures when PT testing is not available. The guideline includes examples with statistical analyses.

Proficiency Testing for Molecular Methods; Proposed Guideline. NCCLS MM14-P; 2004⁹⁸

- This document provides guidelines for a quality proficiency testing program including reliable data bases; design control in the choice of materials and analytes; good manufacturing processes; documentation procedures; complaint handling; corrective and preventive action plans; and responsive timing of reports.

ISO 15189. 2003. Medical Laboratories – Particular requirements for quality and competence¹³⁵.

- This standard is a general quality management standard for all medical laboratories.

Laboratory Accreditation Standards and Guidelines for nucleic acid detection techniques. National Pathology Accreditation Advisory Council. 2000¹³⁶.

- This standard provides guidelines for conducting nucleic acid detection techniques covering all aspects of services provided by the laboratory and the applied quality management tools (quality system, staff, laboratory facilities).

Requirements for the validation of in-house in vitro diagnostic devices. National Pathology Accreditation Advisory Council. 2003¹⁵.

- This document provides guidance for the validation of in-house in vitro diagnostic devices including clinical and technical requirements for validation.

Guidelines for the Accreditation of Swiss Medical Laboratories Performing Nucleic Acid-Based Diagnostic Procedures. METAS 2004¹³⁷.

- This checklist is based on the requirements of ISO/IEC 17025: 1999 and describes the particularities and requirements in the field of nucleic acid-based diagnostics

CUMITECH 31. Verification and Validation of Procedures in the Clinical Microbiology Laboratory. American Society for Microbiology 1997¹⁴¹.

- This guideline offers information that users of tests may consider in their efforts to improve the general operations or quality of their laboratory services.
- Especially the performance characteristics as required by the Clinical Laboratory Improvement Act (CLIA) are included. Also elements that can be used for validation are described.

Best Practice Guidelines Committee from the Clinical Molecular Genetics Society; <http://www.cmgs.org/BPG/default.htm> ¹⁴⁰

- The IQC document describes guidelines for internal quality control of sample reception and DNA extraction.
- The Sequencing document gives guidelines for DNA sequencing analysis and interpretation.

CAP Checklist for Molecular Pathology¹³⁸

- Checklist from the American College of Pathology (CAP) for their own accreditation scheme. The questions are specifically focused on all quality management aspects for molecular inherent disease testing, parental testing and in situ hybridization

M. Neumaier, A. Brauns and C. Wagener. Fundamentals of quality assessment of molecular amplification methods in clinical diagnosis. Clin. Chem. 44(1): 12-26; 1998¹³⁹.

- General guidelines from sampling to evaluation of molecular assay results when using (RT) PCR and nested PCR.

External Quality Assurance schemes for molecular diagnosis

The EQA organizations listed in appendix 6 have mainly been identified based on contacts with participants to the EQUAL project. EQUAL is a project of the 6th European framework, which enables participants to subscribe to three types of proficiency testing within molecular diagnostics (<http://www.equal.unifi.it/htm/home.php>): EQUAL-qual for monitoring of performance of qualitative PCR based assays; EQUAL-quant for monitoring performance of the 5'-nuclease quantitative PCR based assay; EQUAL-seq for monitoring sequencing based assays.

When subscribing to this project, participants were asked to mention the PT-schemes for molecular biology at which they already participated (with website and or e-mail address of the schemes in question). On this basis, we were able to identify most of the organizations. Some of these organizations mentioned on their website links to other organizations which allowed us to identify additional organizations. However not all organizations had websites which provided all of the necessary information. Moreover, even when contacted via e-mail several organizations did not respond; this explains the existence of "gaps". The findings can be summarized as follows.

- The majority of the organizations focus on PT for genetic testing.
- Different organizations offer however the possibility for PT of molecular biology for microbiological parameters (of which HIV, HCV, HBV, C. trachomatis, N. gonorrhoeae are parameters for which the most PT exist).

- PT for molecular biology of hemato-oncology is less developed.
- Although a large part of these organisations offer PT programs at a regular basis, others are to be considered as projects (mainly of the European Union), which are limited in time. There is no evidence of the continuity of these projects. Nevertheless, some of these projects evolve (or may evolve) into regular-based programs.
- Not all programs are open to “foreign” participants: some organizations limit the participants to laboratories of their own country.
- Costs vary enormously; some projects are free (mainly the temporary projects, sponsored by the European Community); the costs of the programs for which a fee has to be paid show large variations. Additional costs may interfere for shipments abroad (especially “overseas” shipments are usually subjects of additional costs). Not all organizations publish a “pricelist” on their website; some organizations publish pricelists which are only sent to (possible) participants.
- Most of the parameters performed by the Belgian CMD are covered by the existing programs. If a given parameter will be introduced in the nomenclature and the number of laboratories performing the test is insufficient for developing a Belgian EQA, there will be no problem for the laboratory to participate in an international program (in most cases, it will be possible to find a European program, which even may already have a distributor in Belgium).
- Some organizations offer the possibility for “general” proficiency testing of molecular biology (which is not focused on a given parameter but focuses on the evaluation of the performance of molecular biology as such). This can be used as an alternative if no specific program for a given parameter exists.

Worth mentioning also is the initiative of 7 IVD manufacturers who created the Industrial Liaison Committee to further quality in molecular testing independent of commercial interests (chair: James.Reid@scotent.co.uk), a first project concerns the evaluation of synthetic HCV-RNA reference material across platforms.

7.2. QUALITY REQUIREMENTS

Quality requirements as required in the RD of 3-12-1999 are in principal sufficient to cover also molecular biology. However the assessment of these requirements by inspections cannot be organized in such a way to guarantee the implementation of all requirements. Especially the frequent use of “home-brew” test and a lack of validation is a specific concern. For all these reasons it is recommended that every laboratory involved in molecular biology testing must be formally accredited according to ISO 15189. A transition period of 3 years is proposed.

During the transition period the following requirements must be fulfilled immediately for reimbursement and for reference activities:

- Implementation validation file must be available for CE labelled kits
- For “home-brew” test kits a complete validation file must be submitted and accepted
- Participation in a national or international EQA program when available
- The laboratory must run an adequate internal control

Criteria for including molecular biology tests in the nomenclature

If formal accreditation is required for all molecular biology testing every test with proven clinical evidence could pass into the nomenclature.

Reference laboratories using molecular diagnostic examination procedures

A project on the financing of reference laboratories in general is in the phase of realisation between RIZIV/INAMI and the IPH¹⁶. Reference laboratories using molecular diagnostic examination procedures may be considered as one type of reference laboratories. Here also formal accreditation ISO 15189 is required. In some countries exists a DORA (directory of rare analytes) as a unique portal site.

Routine laboratories performing only a part of an examination procedure

Until now a referral laboratory is an external laboratory to which a sample is submitted by a referring laboratory for a supplementary or confirmatory examination procedure and report. The referral laboratory provides results and findings to the referring laboratory. A RIZIV/INAMI procedure exists for the reimbursement by the referring laboratory to the referral laboratory if the examination procedure is included in the nomenclature.

In clinical studies it is already common practice that two laboratories are involved in the same analysis, performing each, a part of the whole process consisting of extraction/isolation of RNA/DNA + amplification + sequencing + interpretation.

- a first laboratory only performs: extraction/isolation of RNA/DNA
- a first laboratory only performs extraction/isolation of RNA/DNA + amplification
- a first laboratory only performs extraction/isolation of RNA/DNA + amplification + sequencing
- only interpretation of genetic variation (sequence or mutation pattern) by dedicated software

For quality reasons, the referral laboratory must provide the procedures for performing the first part of analysis and perform acceptance controls on the received sample materials before further treatment. According to this practice the question arises if the concept of “subcontracting” must be revised.

Guidelines for implementing a quality system for molecular diagnostic examinations

These recommendations give guidance to comply with the ISO 15189 standard and are based on the current use technologies, especially PCR and real time PCR. In a near future, new technologies such as microarray technology (DNA, proteins, carbohydrates, DNA methylation tests, bioarrays, mass spectrometry applications, ...) will need a review and an update. The first commercial microarray tests and DNA methylation tests were FDA cleared in January 2005.

Infrastructural requirements

In order to reduce the risk of cross-contamination or carry-over contamination, most recommendations require three areas: extraction area, a dedicated area for the preparation of reagents, a dedicated contained area for amplification and product detection. The movement of specimens and equipment is to be unidirectional ie from pre-amplification to post-amplification areas. These requirements will be less stringent when fully automated and dedicated analyzers will be available. For microbiology nucleic acid amplifications the laboratory must have adequate facilities according to the legislation on pathogens and the contained area required (L2, L3). Precautions must be taken in order to avoid contamination (laminar flow cabinet for preparation of reaction mix, DNase/RNase free water, cups, tips with filter, validated decontamination procedure, validated configuration of pipetting robots,...)^{92,91,138}.

Analytical requirements

In principle only validated examination procedures may be used. The validation must be performed by the manufacturer and the user. If an IVD kit is used, the same validation requirements as mentioned under “analytical requirements” for routine clinical laboratories are applicable. If an RUO, ASR, or IUO kit is used, the technical validation performed by the manufacturer must be completed by the user. The user is fully responsible for the clinical validation. In-house examination procedures are usually developed to meet a need not provided by commercially available kits or to provide test kits at lower-cost than the commercial available kits. The use of an in-house examination procedure may not be an excuse for adequate and appropriate validation (technical AND clinical validation) prior to use. Modified commercial kits must be validated for the modifications made by comparing results from identical samples or identical types of samples.

For the validation of in-house examination procedures following principles may be used:

- evaluation with known positive and negative samples
- by comparison with EQA examination procedure material, if available
- by comparison with an existing validated method in the laboratory
- validated with all specimen types and conditions that will be used to make laboratory diagnosis

Validation of examination procedure methods (depending on the analysis performed) should include at least:

- Specificity
- Sensitivity

- Linearity and precision within the selected range (only for quantitative tests)
- Reproducibility
- Stability of DNA or RNA in the sample under conditions of the assay
- Ruggedness of the method

Validation protocols can be found in a number of documents^{92,91,141,138} as well as in

- Common Technical Specifications for Annex II, List A analytes
- Harmonised standards in the scope of the IVD directive
- ISO/CD 18113-1¹⁴³

General remarks:

- The sensitivity of a examination procedure, or cut-off values should be set at a level that is relevant to the diagnostic use of the examination procedure.
- RUO, ASR and IUO kits can not be used for reporting clinical results, diagnosis or prognosis in humans if the clinical validation is not done by the laboratory.
- For those examination procedures where an IVD kit is available, the use of RUO, ASR or IUO and in-house kits without clinical validation is not allowed.
- For those examination procedures (either RUO, ASR, IUO or in-house) where the clinical validation is not completed due to lack of sufficient patient samples, reports will be issued with a statement that the examination procedure has not yet been clinically validated¹⁵.

A comprehensive overview of terms and definitions related to performance characteristics of IVD's is given in a number of documents^{92,91,136,141,138}.

Internal Quality Control

IQC^{92,140,138,139}: internal QC samples must cover all steps of the analysis: isolation (recovery and purity); control on the amplification step; positive and negative control for the whole analysis procedure. The exact number of controls required for PCR-based systems depends on the number of samples in each run, although in general, two types of negative control should be included: a sample that is negative for the abnormality or pathogen and a "no nucleic acid" sample (ie all reagents except the DNA/RNA). Negative controls should be placed after the last patient samples. Positive controls should be just above the limits of sensitivity of the examination procedure. For quantitative or semi-quantitative examination procedures further standards may be required to calibrate the examination procedure.

Sample instruction manual

Each laboratory using molecular diagnostic examination procedures must have a sample instruction manual as a part of its own quality system documentation. This manual may be printed or preferably be available on the internet and/or intranet. The sample instruction manual must include: indications and limitations of the test, instructions for patient preparation, sampling, preliminary storage and treatment, time limitations in combination with storage conditions before analysis, transport conditions and instructions, sample acceptance/rejection criteria, contact person with contact possibilities (E-mail, tel, fax), TAT for reply, cut-off or reference values or other relevant information for reporting results. This information must be available in a user friendly format (regularly updated webpages) for referring laboratories and clinicians.

Acceptance of samples

The laboratory must check samples against his own procedure for sample conformity. These procedures must include criteria for acceptance/rejection of samples and a definition of which “critical samples” will not be rejected even if acceptance criteria are not fulfilled. (A laboratory may choose initially to process already such sample but not release the results until more information is available or results can be released under reserve, clearly stated in the report).

Competence of staff

The laboratory must have in his staff experienced supervisors with proven experience on the examination procedures performed and with technical competence appropriate to the complexity of testing methods (it is evident that the competence for making in-house kits is greater than for only using commercial IVD kits).

External QC

Participation in a European or worldwide EQA program for each examination procedure, when available, is mandatory. Examination procedure runs that include EQA samples should be carried out in rotation by all trained staff within a laboratory that is performing examination procedures on a routine basis. If there is no EQA program available: see NCCLS GP29-A¹⁴² for the set up of interlaboratory comparisons. An interlaboratory comparison between only Belgian laboratories is not sufficient.

Reporting

As much as possible the way of reporting results should be standardized (especially in hemato-oncology). A written report must be sent to the requester within the stated TAT.

Quality aspects of laboratory information

Initially, quality of laboratories focused on analytical quality. Today, quality is linked to quality of laboratory information. Within the same laboratory pre- and post-analytical quality is taken into consideration. But for optimum patient care, quality must not only cover the information obtained in one laboratory but for the whole clinical phrasing for an individual patient.

Within the scope of molecular diagnostic examinations, information becomes available for the same patient from the clinical biology laboratory, the pathology lab, the CMD, the CMG. It is recommended that in the same hospital a single coordinator is responsible for referring samples and follow-up of results both for the clinical biology and the pathology laboratory. Direct shipment by clinicians of samples to external laboratories must be avoided.

Recommendations

1. The licensing decree for laboratories performing molecular diagnostic testing must include a formal accreditation ISO15189.
2. Review of the concept of referred tests.
3. National EQA schemes in molecular biology: where possible (availability of adequate sample material) the national EQA organization (IPH) shall organize a scheme for the molecular diagnostic tests included in the nomenclature:
 - if the number of participants is > 50: an own scheme will be organized

- if the number of participants is < 50 the scheme will be organized in collaboration with other scheme organizers.

For the organisation of such schemes, recommendations given in the NCCLS protocol MMI4-P will be followed⁹⁸. Organizers of EQA schemes should preferably be accredited for this activity.

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9. APPENDICES

Appendix 1. CMD activity reports

Appendix 2. List of molecular diagnostic kits

Appendix 3. CMD method questionnaire sample form

Appendix 4. CMD expert questionnaire sample form

Appendix 5. List of invoices (Taq specific) submitted by CMDs

Appendix 6. List of EQA programs for molecular tests

Appendix I: CMD activity reports

AANTAL TESTS

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Totaal (1 februari 2003 - 31 jan 2004)
Micro-organismen																			
Bartonella henselae en Bartonella quintana	34		1	21	47		11									7			121
Bordetella pertussis	5		624				34				39					45			747
Borrelia burgdorferi	35			88	375		61									68			627
Chlamydia pneumoniae	8	15	45				90			327	124	15		45		390	129	67	1255
Corynebacterium diphtheriae			1													5			6
Verocytotoxine-producerende E.coli			190																190
Detectie vanA, vanB, vanC bij enterokokken		3	20		52	43			1		24							3	146
Detectie van macroliden resistentie bij Helicobacter pylori																		94	94
Legionella pneumophila		10	161			8	10			263	61	55				212	39	33	852
Mycoplasma pneumoniae	17	17	111				72			350	413	15	25			411	133	88	1652
Mycobacterium tuberculosis	668	198		300	118		453	1244	49		34	205			605	520	237	164	4795
Detectie van rifampicine resistentie bij Mycobacterium tuberculosis	1	17						66				27					6	2	119
Detectie van mecA bij stafylokokken	232	259	49		18	897	3		23			294	38			75	139	54	2081
Detectie en/of identificatie moeilijk identificeerbare en/of kweekbare bacteriën	76	11	46	36	361	127	145		355			37	24			798	20		2036
Typing nosocomiale pathogenen	31	297	5		409	465			16		136		29				13	95	1496
CMV kwalitatief			736	2489	112	758	557	433	71		1073	290	30	1159		1515		791	10014
CMV kwantitatief	1673	746	27						2963	2017			379	219			425	84	8533
EBV kwalitatief		15	11	54	45	302	14		79		11			717		26	20		1294
EBV kwantitatief					433	91			533	752			79						1888
HBV kwalitatief					48						22					283	104		457
HBV kwantitatief	22	51		385	703	463		336	454	778									3192
HCV kwalitatief	12	423		1375	831		1018	540	32	1551		284		58	563	503	102	76	7368
HCV kwantitatief	100	109		222	600		342	292	415	450	110	170		67	185	106		43	3211
HCV genotypering	76	114		204	505		328	229	324	374	72	114			138	115		34	2627
HPV	1168	860	1859	875	1750	2282	2678	532	1166	2566	984	820	3471		301	1395	1506		24213
Enterovirus	195		367	109	45	435	41	751	163	395	163	94		1		114	51		2924
Herpes simplex	194		133	220	185	756	50		6	729	327	76		64		447	101	27	3315
HHV8 (vanaf 23/09/03)			1			15													16
Parvovirus B19					114	130								35		18			297
Polyomavirussen JCV en BKV			40							1261				734			8		2043
Rubella virus						14													14
VZV	188		25		75	131	48			598	157			3		74	23	27	1349
Toxoplasma gondii	34		113	188		240				524	57			31		89			1276
Aspergillus				337												456			793
Candida																470			470
Pneumocystis carinii	103	10				19				356				83					571
Identificatie gekweekte fungi	1								141				63				52		257
Subtotaal micro-organismen	4873	3155	4565	6903	6826	7176	5955	4423	6791	13291	3807	2496	4059	3295	1792	8142	3108	1682	92339

AANTAL TESTS

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Totaal (1 februari 2003 - 31 jan 2004)
Genherschikkingen (diagnostiek)																			
Herschikking immunoglobulinenegenen	659	311		60	278		967		239	981	230	495	322	248		54	20		4864
Herschikking β en γ genen T receptor van lymfocyten	113	81		29	203		160		91	539	193	150	229	47		12			1847
Chromosomale translocaties en inversies (diagnostiek)																			
t (1;14) SIL-TAL	14	33	47		28				93		8	33							256
t (1;19) E2A-PBX	14	33	47		28		1		95	31	16	40							305
t (2;5) NPM-ALK	3	10					1		3	1			9						27
t (4;11) MLL-AF4	56	33	47		28				91	11	12								278
t (8;14) JH-MYC		7					9			466									482
t (8;21) ETO-AML1	45	33	47		58		8		91	162	43	38							525
t (9;11) MLL-AF9		33	47						91	4		33							208
t (9;22) BCR-ABL	122	78	72		315		202		141	500	91	139	71				26		1757
t (10;11) MLL-AF10		33	48						91			33							205
t (11;14) JH-BCL1	78	12		5			42			480	89	221	99	5		1			1032
t (12;21) TEL-AML1	14	33	48		28		1		97	31	13	42							307
t (14;18) JH-BCL2	138	133		18	8		110		61	515	95	239	91	27		13			1448
t (15;17) PML-RAR	45	33	51		58		6		91	381	43	39							747
inv, 16 MYH11-CBCF	45	33	47		58		8		91	284	43	38							647
afwijking 11q23			47				9			101		38							195
afwijkingen chromosoom 11							84			30			69						183
afwijkingen chromosoom 13		20					32			31		25	129						237
opsporen trisomie 12		19					85			11		20	75						210
aneuploidie TCC (vanaf 23/09/03)			4																4
LOH 1p/19q (vanaf 23/09/03)																			0
EGFR gen amplificatie (vanaf 23/09/03)			7																7
LOH 17p, 13q, 9p, 8p, 3p en p53 (vanaf 23/09/03)																			0
Genetische mutaties, amplificaties en overexpressie (diagnostiek)																			
p53																			0
cycline D1		98					123		20			15	25						281
myc																			0
Neu/Her2	34	119	237		112	30	214		209		114	280	138	351			90		1928
Ki-ras							1			201									202
kappa en lambda keten immunoglobulinen																	60		60
Hormonen: insuline, glucagon, somatostatine, gastrine calcitonine, thyroglobuline en PTH gerelateerd peptide					77			18											95
Andere testen (diagnostiek)																			
apoptose in situ																			0
micrometastasen in kankers van de pancreas						135													135
chimerisme voor allogene transplantaties	29		42		273		4			57									405
HUMARA		11																	11
Subtotaal GEN Diagnostiek	1409	1196	838	112	1552	165	2067	18	1595	4817	990	1918	1257	678	0	80	196	0	18888

AANTAL TESTS

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Totaal (1 februari 2003 - 31 jan 2004)
Genherschikkingen (opvolging)																			
Herschikking immunoglobulinegenen	381	258					9		25	230	43	358	102	25			3		1434
Herschikking β en γ genen T receptor van lymfocyten	52	80					21		20	151	46	65	25						460
Chromosomale translocaties en inversies (opvolging)																			0
t (1;14) SIL-TAL			13						7	1		7							28
t (1;19) E2A-PBX	4		8						4			7							23
t (2;5) NPM-ALK									1										1
t (4;11) MLL-AF4	14		8						1	1	3								27
t (8;14) JH-MYC										151									151
t (8;21) ETO-AML1	7	28	9				5		29	35	15	10							138
t (9;11) MLL-AF9			8						2			7							17
t (9;22) BCR-ABL	69	165	43				89		256	267	55	86	45	6			4		1085
t (10;11) MLL-AF10			8						2			7							17
t (11;14) JH-BCL1	28									151	14	95	20						308
t (12;21) TEL-AML1	6		24						10		3	7							50
t (14;18) JH-BCL2	44	48					24		20	151	15	105	25						432
t (15;17) PML-RAR	9	41	11				16		32	42	18	8							177
inv, 16 MYH11-CBCF	13	37	8				5		13	24	13	9							122
afwijking 11q23			8				4					7							19
afwijkingen chromosoom 11										26									26
afwijkingen chromosoom 13										27		5							32
opsporen trisomie 12										8		4							12
aneuploidie TCC (vanaf 23/09/03)																			0
LOH 1p/19q (vanaf 23/09/03)																			0
EGFR gen amplificatie (vanaf 23/09/03)																			0
LOH 17p, 13q, 9p, 8p, 3p en p53 (vanaf 23/09/03)																			0
Genetische mutaties, amplificaties en overexpressie (opvolging)																			
p53																			0
cycline D1							10					10							20
myc																			0
Neu/Her2												7					6		13
Ki-ras																			0
kappa en lambda keten immunoglobulinen																			0
Hormonen: insuline, glucagon, somatostatine, gastrine, calcitonine, thyroglobuline en PTH gerelateerd peptide								18											18
Andere testen (opvolging)																			
apoptose in situ																			0
micrometastasen in kankers van de pancreas			68																68
chimerisme voor allogene transplantaties	153	164					14												331
HUMARA																			0
Subtotaal GEN opvolging	780	821	216	0	0	0	197	18	422	1265	225	804	217	31	0	0	13	0	5009
Subtotaal GEN (diagnostiek + opvolging)	2189	2017	1054	112	1552	165	2264	36	2017	6082	1215	2722	1474	709	0	80	209	0	23897

AANTAL TESTS

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Totaal (1 februari 2003 - 31 jan 2004)
TOTAAL (periode 1 februari 2003 - 31 januari 2004)	7062	5172	5619	7015	8378	7341	8219	4459	8808	19373	5022	5218	5533	4004	1792	8222	3317	1682	116236

AANTAL POSITIEVEN

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Totaal (1 februari 2003 - 31 jan 2004)
Micro-organismen																			
Bartonella henselae en Bartonella quintana	10		1	1	12		4									0			28
Bordetella pertussis	2		55				3				9					6			75
Borrelia burgdorferi	0			1	5		2									4			12
Chlamydia pneumoniae	0	0	3				0			0	0	5		0		7	3	0	18
Corynebacterium diphtheriae			0													0			0
Verocytotoxine-producerende E.coli			48																48
Detectie vanA, vanB, vanC bij enterokokken		3	12		12	24			1		7							2	61
Detectie van macroliden resistentie bij Helicobacter pylori																		67	67
Legionella pneumophila		1	12			4	0			9	13	12				10	3	2	66
Mycoplasma pneumoniae	0	0	3				0			5	3	5	3			4	4	1	28
Mycobacterium	62	31		68	104		35	121	ident		6	55			132	31	54	33	732
Detectie van rifampicine resistentie bij Mycobacterium tuberculosis	0	0						4				0					5	0	9
Detectie van mecA bij stafylokokken	208	26	11		8	398	3		15			231	9			35	46	29	1019
Detectie en/of identificatie moeilijk identificeerbare en/of kweekbare bacteriën	nvt	11	27	2	361	127	nvt		ident			37	24			92	nvt		681
Typing nosocomiale pathogenen	nvt	297	NVT		100	NVT			typering		NVT		29				nvt	95	521
CMV kwalitatief			27	257	9	149	49	96	30		300	98	8	253		152		114	1542
CMV kwantitatief	414	126	27						150	643			83	219			93	83	1838
EBV kwalitatief		2	1	18	21	91	2		60		4			97		16	4		316
EBV kwantitatief					179	91			122	384				79					855
HBV kwalitatief					20						11					207	47		285
HBV kwantitatief	12	26		275	266	308		250	263	410									1810
HCV kwalitatief	2	215		594	401		582	330	16	495		112		42	270	211	56	33	3359
HCV kwantitatief	97	109		nvt	528		nvt	272	348	450	85	125		54	171	103		41	2383
HCV genotyping	76	113		nvt			nvt	229	typering	371	72	85			127	112		34	1219
HPV	757	344	720	411	473	1715	1339	243	684	611	473	483	1875		135	598	520		11381
Enterovirus	16		46	10	7	57	1	91	9	69	13	34		0		16	4		373
Herpes simplex	12		9	15	7	45	2		2	134	19	13		3		79	17	4	361
HHV8 (vanaf 23/09/03)			0			3													3
Parvovirus B19					18	4								1		3			26
Polyomavirussen JCV en BKV			28							598				307			2		935
Rubella virus						0													0
VZV	15		1		0	12	1			119	30			1		23	5	2	209
Toxoplasma gondii	6		4	3		13				19	2			0		5			52
Aspergillus				31												19			50
Candida																114			114
Pneumocystis carinii	8	3				6				59				1					77
Identificatie gekweekte fungi	nvt								ident				63						63
Subtotaal micro-organismen	1697	1307	1035	1686	2531	3047	2023	1636	1700	4376	1047	1295	2094	1057	835	1847	863	540	30616

AANTAL POSITIEVEN

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Totaal (1 februari 2003 - 31 jan 2004)
Genherschikkingen (diagnostiek)																			
Herschikking immunoglobulinegenen	263	141		37	61		371		102	368	117	146	166	248		34	11		2065
Herschikking β en γ genen T receptor van lymfocyten	29	11		4	24		31		51	78	60	35	57	47		2			429
Chromosomale translocaties en inversies (diagnostiek)																			
t (1;14) SIL-TAL	0	0	1		0				4		0	0							5
t (1;19) E2A-PBX	0	0	0		0		0		1	0	0	0							1
t (2;5) NPM-ALK	0	1					0		1	0			2						4
t (4;11) MLL-AF4	0	0	0		0				1	0	0								1
t (8;14) JH-MYC		0					6			3									9
t (8;21) ETO-AML1	1	0	0		3		4		3	2	0	2							15
t (9;11) MLL-AF9		0	0						0	0		0							0
t (9;22) BCR-ABL	16	6	7		91		31		24	18	24	49	6				3		275
t (10;11) MLL-AF10		0	0						2			0							2
t (11;14) JH-BCL1	6	3		3			17			13	9	26	13	1		0			91
t (12;21) TEL-AML1	1	0	3		4		0		2	3	1	2							16
t (14;18) JH-BCL2	34	34		4	2		33		15	43	27	43	14	5		1			255
t (15;17) PML-RAR	2	0	2		0		1		1	5	0	4							15
inv, 16 MYH11-CBCF	3	1	0		0		4		2	6	0	4							20
afwijking 11q23			1				3			0		4							8
afwijkingen chromosoom 11							3			9			3						15
afwijkingen chromosoom 13		6					7			15		5	69						102
opsporen trisomie 12		3					9			0		5	12						29
aneuploidie TCC (vanaf 23/09/03)			1																1
LOH 1p/19q (vanaf 23/09/03)																			0
EGFR gen amplificatie (vanaf 23/09/03)			0																0
LOH 17p, 13q, 9p, 3p en p53 (vanaf 23/09/03)																			0
Genetische mutaties, amplificaties en overexpressie (diagnostiek)																			
p53																			0
cycline D1		7					34		2			14	8						65
myc																			0
Neu/Her2	18	45	60		47	15	103		98		76	56	57	214			30		819
Ki-ras							0			4									4
kappa en lambda keten immunoglobulinen																	4		4
Hormonen: insuline, glucagon, somatostatine, gastrine calcitonine, thyroglobuline en PTH gerelateerd peptide					37			10											47
Andere testen (diagnostiek)																			
apoptose in situ																			0
micrometastasen in kankers van de pancreas						54													54
chimerisme voor allogene transplantaties	29		NVT		262		4												295
HUMARA		3																	3
Subtotaal GEN Diagnostiek	402	261	75	48	531	69	661	10	309	567	314	395	407	515	0	37	48	0	4649

AANTAL POSITIEVEN

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Totaal (1 februari 2003 - 31 jan 2004)
Generschikkingen (opvolging)																			
Herschikking immunoglobulinegenen	172	86					5		12	90	32	125	36	12			2		572
Herschikking β en γ genen T receptor van lymfocyten	17	20					5		9	17	24	22	13						127
Chromosomale translocaties en inversies (opvolging)																			
t (1;14) SIL-TAL			1						2	0		0							3
t (1;19) E2A-PBX	0		0						0			0							0
t (2;5) NPM-ALK									0										0
t (4;11) MLL-AF4	2		0						0	0	1								3
t (8;14) JH-MYC										0									0
t (8;21) ETO-AML1	0	18	0				0		5	12	1	2							38
t (9;11) MLL-AF9			0						0			0							0
t (9;22) BCR-ABL	45	99	18				61		212	96	26	53	26	3			3		642
t (10;11) MLL-AF10			0						0			0							0
t (11;14) JH-BCL1	0									9	1	17	4						31
t (12;21) TEL-AML1	1		1						0		1	0							3
t (14;18) JH-BCL2	7	24					4		8	9	9	18	10						89
t (15;17) PML-RAR	0	4	1				4		8	6	0	0							23
inv, 16 MYH11-CBCF	6	24	0				2		9	9	2	0							52
afwijking 11q23			0				0					0							0
afwijkingen chromosoom 11										11									11
afwijkingen chromosoom 13										12		2							14
opsporen trisomie 12										2		2							4
aneuploidie TCC (vanaf 23/09/03)																			0
LOH 1p/19q (vanaf 23/09/03)																			0
EGFR gen amplificatie (vanaf 23/09/03)																			0
LOH 17p, 13q, 9p, 3p en p53 (vanaf 23/09/03)																			0
Genetische mutaties, amplificaties en overexpressie (opvolging)																			
p53																			0
cycline D1							4					5							9
myc																			0
Neu/Her2												3					2		5
Ki-ras																			0
kappa en lambda keten immunoglobulinen																			0
Hormonen: insuline, glucagon, somatostatine, gastrine, calcitonine, thyroglobuline en PTH gerelateerd peptide								10											10
Andere testen (opvolging)																			
apoptose in situ																			0
micrometastasen in kankers van de pancreas																			0
chimerisme voor allogene transplantaties	153	36	NVT				14												203
HUMARA																			0
Subtotaal GEN opvolging	403	311	21	0	0	0	99	10	265	273	97	249	89	15	0	0	7	0	1839
Subtotaal GEN (diagnostiek + opvolging)	805	572	96	48	531	69	760	20	574	840	411	644	496	530	0	37	55	0	6488

AANTAL POSITIEVEN

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Totaal (1 februari 2003 - 31 jan 2004)
TOTAAL (periode 1 februari 2003 - 31 januari 2004)	2502	1879	1131	1734	3062	3116	2783	1656	2274	5216	1458	1939	2590	1587	835	1884	918	540	37104

RATIO POSITIEVE OP # TESTS

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Micro-organismen																		
Bartonella henselae en Bartonella quintana	29%		100%	5%	26%		36%							0%		0%		
Bordetella pertussis	40%		9%				9%				23%					13%		
Borrelia burgdorferi	0%			1%	1%		3%									6%		
Chlamydia pneumoniae	0%	0%	7%				0%			0%	0%	33%				2%	2%	0%
Corynebacterium diphtheriae																0%		
Verocytotoxine-producerende E.coli			25%															
Detectie vanA, vanB, vanC bij enterokokken		100%	60%		23%	56%			100%		29%							67%
Detectie van macroliden resistentie bij Helicobacter pylori																		71%
Legionella pneumophila		10%	7%			50%	0%			3%	21%	22%				5%	8%	6%
Mycoplasma pneumoniae	0%	0%	3%				0%			1%	1%	33%	12%			1%	3%	1%
Mycobacterium tuberculosis	9%	16%		23%	88%		8%	10%	nvt		18%	27%			22%	6%	23%	20%
Detectie van rifampicine resistentie bij Mycobacterium tuberculosis	0%	0%						6%				0%					83%	0%
Detectie van mecA bij stafylokokken	90%	10%	22%		44%	44%	100%		65%			79%	12%			47%	33%	54%
Detectie en/of identificatie moeilijk identificeerbare en/of kweekbare bacteriën	nvt	100%	59%	6%	100%	100%	nvt		nvt			100%	100%			12%		
Typering nosocomiale pathogenen	nvt	100%	NVT		24%	NVT			nvt		nvt		100%					100%
CMV kwalitatief			4%	10%	8%	20%	9%	22%	42%		28%	34%	27%	22%		10%		14%
CMV kwantitatief	25%	17%	100%						5%	32%			22%	100%			22%	99%
EBV kwalitatief		13%	9%	33%	47%	30%	14%		76%		36%			14%		61%	20%	
EBV kwantitatief					41%	100%			23%	51%				100%				
HBV kwalitatief					42%						50%					73%	45%	
HBV kwantitatief	55%	51%		71%	38%	67%		74%	58%	53%								
HCV kwalitatief	17%	51%		43%	48%		57%	61%	50%	32%		39%		72%	48%	42%	55%	43%
HCV kwantitatief	97%	100%			88%		nvt	93%	84%	100%	77%	74%		81%	92%	97%		95%
HCV genotypering	100%	99%					nvt	100%	nvt	99%	100%	75%			92%	97%		
HPV	65%	40%	39%	47%	27%	75%	50%	46%	59%	24%	48%	59%	54%		42%	43%	35%	
Enterovirus	8%		13%	9%	16%	13%	2%	12%	6%	17%	8%	36%		0%		14%	8%	
Herpes simplex	6%		7%	7%	4%	6%	4%		33%	18%	6%	17%		5%		18%	17%	18%
HHV8 (vanaf 23/09/03)			0%			20%												
Parvovirus B19					16%	3%								3%		17%		
Polyomavirussen JCV en BKV			70%							47%				42%			25%	
Rubella virus						0%												
VZV	8%		4%		0%	9%	2%			20%	19%			33%		31%	22%	9%
Toxoplasma gondii	18%		4%	2%		5%				4%	4%			0%		6%		
Aspergillus				9%												4%		
Candida																24%		
Pneumocystis carinii	8%	30%				32%				17%				1%				
Identificatie gekweekte fung	nvt								nvt				100%					
Subtotaal micro-organismen	35%	41%	23%	24%	37%	43%	34%	37%	25%	33%	28%	52%	52%	32%	47%	23%	28%	32%

RATIO POSITIEVE OP # TESTS

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Genherschikkingen (diagnostiek)																		
Herschikking immunoglobulinegenen	40%	45%		62%	22%		38%		43%	38%	51%	29%	52%	100%		63%	55%	
Herschikking β en γ genen T receptor van lymfocyten	26%	14%		14%	12%		19%		56%	14%	31%	23%	25%	100%		16%		
Chromosomale translocaties en inversies (diagnostiek)																		
t (1;14) SIL-TAL	0%	0%	2%		0%				4%		0%	0%						
t (1;19) E2A-PBX	0%	0%	0%		0%		0%		1%	0%	0%	0%						
t (2;5) NPM-ALK	0%	10%					0%		33%	0%			22%					
t (4;11) MLL-AF4	0%	0%	0%		0%				1%	0%	0%							
t (8;14) JH-MYC		0%					67%			1%								
t (8;21) ETO-AML1	2%	0%	0%		5%		50%		3%	1%	0%	5%						
t (9;11) MLL-AF9		0%	0%						0%	0%		0%						
t (9;22) BCR-ABL	13%	8%	10%		29%		15%		17%	4%	26%	35%	8%				12%	
t (10;11) MLL-AF10		0%	0%						2%			0%						
t (11;14) JH-BCL1	8%	25%		60%			40%			3%	10%	12%	13%	20%		0%		
t (12;21) TEL-AML1	7%	0%	6%		14%		0%		2%	10%	8%	5%						
t (14;18) JH-BCL2	25%	26%		22%	25%		30%		25%	8%	28%	18%	15%	19%		8%		
t (15;17) PML-RAR	4%	0%	4%		0%		17%		1%	1%	0%	10%						
inv, 16 MYH11-CBCF	7%	3%	0%		0%		50%		2%	2%	0%	11%						
afwijking 11q23			2%				33%			0%		11%						
afwijkingen chromosoom 11							4%			30%			4%					
afwijkingen chromosoom 13		30%					22%			48%		20%	53%					
opsoren trisomie 12		16%					11%			0%		25%	16%					
aneuploidie TCC (vanaf 23/09/03)			25%															
LOH 1p/19q (vanaf 23/09/03)																		
EGFR gen amplificatie (vanaf 23/09/03)			0%															
LOH 17p, 13q, 9p, 8p, 3p en p53 (vanaf 23/09/03)																		
Genetische mutaties, amplificaties en overexpressie (diagnostiek)																		
p53																		
cycline D1		7%					28%		10%			93%	32%					
myc																		
Neu/Her2	53%	38%	25%		42%	50%	48%		47%		67%	20%	41%	60%			33%	
Ki-ras							0%			2%								
kappa en lambda keten immunoglobulinen																	7%	
Hormonen: insuline, glucagon, somatostatine, gastrine, calcitonine, thyroglobuline en PTH gerelateerd peptide					48%			56%										
Andere testen (diagnostiek)																		
apoptose in situ																		
micrometastasen in kankers van de pancreas						40%												
chimerisme voor allogene transplantaties	100%		NVT		96%		100%											
HUMARA		27%																
Subtotaal GEN Diagnostiek	29%	22%	9%	44%	34%	42%	32%	56%	19%	12%	32%	21%	32%	76%	nvt	46%	25%	nvt

RATIO POSITIEVE OP # TESTS

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Genherschikkingen (opvolging)																		
Herschikking immunoglobulinegenen	45%	33%					56%		48%	39%	74%	35%	35%	48%			67%	
Herschikking β en γ genen T receptor van lymfocyten	33%	25%					24%		45%	11%	52%	34%	52%					
Chromosomale translocaties en inversies (opvolging)																		
t (1;14) SIL-TAL			8%						29%	0%		0%						
t (1;19) E2A-PBX	0%		0%						0%			0%						
t (2;5) NPM-ALK									0%									
t (4;11) MLL-AF4	14%		0%						0%	0%	33%							
t (8;14) JH-MYC										0%								
t (8;21) ETO-AML1	0%	64%	0%				0%		17%	34%	7%	20%						
t (9;11) MLL-AF9			0%						0%			0%						
t (9;22) BCR-ABL	65%	60%	42%				69%		83%	36%	47%	62%	58%	50%			75%	
t (10;11) MLL-AF10			0%						0%			0%						
t (11;14) JH-BCL1	0%									6%	7%	18%	20%					
t (12;21) TEL-AML1	17%		4%						0%		33%	0%						
t (14;18) JH-BCL2	16%	50%					17%		40%	6%	60%	17%	40%					
t (15;17) PML-RAR	0%	10%	9%				25%		25%	14%	0%	0%						
inv, 16 MYH11-CBCF	46%	65%	0%				40%		69%	38%	15%	0%						
afwijking 11q23			0%				0%					0%						
afwijkingen chromosoom 11										42%								
afwijkingen chromosoom 13										44%		40%						
opsporen trisomie 12										25%		50%						
aneuploidie TCC (vanaf 23/09/03)																		
LOH 1p/19q (vanaf 23/09/03)																		
EGFR gen amplificatie (vanaf 23/09/03)																		
LOH 17p, 13q, 9p, 8p, 3p en p53 (vanaf 23/09/03)																		
Genetische mutaties, amplificaties en overexpressie (opvolging)																		
p53																		
cycline D1							40%					50%						
myc																		
Neu/Her2												43%					33%	
Ki-ras																		
kappa en lambda keten immunoglobulinen																		
Hormonen: insuline, glucagon, somatostatine, gastrine, calcitonine, thyroglobuline en PTH gerelateerd peptide								56%										
Andere testen (opvolging)																		
apoptose in situ																		
micrometastasen in kankers van de pancreas																		
chimerisme voor allogene transplantaties	100%	22%	NVT				100%											
HUMARA																		
Subtotaal GEN opvolging	52%	38%	10%	nvt	nvt	nvt	50%	56%	63%	22%	43%	31%	41%	48%	nvt	nvt	54%	nvt
TOTAAL (periode 1 februari 2003- 31 januari 2004)	35%	36%	20%	25%	33%	42%	34%	37%	26%	27%	29%	37%	47%	40%	47%	23%	28%	32%

AANTAL PATIËNTEN

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Totaal (1 februari 2003 - 31 jan 2004)
Micro-organismen																			
Bartonella henselae en Bartonella quintana	28		1	21	45		10									7			112
Bordetella pertussis	5		564				30				38					44			681
Borrelia burgdorferi	35			84	355		55									66			595
Chlamydia pneumoniae	8	15	45				81			327	110	7		38		354	110	52	1147
Corynebacterium diphtheriae			1													5			6
Verocytotoxine-producerende E.coli			115																115
Detectie vanA, vanB, vanC bij enterokokken		3	14		51	30			1		12							3	114
Detectie van macroliden resistentie bij Helicobacter pylori																		88	88
Legionella pneumophila		10	111			8	8			263	38	36				210	32	33	749
Mycoplasma pneumoniae	16	17	111				65			350	343	7	24			379	113	64	1489
Mycobacterium tuberculosis	449	143		258	97		386	1043	42		24	150			268	407	174	102	3543
Detectie van rifampicine resistentie bij Mycobacterium tuberculosis	1	17						66				27					5	2	118
Detectie van mecA bij stafylokokken	215	257	35		12	482	3		20			200	38			55	121	52	1490
Detectie en/of identificatie moeilijk identificeerbare en/of kweekbare bacteriën	76	11	45	34	350	55	145		345			33	24			665	18		1801
Typing nosocomiale pathogenen	31	297	5		376	421			12		136		29				12	88	1407
CMV kwalitatief			221	1080	108	467	188	195	69		324	200	25	153		396		237	3663
CMV kwantitatief	261	203	19						330	2017			71	38			225	26	3190
EBV kwalitatief		14	11	51	41	170	11		79		11			92		24	18		522
EBV kwantitatief					121	47			354	752				16					1290
HBV kwalitatief					45						21					261	89		416
HBV kwantitatief	20	44		313	575	397		292	380	778									2799
HCV kwalitatief	12	353		1089	720		988	447	31	1551		190		56	474	422	91	62	6486
HCV kwantitatief	83	104		203	575		324	270	358	450	101	105		40	136	95		39	2883
HCV genotypering	76	104		204			320	229	321	374	67	73			113	103		34	2018
HPV	956	742	1662	622	540	2282	2487	530	1088	2566	984	818	3054		213	940	1436		20920
Enterovirus	180		336	104	43	418	34	715	145	395	148	74		1		111	47		2751
Herpes simplex	181		127	185	177	673	46		6	729	312	74		25		403	90	22	3050
HHV8 (vanaf 23/09/03)			1			11													12
Parvovirus B19					94	115								24		15			248
Polyomavirussen JCV en BKV			15							1261				138			8		1422
Rubella virus						12													12
VZV	169		24		73	116	42			598	149			2		72	20	22	1287
Toxoplasma gondii	28		113	105		186				524	52			23		88			1119
Aspergillus				151												129			280
Candida																94			94
Pneumocystis carinii	92	10				17				356				63					538
Identificatie gekweekte fungi	1								135				63				40		239
Subtotaal micro-organismen	2923	2344	3576	4504	4398	5907	5223	3787	3716	13291	2870	1994	3328	709	1204	5345	2649	926	68694

AANTAL PATIËNTEN

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Totaal (1 februari 2003 - 31 jan 2004)
Genherschikkingen (diagnostiek)																			
Herschikking immunoglobulinegenen	478	243		58	263		497		205	832	180	399	239	121		52	19		3586
Herschikking β en γ genen T receptor van lymfocyten	94	72		29	73		85		81	495	133	97	164	44		12			1379
Chromosomale translocaties en inversies (diagnostiek)																			
t (1;14) SIL-TAL	12	33	43		28				92		4	26							238
t (1;19) E2A-PBX	12	33	43		28		1		93	29	8	32							279
t (2;5) NPM-ALK	3	8					1		3	1			5						21
t (4;11) MLL-AF4	50	33	43		28				91	6	7								258
t (8;14) JH-MYC		6					9			387									402
t (8;21) ETO-AML1	41	33	43		58		8		91	153	27	30							484
t (9;11) MLL-AF9		33	43						91	2		25							194
t (9;22) BCR-ABL	113	74	65		315		191		138	236	58	87	63				25		1365
t (10;11) MLL-AF10		33	44						91			25							193
t (11;14) JH-BCL1	61	11		5			29			399	58	153	65	5		1			787
t (12;21) TEL-AML1	12	33	44		28		1		97	29	7	34							285
t (14;18) JH-BCL2	99	115		16	4		96		60	427	62	177	65	27		13			1161
t (15;17) PML-RAR	40	33	45		58		6		91	153	27	31							484
inv. 16 MYH11-CBCF	40	33	43		58		8		91	153	27	30							483
afwijking 11q23			43				9			92		30							174
afwijkingen chromosoom 11							81			30			69						180
afwijkingen chromosoom 13		20					32			31		20	66						169
opsporen trisomie 12		19					82			11		17	75						204
aneuploidie TCC (vanaf 23/09/03)			4																4
LOH 1p/19q (vanaf 23/09/03)																			0
EGFR gen amplificatie (vanaf 23/09/03)			7																7
LOH 17p, 13q, 9p, 8p, 3p en p53 (vanaf 23/09/03)																			0
Genetische mutaties, amplificaties en overexpressie (diagnostiek)																			
p53																			0
cycline D1		89					110		20			11	20						250
myc																			0
Neu/Her2	32	103	232		93	30	208		209		114	270	138	343			87		1859
Ki-ras							1			192									193
kappa en lambda keten immunoglobulinen																	30		30
Hormonen: insuline, glucagon, somatostatine, gastrine, calcitonine, thyroglobuline en PTH gerelateerd peptide					23			9											32
Andere testen (diagnostiek)																			
apoptose in situ																			0
micrometastasen in kankers van de pancreas						11													11
chimerisme voor allogene transplantaties	29		14		273		2			57									375
HUMARA		11																	11
Subtotaal GEN Diagnostiek	1116	1068	756	108	1330	41	1457	9	1544	3715	712	1494	969	540	0	78	161	0	15098

AANTAL PATIËNTEN

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Totaal (1 februari 2003 - 31 jan 2004)
Generschikkingen (opvolging)																			
Herschikking immunoglobulinegenen	180	145					4		18	210	27	228	57	4			3		876
Herschikking β en γ genen T receptor van lymfocyten	33	25					11		15	117	27	40	14						282
Chromosomale translocaties en inversies (opvolging)																			
t (1;14) SIL-TAL			12						3	1		5							21
t (1;19) E2A-PBX	1		7						2			5							15
t (2;5) NPM-ALK									1										1
t (4;11) MLL-AF4	11		7						1	1	1								21
t (8;14) JH-MYC										138									138
t (8;21) ETO-AML1	7	4	8				4		9	24	10	8							74
t (9;11) MLL-AF9			7						1			5							13
t (9;22) BCR-ABL	43	37	31				42		80	157	27	50	10	4			4		485
t (10;11) MLL-AF10			7						1			5							13
t (11;14) JH-BCL1	17									138	10	65	11						241
t (12;21) TEL-AML1	2		15						8		1	5							31
t (14;18) JH-BCL2	36	42					12		15	138	10	65	13						331
t (15;17) PML-RAR	7	14	9				4		9	34	11	6							94
inv, 16 MYH11-CBCF	10	6	7				3		6	17	9	7							65
afwijking 11q23			7				1					5							13
afwijkingen chromosoom 11										25									25
afwijkingen chromosoom 13										26		3							29
opsporen trisomie 12										8		3							11
aneuploidie TCC (vanaf 23/09/03)																			0
LOH 1p/19q (vanaf 23/09/03)																			0
EGFR gen amplificatie (vanaf 23/09/03)																			0
LOH 17p, 13q, 9p, 8p, 3p en p53 (vanaf 23/09/03)																			0
Genetische mutaties, amplificaties en overexpressie (opvolging)																			
p53																			0
cycline D1							7					7							14
myc																			0
Neu/Her2												7					6		13
Ki-ras																			0
kappa en lambda keten immunoglobulinen																			0
Hormonen: insuline, glucagon, somatostatine, gastrine, calcitonine, thyroglobuline en PTH gerelateerd peptide								9											9
Andere testen (opvolging)																			
apoptose in situ																			0
micrometastasen in kankers van de pancreas																			0
chimerisme voor allogene transplantaties	33	47	24				2												106
HUMARA																			0
Subtotaal GEN opvolging	380	320	141	0	0	0	90	9	169	1034	133	519	105	8	0	0	13	0	2921
Subtotaal GEN (diagnostiek + opvolging)	1496	1388	897	108	1330	41	1547	18	1713	4749	845	2013	1074	548	0	78	174	0	18019

AANTAL PATIËNTEN

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Totaal (1 februari 2003 - 31 jan 2004)
TOTAAL (periode 1 februari 2003 - 31 januari 2004)	4419	3732	4473	4612	5728	5948	6770	3805	5429	18040	3715	4007	4402	1257	1204	5423	2823	926	86713

TESTS PER PATIËNT

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Micro-organismen																		
Bartonella henselae en Bartonella quintana	1,21		1,00	1,00	1,04		1,10							1,18		1,00		
Bordetella pertussis	1,00		1,11				1,13				1,03					1,02		
Borrelia burgdorferi	1,00			1,05	1,06		1,11									1,03		
Chlamydia pneumoniae	1,00	1,00	1,00				1,11			1,00	1,13	2,14				1,10	1,17	1,29
Corynebacterium diphtheriae			1,00													1,00		
Verocytotoxine-producerende E.coli			1,65															
Detectie vanA, vanB, vanC bij enterokokken		1,00	1,43		1,02	1,43			1,00		2,00							1,00
Detectie van macroliden resistentie bij Helicobacter pylori																		1,07
Legionella pneumophila		1,00	1,45			1,00	1,25			1,00	1,61	1,53				1,01	1,22	1,00
Mycoplasma pneumoniae	1,06	1,00	1,00				1,11			1,00	1,20	2,14	1,04			1,08	1,18	1,37
Mycobacterium tuberculosis	1,49	1,38		1,16	1,22		1,17	1,19	1,17		1,42	1,37			2,26	1,28	1,36	1,61
Detectie van rifampicine resistentie bij Mycobacterium tuberculosis	1,00	1,00						1,00				1,00					1,20	1,00
Detectie van mecA bij stafylokokken	1,08	1,01	1,40		1,50	1,86	1,00		1,15			1,47	1,00			1,36	1,15	1,04
Detectie en/of identificatie moeilijk identificeerbare en/of kweekbare bacteriën	1,00	1,00	1,02	1,06	1,03	2,31			1,03			1,12	1,00			1,20	1,11	
Typering nosocomiale pathogenen	1,00	1,00	1,00		1,09	1,10			1,33		1,00		1,00				1,08	1,09
CMV kwalitatief			3,33	2,31	1,03	1,62	2,96	2,22	1,03		3,31	1,45	1,20	7,58		3,83		3,33
CMV kwantitatief	6,41	3,67	1,42						8,98	1,00			5,34	5,76			1,89	3,23
EBV kwalitatief		1,07	1,00	1,06	1,01	1,78	1,27		1,00		1,00			7,79		1,08	1,11	
EBV kwantitatief					3,58	1,94			1,51	1,00				4,94				
HBV kwalitatief					1,07						1,05					1,08	1,17	
HBV kwantitatief	1,10	1,16		1,23	1,22	1,17		1,15	1,19	1,00								
HCV kwalitatief	1,00	1,20		1,26	1,15		1,03	1,21	1,03	1,00		1,49		1,04	1,19	1,19	1,12	1,23
HCV kwantitatief	1,20	1,05		1,09	1,04		1,06	1,08	1,16	1,00	1,09	1,62		1,68	1,36	1,12		1,10
HCV genotypering	1,00	1,10		1,00			1,03	1,00	1,01	1,00	1,07	1,56			1,22	1,12		1,00
HPV	1,22	1,16	1,12	1,41	3,20	1,00	1,08	1,00	1,07	1,00	1,00	1,00	1,14		1,41	1,48	1,05	
Enterovirus	1,08		1,09	1,05	1,05	1,04	1,21	1,05	1,12	1,00	1,10	1,27		1,00		1,03	1,09	
Herpes simplex	1,07		1,05	1,19	1,04	1,12	1,09		1,00	1,00	1,05	1,03		2,56		1,11	1,12	1,22
HHV8 (vanaf 23/09/03)			1,00			1,36												
Parvovirus B19					1,21	1,13								1,46		1,20		
Polyomavirussen JCV en BKV			2,67							1,00				5,32			1,00	
Rubella virus						1,17												
VZV	1,11		1,04		1,03	1,13	1,14			1,00	1,05			1,50		1,03	1,15	1,22
Toxoplasma gondii	1,21		1,00	1,79		1,29				1,00	1,10			1,35		1,01		
Aspergillus				2,23												3,53		
Candida																5,00		
Pneumocystis carinii	1,12	1,00				1,12				1,00				1,32				
Identificatie gekweekte fungi	1,00								1,04				1,00				1,30	
Subtotaal micro-organismen	1,67	1,35	1,28	1,53	1,55	1,21	1,14	1,17	1,83	1,00	1,33	1,25	1,22	4,65	1,49	1,52	1,17	1,82

TESTS PER PATIËNT

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Genherschikkingen (diagnostiek)																		
Herschikking immunoglobulinegenen	1,38	1,28		1,03	1,06		1,95		1,17	1,18	1,28	1,24	1,35	2,05		1,00	1,05	
Herschikking β en γ genen T receptor van lymfocyten	1,20	1,13		1,00	2,78		1,88		1,12	1,09	1,45	1,55	1,40	1,07		1,00		
Chromosomale translocaties en inversies (diagnostiek)																		
t (1;14) SIL-TAL	1,17	1,00	1,09		1,00				1,01		2,00	1,27						
t (1;19) E2A-PBX	1,17	1,00	1,09		1,00		1,00		1,02	1,07	2,00	1,25						
t (2;5) NPM-ALK	1,00	1,25					1,00		1,00	1,00			1,80					
t (4;11) MLL-AF4	1,12	1,00	1,09		1,00				1,00	1,83	1,71							
t (8;14) JH-MYC		1,17					1,00			1,20								
t (8;21) ETO-AML1	1,10	1,00	1,09		1,00		1,00		1,00	1,06	1,59	1,27						
t (9;11) MLL-AF9		1,00	1,09						1,00	2,00		1,32						
t (9;22) BCR-ABL	1,08	1,05	1,11		1,00		1,06		1,02	2,12	1,57	1,60	1,13				1,04	
t (10;11) MLL-AF10		1,00	1,09						1,00			1,32						
t (11;14) JH-BCL1	1,28	1,09		1,00			1,45			1,20	1,53	1,44	1,52	1,00		1,00		
t (12;21) TEL-AML1	1,17	1,00	1,09		1,00		1,00		1,00	1,07	1,86	1,24						
t (14;18) JH-BCL2	1,39	1,16		1,13	2,00		1,15		1,02	1,21	1,53	1,35	1,40	1,00		1,00		
t (15;17) PML-RAR	1,13	1,00	1,13		1,00		1,00		1,00	2,49	1,59	1,26						
inv, 16 MYH11-CBCF	1,13	1,00	1,09		1,00		1,00		1,00	1,86	1,59	1,27						
afwijking 11q23			1,09				1,00			1,10		1,27						
afwijkingen chromosoom 11							1,04			1,00			1,00					
afwijkingen chromosoom 13		1,00						1,00		1,00		1,25	1,95					
opsoren trisomie 12		1,00						1,04		1,00		1,18	1,00					
aneuploidie TCC (vanaf 23/09/03)			1,00															
LOH 1p/19q (vanaf 23/09/03)																		
EGFR gen amplificatie (vanaf 23/09/03)			1,00															
LOH 17p, 13q, 9p, 8p, 3p en p53 (vanaf 23/09/03)																		
Genetische mutaties, amplificaties en overexpressie (diagnostiek)																		
p53																		
cycline D1		1,10					1,12		1,00			1,36	1,25					
myc																		
Neu/Her2	1,06	1,16	1,02		1,20	1,00	1,03		1,00		1,00	1,04	1,00	1,02			1,03	
Ki-ras							1,00			1,05								
kappa en lambda keten immunoglobulinen																	2,00	
Hormonen: insuline, glucagon, somatostatine, gastrine, calcitonine, thyroglobuline en PTH gerelateerd peptide					3,35			2,00										
Andere testen (diagnostiek)																		
apoptose in situ																		
micrometastasen in kankers van de pancreas						12,27												
chimerisme voor allogene transplantaties	1,00		3,00		1,00		2,00			1,00								
HUMARA		1,00																
Subtotaal GEN Diagnostiek	1,26	1,12	1,11	1,04	1,17	4,02	1,42	2,00	1,03	1,30	1,39	1,28	1,30	1,26	0,00	1,00	1,22	0,00

TESTS PER PATIËNT

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Genherschikkingen (opvolging)																		
Herschikking immunoglobulinegenen	2,12	1,78					2,25		1,39	1,10	1,59	1,57	1,79	6,25			1,00	
Herschikking β en γ genen T receptor van lymfocyten	1,58	3,20					1,91		1,33	1,29	1,70	1,63	1,79					
Chromosomale translocaties en inversies (opvolging)																		
t (1;14) SIL-TAL			1,08						2,33	1,00		1,40						
t (1;19) E2A-PBX	4,00		1,14						2,00			1,40						
t (2;5) NPM-ALK									1,00									
t (4;11) MLL-AF4	1,27		1,14						1,00	1,00	3,00							
t (8;14) JH-MYC										1,09								
t (8;21) ETO-AML1	1,00	7,00	1,13				1,25		3,22	1,46	1,50	1,25						
t (9;11) MLL-AF9			1,14						2,00			1,40						
t (9;22) BCR-ABL	1,60	4,46	1,39				2,12		3,20	1,70	2,04	1,72	4,50	1,50			1,00	
t (10;11) MLL-AF10			1,14						2,00			1,40						
t (11;14) JH-BCL1	1,65									1,09	1,40	1,46	1,82					
t (12;21) TEL-AML1	3,00		1,60						1,25		3,00	1,40						
t (14;18) JH-BCL2	1,22	1,14					2,00		1,33	1,09	1,50	1,62	1,92					
t (15;17) PML-RAR	1,29	2,93	1,22				4,00		3,56	1,24	1,64	1,33						
inv. 16 MYH11-CBCF	1,30	6,17	1,14				1,67		2,17	1,41	1,44	1,29						
afwijking 11q23			1,14				4,00					1,40						
afwijkingen chromosoom 11										1,04								
afwijkingen chromosoom 13										1,04		1,67						
opsporen trisomie 12										1,00		1,33						
aneuploidie TCC (vanaf 23/09/03)																		
LOH 1p/19q (vanaf 23/09/03)																		
EGFR gen amplificatie (vanaf 23/09/03)																		
LOH 17p, 13q, 9p, 8p, 3p en p53 (vanaf 23/09/03)																		
Genetische mutaties, amplificaties en overexpressie (opvolging)																		
p53																		
cycline D1							1,43					1,43						
myc																		
Neu/Her2												1,00					1,00	
Ki-ras																		
kappa en lambda keten immunoglobulinen																		
Hormonen: insuline, glucagon, somatostatine, gastrine, calcitonine, thyroglobuline en PTH gerelateerd peptide								2,00										
Andere testen (opvolging)																		
apoptose in situ																		
micrometastasen in kankers van de pancreas																		
chimerisme voor allogene transplantaties	4,64	3,49	2,83				7,00											
HUMARA																		
Subtotaal GEN opvolging	2,05	2,57	1,53	0,00	0,00	0,00	2,19	2,00	2,50	1,22	1,69	1,55	2,07	3,88	0,00	0,00	1,00	0,00
TOTAAL (periode 1 februari 2003- 31 januari 2004)	1,60	1,39	1,26	1,52	1,46	1,23	1,21	1,17	1,62	1,07	1,35	1,30	1,26	3,19	1,49	1,52	1,18	1,82

OVERZICHT PLAATS UITVOERING TESTS

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Micro-organismen																		
Bartonella henselae en Bartonella quintana	NG		MB	AP	MB		MB									AP		
Bordetella pertussis	NG		MB				MB				MB					MB		
Borrelia burgdorferi	NG			MB	MB		MB									MB	MB	MB
Chlamydia pneumoniae	NG	MB	MB				MB			NG	MB	NG		MB		MB	MB	MB
Corynebacterium diphtheriae			MB													MB		
Verocytotoxine-producerende E.coli			MB															
Detectie vanA, vanB, vanC bij enterokokken		MB	MB		MB	MB			MB		MB							MB
Detectie van macroliden resistentie bij Helicobacter pylori																		MB
Legionella pneumophila		MB	MB			MB	MB			NG	MB	NG				MB	MB	MB
Mycoplasma pneumoniae	NG	MB	MB				MB			NG	MB	NG	NG			MB	MB	MB
Mycobacterium	NG	MB		MB/AP	MB		MB	MB	MB		MB	NG			MB	MB/AP	MB	MB
Detectie van rifampicine resistentie bij Mycobacterium tuberculosis	NG	MB						MB				NG					MB	MB
Detectie van mecA bij stafylokokken	NG	MB	MB		MB	MB	MB		MB		MB	NG	NG			MB	MB	MB
Detectie en/of identificatie moeilijk identificeerbare en/o kweekbare bacteriën	NG	MB	MB	MB	MB	MB	MB		MB			NG	NG			MB	MB	
Typering nosocomiale pathogenen	NG	MB	MB		MB	MB			MB		MB		NG				MB	MB
CMV kwalitatief			MB	MB	MB	MB	MB	MB	AP		MB	NG	NG	MB		MB		MB
CMV kwantitatief	NG	MB	MB						MB	NG			NG	MB			MB	MB
EBV kwalitatief		AP/MB	AP	AP	AP	MB	MB		AP		MB/AP			MB/AP		AP	MB	
EBV kwantitatief					MB	MB			MB	NG				MB				
HBV kwalitatief					MB						MB			MB		MB	MB	
HBV kwantitatief	NG	MB		MB	MB	MB		MB	MB	NG				MB				
HCV kwalitatief	NG	MB		MB	MB		MB	MB	AP	NG		NG		MB	MB	MB	MB	MB
HCV kwantitatief	NG	MB		MB	MB		MB	MB	MB	NG	MB	NG		MB	MB	MB		MB
HCV genotypering	NG	MB		MB	MB		MB	MB	MB	NG	MB	NG			MB	MB		MB
HPV	NG	MB/AP	AP	AP	AP	AP	MB	MB	MB/AP	NG	AP	NG	NG		AP	AP	MB/AP	
Enterovirus	NG		MB	MB	MB	MB	MB	MB	MB	NG	MB	NG		MB		MB	MB	
Herpes simplex	NG		MB	MB	MB	MB	MB		AP	NG	MB	NG		MB		MB	MB	MB
HHV8			MB			MB												
Parvovirus B19					MB	MB								MB		MB		
Polyomavirussen JCV en BKV			MB							NG				MB			MB	
Rubella virus			MB			MB												
VZV	NG		MB		MB	MB	MB			NG	MB			MB		MB	MB	MB
Toxoplasma gondii	NG		MB	MB		MB				NG	MB			MB		MB		
Aspergillus				MB												MB		
Candida																MB		
Pneumocystis carinii	NG	MB				MB				NG				MB				
Identificatie gekweekte fungi	NG								MB								MB	

OVERZICHT PLAATS UITVOERING TESTS

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Genherschikkingen																		
Herschikking immunoglobulinegenen	NG	HO		AP	HO/AP		HO		HO	NG	HO	NG	NG	HO/AP		AP	HO	
Herschikking β en γ genen T receptor van lymfocyten	NG	HO		AP	HO/AP		HO		HO	NG	HO	NG	NG	AP		AP		
Chromosomale translocaties en inversies																		
t (1;14) SIL-TAL	NG	HO	HO		HO				HO	NG	HO	NG						
t (1;19) E2A-PBX	NG	HO	HO		HO		HO		HO	NG	HO	NG						
t (2;5) NPM-ALK	NG	HO					HO		HO	NG			NG					
t (4;11) MLL-AF4	NG	HO	HO		HO				HO	NG	HO							
t (8;14) JH-MYC		HO/AP					HO			NG								
t (8;21) ETO-AML1	NG	HO	HO		HO		HO		HO	NG	HO	NG						
t (9;11) MLL-AF9		HO	HO						HO	NG		NG						
t (9;22) BCR-ABL	NG	HO	HO		HO		HO		HO	NG	HO	NG	NG	HO			HO	
t (10;11) MLL-AF10		HO	HO						HO			NG						
t (11;14) JH-BCL1	NG	HO/AP		AP			HO			NG	HO	NG	NG	AP		AP		
t (12;21) TEL-AML1	NG	HO	HO		HO		HO		HO	NG	HO	NG						
t (14;18) JH-BCL2	NG	HO/AP		AP	AP		HO		HO	NG	HO	NG	NG	AP		AP		
t (15;17) PML-RAR	NG	HO	HO		HO		HO		HO	NG	HO	NG						
inv, 16 MYH11-CBCF	NG	HO	HO		HO		HO		HO	NG	HO	NG						
afwijking 11q23			HO				HO			NG		NG						
afwijkingen chromosoom 11							HO			NG			NG					
afwijkingen chromosoom 13		HO					HO			NG		NG	NG					
opsporen trisomie 12		HO					HO			NG		NG	NG					
aneuploidie TCC			AP															
LOH 1p/19q																		
EGFR gen amplificatie			AP															
LOH 17p, 13q, 9p, 8p, 3p en p53																		
Genetische mutaties, amplificaties en overexpressie																		
p53																		
cycline D1		HO					HO		HO			NG	NG					
myc																		
Neu/Her2	NG	AP	AP		AP	AP	AP		AP		AP	NG	NG	AP			AP	
Ki-ras							HO			NG								
kappa en lambda keten immunoglobulinen																	AP	
Hormonen: insuline, glucagon, somatostatine, gastrine, calcitonine, thyroglobuline en PTH gerelateerd peptide					AP			HO										
Andere testen																		
apoptose in situ																		
micrometastasen in kankers van de pancreas						AP												
chimerisme voor allogene transplantaties	NG	HO	HO		HO		HO			NG								
HUMARA		HO																
Microbiologie (MB), hemato-oncologie (HO), anatomo-pathologie (AP) of niet gespecificeerd (NG)																		

Appendix 2: List of molecular diagnostic kits

Nr	WG	Test	Kit	Manufacturer	EU /FDA	Description
1	MB	Bartonella henselae, Bartonella quintana	OLIGODETECT® BARTONELLA	CHEMICON (www.chemicon.com)	CE /ASR	Qualitative detection of Bartonella spp. DNA generated by an in-house validated in vitro nucleic acid amplification of a portion of the 16S ribosomal RNA(rRNA) gene of B. quintana, B. henselae, B. clarridgea, B. elizabethae.
2	MB	Bordetella pertussis	OLIGODETECT® BORDETELLA PERTUSSIS	CHEMICON (www.chemicon.com)	CE /ASR	Qualitative detection of Bordetella pertussis DNA generated by an in-house validated in vitro nucleic acid amplification of the IS481.
3	MB	Bordetella pertussis	Bordetella Pertussis Real Time Kit	PRODESSE (www.prodesse.com)	CE /ASR	Detects Bordetella pertussis
4	MB	Bordetella pertussis	ProDect Bordetella Pertussis	bcs Biotech S.p.A. (www.biocs.it)		PCR qualitative, region: toxin-operon
5	MB	Bordetella pertussis	B. pertussis ASR	Cepheid (www.cepheid.com)	RUO /ASR	Real-time PCR primers and FAM-labeled probe to detect 103bp region of IS481 gene plus Texas Red-labeled probe and DNA for an internal control sequence.
6	MB	Borrelia burgdorferi	Borrelia burgdorferi	CLONIT (www.clonit.it)	CE /NA	PCR qualitative, sequence of flagellin gene.
7	MB	Borrelia burgdorferi	attomol® Borrelia burgdorferi-DNA-LINA	Attomol GmbH (www.attomol.de)		Reverse hybridization strip for detection of Borrelia burgdorferi
8	MB	Borrelia burgdorferi	ProDect Borrelia burgdorferi	bcs Biotech S.p.A. (www.biocs.it)		PCR qualitative, region: flagellin gene
9	MB	Chlamydia pneumoniae	ProbeTec ET Chlamydiaceae Family (CF) Amplified DNA Assay	BD Diagnostics (www.bd.com)	CE /NA	SDA technology for direct qualitative detection of DNA from organisms belonging to the Chlamydiaceae Family (incl but not limited to C. pneumoniae, C. trachomatis and C. psittaci), from lower respiratory specimens and throat swabs from patients in a clinical suspicion of pneumonia.
10	MB	Chlamydia pneumoniae	OLIGODETECT® CHLAMYDIA PNEUMONIAE	CHEMICON (www.chemicon.com)	CE /ASR	Qualitative detection of Chlamydia pneumoniae DNA generated by an in-house validated in vitro nucleic acid amplification of the KDTA gene.
11	MB	Chlamydia pneumoniae	Chlamydia pneumoniae ("nested")	CLONIT (www.clonit.it)	CE /NA	PCR qualitative, detection of RNA beta polymerase gene.
12	MB	Chlamydia pneumoniae	ProDect Chlamydia pneumoniae	bcs Biotech S.p.A. (www.biocs.it)		PCR qualitative, detection region: HR1/HM1.
13	MB	Corynebacterium diphtheriae				
14	MB	Escherichia coli (Verotoxin-producing)	GenoType® EHEC	HAIN-LIFESCIENCE (www.hain-lifescience.com)		Molecular genetic assay for the identification of the Shiga Toxin genes stx1 and stx2, the eae gene, and the ipaH gene
15	MB	Escherichia coli (Verotoxin-producing)	E. coli 0157:H7 (EHEC/EPEC)	CLONIT (www.clonit.it)	CE /NA	PCR qualitative, amplification A/E gene
16	MB	Enterococci (resistance genes)	LightCycler VRE Detection Kit	Roche Diagnostics (www.roche-diagnostics.com)	RUO /NA	Real-time PCR, detection of the vanA and vanB genes from bacterial culture or colonies, for research applications
17	MB	Helicobacter pylori (macrolide resistance)				
18	MB	Legionella pneumophila	BD ProbeTec™ Legionella pneumophila (LP) Amplified DNA Assay	BD Diagnostics (www.bd.com)	CE /IVD	Strand Displacement Amplification (SDA), designed for the detection of L. pneumophila (serogroups 1-14) in sputum specimens from patients with clinical suspicion of pneumonia.
19	MB	Legionella pneumophila	OLIGODETECT® LEGIONELLA PNEUMOPHILA	CHEMICON (www.chemicon.com)	NA /ASR	Qualitative detection of Legionella pneumophila DNA generated by an in-house validated in vitro nucleic acid amplification of the 16S rRNA gene.
20	MB	Legionella pneumophila	Onar® Lp (QP)	MINERVA-BIOLABS (www.minerva-biolabs.com)	CE /NA	Qualitative and quantitative diagnosis of Legionella pneumophila serogroups 1-14, as well as 16 other human pathogenic Legionella species. Onar® Lp is available as a conventional PCR assay with result evaluation by agarose gel, as well as a quantitative PCR probe system for use on all real-time instruments (Onar® Lp-QP)
21	MB	Legionella pneumophila	Legionella pneumophila ("nested")	CLONIT (www.clonit.it)	CE /NA	PCR qualitative, sequence within macrophage infectivity potentiator (mip) gene.

Nr	WG	Test	Kit	Manufacturer	EU /FDA	Description
22	MB	Legionella pneumophila, Legionella micdadei, Mycoplasma pneumoniae, Chlamydomphila pneumoniae	Pneumoplex Real Time Kit	PRODESSE (www.prodesse.com)	CE /ASR	The Real Time product detects four targets, and reports in three channels – one channel is Mycoplasma pneumoniae, one channel is Chlamydomphila pneumoniae, one channel is Legionella (both pneumophila and micdadei) – with the fourth channel used for an internal control.
23	MB	Legionella pneumophila and micdadei, Mycoplasma pneumoniae, Chlamydomphila pneumoniae, Bordetella Pertussis	Pneumoplex®	PRODESSE (www.prodesse.com)	CE /ASR	Pneumoplex® simultaneously detects the most common bacterial causes of atypical pneumonia (Legionella pneumophila, Legionella micdadei, Mycoplasma pneumoniae and Chlamydomphila pneumoniae) while also detecting pertussis. The Standard Platform product detects all five targets.
24	MB	Legionella, Mycoplasma pneumoniae, Chlamydiae pneumoniae	CHLAMYLEGE	ARGENE-BIOSOFT (www.argene.com)	CE05 /NA	PCR qualitative, screening and identification of Legionella, Mycoplasma pneumoniae, Chlamydiae pneumoniae
25	MB	Legionella, Mycoplasma pneumoniae, Chlamydiae pneumoniae	ProDect BCS RB2 Chip	bcs Biotech S.p.A. (www.biocs.it)		PCR qualitative, detection of Legionella, Mycoplasma pneumoniae, Chlamydiae pneumoniae on chip
26	MB	Mycoplasma pneumoniae	ProbeTec ET Mycoplasma pneumoniae (MP) Amplified DNA Assay	BD Diagnostics (www.bd.com)	CE /NA	Strand Displacement Amplification (SDA), designed for the detection of Mycoplasma pneumoniae DNA in throat swabs from patients with clinical suspicion of pneumonia.
27	MB	Mycoplasma pneumoniae	OLIGODETECT® MYCOPLASMA PNEUMONIAE	CHEMICON (www.chemicon.com)	CE /ASR	Qualitative detection of Mycoplasma pneumoniae DNA generated by an in-house validated in vitro nucleic acid amplification of the ATPase operon gene.
28	MB	Mycoplasma pneumoniae	Venor®Mp (QP)	MINERVA-BIOLABS (www.minerva-biolabs.com)	CE /NA	Qualitative and quantitative diagnosis of <i>Mycoplasma pneumoniae</i> in clinical samples. Venor®Mp is available as a conventional PCR assay with result evaluation by agarose gel, as well as a quantitative PCR probe system for use on all real-time instruments (Venor®Mp-QP).
29	MB	Mycoplasma pneumoniae	Mycoplasma pneumoniae ("nested")	CLONIT (www.clonit.it)	CE /NA	PCR qualitative, sequence of the D02 gene.
30	MB	Mycoplasma pneumoniae	ProDect Mycoplasma pneumoniae	bcs Biotech S.p.A. (www.biocs.it)		PCR qualitative, sequence of the P1 gene.
31	MB	Mycobacterium tuberculosis (culture)	INNO-LiPA MYCOBACTERIA v2	INNOGENETICS (www.innogenetics.com)	CE /RUO	Line Probe Assay for the detection and identification of clinically relevant Mycobacterium species from liquid and solid culture, based on the nucleotide differences in the 16S-23S rRNA spacer region.
32	MB	Mycobacterium tuberculosis (culture)	Atypical Mycobacteria (22 Types)	Symbiosis (www.symbiosis.it)		Reverse hybridisation kit for detection of mycobacteria and differentiation.
33	MB	Mycobacterium tuberculosis (culture)	GenoType® Mycobacterium CM	HAIN-LIFESCIENCE (www.hain-lifescience.com)		Identification of the clinical most relevant mycobacteria species from cultured material
34	MB	Mycobacterium tuberculosis (culture)	GenoType® Mycobacterium AS	HAIN-LIFESCIENCE (www.hain-lifescience.com)		Identification of further clinical relevant mycobacteria species from cultured material

Nr	WG	Test	Kit	Manufacturer	EU /FDA	Description
35	MB	Mycobacterium tuberculosis (culture)	ACCUPROBE Mycobacterium (avium /IC/ avium complex/ gordonae/ kansasii / MTBC)	BIOMérieux / GEN-PROBE (www.biomerieux.com)	CE /IVD	Rapid DNA probe tests for culture identification.
36	MB	Mycobacterium tuberculosis (culture)	BD ProbeTec ET Mycobacterium tuberculosis Complex (ctb) Culture Identification	BD Diagnostics (www.bd.com)	CE /IVD	Simultaneous amplification and real-time detection for M. tuberculosis complex, M. avium complex and M. kansasii from positive culture media
37	MB	Mycobacterium tuberculosis (direct)	BD ProbeTec ET Mycobacterium tuberculosis Complex (DTB) Direct Detection	BD Diagnostics (www.bd.com)	CE /NA	Uses SDA for direct qualitative detection of M. tuberculosis complex DNA from decontaminated, digested clinical respiratory samples.
38	MB	Mycobacterium tuberculosis (direct)	(COBAS) AMPLICOR MTB	Roche Diagnostics (www.roche-diagnostics.com)	CE /IVD	Detects the presence of M. tuberculosis in liquefied, decontaminated and concentrated human respiratory specimens.
39	MB	Mycobacterium tuberculosis (direct)	RealArt M. tuberculosis (LC/RG/TM) PCR kit	ARTUS (www.artus-biotech2.com)	CE /NA	Real-time PCR (FAM fluorescence), for use with the ABI PRISM 7000, 7700, 7900HT sequence detection systems (Applied Biosystems) for detection of all members of M. tuberculosis complex.
40	MB	Mycobacterium tuberculosis (direct)	M. tuberculosis PCR kit	ABBOTT/ARTUS (www.artus-biotech2.com)	CE /NA	Real-time PCR (FAM fluorescence), for use with the ABI PRISM 7000, 7700, 7900HT sequence detection systems (Applied Biosystems) for detection of all members of M. tuberculosis complex.
41	MB	Mycobacterium tuberculosis (direct)	AMPLIFIED MTB	BIOMérieux / GEN-PROBE (www.biomerieux.com)	CE /IVD	Target-amplified nucleic acid probe test for detection of Mycobacterium tuberculosis complex rRNA in sediments prepared from sputum (induced or expectorated), bronchial specimens (e.g., bronchoalveolar lavages or bronchial aspirates) or tracheal aspirates.
42	MB	Mycobacterium tuberculosis (direct)	GenoType® Mycobacteria Direct	HAIN-LIFESCIENCE (www.hain-lifescience.com)		Molecular genetic assay for identification of the M. tuberculosis complex and four clinical relevant mycobacteria species from patient specimens
43	MB	Mycobacterium tuberculosis (direct)	M. tuberculosis IS6110 region	CLONIT (www.clonit.it)	RUO /NA	PCR qualitative, amplifies sequence of the IS6110 region
44	MB	Mycobacterium tuberculosis (direct)	ProDect Mycobacterium tuberculosis	bcs Biotech S.p.A. (www.biocs.it)		PCR qualitative, amplifies sequence of the IS6110 region
45	MB	Mycobacterium tuberculosis (direct)	ProDect Mycobacterium HSP65	bcs Biotech S.p.A. (www.biocs.it)		PCR qualitative, detection M. tuberculosis, avium and fortuitum, amplifies sequence of the HSP65 gene
46	MB	Mycobact. tubercul. (resistance genes)	INNO-LiPA Rif. TB	INNOGENETICS (www.innogenetics.com)	CE /RUO	Line Probe Assay for the detection of Mycobacterium tuberculosis complex and its resistance to rifampicin
47	MB	Staphylococci (resistance genes)	GenoType® MRSA	HAIN-LIFESCIENCE (www.hain-lifescience.com)		Molecular genetic assay for fast identification of methicillin-resistant staphylococci
48	MB	Staphylococci (resistance genes)	IDI-MRSA	Cepheid (www.cephid.com)	CE /NA	Rapid, highly sensitive test that specifically detects methicillin-resistant Staphylococcus aureus directly from a single nasal swab specimen — in less than two hours; designed specifically for use on the Cepheid SmartCycler® System.
49	MB	Staphylococci (resistance genes)	LightCycler MRSA Detection Kit	Roche Diagnostics (www.roche-diagnostics.com)	RUO /NA	Real-time PCR, rapid detection of the mecA gene from biological specimens, including cultured bacteria or isolated colonies in research applications
50	MB	Staphylococci (resistance genes)	hyplex StaphyloResist®	Biologische Analysensystem GmbH (www.baq-germany.com)	CE /NA	Multiplex PCR, results after 4.5hrs.
51	MB	Staphylococci (resistance genes)	MRSA EVIGENE	AdvanDx (www.advandx.com)	RUO /NA	Qualitative nucleic acid probe-based colorimetric test, detects mecA and nuc genes found in MRSA (from cultures).
52	MB	Identification of bacteria difficult to identify				

Nr	WG	Test	Kit	Manufacturer	EU /FDA	Description
53	MB	Molecular typing of nosocomial pathogens	GenePath™ Strain Typing System	BIO-RAD (www.bio-rad.com)	CE /NA	The GenePath™ Strain Typing System includes the GenePath power module with a menu of 22 pre-optimized PFGE programs, an electrophoresis cell, cooling module, variable speed pump, Tygon™ tubing, casting stand and frame (14 cm wide by 13 cm long), 10-well comb and comb holder, 50-well disposable plug mold, leveling bubble, cables, tubing connectors, 0.5A FB fuses and instruction manual.
54	MB	Cytomegalovirus (CMV) qualitative	Amplicor CMV test	Roche Diagnostics (www.roche-diagnostics.com)	CE /NA	Detect the presence of human CMV DNA in clinical specimens, in particular from solid organ transplant recipients. Diagnosis of disseminated infection and active visceral disease usually involves detection of CMV in peripheral blood leukocytes.
55	MB	Cytomegalovirus (CMV) qualitative	OLIGODETECT® CMV	CHEMICON (www.chemicon.com)	CE /ASR	The Light Diagnostics CMV OligoDetect® Assay is applicable for the qualitative detection of human cytomegalovirus (CMV) DNA generated by an in-house validated in vitro nucleic acid amplification of a portion of the major immediate early (MIE) antigen gene.
56	MB	Cytomegalovirus (CMV) qualitative	CMV pp67 mRNA	BIOMérieux (www.biomerieux.com)	CE /IVD	NASBA based amplification of isolated CMV pp67 mRNA (indicating viral replication) in anticoagulated human whole blood of immunosuppressed individuals.
57	MB	Cytomegalovirus (CMV) qualitative	HC1 CMV DNA test	DIGENE (www.digene.com)	CE /IVD	Hybrid capture, qualitative detection of human CMV in peripheral white blood cells isolated from whole blood.
58	MB	Cytomegalovirus (CMV) qualitative	CMV ("nested")	CLONIT (www.clonit.it)	RUO /NA	Nested PCR, qualitative or semi-quantitative detection of "immediate early region" sequence.
59	MB	Cytomegalovirus (CMV) qualitative	ProDect Cytomegalovirus	bcs Biotech S.p.A. (www.biocs.it)		PCR qualitative, region: major intermediate early antigen
60	MB	Cytomegalovirus (CMV) qualitative	INFORM® CMV	VENTANA (www.ventanamed.com)	CE /NA	ISH for immediate early RNA of an active CMV infection in cytoplasmic and nuclear inclusions
61	MB	Cytomegalovirus (CMV) quantitative	Cobas Amplicor CMV Monitor	Roche Diagnostics (www.roche-diagnostics.com)	CE /NA	Quantitates the amount of CMV DNA in human plasma
62	MB	Cytomegalovirus (CMV) quantitative	affigene CMV VL	Sangtec Molecular Diagnostics AB (www.sangtec.se)	CE /NA	PCR quantitative
63	MB	Cytomegalovirus (CMV) quantitative	affigene CMV trender	Sangtec Molecular Diagnostics AB (www.sangtec.se)	CE05 /NA	Real-time PCR quantitative
64	MB	Epstein-Barr virus (EBV) qualitative	OLIGODETECT® EBV	CHEMICON (www.chemicon.com)	CE /ASR	Qualitative detection of Epstein-Barr Virus (EBV) DNA generated by in-house validated in vitro nucleic acid amplification of the epstein-barr nuclear antigen gene.
65	MB	Epstein-Barr virus (EBV) qualitative	EBV ("nested")	CLONIT (www.clonit.it)	CE /NA	Nested PCR, qualitative or semi-quantitative detection of Bam-HIIV region sequence.
66	MB	Epstein-Barr virus (EBV) qualitative	ProDect Epstein Barr	bcs Biotech S.p.A. (www.biocs.it)		PCR qualitative, region: capsid protein gp220
67	MB	Epstein-Barr virus (EBV) qualitative	RealArt™ EBV (LC/RG/TM) PCR Kit	ARTUS (www.artus-biotech2.com)	CE /ASR	Qualitative detection (PCR) of the DNA sequence of the Epstein-Barr Virus genome.
68	MB	Epstein-Barr virus (EBV) qualitative	EBV PCR kit	ABBOTT/ARTUS (www.artus-biotech2.com)	CE /NA	Real-time PCR (FAM fluorescence), for use with the ABI PRISM 7000, 7700, 7900HT sequence detection systems (Applied Biosystems).
69	MB	Epstein-Barr virus (EBV) quantitative	affigene EBV VL	Sangtec Molecular Diagnostics AB (www.sangtec.se)	CE /NA	PCR quantitative
70	MB	Epstein-Barr virus (EBV) quantitative	affigene EBV trender	Sangtec Molecular Diagnostics AB (www.sangtec.se)	CE05 /NA	Real-time PCR quantitative
71	MB	Hepatitis B virus DNA qualitative	HBV-HBsAg gene	CLONIT (www.clonit.it)	RUO /NA	PCR, qualitative or semi-quantitative detection of the HBV HBsAg gene.
72	MB	Hepatitis B virus DNA qualitative	HBV-HBcAg gene	CLONIT (www.clonit.it)	RUO /NA	PCR, qualitative detection of the HBV HBcAg gene.

Nr	WG	Test	Kit	Manufacturer	EU /FDA	Description
73	MB	Hepatitis B virus DNA qualitative	ProDect Hepatitis B virus	bcs Biotech S.p.A. (www.biocs.it)		PCR, qualitative detection of the HBV HBcAg gene.
74	MB	Hepatitis B virus DNA quantitative dosage	COBAS Amplicor HBV Monitor	Roche Diagnostics (www.roche-diagnostics.com)	CE /NA	Quantitation of HBV DNA in human serum or plasma
75	MB	Hepatitis B virus DNA quantitative dosage	Cobas Amplicor HBV Monitor	Roche Diagnostics (www.roche-diagnostics.com)	CE /NA	Quantitation of HBV DNA in human serum or plasma
76	MB	Hepatitis B virus DNA quantitative dosage	Cobas TaqMan HBV Monitor	Roche Diagnostics (www.roche-diagnostics.com)		Quantitation of HBV DNA in human serum or plasma
77	MB	Hepatitis B virus DNA quantitative dosage	VERSANT HBV DNA Quantitative assay (bDNA)	BAYER DIAGNOSTICS (www.bayerdiag.com)		bDNA quantitation of all six different HBV genotypes. Can be performed on a manual system and the System 340
78	MB	Hepatitis B virus DNA quantitative dosage	affigene HBV VL	Sangtec Molecular Diagnostics AB (www.sangtec.se)	CE /NA	PCR quantitative
79	MB	Hepatitis B virus DNA quantitative dosage	affigene HBV trender	Sangtec Molecular Diagnostics AB (www.sangtec.se)	CE05 /NA	Real-time PCR quantitative
80	MB	Hepatitis B virus DNA quantitative dosage	HBV PCR kit	ABBOTT/ARTUS (www.artus-biotech2.com)	CE /NA	Real-time PCR (FAM fluorescence), for use with the ABI PRISM 7000, 7700, 7900HT sequence detection systems (Applied Biosystems).
81	MB	Hepatitis B virus DNA quantitative	RealArt™ HBV (LC/RG/TM) PCR Kit	ARTUS (www.artus-biotech2.com)	CE /ASR	Real-time PCR, quantitative detection (PCR) of the HBV DNA.
82	MB	Hepatitis B virus DNA resistance	INNO-LiPA HBV DR	INNOGENETICS (www.innogenetics.com)	CE /RUO	Line probe assay test for the simultaneous detection of hepatitis B virus wild-type and mutations or polymorphisms at codon 180,204 and 207. Also changes at codon position 171,172,195,198 and 199 of the HbsAg can be identified due to the overlapping reading frame.
83		Hepatitis B virus DNA resistance	INNO-LiPA HBV DR v2	INNOGENETICS (www.innogenetics.com)	RUO05 /NA	INNO-LiPA HBV DR v2is an in vitro, reverse hybridization line probe assay used on human serum or plasma. Using INNO-LiPA HBV DR v2, wild type and mutations or polymorphisms at codons 80, 173, 180, 181, 204 and 236 of the HBV polymerase gene can be detected simultaneously.
84	MB	Hepatitis B virus DNA resistance	affigene HBV DE/3TC	Sangtec Molecular Diagnostics AB (www.sangtec.se)	CE /NA	PCR and mini-sequencing, detection of lamivudine resistance
85	MB	Hepatitis B virus DNA resistance	TRUGENE® HBV Genotyping Kit	BAYER DIAGNOSTICS (www.bayerdiag.com)		Amplifies the viral genome directly from serum samples and sequences the region coding for the viral RT gene and the central portion of HBsAG
86	MB	Hepatitis B virus DNA resistance	ProDect HBV Lamivudina R	bcs Biotech S.p.A. (www.biocs.it)		PCR detection POL gene codon 550 mutations
87	MB	Hepatitis C virus (HCV) qualitative	Amplicor HCV Test v 2.0	Roche Diagnostics (www.roche-diagnostics.com)	CE /IVD	PCR detection of HCV-RNA in clinical specimens.
88	MB	Hepatitis C virus (HCV) qualitative	VERSANT® HCV RNA Qualitative Assay	BAYER DIAGNOSTICS / GEN-PROBE (www.bayerdiag.com)	CE /IVD	Transcription-Mediated Amplification (TMA) technology. Maximum sensitivity needed to detect minute amounts of virus. This HCV RNA qualitative assay is utilized to detect HCV in infected patients before, during and after antiviral therapy.
89	MB	Hepatitis C virus (HCV) qualitative	HCV-RNA 5'UTR (RT+nested)	CLONIT (www.clonit.it)	RUO /NA	PCR, qualitative and semi-quantitative kit
90	MB	Hepatitis C virus (HCV) qualitative	ProDect HCV PLUS or NESTED	bcs Biotech S.p.A. (www.biocs.it)		PCR, qualitative
91	MB	Hepatitis C virus (HCV) qualitative	COBAS AMPLICOR® HCV Test, v2.0	Roche Diagnostics (www.roche-diagnostics.com)	CE /IVD	PCR, qualitative detection. Automated Sample preparation is possible with the Cobas Ampliprep, PCR and detection with the Cobas Amplicor.
92	MB	Hepatitis C virus (HCV) quantitative	(Cobas) Amplicor HCV Monitor 2.0	Roche Diagnostics (www.roche-diagnostics.com)	CE /NA	PCR, quantitation of Hepatitis C Virus RNA in human serum or plasma. The test is intended for use in conjunction with clinical presentation and other laboratory markers as an aid in assessing vital response to antiviral treatment as measured by changes in serum or plasma HCV RNA levels.
93	MB	Hepatitis C virus (HCV) quantitative	Cobas TaqMan HCV Monitor	Roche Diagnostics (www.roche-diagnostics.com)		Quantitates the amount of HCV RNA in human plasma

Nr	WG	Test	Kit	Manufacturer	EU /FDA	Description
94	MB	Hepatitis C virus (HCV) quantitative	VERSANT® HCV RNA 3.0 Assay (bDNA)	BAYER DIAGNOSTICS (www.bayerdiag.com)	CE /IVD	bDNA, signal amplification nucleic acid probe assay for the quantitation of human hepatitis C viral RNA (HCV RNA) in the serum or plasma (EDTA and ACD) of HCV-infected individuals using the Bayer System 340 bDNA Analyzer.
95	MB	Hepatitis C virus (HCV) quantitative	RealTime HCV Viral Load kit	ABBOTT LABORATORIES (www.abbottdiagnostics.com)	CE05 /NA	Real Time PCR Assay (FAM Fluorescence), for use with the IVD Abbott m2000rt RealTime PCR Thermalcycler and Detection system.
96	MB	Hepatitis C virus (HCV) genotyping	VERSANT® Genotype Assay (LiPA)	BAYER DIAGNOSTICS / INNOGENETICS (www.bayerdiag.com)	CE /NA	Starting from a biotinylated PCR amplicon it has been designed to detect the six most prevalent HCV genotypes and provides subtype information for the majority of samples.
97	MB	Hepatitis C virus (HCV) genotyping	TRUGENE® HCV 5'NC Genotyping Kit	BAYER DIAGNOSTICS (www.bayerdiag.com)		Determines HCV type and subtype based on nucleotide sequence analysis of the 5' NC region of the genome
98	MB	Hepatitis C virus (HCV) genotyping	HCV Genotyping ASR (NS5b)	ABBOTT LABORATORIES (www.abbottdiagnostics.com)	CE05 /ASR	Real time PCR assay for the identification of HCV Genotypes 1-6 on the ABI PRISM 7000, 7700 Sequence Detection Systems
99	MB	Hepatitis C virus (HCV) genotyping	ProDect HCV GENOTYPING	bcs Biotech S.p.A. (www.biocs.it)		Detection and typing of HCV genome, types and subtypes 1-6, region: CORE
100	MB	Human Papillomavirus (HPV)	Hybrid Capture® 2 HPV DNA Test	DIGENE (www.digene.com)	CE /IVD	Detection of HPV. The hc2 HPV Test uses two RNA probe cocktails to differentiate between carcinogenic and low-risk HPV types.
101	MB	Human Papillomavirus (HPV)	Amplicor HPV	Roche Diagnostics (www.roche-diagnostics.com)	CE /IVD	PCR-based reagent to amplify HPV DNA from 13 high risk genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68)
102	MB	Human Papillomavirus (HPV)	INFORM® HPV	VENTANA (www.ventanamed.com)	CE /NA	Sample types: tissue, monolayer-based preparations, and conventional Pap. Utilizes proprietary probe formulations. The Family 16 probe cocktail has an affinity to HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. The Family 6 Probe cocktail has an affinity to HPV genotypes 6, 11, 42, 43, and 44
103	MB	Human Papillomavirus (HPV)	HPV Consensus kit	ARGENE-BIOSOFT (www.argene.com)	RUO /NA	PCR, screening and identification of HPV
104	MB	Human Papillomavirus (HPV)	HPV total and serotypes	CLONIT (www.clonit.it)	CE /NA	PCR, kits for detection of HPV serotypes 6, 11, 16, 18, 33
105	MB	Human Papillomavirus (HPV)	ProDect HPV extraction, primers and separation	bcs Biotech S.p.A. (www.biocs.it)		Kits for extraction, PCR detection of L1 and E6/E7 regions, and agarose gel separation.
106	MB	Human Papillomavirus (HPV)	HPV screening and typing	Genome Identification Diagnostics GmbH (www.aid-diagnostika.com)		Reverse hybridisation kit for detection of HPV and differentiation into high and low risk genotypes
107	MB	Human Papillomavirus (HPV)	HPV screening and typing	Symbiosis (www.symbiosis.it)		Reverse hybridisation kit for detection of HPV and differentiation into high and low risk genotypes
108	MB	Human Papillomavirus (HPV)	PapVirID	GenoID (www.genoid.hu)	CE05 /NA	PCR based, identifies 51 genotypes
109	MB	Enterovirus	NucliSens EasyQ® Enterovirus	BIOMérieux (www.biomérieux.com)	CE /ASR	NASBA, after isolating the target RNA from the sample using the NucliSens magnetic extraction platforms, the NucliSens EasyQ® Enterovirus assay is run directly on the automated NucliSens EasyQ analyzer (NASBA)
110	MB	Enterovirus	OLIGODETECT® PAN-ENTEROVIRUS	CHEMICON (www.chemicon.com)	CE /ASR	Qualitative detection of enterovirus RNA generated by an in-house validated in vitro nucleic acid amplification of the 5' untranslated region (UTR).
111	MB	Enterovirus	ENTEROVIRUS CONSENSUS	ARGENE-BIOSOFT (www.argene.com)	CE05 /NA	RT-PCR qualitative, amplification and detection of all serotypes.
112	MB	Enterovirus	EnteroVision	BIO-RAD / DNA TECHNOLOGY (www.dna-technology.dk)	CE /NA	Detection of enteroviruses in cerebrospinal fluid, feces, throat swabs and plasma, based on enterovirus amplification using a one-tube Reverse Transcription Polymerase Chain Reaction (RT-PCR) followed by nucleic acid hybridization and detection by color formation in a microtitre well

Nr	WG	Test	Kit	Manufacturer	EU /FDA	Description
113	MB	Enterovirus	Enterovirus ASR	Cepheid (www.cephheid.com)	RUO /ASR	Real-time PCR primers and FAM and Alexa 532-labeled probe to detect a 115 bp region of the 5' UTR and a Texas-Red labeled probe for a separate sample processing control (SPC) sequence. <u>This SPC is a separate control for the extraction procedure.</u>
114	MB	Enterovirus	ProDect BCS EV Chip	bcs Biotech S.p.A. (www.biocs.it)		RT-PCR followed by reverse hybridization on chip. Detection of pan-enterovirus and Enterovirus 71 and Coxsackie A16.
115	MB	Herpes simplex virus	OLIGODETECT® HSV	CHEMICON (www.chemicon.com)	CE /ASR	Qualitative detection of herpes simplex virus (HSV) DNA generated by an in-house validated in vitro nucleic acid amplification of the pol gene. This assay is not intended for use in type specific determination of HSV infection.
116	MB	Herpes simplex virus	OLIGODETECT® HSV 1/2 TYPING	CHEMICON (www.chemicon.com)	CE /ASR	Qualitative detection of herpes simplex virus type 1 (HSV) and herpes simplex virus type 2 (HSV) DNA generated by an in-house validated in vitro nucleic acid amplification of the pol gene.
117	MB	Herpes simplex virus	RealArt™ HSV 1/2 LC PCR Kit	ARTUS (www.artus-biotech2.com)	CE /ASR	PCR detection of the DNA sequence of Herpes Simplex Virus 1 and Herpes Simplex Virus 2 genomes. Following the amplification in a real time cycler instrument, the closely related Herpes Simplex 1 and 2 viruses can be distinguished from one another by melting curve analysis.
118	MB	Herpes simplex virus	attomol® HSV1, 2-DNA-LINA	Attomol GmbH (www.attomol.de)		Reverse hybridization strip for detection of HSV 1, 2
119	MB	Herpes simplex virus	HSV 1	CLONIT (www.clonit.it)	CE /NA	PCR qualitative, gene coding for gD protein
120	MB	Herpes simplex virus	ProDect Herpes S.V.	bcs Biotech S.p.A. (www.biocs.it)		PCR qualitative, region: DNA polymerase
121	MB	Herpes simplex virus	HSV Non-Typing	Cepheid (www.cephheid.com)	RUO /ASR	Real-time PCR primers and FAM-labeled probe to detect 92bp region of polymerase gene.
122	MB	Herpes simplex virus	Herpes Mplex Kit	PRODESSE (www.prodesse.com)	CE /ASR	Multiplex PCR, detects Herpes Simplex Virus-1, Herpes Simplex Virus-2, Epstein-Barr Virus, Varicella Zoster Virus, HHV-6
123	MB	HSV 1/2, CMV, VZV, EBV, HHV-6	HERPES CONSENSUS GENERIC and HYBRIDOWELL HERPES Identification	ARGENE-BIOSOFT (www.argene.com)	CE05 /NA	PCR qualitative, generic probe, amplified product to hybridize in second stage with specific probes
124	MB	HSV 1/2	Herpes Simplex 1/2 Consensus	ARGENE-BIOSOFT (www.argene.com)	RUO /NA	PCR qualitative detection and identification
125	MB	Human herpesvirus type 8 (HHV8)	ProDect Herpes 8	bcs Biotech S.p.A. (www.biocs.it)		PCR qualitative, detection HHV-8-Kaposi Sarcoma Virus, region: capsid protein
126	MB	Parvovirus B19	OLIGODETECT® PARVO B19	CHEMICON (www.chemicon.com)	CE /ASR	Qualitative detection of parvovirus B19 (Parvo B19) DNA generated by an in-house validated in vitro nucleic acid amplification of the VP1 and VP2 gene.
127	MB	Parvovirus B19	RealArt™ Parvo B19 (LC/RG/TM) PCR Kit	ARTUS (www.artus-biotech2.com)	CE /ASR	Direct detection of the DNA sequence of the Parvo B19 virus genome
128	MB	Parvovirus B19	Parvo B19 PCR kit	ABBOTT/ARTUS (www.artus-biotech2.com)	CE /NA	Real-time PCR (FAM fluorescence), for use with the ABI PRISM 7000, 7700, 7900HT sequence detection systems (Applied Biosystems).
129	MB	Parvovirus B19	LightCycler Parvovirus B19 Quantification Kit	Roche Diagnostics (www.roche-diagnostics.com)	RUO /RUO	Real-time PCR, quantification of DNA encoding human Parvovirus B19 and simultaneous detection of a Parvovirus B19- specific internal control, by dual color detection.
130	MB	Parvovirus B19	Parvovirus B19	CLONIT (www.clonit.it)	CE /NA	PCR qualitative, region encoding structural proteins VP1
131	MB	Parvovirus B19	ProDect Parvovirus B19	bcs Biotech S.p.A. (www.biocs.it)		PCR qualitative, region encoding structural proteins VP1/VP2
132	MB	Polyomaviruses JC and BK	JC/BK CONSENSUS	ARGENE-BIOSOFT (www.argene.com)	CE05 /NA	PCR qualitative detection and identification in urine, serum, plasma, CSF
133	MB	Polyomaviruses JC and BK	ProDect JC/BK	bcs Biotech S.p.A. (www.biocs.it)		PCR qualitative, region: EARLY
134	MB	Rubella virus	ProDect Rubella	bcs Biotech S.p.A. (www.biocs.it)		PCR qualitative, region: E1

Nr	WG	Test	Kit	Manufacturer	EU /FDA	Description
135	MB	Varicella Zoster Virus (VZV)	OLIGODETECT® VZV	CHEMICON (www.chemicon.com)	CE /ASR	Qualitative detection of varicella-zoster virus (VZV) DNA generated by an in-house validated in vitro nucleic acid amplification of gene 29.
136	MB	Varicella Zoster Virus (VZV)	VZV PCR kit	ABBOTT/ARTUS (www.artus-biotech2.com)	CE /NA	Real-time PCR (FAM fluorescence), for use with the ABI PRISM 7000, 7700, 7900HT sequence detection systems (Applied Biosystems).
137	MB	Varicella Zoster Virus (VZV)	RealArt™ VZV (LC/TM) PCR Kit	ARTUS (www.artus-biotech2.com)	CE /ASR	CE marked for the use with the LightCycler®, ABI Prism® 7000 / 7700 / 7900HT. PCR-based, enable the direct detection of the DNA sequence of the Varicella-Zoster Virus genome.
138	MB	Varicella Zoster Virus (VZV)	VZV	CLONIT (www.clonit.it)	CE /NA	PCR qualitative, amplifies sequence of the C region
139	MB	Varicella Zoster Virus (VZV)	VZV	CLONIT (www.clonit.it)	RUO /NA	PCR qualitative, amplifies sequence of the POL region
140	MB	Varicella Zoster Virus (VZV)	attomol® VZV-DNA-LINA	Attomol GmbH (www.attomol.de)		Reverse hybridization strip for detection of VZV
141	MB	Toxoplasma gondii	Toxoplasma gondii	CLONIT (www.clonit.it)	RUO /NA	PCR qualitative, amplifies sequence of the B1 region
142	MB	Toxoplasma gondii	ProDect Toxo B1	bcs Biotech S.p.A. (www.biocs.it)		PCR qualitative, amplifies sequence of the B1 region
143	MB	Toxoplasma gondii	ProDect Toxo P30	bcs Biotech S.p.A. (www.biocs.it)		PCR qualitative, amplifies sequence of the surface antigen P30 gene
144	MB	Aspergillus				
145	MB	Candida	LightCycler Candida Kit MGRADE	Roche Diagnostics (www.roche-diagnostics.com)	RUO /RUO	Real-time on-line PCR, C. albicans identification from biological specimens.
146	MB	Identification fungi				
147	MB	Pneumocystis jiroveci (carinii)				
148	HO	Ig rearrangements	IGH Gene Clonality Assay (BIOMED-2)	Invivoscribe (www.invivoscribe.com)	RUO /RUO	PCR-based assay, identifies clonal rearrangements of the Immunoglobulin heavy chain locus testing genomic DNA extracted from a wide variety of sources. The sensitivity of this assay permits testing of very small and archival samples (e.g., paraffin-embedded formalin fixed tissue sections). Five master mixes target conserved regions within the variable (V), diversity (D), and the joining (J) regions that flank the unique hypervariable antigen-binding region 3 (CDR3).
149	HO	Ig rearrangements	IGK Gene Clonality Assay (BIOMED-2)	Invivoscribe (www.invivoscribe.com)	RUO /RUO	PCR-based assay, identifies clonal rearrangements of the Immunoglobulin kappa light chain locus testing genomic DNA extracted from a wide variety of sources.
150	HO	Ig rearrangements	IGL Gene Clonality Assay (BIOMED-2)	Invivoscribe (www.invivoscribe.com)	RUO /RUO	PCR-based assay, identifies clonal rearrangements of the Immunoglobulin lambda light chain locus testing genomic DNA extracted from a wide variety of sources.
151	HO	Ig rearrangements	ProDect B-limph extraction, primers and separation	bcs Biotech S.p.A. (www.biocs.it)		Kits for extraction and detection of B-cell lymphoma, region: FRIII, FRII and Ig heavy chain, separation on agarose gel
152	HO	Ig rearrangements	INFORM® Cytoplasmic Kappa Probe	VENTANA (www.ventanamed.com)	CE /NA	cocktail of oligonucleotide probes labeled with fluorescein. The intended target is the Kappa or Lambda light chain immunoglobulin messenger RNA (mRNA) in the cytoplasm of immunoblastic cells, plasma cells, and plasmacytoid cells.
153	HO	Ig rearrangements	INFORM® Cytoplasmic Lambda Probe	VENTANA (www.ventanamed.com)	CE /NA	cocktail of oligonucleotide probes labeled with fluorescein. The intended target is the Kappa or Lambda light chain immunoglobulin messenger RNA (mRNA) in the cytoplasm of immunoblastic cells, plasma cells, and plasmacytoid cells.
154	HO	t(2;5)				
155	HO	TCR rearrangements	TCRB Gene Clonality Assay (BIOMED-2)	Invivoscribe (www.invivoscribe.com)	RUO /RUO	PCR-based assay, identifies clonal rearrangements of the T cell receptor beta chain locus testing genomic DNA extracted from a wide variety of sources. The sensitivity of this assay permits testing of very small and archival samples (e.g., paraffin-embedded formalin fixed tissue sections).
156	HO	TCR rearrangements	TCRD Gene Clonality Assay (BIOMED-2)	Invivoscribe (www.invivoscribe.com)	RUO /RUO	PCR-based assay, identifies clonal rearrangements of the T cell receptor delta chain locus testing genomic DNA extracted from a wide variety of sources.

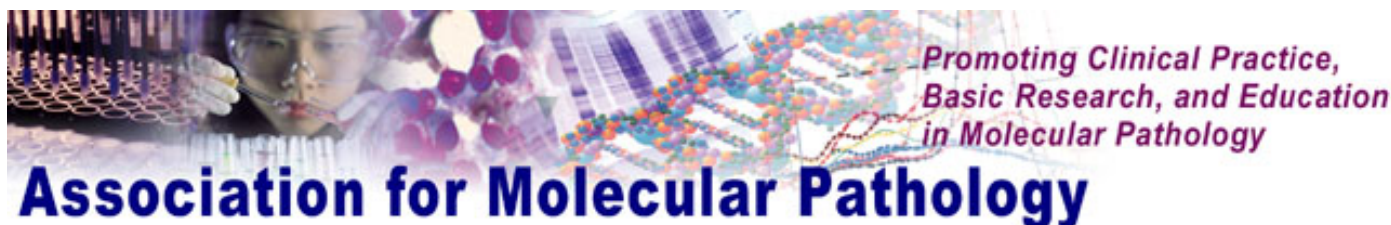
Nr	WG	Test	Kit	Manufacturer	EU /FDA	Description
157	HO	TCR rearrangements	TCRG Gene Clonality Assay (BIOMED-2)	Invivoscribe (www.invivoscribe.com)	RUO /RUO	PCR-based assay, identifies clonal rearrangements of the T cell receptor gamma chain locus testing genomic DNA extracted from a wide variety of sources.
158	HO	VH sequencing				
159	HO	Patient-specific PCR (no method question.)				
160	HO	Leukemia	HemaVision Screen	BIO-RAD / DNA TECHNOLOGY (www.dna-technology.dk)	CE /NA	Qualitative multiplex RT-PCR to screen for 28 different translocations or chromosomal rearrangements, including more than 80 breakpoints or mRNA splice variants, that have been found to be specific markers for particular subtypes of leukemia. Translocations detected by the HemaVision®-Screen Kit require further characterization by the Split-out reactions found in the HemaVision® Kit
161	HO	Leukemia	HemaVision (Typing/Subtyping)	BIO-RAD / DNA TECHNOLOGY (www.dna-technology.dk)	CE /NA	Qualitative multiplex RT-PCR to screen for 28 different translocations or chromosomal rearrangements, including more than 80 breakpoints or mRNA splice variants, that have been found to be specific markers for particular subtypes of leukemia.
162	HO	Leukemia	HemaVision-7	BIO-RAD / DNA TECHNOLOGY (www.dna-technology.dk)	CE /NA	Qualitative multiplex RT-PCR to detect seven of the most frequent translocations involved in and having a prognostic value in acute leukemias: t(1;19), t(12;21), inv(16), t(15;17), t(9;22), t(8;21), and t(4;11)
163	HO	t(1;14) SIL-TAL	SIL-TAL1 FISH DNA Probe, Sub-Deletion Signal	DAKOCYTOMATION (www.dakocytomation.com)		SIL-TAL1 FISH DNA Probe, Sub-Deletion Signal, is intended for the detection of deletion involving the SIL gene at chromosome 1p32 by fluorescence in situ hybridization (FISH).
164	HO	t(1;19) E2A-PBX	HemaVision® - 1;19	BIO-RAD / DNA TECHNOLOGY (www.dna-technology.dk)	CE /NA	RT-PCR test designed for the detection of translocation t(1;19) involved in and having a prognostic value in acute leukemias.
165	HO	t(1;19) E2A-PBX	TCF3 FISH DNA Probe, Split Signal	DAKOCYTOMATION (www.dakocytomation.com)		FISH, detects translocations involving the TCF3 (E2A) gene at chr 19p13.
166	HO	t(12;21) TEL-AML1	HemaVision® - 12;21	BIO-RAD / DNA TECHNOLOGY (www.dna-technology.dk)	CE /NA	RT-PCR test designed for the detection of translocation t(12;21) involved in and having a prognostic value in acute leukemias.
167	HO	t(12;21) TEL-AML2	ETV6 FISH DNA Probe, Split Signal	DAKOCYTOMATION (www.dakocytomation.com)		FISH, detects translocations involving the ETV6 (TEL) gene at chr 12p13.
168	HO	t(12;21) TEL-AML2	LSI TEL/AML1 ES Dual Color Translocation Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	Detect the TEL (ETV6)/AML1 gene fusion that occurs as a result of a translocation between chromosomes 12p13 and 21q22.
169	HO	MLL translocations in ALL t(4;11) MLL-AF4	HemaVision® - 4;11	BIO-RAD / DNA TECHNOLOGY (www.dna-technology.dk)	CE /NA	RT-PCR test designed for the detection of translocation t(4;11) involved in and having a prognostic value in acute leukemias.
170	HO	MLL translocations in ALL	MLL FISH DNA Probe, Split Signal	DAKOCYTOMATION (www.dakocytomation.com)		MLL FISH DNA Probe, Split Signal, is intended for the detection of translocations involving the MLL gene at chromosome 11q23 by fluorescence in situ hybridization (FISH).
171	HO	MLL translocations in ALL	LSI MLL Dual Color, Break Apart Rearrangement Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	Detects the 11q23 rearrangement associated with various translocations involving the MLL gene. Translocations disrupting the MLL (ALL-1, HRX) gene are among the most common cytogenetic abnormalities observed in hematopoietic malignancies. Although over 30 variant translocations have been seen involving MLL translocations, the most common abnormalities are t(4;11)(q21;q23), t(9;11)(p22;q23) and t(11;19)(q23;p13)
172	HO	Trisomy 8	CEP 8 DNA Probe kit	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	CE /IVD	CEP 8 is a SpectrumOrange labeled probe specific for the alpha satellite (centromeric) region, 8p11.1-q11.1. Trisomy 8 is related to CML, AML, MPD, MDS, and other hematological disorders.
173	HO	t(8;21) ETO-AML1	HemaVision® - 8;21	BIO-RAD / DNA TECHNOLOGY (www.dna-technology.dk)	CE /NA	RT-PCR test designed for the detection of translocation t(8;21) involved in and having a prognostic value in acute leukemias.
174	HO	t(8;21) ETO-AML2	LSI AML1/ETO Dual Color, Dual Fusion Translocation Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	Detect the juxtaposition of the AML1 gene locus on chromosome 21q22 with the ETO gene locus on chromosome 8q22.

Nr	WG	Test	Kit	Manufacturer	EU /FDA	Description
175	HO	t(15;17) PML-RARa	HemaVision® - 15;17	BIO-RAD / DNA TECHNOLOGY (www.dna-technology.dk)	CE /NA	RT-PCR test designed for the detection of translocation t(15;17) involved in and having a prognostic value in acute and chronic leukemias.
176	HO	t(15;17) PML-RARa	ProDect PML-RARA	bcs Biotech S.p.A. (www.biocs.it)		Kit for the detection of translocation t(15;17) PML-RARA.
177	HO	t(15;17) PML-RARa	FusionQuant® PML-RARA bcr1	Ipsogen (www.ipsogen.com)	CE /NA	Kit for quantitative detection of PML-RARA type bcr1 fusion transcripts using ABI Prism TaqMan®, LightCycler® or SmartCycler® instruments.
178	HO	t(15;17) PML-RARa	PML/RAR t(15;17) Translocation Assay	Invivoscribe (www.invivoscribe.com)	RUO /RUO	RT-PCR assay detects all of the major t(15;17) translocations associated with acute promyelocytic leukemia
179	HO	t(15;17) PML-RARa	LSI PML/RARA Dual Color Translocation Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	Detect the common t(15;17).The t(15;17) involving the PML (15q22) and RARA (17q12-q21) genes results in the formation of the PML/RARA gene fusion product.
180	HO	t(15;17) PML-RARa	LSI PML/RARA Dual Color, Dual Fusion Translocation Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	Detect the common t(15;17).The t(15;17) involving the PML (15q22) and RARA (17q12-q21) genes results in the formation of the PML/RARA gene fusion product.
181	HO	inv16 MYH11-CBF	HemaVision® - inv(16)	BIO-RAD / DNA TECHNOLOGY (www.dna-technology.dk)	CE /NA	RT-PCR test designed for the detection of translocation inv(16) involved in and having a prognostic value in acute and chronic leukemias.
182	HO	t(9;11) MLL-AF9				
183	HO	FLT3	FLT3 Mutation Assay	Invivoscribe (www.invivoscribe.com)	RUO /RUO	PCR, includes FLT3 ITD Master Mix and FLT3 D835 Master Mix
184	HO	WT1				
185	HO	MLL translocations in AML (see above)				
186	HO	t(11;14) JH-BCL1 (qualit)	BCL1/JH Translocation Assay (BIOMED-2)	Invivoscribe (www.invivoscribe.com)	RUO /RUO	Multiplex PCR assays, based on BIOMED-2 primers.
187	HO	t(11;14) JH-BCL1 (qualit)	LSI IGH/CCND1 Dual Color, Dual Fusion Translocation Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	Detects the juxtaposition of the immunoglobulin heavy chain (IGH) locus and the Cyclin D1 gene (CCND1).
188	HO	t(11;14) JH-BCL1 (qualit)	LSI IGH/CCND1 XT Dual Color, Dual Fusion Translocation Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	Detects the juxtaposition of the immunoglobulin heavy chain (IGH) locus and the Cyclin D1 gene (CCND1).
189	HO	t(14;18) JH-BCL2 (qualit)	ProDect BCL2	bcs Biotech S.p.A. (www.biocs.it)		PCR kit for detection of translocation t(14;18) BCL-2
190	HO	t(14;18) JH-BCL2 (qualit)	BCL2/JH Translocation Assay (BIOMED-2)	Invivoscribe (www.invivoscribe.com)	RUO /RUO	Multiplex PCR assays, one is based on BIOMED-2 primers.
191	HO	t(14;18) JH-BCL2 (qualit)	LSI IGH/BCL2 Dual Color, Dual Fusion Translocation Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	Detects the juxtaposition of immunoglobulin heavy chain (IGH) locus and BCL gene sequences. The translocation involving IGH at 14q32 and BCL2 at 18q21, t(14;18)(q32;q21) is common.
192	HO	t(14;18) JH-BCL2 (qualit)	LSI IGH/MALT1 Dual Color, Dual Fusion Translocation Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	This probe is provided for those researchers interested in identifying the IGH/MALT1 t(14;18)(q32;q21) translocation.
193	HO	t(11;14) JH-BCL1 (quantit)				
194	HO	t(14;18) JH-BCL2 (quantit)				
195	HO	t(8;14) JH-MYC (and variants)	LSI IGH/MYC, CEP 8 Tri-color, Dual Fusion Translocation Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	Detects the juxtaposition of immunoglobulin heavy chain (IGH) locus and MYC gene region sequences. The translocation t(8;14) (q24;q32) involving IGH at 14q32 and the MYC region at 8q24, is the most frequently observed MYC region translocation.

Nr	WG	Test	Kit	Manufacturer	EU /FDA	Description
196	HO	cyclin-D1 overexpression				
197	HO	trisomy 12	CEP 12 DNA Probe Kit	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	CE /IVD	Adjunct to standard karyotyping to identify and enumerate chromosome 12 in nuclei of cells obtained from peripheral blood lymphocytes in patients with B-cell chronic lymphocytic leukemia (B-CLL).
198	HO	t(9;22) BCR-ABL (qualit, diagn)	HemaVision® - 9;22	BIO-RAD / DNA TECHNOLOGY (www.dna-technology.dk)	CE /NA	RT-PCR test designed for the detection of translocation t(9;22) involved in and having a prognostic value in acute and chronic leukemias.
199	HO	t(9;22) BCR-ABL (qualit, diagn)	ProDect Philadelphia Chromosome	bcs Biotech S.p.A. (www.biocs.it)		RT-PCR Kit for detection of t(9;22) bcr/abl fusion transcript.
200	HO	t(9;22) BCR-ABL (qualit, diagn)	ProDect BCR-ABL	bcs Biotech S.p.A. (www.biocs.it)		RT-PCR Kit for detection of t(9;22) bcr/abl fusion transcripts p190 and p210.
201	HO	t(9;22) BCR-ABL (qualit, diagn)	m-bcr FusionQuant® Kit	Ipsogen (www.ipsogen.com)	CE /NA	Kit for quantitative detection of m-bcr fusion transcripts using either ABI Prism TaqMan®, LightCycler® or SmartCycler® instruments.
202	HO	t(9;22) BCR-ABL (qualit, diagn)	M-bcr FusionQuant® Kit	Ipsogen (www.ipsogen.com)	CE /NA	Kit for quantitative detection of M-bcr fusion transcripts using either ABI Prism TaqMan®, LightCycler® or SmartCycler® instruments.
203	HO	t(9;22) BCR-ABL (qualit, diagn)	BCR FISH DNA Probe, Split Signal	DAKOCYTOMATION (www.dakocytomation.com)		BCR FISH DNA Probe, Split Signal, is intended for the detection of translocations involving the BCR gene at chromosome 22q11 by fluorescence in situ hybridization (FISH).
204	HO	t(9;22) BCR-ABL (qualit, diagn)	LSI BCR/ABL Dual Color, Single Fusion Translocation Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	Detects the 5' BCR/3' ABL gene fusion.
205	HO	t(9;22) BCR-ABL (qualit, diagn)	BCR/ABL ES Dual Color Translocation Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	The BCR/ABL ES Dual Color Translocation Probe is a mixture of the LSI ABL probe labeled with SpectrumOrange™ and the LSI BCR probe labeled with SpectrumGreen(TM).
206	HO	t(9;22) BCR-ABL (qualit, diagn)	LSI BCR/ABL Dual Color, Dual Fusion Translocation Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	The LSI BCR/ABL Dual Color, Dual Fusion Translocation Probe is a mixture of the LSI BCR probe labeled with SpectrumGreen™ and the LSI ABL probe labeled with SpectrumOrange(TM).
207	HO	t(9;22) BCR-ABL (qualit, diagn)	BCR/ABL t(9;22) Translocation Assay	Invivoscribe (www.invivoscribe.com)	RUO /RUO	RT-PCR assay identifies all of most common BCR/ABL t(9;22) chromosomal translocations (p210 b3a3; p210 b2a2; p190 e1a2; p230 e19a2) that are a hallmark of chronic myeloid leukemia (CML) and are associated to a lesser extent with acute lymphoblastic leukemia (ALL) and other hematologic malignancies.
208	HO	t(9;22) BCR-ABL (quantit, follow-up)	affigene bcr-abl trender	Sangtec Molecular Diagnostics AB (www.sangtec.se)	CE05 /NA	Monitoring tumor load in CML
209	HO	t(9;22) BCR-ABL (quantit, follow-up)	LightCycler® t(9;22) Quantification Kit	Roche Diagnostics (www.roche-diagnostics.com)	RUO /NA	Real-time PCR, quantification of BCR-ABL mRNA, determines relative BCR-ABL expression levels by comparing them to the expression levels of the housekeeping gene G6PDH, the PCR can detect fusion transcripts resulting from the breakpoints b3a2, b2a2, b2a3, b3a3 and e1a2.
210	HO	HUMARA				
211	HO	PRV1				
212	HO	Chimerism	CEP X/Y DNA Probe Kit	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	CE /IVD	FISH, sex mismatched bone marrow transplantation
213	HO	t(6;9)				
214	HO	t(11;18)				
215	AP	HPV (see also microbiol)				

Nr	WG	Test	Kit	Manufacturer	EU /FDA	Description
216	AP	EBV (see also microbiol)	Epstein-Barr virus probe ISH kit	www.novocastra.co.uk		The Epstein-Barr virus (EBV) probe demonstrates cells latently infected with EBV. The probe hybridises to abundantly expressed Epstein-Barr virus-encoded RNA (EBER) transcripts which are concentrated in the nuclei of latently infected cells.
217	AP	EBV (see also microbiol)	Epstein-Barr virus (EBER) PNA Probe/Fluorescein	DAKOCYTOMATION (www.dakocytomation.com)		ISH detection kit for latent EBV infection on formalin-fixed, paraffin-embedded tissue sections.
218	AP	EBV (see also microbiol)	Epstein-Barr virus (Lytic) PNA Probe/Fluorescein	DAKOCYTOMATION (www.dakocytomation.com)		ISH detection kit for EBV infection on formalin-fixed, paraffin-embedded tissue sections.
219	AP	EBV (see also microbiol)	INFORM® EBER (Epstein-Barr Virus Early RNA)	VENTANA (www.ventanamed.com)	CE /NA	ISH, cocktail of oligonucleotide probes labeled with fluorescein in a formamide based diluent. Target is the early RNA transcript of Epstein-Barr Virus (EBV) infection.
220	AP	EBV (see also microbiol)	Epstein-Barr Virus Early RNA (EBER)	Biogenex (www.biogenex.com)		The EBER probe detects the Epstein-Barr Early RNA transcript characteristic of the latent phase of infection.
221	AP	B cell monoclon. in Lymphoma (see also hem)				
222	AP	T cell monoclon. in Lymphoma (see also hem)				
223	AP	t(14;18) in follic lymph (see also hem)				
224	AP	t(1;14) in mantle cell and anapl lymph				
225	AP	t(11;14) in mantle cell lymph (see also hem)				
226	AP	t(11;14) in mantle cell lymph (see also hem)				
227	AP	t(8;14), t(8;22), t(2;8) in Burkitt lymphoma	LSI MYC Dual Color, Break Apart Rearrangement Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	The LSI MYC Dual Color, Break Apart Rearrangement Probe is a mixture of two probes that hybridize to opposite sides of the region located 3' of MYC. This region is involved in the vast majority of breakpoints for t(8;22)(q24;q11) and t(2;8)(p11;q24).
228	AP	t(2;5) in anaplastic lymphoma (see also hem)	LSI ALK Dual Color, Break Apart Rearrangement Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	The LSI ALK (Anaplastic Lymphoma Kinase) Dual Color, Break Apart Rearrangement Probe is designed to detect the known 2p23 rearrange-ments that occur in t(2;5) and its variants.
229	AP	inv(2) in anaplastic lymphoma				
230	AP	t(11;18) in MALT lymphoma	LSI MALT1 Dual Color, Break Apart Rearrangement Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	In t(11;18) (q21;q21), a well-documented translocation involving the MALT1 gene, a gene fusion is produced on the der(11) chromosome between a 5' portion of the API2 (11q21) gene and a 3' portion of the MALT1 gene (18q21). Hybridization with the LSI MALT1 (18q21) Break Apart Rearrangement Probe will identify t(18q21) but not the specific translocation partner.
231	AP	t(11;18) in MALT lymphoma	LSI API2/MALT1 Dual Color, Dual Fusion Translocation Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	This probe is provided for those researchers interested in identifying the t(11;18)(q21;q21) translocation.

Nr	WG	Test	Kit	Manufacturer	EU /FDA	Description
232	AP	Neu/HER2	PathVysion Kit	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	CE /IVD	FISH. The PathVysion Kit is designed to detect amplification of the HER-2/neu gene via fluorescence in situ hybridization (FISH) in formalin-fixed, paraffin-embedded human breast cancer tissue specimens. Results from the PathVysion Kit are intended for use as an adjunct to existing clinical and pathologic information currently used as prognostic factors in stage II, node-positive breast cancer patients. The PathVysion Kit is further indicated as an aid to predict disease-free and overall survival in patients with stage II, node positive breast cancer treated with adjuvant cyclophosphamide, doxorubicin, and 5-fluorouracil (CAF) chemotherapy. The PathVysion Kit is indicated as an aid in the assessment of patients for whom HERCEPTIN® (Trastuzumab) treatment is considered.
233	AP	Neu/HER2	HER2 FISH pharmDx™	DAKOCYTOMATION (www.dakocytomation.com)		FISH, quantitatively determine HER2 gene amplification in formalin-fixed, paraffin-embedded breast cancer tissue specimens. Indicated as an aid in the assessment of patients for whom Herceptin treatment is considered.
234	AP	Neu/HER2	Ventana® Medical Systems' INFORM HER-2/neu Probe	VENTANA (www.ventanamed.com)	CE /NA	FISH, reagents to detect the HER-2/neu sequence in genomic DNA in formalin fixed, paraffin embedded tissue on the BenchMark® or BenchMark XT automated slide stainers.
235	AP	Neu/HER2	SPOT-Light® HER2 CISH™ Kit	ZYMED (www.zymed.com)	NA /ASR	Intended to detect HER2 gene amplification in formalin-fixed, paraffin-embedded (FFPE) tissue sections using Chromogenic In Situ Hybridization (CISH™).
236	AP	Neu/HER3	LightCycler HER2/neu DNA Quantification Kit	Roche Diagnostics (www.roche-diagnostics.com)	RUO /NA	Real-time PCR, simultaneous quantitative detection of DNA encoding human HER2/neu relative to a reference gene, by dual color detection.
237	AP	m-RNA neuroendocrine products - nesidioblastosis				
238	AP	m-RNA neuroendocrine products - graft				
239	AP	m-RNA receptors - nesidioblastosis				
240	AP	m-RNA somatostatin receptor				
241	AP	Aneuploidy TCC	UroVysion™ Kit	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	CE /IVD	FISH. The UroVysion™ Kit is designed to detect aneuploidy for chromosomes 3, 7, 17, and loss (deletion) of the 9p21 locus via fluorescence in situ hybridization (FISH) in urine specimens from subjects with transitional cell carcinoma of the bladder. Results from the UroVysion Kit are intended for use as a noninvasive method for monitoring for tumor recurrence in conjunction with cystoscopy in patients previously diagnosed with bladder cancer. New indication: aid in initial diagnosis of patients suspected of having TCC.
242	AP	LOH 1p-19q	LSI® 1p36 / LSI 1q25 and LSI 19q13/19p13 Dual-Color Probe Sets	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	Diffuse gliomas of the central nervous system are classified based on their histological appearance as astrocytomas, oligodendrogliomas and mixed oligoastrocytomas. Chromosomal deletions involving the 1p36 and 19q13 regions are characteristic molecular features of certain types of solid tumors.
243	AP	t(X;18) synoviosarcoma	LSI® SYT (18q11.2) Dual Color, Break Apart Rearrangement Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	Several studies have indicated that the t(X;18) (p11.2;q11.2) translocation arises exclusively in synovial sarcoma.
244	AP	t(11;22) EWS	LSI® EWSR1 (22q12) Dual Color, Break Apart Rearrangement Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	Identifying chromosomal rearrangements of the EWSR1 gene region on chromosome 22q12.
245	AP	t(X;13) alveol. Rhabdomyosarcoma (ARMS)	LSI FKHR (13q14) Dual Color Break Apart Rearrangement Probe	ABBOTT LABORATORIES / VYSIS (www.abbottdiagnostics.com)	RUO /ASR	The LSI FKHR Probe Set detects rearrangements of the FKHR gene located in the breakpoint region of 13q14. Translocations with the FKHR gene at 13q14 involved are associated with Alveolar Rhabdomyosarcoma (ARMS).



FDA-APPROVED MOLECULAR DIAGNOSTICS TESTS

The following table is a listing of the *in vitro* molecular diagnostics tests that are cleared for diagnostic use in the United States by the Food and Drug Administration. Such tests are classified as "biological devices" and they are listed by year of approval at: <http://www.fda.gov/cber/products.htm>.

This list is for informational purposes only and is not intended as an endorsement or recommendation in any way of the products and manufacturers listed, by the Association for Molecular Pathology or any of its officers or members. Companies are listed alphabetically within each category.

This table is current through November 2, 2004. Every effort will be made to keep the list error-free and current. Please email comments, corrections, additions, etc. to Carol Holland-Staley, Ph.D. (chollandstaley@beaumont.edu).

Abbreviations:

bdDNA: Branched Chain DNA Signal Amplification

DKA: Dual Kinetic Assay

ELISA: Enzyme-linked Immunosorbent Assay

FISH: Fluorescent *in situ* Hybridization

HPA: Hybridization Protection Assay

NASBA: Nucleic Acid Sequence Based Amplification

PCR: Polymerase Chain Reaction

QC: Quality control

RT-PCR: Reverse-Transcription PCR

SDA: Strand Displacement Amplification

TMA: Transcription Mediated Amplification

INFECTIOUS DISEASE TESTS - BACTERIAL

TEST	MANUFACTURER	TEST NAME	METHOD
<i>Chlamydia trachomatis</i> detection (single organism)	Digene Corporation Gaithersburg, MD	HC2 [®] CT ID	Hybrid Capture
	Gen-Probe, Inc. San Diego, CA	PACE [®] 2 CT	HPA
	Gen-Probe, Inc. San Diego, CA	Probe Competition Assay (Ct-confirmation test)	HPA
	Roche Molecular Diagnostics Pleasanton, CA	AMPLICOR [®] CT/NG Test for <i>Chlamydia trachomatis</i>	PCR
	Roche Molecular Diagnostics Pleasanton, CA	COBAS AMPLICOR [®] CT/NG Test for <i>Chlamydia trachomatis</i> ¹	PCR
<i>Neisseria gonorrhoeae</i> detection (single organism)	Digene Corporation Gaithersburg, MD	HC2 [®] GC ID	Hybrid Capture
	Gen-Probe, Inc. San Diego, CA	PACE [®] 2 GC	HPA
	Gen-Probe, Inc. San Diego, CA	Probe Competition Assay (GC-confirmation test)	HPA
	Roche Molecular Diagnostics Pleasanton, CA	AMPLICOR [®] CT/NG Test for <i>Neisseria gonorrhoeae</i>	PCR
	Roche Molecular Diagnostics Pleasanton, CA	COBAS AMPLICOR [®] CT/NG Test for <i>Neisseria gonorrhoeae</i> ¹	PCR
<i>Chlamydia trachomatis</i> and <i>Neisseria gonorrhoeae</i> detection	Becton Dickinson Microbiology Systems Franklin Lakes, NJ	BD ProbeTec [™] ET C. <i>trachomatis</i> and <i>N. gonorrhoeae</i> amplified DNA Assay	SDA
	Digene Corporation Gaithersburg, MD	HC2 [®] CT/GC Combo Test	Hybrid Capture
	Gen-Probe, Inc. San Diego, CA	PACE [®] 2C CT/GC	HPA

	Gen-Probe, Inc. San Diego, CA	APTIMA® Combo 2 Assay	Target Capture, TMA and DKA
	Roche Molecular Diagnostics Pleasanton, CA	AMPLICOR® CT/NG Test	PCR
	Roche Molecular Diagnostics Pleasanton, CA	COBAS AMPLICOR™ CT/NG Test ¹	PCR
<i>Gardnerella</i> , <i>Trichomonas vaginalis</i> and <i>Candida spp.</i> detection	Becton Dickinson Microbiology Systems Franklin Lakes, NJ	BD Affirm™ VPIII Microbial Identification Test	Hybridization
Group A <i>Streptococci</i> detection	Gen-Probe, Inc. San Diego, CA	Group A Strep direct (GASD)	HPA
Group B <i>Streptococci</i> detection	Gen-Probe, Inc. San Diego, CA	Group B AccuProbe®	HPA
	Infectio Diagnostics, Inc. Quebec, Canada (distributed by Cepheid US, Sunnyvale, CA)	IDI-Strep B™ Assay	Real Time PCR
MRSA for <i>Staphylococcus aureus</i>	Infectio Diagnostics, Inc. Quebec, Canada (distributed by Cepheid US, Sunnyvale, CA)	IDI-MRSA™ Assay	Real Time PCR
<i>Mycobacterium tuberculosis</i> detection	Gen-Probe, Inc. San Diego, CA	AMPLIFIED™ Mycobacterium tuberculosis Direct Test (MTD)	TMA
	Roche Molecular Diagnostics Pleasanton, CA	AMPLICOR™ Mycobacterium tuberculosis Test	PCR
<i>Mycobacteria spp.</i> , different fungi and bacteria culture confirmation ²	Gen-Probe, Inc. San Diego, CA	AccuProbe® Culture Identification Tests	HPA

¹*C. trachomatis* and *N. gonorrhoeae* detection may now be done using the Roche COBAS Amplicor system directly from Cytoc Corporation's ThinPrep Pap test collection kit; this use is FDA Approved.

²*Campylobacter spp.*, *Enterococcus spp.*, Group B *Streptococcus*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Listeria monocytogenes*, Group A *Streptococcus*, *Mycobacterium avium*, *Mycobacterium intracellulare*, *Mycobacterium avium* complex, *Mycobacterium gordonae*, *Mycobacterium tuberculosis* complex, *Mycobacterium kansasii*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*.

INFECTIOUS DISEASE TESTS - VIRAL

TEST	MANUFACTURER	TEST NAME	METHOD
Cytomegalovirus detection	Digene Corporation Gaithersburg, MD	HC1® CMV DNA Test	Hybrid Capture
	bioMerieux, Inc. Durham, NC	CMV pp67 mRNA	NASBA
HCV Qualitative detection	Gen-Probe, Inc. San Diego, CA (distributed by Bayer HealthCare, Berkeley, CA)	VERSANT® HCV RNA	TMA
	Roche Molecular Diagnostics Pleasanton, CA	AMPLICOR™ HCV Test, v2.0	PCR
	Roche Molecular Diagnostics Pleasanton, CA	COBAS AMPLICOR™ HCV Test, v2.0	PCR
HCV Quantitation	Bayer HealthCare Berkeley, CA	VERSANT® HCV RNA 3.0 Assay (bDNA)	bDNA
HIV drug resistance testing	Celera Diagnostics Alameda, CA (distributed by Abbott Laboratories, Abbott Park, IL)	ViroSeq™ HIV-1 Genotyping System	Sequencing
	Bayer HealthCare Berkeley, CA	TruGene™ HIV-1 Genotyping and Open Gene DNA Sequencing System	Sequencing

HIV Qualitative	bioMerieux, Inc. Durham, NC	NucliSens® HIV-1 QL	NASBA
HIV Quantitation	Bayer HealthCare Berkeley, CA	VERSANT® HIV-1 RNA 3.0 Assay (bDNA)	bDNA
	bioMerieux, Inc. Durham, NC	NucliSens® HIV-1 QT	NASBA
	Roche Molecular Diagnostics Pleasanton, CA	AMPLICOR HIV-1 MONITOR™ Test, v1.5	RT-PCR
	Roche Molecular Diagnostics Pleasanton, CA	COBAS AMPLICOR HIV-1 MONITOR™ Test, v1.5	RT-PCR
HCV/HIV for blood donations	Gen-Probe, Inc. San Diego, CA (distributed by Chiron)	Procleix™	TMA
	Gen-Probe, Inc. San Diego, CA (distributed by Chiron)	Chiron Procleix™ HIV-1/HCV Controls	QC controls
	Gen-Probe, Inc. San Diego, CA (distributed by Chiron)	Chiron Procleix™ HIV/HCV Proficiency Panels	QC proficiency panel
	National Genetics Institute Los Angeles, CA	UltraQual™ HCV RT-PCR Assay	RT-PCR
	National Genetics Institute Los Angeles, CA	UltraQual™ HIV-1 RT-PCR Assay	RT-PCR
	Roche Molecular Diagnostics Pleasanton, CA	COBAS AmpliScreen™ HCV Test, v2.0	RT-PCR
	Roche Molecular Diagnostics Pleasanton, CA	COBAS AmpliScreen™ HiV Test, v1.5	RT-PCR
Human Papillomavirus Testing	Digene Corporation Gaithersburg, MD	HC2® HR and LR	Hybrid Capture
	Digene Corporation Gaithersburg, MD	HC2® HPV HR	Hybrid Capture
	Digene Corporation Gaithersburg, MD	HC2® DNAwithPap	Hybrid Capture

MOLECULAR DIAGNOSTIC TESTS - HUMAN

TEST	MANUFACTURER	TEST NAME	METHOD
B-Cell Chronic Lymphocytic Leukemia (B-CLL)	Vysis/Abbott Laboratories Downers Grove, IL	CEP®12 DNA Probe Kit	FISH
Chromosome 8 Enumeration (CML, AML, MPD, MDS)	Vysis/Abbott Laboratories Downers Grove, IL	CEP®8 DNA Probe Kit	FISH
Factor II (prothrombin)	Roche Diagnostics Pleasanton, CA	Factor II (prothrombin) G20210A kit	Real-Time PCR
Factor V Leiden	Roche Diagnostics Pleasanton, CA	Factor V Leiden kit	Real-Time PCR
HLA Typing	Biotest Diagnostics Corp. Denville, NJ	Biotest HLA SSP	PCR
	Biotest Diagnostics Corp. Denville, NJ	Biotest ELPHA SSP	Enzyme linked DNA Probe Hybridization
	Genetic Testing Institute Brookfield, WI	GTI PAT HPA-1 (P1) Genotyping kit	PCR, ELISA
	Pel-Freez Clinical Systems, LLC Brown Deer, WI	Pel Freez HLA High Resolution SSP UniTray	PCR
HER-2 Status	Vysis/Abbott Laboratories Downers Grove, IL	PathVysion®	FISH

Monitoring recurrence of bladder cancer	Vysis/Abbott Laboratories Downers Grove, IL	UroVysion®	FISH
Prenatal (Chromosome 13, 18, 21, X & Y)	Vysis/Abbott Laboratories Downers Grove, IL	AneuVysion®	FISH
Sex Mismatched Bone-Marrow Transplantation	Vysis/Abbott Laboratories Downers Grove, IL	CEP®X/Y DNA Probe Kit	FISH

Last updated: November 2, 2004

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Appendix 3: CMD method questionnaire sample form

#	Please use separate form for each test / indication pair if appropriate
	Centre: Questions completed by:..... Date:

1	Name / description of the diagnostic test	
2	Does the lab follow a SOP for performing the test? If yes, please provide SOP.	☐ Yes, SOP is attached ☐ No
3	What is the method used in your lab for extraction of DNA or RNA for this test?	☐ Commercial IVD Kit : ☐ Modification of IVD Kit : ☐ In-house method
4	What is the analytic method used in your lab for nucleic acid / probe / signal amplification.	☐ Commercial IVD Kit : ☐ Modification of IVD Kit : ☐ In-house method
5	Do you use automation for result read-out?	☐ Yes: ☐ No
6	How frequently is the test run per week? What are the minimum and average test turnaround time (from request to result communicated).	Test is run ... times per week. Minimum test turn around time: days Mean test turn around time: days
7	Does the test require expert knowledge for interpretation of results? If yes, how is it done: by the lab, by the requesting physician, or always in dialogue?	☐ No ☐ Yes, interpretation by lab ☐ Yes, interpretation by requesting physician ☐ Yes, always in dialogue
8	Have the analytical performance characteristics been validated for the test method (e.g. analytical sensitivity, analytical specificity, reproducibility, limits of detection and measurement range, including information needed for the control of known relevant interferences)? If yes, please provide validation report summary.	☐ No formal validation report available ☐ Validation report summary attached
9	What is the proportion (%) of tests performed routinely which can not be interpreted? If known, please give the main reason?	... %, main reason:
10	Did your lab already participate in an external proficiency testing program / external quality assessment scheme for this test, national or international? If yes, please detail.	☐ CMD QA Round ☐ Non-CMD EQA: year:

Appendix 4: CMD expert questionnaire sample form

#	Please use separate form for each test / indication pair if appropriate
	Technical questions completed by:..... Date: Clinical questions completed by: Date:

Test Method / Indication and Analytical Performance Characteristics

1	Name / description of the diagnostic test	
2	What are the target population and the used indication for this test method? Please mention specific selection criteria and clinical setting. Please also indicate whether this test is used to study outbreaks.	
3	Please compare the alternative test methods for extraction, amplification and read-out used in the CMDs or methods you consider of particular relevance. For each of these methods please mention whether equivalence has been tested. Also indicate those methods which have been found 'substantially equivalent'.	
4	What type and amount of biological material are needed for this test method? Are there any specific precautions with respect to sample handling/transport or storage? Any differences between the 'substantially equivalent' methods?	
5	Has between-lab reproducibility of the test method been analyzed independent of EQA schemes? If yes, please add report. Any differences between the 'substantially equivalent' methods?	☐ No ☐ Yes, please detail.

Diagnostic accuracy

6	What is the 'gold standard' or reference standard or what are the criteria of the well-defined clinical disorder? Remark: the clinical disorder must be defined by criteria independent of the test under consideration. In absence of a 'gold standard', what does this test add in comparison with other tests?	
7	Please indicate references and critically appreciate the diagnostic accuracy/performance (diagnostic sensitivity, diagnostic specificity, ...) of the used test method in the indication. The term clinical validation is also used for genetic testing (ACMG Standards and Guidelines, www.ACMG.net). Diagnostic sensitivity is defined as the proportion of patients with a well-defined clinical disorder whose test values are positive or exceed a defined decision limit (i.e., a positive result and identification of the patients who have a disease). The term diagnostic sensitivity (Europe) is equivalent to clinical sensitivity	

	(U.S.). Diagnostic specificity is defined as the proportion of subjects who do not have a specified clinical disorder whose test results are negative or within the defined decision limit. The term diagnostic specificity (Europe) is equivalent to clinical specificity (U.S.). The STARD list may be of help to check the quality of the studies (Bossuyt PM et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. BMJ 2003; 326: 41-4). Any differences between the 'substantially equivalent' methods?	
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Aspects of Clinical Utility and Cost-benefit

8	What is on average the proportion of tests performed routinely which can not be interpreted? Any differences between the 'substantially equivalent' methods?	
9	Does such result have a serious negative impact on patient health?	☐ No ☐ Yes, please detail.
10	Does such result have a significant impact on health care costs, eg >1 extra tests run or >1 extra patient visit?	☐ No ☐ Yes, please detail.
11	For qualitative tests: what proportion of the tests performed is positive? For genetic markers: in what proportion of the tests performed can a relevant genetic variation be demonstrated?	
12	What are the clinical decisions/actions that follow a positive test result (or typing result)?	
13	What is the proportion of false-positive test results among the test-positives in routine? For typing assays: what is the proportion of incorrect typing results? For quantitative tests: what is the proportion of incorrect measures, eg error > 1 log10? What is the linearity of the assay? Any differences between the 'substantially equivalent' methods? Please provide source of information.	
14	Are false-positive results (or incorrect typing result or incorrect measure) associated with any serious negative impact on patient health?	☐ No ☐ Yes, please detail.
15	Are false-positive results (or incorrect typing result or incorrect measure) associated with any serious impact on health care costs (>1 extra tests or >1 extra visits)?	☐ No ☐ Yes, please detail.
16	What is the proportion of false-negative test results among test negatives in routine? Any differences between the 'substantially equivalent' methods? Please provide source of information.	

17	Are false-negative results associated with any serious negative impact on patient health?	☐ No ☐ Yes, please detail.
18	Are false-negative results associated with any serious impact on health care costs (>1 extra tests of >1 extra visits), eg because of missed diagnosis or delay in diagnosis?	☐ No ☐ Yes, please detail.
19	Based on epidemiology, how many samples are expected to get tested in Belgium per year, how many are expected to test positive?	
20	Please indicate references and critically appreciate the clinical utility of the used test method/indication in comparison with existing method (eg added value versus currently used non-molecular diagnostic test) or no testing, whatever is appropriate. If available, please add consensus guidelines or reviews of relevance. Please differentiate between clinical utility demonstrated for equivalent test methods from studies based on test methods, which were not shown to be equivalent to the analytic method used.	
21	Please calculate the cost per test performed using this method (personnel, reagents, write-off of instruments, housing,...). Do not include R&D. If calculated per test performed, what would be the R&D cost, if any. Any differences between the 'substantially equivalent' methods?	
23	Please indicate references and critically appreciate the cost-effect of the used test method/indication. Please differentiate between cost-effect demonstrated for equivalent test methods from studies based on test methods, which were not shown to be equivalent to the analytic method used.	
24	What would be the impact of fully reimbursing this test via the 'nomenclature'? Please suggest criteria (diagnostic rules, accreditation of laboratories performing tests, limitation of requesting physicians, ..) which could help prevent inappropriate testing while allowing access to the test for those who need it. Is the test most frequently ordered together with or after other tests? If yes, which tests and where are those run?	

Expected 5 year evolution

25	Do you expect any major change in indication, number of tests performed, or in test method (kit, automation, ..) within the next 5 years? If yes, please detail.	☐ No ☐ Yes, please detail.
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References

Appendix 5: List of invoices (*Taq* specific) submitted by CMDs

Depart.	Date	Invoice's descriptor	Tests
HO	19-mei-03	3*200 TaqMan Universal PCR Master Mix, MNL (4 304 437)	600
HO	27-mei-03	3*200 TaqMan Universal PCR Master Mix, MNL (4 304 437)	600
HO	26-sep-03	3*200 TaqMan Universal PCR Master Mix, MNL (4 304 437)	600
HO	2-dec-03	3*200 TaqMan Universal PCR Master Mix, MNL (4 304 437)	600
HO	5-jan-04	3*200 TaqMan Universal PCR Master Mix, MNL (4 304 437)	600
HO	19-mei-03	1*1,200 tests AmpliTaq Gold DNA Polymerase 6*250 U, Buffer II (N8 080 243)	1 200
HO	14-jan-04	2*80 tests Taq DNA Polymerase (18 038 034)	160
HO	08-apr-03	2*80 tests Taq DNA Polymerase (18 038 034)	160
MB	28-jul-03	1*(10*200) tests TaqMan Universal PCR Master Mix (4 305 719)	2 000
MB	13-feb-03	1*(10*200) tests TaqMan Universal PCR Master Mix (4 305 719)	2 000
MB	21-nov-03	2*200 tests AmpliTaq Gold PCR Master Mix, 250 U/ 5mL (4 318 739)	400
MB	08-apr-03	2*200 tests AmpliTaq Gold PCR Master Mix, 250 U/ 5mL (4 318 739)	400
MB	13-jun-03	1*200 tests AmpliTaq Gold PCR Master Mix, 250 U/ 5mL (4 318 739)	200
MB	13-feb-03	1*200 tests AmpliTaq Gold PCR Master Mix, 250 U/ 5mL (4 318 739)	200
MB	27-mrt-03	3*200 tests TaqMan Universal PCR Master Mix, MNL (4 304 437)	600
1		PCR assays achievable from the Taq DNA polymerase bought	10 320

Depart.	Date	Invoice's descriptor	Tests
MB	01-apr-03	1*1000 tests TaqMan 1000 RXN Gold with Buffer A Pack (4 304 441)	1.000
MB	27-mei-03	1*1000 tests TaqMan 1000 RXN Gold with Buffer A Pack (4 304 441)	1 000
MB	24-jul-03	1*1000 tests TaqMan 1000 RXN Gold with Buffer A Pack (4 304 441)	1 000
MB	16-sep-03	1*1000 tests TaqMan 1000 RXN Gold with Buffer A Pack (4 304 441)	1 000
MB	22-okt-03	1*1000 tests TaqMan 1000 RXN Gold with Buffer A Pack (4 304 441)	1 000
MB	09-mei-03	3*200 tests TaqMan One-Step RT-PCR Master Mix Reagents Kit (4 309 169)	600
MB	01-okt-03	3*200 tests TaqMan One-Step RT-PCR Master Mix Reagents Kit (4 309 169)	600
MB	27-mei-03	1*(5*800) tests AmpliTaq Gold 5 x 1000 U Buffer I (N8 080 248)	4 000
MB	11-feb-03	1*(5*800) tests AmpliTaq Gold 5 x 1000 U Buffer II (N8 080 249)	4 000
MB	01-apr-03	1*(5*800) tests AmpliTaq Gold 5 x 1,000 U Buffer II (N8 080 249)	4 000
MB	25-sep-03	1*(5*800) tests AmpliTaq Gold 5 x 1000 U Buffer II (N8 080 249)	4 000
MB	25-apr-03	5*(2*50) tests PCR Master Mix (M7 502) # 214 473	500
MB	11-jun-03	3*200 tests PCR Core System I (M7 660) # 215 497	600
2		PCR assays achievable from the Taq DNA polymerase bought	23 300

Depart.	Date	Invoice's descriptor	Tests
MB	27-mrt-03	1*800 tests AmpliTaq Gold 1,000 U & 10X PCR Buffer (N8 080 246)	800
MB	03-jun-03	1*4,000 tests AmpliTaq Gold DNA Polymerase, 5x1,000 U, Buffer II & Mg (N8 080 249)	4 000
MB	07-feb-03	1*200 tests FG SYBR GREEN PCR Master Mix (4 309 155)	200
MB	10-mrt-03	1*200 tests iTAQ DNA Polymerase (D) 250 U/50 µl (1 708 870)	200
MB	28-apr-03	1*200 tests iTAQ DNA Polymerase (D) 250 U/50 µl (1 708 870)	200
MB	03-jun-03	1*200 tests iTAQ DNA Polymerase (D) 250 U/50 µl (1 708 870)	200
MB	01-jul-03	3*200 tests iTAQ DNA Polymerase (D) 250 U/50 µl (1 708 870)	600
MB	22-jul-03	3*200 tests iTAQ DNA Polymerase (D) 250 U/50 µl (1 708 870)	600
MB	16-dec-03	3*200 tests iTAQ DNA Polymerase (D) 250 U/50 µl (1 708 870)	600
MB	29-dec-03	3*200 tests iTAQ DNA Polymerase (D) 250 U/50 µl (170-8870)	600
3		PCR assays achievable from the Taq DNA polymerase bought	8 000

Depart.	Date	Invoice's descriptor	Tests
MB	31-okt-03	3*200 TaqMan Universal PCR Master Mix, MNL (4 304 437)	600
MB	23-mei-03	4*200 tests SYBR® Green PCR Master Mix (4 309 155)	800
HO	03-feb-03	1*100 tests AmpFLSTR Profiler Plus PCR Amplification Kit (4 303 326)	100
HO	22-apr-03	1*2,000 tests SYBR® Green PCR Master Mix 10-Pack (4 312 704)	2 000
HO	05-mei-03	1*2,000 tests SYBR® Green PCR Master Mix 10-Pack (4 312 704)	2 000
HO	20-mei-03	1*2,000 tests SYBR® Green PCR Master Mix 10-Pack (4 312 704)	2 000
HO	23-sep-03	1*2,000 tests SYBR® Green PCR Master Mix 10-Pack (4 312 704)	2 000
HO	21-nov-03	1*2,000 tests SYBR® Green PCR Master Mix 10-Pack (4 312 704)	2 000
HO	26-jan-04	1*2,000 tests TaqMan Universal PCR Master Mix, No AmpErase UNG (4 324 020)	2 000
HO	28-nov-03	1*200 TaqMan Universal PCR Master Mix, No AmpErase UNG (4 324 018)	200
HO	26-jan-04	1*200 tests AmpliTaq Gold DNA Polymerase, 250 U, Gold Buffer & Mg (4 311 806)	200
HO	13-aug-03	3*1,000 tests TaqMan 1000 RXN Gold with Buffer A Pack (4 304 441)	3 000
HO	20-jan-03	4*200 tests SYBR Green PCR Core Reagents 10-Pack (4 304 886)	800
HO	12-nov-03	1*100 tests AmpFLSTR Profiler Plus PCR Amplification Kit (4 303 326)	100
AP	21-feb-03	1*1,200 tests AmpliTaq Gold DNA Polymerase 6*250 U, Buffer II (N8 080 243)	1 200
AP	19-nov-03	1*1,200 tests AmpliTaq Gold DNA Polymerase 6*250 U, Buffer II (N8 080 243)	1 200
AP	29-aug-03	1*1,200 tests AmpliTaq Gold DNA Polymerase 6*250 U, Buffer II (N8 080 243)	1 200
4		PCR assays achievable from the Taq DNA polymerase bought	21 400

Depart.	Date	Invoice's descriptor	Tests
MB/HO	05-nov-03	1*1,200 tests AmpliTaq DNA Polymerase, 6x250 U, Buffer II (N8 080 167)	1 200
MB/HO	08-sept-03	1*1,200 tests AmpliTaq DNA Polymerase, 6x250 U, Buffer II (N8 080 167)	1 200
MB/HO	19-mai-03	1*1,200 tests AmpliTaq DNA Polymerase, 6x250 U, Buffer II (N8 080 167)	1 200
MB/HO	22-mai-03	1*(10*200) tests TaqMan Universal PCR Master Mix (4 305 719)	2 000
MB/HO	11-feb-03	1*(10*200) tests TaqMan Universal PCR Master Mix (4 305 719)	2 000
MB/HO	08-sept.-03	2*(10*200) tests TaqMan Universal PCR Master Mix (4 305 719)	4 000
MB/HO	05-nov-03	2*(10*200) tests TaqMan Universal PCR Master Mix (4 305 719)	4 000
MB/HO	11-feb-03	8*200 tests TaqMan Universal PCR Master Mix, MNL (4 304 437)	1 600
MB/HO	09-sept-03	2*2,400 tests AmpliTaq Gold DNA Polymerase, 12x250 U, Buffer I (N8 080 244)	4 800
5		PCR assays achievable from the Taq DNA polymerase bought	22 000

Depart.	Date	Invoice's descriptor	Tests
MB	01-mrt-03	3*200 tests TaqMan Universal PCR Master Mix, MNL (4 304 437)	600
MB	14-apr-03	3*200 tests TaqMan Universal PCR Master Mix, MNL (4 304 437)	600
MB	16-jun-03	2*200 tests TaqMan Universal PCR Master Mix, MNL (4 304 437)	400
MB	07-jul-03	3*200 tests TaqMan Universal PCR Master Mix, MNL (4 304 437)	600
MB	08-sep-03	2*200 tests TaqMan Universal PCR Master Mix, MNL (4 304 437)	400
MB	09-okt-03	2*200 tests TaqMan Universal PCR Master Mix, MNL (4 304 437)	400
MB	16-jan-04	3*200 tests TaqMan Universal PCR Master Mix, MNL (4 304 437)	600
MB	14-apr-03	2*200 tests SYBR Green PCR Master Mix (4 309 155)	400
MB	16-jun-03	2*200 tests SYBR Green PCR Master Mix (4 309 155)	400
MB	15-dec-03	1*200 tests SYBR Green PCR Core Reagents (4 304 886)	200
MB	05-mei-03	4*200 tests Jumpstart Taq DNA Polymerase (D 9 307-250U)	800
MB	23-dec-03	8*200 tests Jumpstart Taq DNA Polymerase (D 9 307-250U)	1 600
MB	28-jan-04	1*200 tests TaqMan One-Step RT-PCR Master Mix (4 309 169)	200
6*		PCR assays achievable from the Taq DNA polymerase bought	7 200

[illegible]

Depart.	Date	Invoice's descriptor	Tests
MB/HO	1-feb-03	4*200 tests Taqman PCR Mastermix 250 Units (4 304 437)	800
MB/HO	1-feb-03	1*200 tests Taqman PCR Mastermix 250 Units (4 304 437)	200
MB/HO	1-feb-03	4*200 tests Taqman PCR Mastermix 250 Units (4 304 437)	800
MB/HO	1-feb-03	1*200 tests Taqman PCR Mastermix 250 Units (4 304 437)	200
MB/HO	1-feb-03	3*200 tests Taqman PCR Mastermix 250 Units (4 304 437)	600
MB/HO	01 feb 03	1*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	100
MB/HO	01 feb 03	3*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	300
MB/HO	01 feb 03	1*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	100
MB/HO	01 feb 03	3*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	300
MB/HO	01 feb 03	3*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	300
MB/HO	01 feb 03	2*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	200
MB/HO	01 feb 03	1*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	100
MB/HO	01 feb 03	2*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	200
MB/HO	01 feb 03	1*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	100
MB/HO	01 feb 03	2*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	200
MB/HO	01 feb 03	4*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	400
MB/HO	01 feb 03	1*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	100
MB/HO	01 feb 03	3*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	300
MB/HO	01 feb 03	2*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	200
MB/HO	01 feb 03	2*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	200
MB/HO	01 feb 03	2*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	200
MB/HO	01 feb 03	3*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	300
MB/HO	01 feb 03	1*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	100
MB/HO	01 feb 03	1*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	100
MB/HO	01 feb 03	1*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	100
MB/HO	01 feb 03	4*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	400
MB/HO	01 feb 03	3*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	300
MB/HO	01 feb 03	4*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	400
MB/HO	01 feb 03	6*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	600
MB/HO	01 feb 03	1*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	100
MB/HO	01 feb 03	3*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	300
MB/HO	01 jan 04	3*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	300
MB/HO	01 jan 04	1*100 tests QIAGEN OneStep RT-PCR Kit (QI 210 212) #	100
HO/AP	8/dec/03	2*4,000 tests AmpliTaq Gold DNA Polymerase (5x1000 U) & Buffer II (N8 080 249)	8 000
HO/AP	5/jan/04	2*4,000 tests AmpliTaq Gold DNA Polymerase (5x1000 U) & Buffer II (N8 080 249)	8 000
HO/AP	8/apr/03	6*100 tests TAKARA LA Taq 125 U	600
HO/AP	4/mrt/03	1*(2*400 tests) SYBR GREEN I (2 X 500 U)	800
HO/AP	11/aug/03	1*(2*400 tests) SYBR GREEN I (2 X 500 U)	800
HO/AP	17/nov/03	1*(2*400 tests) SYBR GREEN I (2 X 500 U)	800
AP	13-feb-03	1*6 x 200 tests AmpliTaq Gold DNA Polymerase 6x250 U, Buffer II & Mg (N8 080 243)	1 200
AP	3-feb-03	1*6 x 200 tests AmpliTaq Gold DNA Polymerase 6x250 U, Buffer II & Mg (N8 080 243)	1 200
AP	27-aug-03	1*6 x 200 tests AmpliTaq Gold DNA Polymerase 6x250 U, Buffer II & Mg (N8 080 243)	1 200
AP	9-dec-03	1*6 x 200 tests AmpliTaq Gold DNA Polymerase 6x250 U, Buffer II & Mg (N8 080 243)	1 200
AP	24-jul-03	3*200 tests Taqman PCR Mastermix 250 Units (4 304 437)	600
AP	21-okt-03	2*200 tests Taqman PCR Mastermix 250 Units (4 304 437)	400
7		PCR assays achievable from the Taq DNA polymerase bought	65 800

Depart.	Date	Invoice's descriptor	Tests
MB	16-jan-03	1*200 tests TaqMan One-Step RT-PCR Master Mix (4 309 169)	200
MB	09-jan-03	1*2000 tests TaqMan Universal PCR Master Mix (4 318 157)	2 000
MB	18-mrt-03	1*2000 tests TaqMan Universal PCR Master Mix (4 318 157)	2 000
MB	07-apr-03	2*2000 tests TaqMan Universal PCR Master Mix (4 318 157)	4 000
MB	28-feb-03	1*300 tests RT Mix 2x (Q-PCR mastermix) (RT-QP2X-03)	300
MB	01-jul-03	1*2,000 tests TaqMan Universal PCR Master Mix (4 318 157)	2 000
MB	16-dec-03	1*2,000 tests TaqMan Universal PCR Master Mix (4 318 157)	2 000
MB	04-dec-03	2*6,000 tests TaqMan Universal PCR Master Mix (Oracle)	12 000
MB	28-feb-03	2*400 tests FastStar Taq DNA polymerase 2*250 U (2 032 929)	800
8		PCR assays achievable from the Taq DNA polymerase bought	25 300

Depart .	Date	Invoice's descriptor	Tests
6 MB	21-jan-03	1*2,000 tests TaqMan® Universal PCR Master Mix (4 305 719)	2 000
6 MB	07-apr-03	1*2,000 tests TaqMan® Universal PCR Master Mix (4 305 719)	2 000
6 MB	02-jun-03	1*2,000 tests TaqMan® Universal PCR Master Mix (4 305 719)	2 000
6 MB	04-aug-03	1*2,000 tests TaqMan® Universal PCR Master Mix (4 305 719)	2 000
6 MB	27-aug-03	1*2,000 tests TaqMan® Universal PCR Master Mix (4 305 719)	2 000
6 MB	07-okt-03	2*2,000 tests TaqMan® Universal PCR Master Mix (4 305 719)	4 000
6 MB	03-apr-03	1*200 tests ACCUPRIME SUPERMIX II (12 341 012)	200
6 MB	08-jul-03	3*200 tests ACCUPRIME SUPERMIX II (12 341 012)	600
6 MB	07-jul-03	1*1,000 reactions PCR Master Mix (M7 505)	1 000
6 MB	04-aug-03	1*200 tests TaqMan® One-Step RT-PCR Master Mix (4 309 169)	200
6 MB	21-okt-03	10*400 tests FIREPoL DNA Polymerase 500 U ()	4 000
6 MB	13-feb-03	2*4,000 tests AMPLITAQ Gold 5000 U + Gold Buffer (4 311 818)	8 000
9*		PCR assays achievable from the Taq DNA polymerase bought	28 000

Depart.	Date	Invoice's descriptor	Tests
MB	19-feb-03	1*480 reactions Lightcycler faststart DNA master hybridization (2 239 272)	480
MB	20-feb-03	1*480 reactions Lightcycler faststart DNA master hybridization (2 239 272)	480
MB	13-mrt-03	1*480 reactions Lightcycler faststart DNA master hybridization (2 239 272)	480
MB	10-apr-03	2*480 reactions Lightcycler faststart DNA master hybridization (2 239 272)	960
MB	05-mei-03	2*480 reactions Lightcycler faststart DNA master hybridization (2 239 272)	960
MB	16-mei-03	2*480 reactions Lightcycler faststart DNA master hybridization (2 239 272)	960
MB	02-okt-03	1*480 reactions Lightcycler faststart DNA master hybridization (2 239 272)	480
MB	05-nov-03	2*480 reactions Lightcycler faststart DNA master hybridization (2 239 272)	960
MB	15-jan-04	2*480 reactions Lightcycler faststart DNA master hybridization (12 239 272 001)	960
MB	04-sep-03	2*96 reactions Lightcycler faststart DNA master hybridization (3 003 248)	192
MB	27-jan-04	1*1,200 tests AmpliTaq Gold DNA Polymerase 6*250 U, Buffer II (N8 080 243)	1 200
MB	27-jan-04	3*200 tests AmpliTaq DNA Polymerase Recomb. 250 U, LD, Buffer II (N8 080 158)	600
MB	26-mei-03	2*400 tests Yellowstar Licensed Taq Pol. 500 U (ME-0062-05)	800
MB	29-apr-03	2*400 tests Taq DNA Polym. Rec. 500 U (EP0402)	800
MB	26-feb-03	1*400 tests Taq DNA Polymerase in Storage Buffer B (M1665)	400
MB	22-mei-03	5*200 tests PCR core system I (M7660)	1 000
MB	17-mrt-03	1*800 tests HotStarTaq Master Mix 1000 U (QI 203 445)	800
MB	24-mrt-03	1*800 tests HotStarTaq Master Mix 1000 U (QI 203 445)	800
MB	21-jul-03	2*800 tests HotStarTaq Master Mix 1000 U (QI 203 445)	1 600
MB	29-okt-03	2*800 tests HotStarTaq Master Mix 1000 U (QI 203 445)	1 600
MB	12-jan-04	2*800 tests HotStarTaq Master Mix 1000 U (QI 203 445)	1 600
HO	11-feb-03	1*500 tests iQ SYBR Green Supermix, 500 x 50 µl reactions (1 708 882)	500
HO	18-jun-03	1*500 tests iQ SYBR Green Supermix, 500 x 50 µl reactions (1 708 882)	500
HO	06-aug-03	2*500 tests iQ SYBR Green Supermix, 500 x 50 µl reactions (1 708 882)	1 000
HO	27-okt-03	1*500 tests iQ SYBR Green Supermix, 500 x 50 µl reactions (1 708 882)	500
HO	09-dec-03	2*500 tests iQ SYBR Green Supermix, 500 x 50 µl reactions (1 708 882)	1 000
HO	27-feb-03	1*200 tests AmpliTaq DNA Polymerase Recomb. 250 U, LD, Buffer II (N8 080 158)	200
HO	31-mrt-03	4*200 tests AmpliTaq DNA Polymerase Recomb. 250 U, LD, Buffer II (N8 080 158)	800
HO	31-mrt-03	4*1,200 tests AmpliTaq Gold DNA Polymerase 6*250 U, Buffer II (N8 080 243)	4 800
10		PCR assays achievable from the Taq DNA polymerase bought	27 412

Depart.	Date	Invoice's descriptor	Tests
MB	06-mei-03	1*1000 tests TaqMan 1000 RXN Gold with Buffer A Pack (4 304 441)	1 000
MB	19-mrt-03	1*1000 tests TaqMan 1000 RXN Gold with Buffer A Pack (4 304 441)	1 000
MB	06-mei-03	CN 1*1000 tests TaqMan 1000 RXN Gold with Buffer A Pack (4 304 441)	-1 000
MB	25-jun-03	1*1000 tests TaqMan 1000 RXN Gold with Buffer A Pack (4 304 441)	1 000
MB	08-okt-03	1*1000 tests TaqMan 1000 RXN Gold with Buffer A Pack (4 304 441)	1 000
MB	08-okt-03	1*1,200 tests AmpliTaq Gold DNA Polymerase 6x250 U, Buffer II (N8 080 243)	1 200
MB	25-jun-03	3*100 tests GeneAmp dNTP Blend, 12.5 mM with dUTP (N8 080 270)	300
MB/HO	10-apr-03	CN 1*1200 tests AmpliTaq DNA Polymerase, 6x250 U, Buffer II (N8 080 167)	-1 200
MB/HO	11-feb-03	1*(6*200 tests) AmpliTaq DNA Polymerase , 6x250 U, Buffer II (N8 080 167)	1 200
MB/HO	25-feb-03	1*(6*200 tests) AmpliTaq DNA Polymerase , 6x250 U, Buffer II (N8 080 167)	1 200
MB/HO	10-apr-03	1*(6*200 tests) AmpliTaq DNA Polymerase , 6x250 U, Buffer II (N8 080 167)	1 200
MB/HO	15-jul-03	1*6 x 250 U AmpliTaq Gold DNA Polymerase, Buffer II & Mg (N8 080 243)	1 200
MB/HO	08-sep-03	1*6 x 250 U AmpliTaq Gold DNA Polymerase, Buffer II & Mg (N8 080 243)	1 200
MB/HO	29-apr-03	1*6 x 250 U AmpliTaq Gold DNA Polymerase, Buffer II & Mg (N8 080 243)	1 200
MB/HO	05-nov-03	1*6 x 250 U AmpliTaq Gold DNA Polymerase, Buffer II & Mg (N8 080 243)	1 200
MB/HO	20-feb-03	2*6 x 250 U AmpliTaq Gold DNA Polymerase, Buffer II & Mg (N8 080 243)	2 400
HO	07-mei-03	3*200 tests TaqMan Universal PCR Master Mix (4 304 437)	600
HO	03-apr-03	3*200 tests TaqMan Universal PCR Master Mix (4 304 437)	600
HO	12-mrt-03	1*200 tests TaqMan Universal PCR Master Mix (4 304 437)	100
HO	03-apr-03	2*100 tests GeneAmp dNTP Blend, 12.5 mM with dUTP (N8 080 270)	200
11		PCR assays achievable from the Taq DNA polymerase bought	15 600

Depart.	Date	Invoice's descriptor	Tests
MB/HO	05-mrt-03	1*200 tests TaqMan Universal PCR Master Mix, No AmpErase UNG (4 324 018)	200
MB/HO	28-apr-03	2*200 tests TaqMan Universal PCR Master Mix, No AmpErase UNG (4 324 018)	400
MB/HO	09-mei-03	2*200 tests TaqMan Universal PCR Master Mix, No AmpErase UNG (4 324 018)	400
MB/HO	16-mei-03	2*200 tests TaqMan Universal PCR Master Mix, No AmpErase UNG (4 324 018)	400
MB/HO	05-jun-03	3*200 tests TaqMan Universal PCR Master Mix, No AmpErase UNG (4 324 018)	600
MB/HO	24-jun-03	3*200 tests TaqMan Universal PCR Master Mix, No AmpErase UNG (4 324 018)	600
MB/HO	24-jun-03	3*200 tests TaqMan Universal PCR Master Mix, No AmpErase UNG (4 324 018)	600
MB/HO	08-aug-03	1*200 tests AmpliTaq Gold DNA Polymerase 250 U, Buffer II & Mg (N8 080 241)	200
MB/HO	07-mei-03	1*480 tests LC Faststart DNA Master Hybridization (2 239 272)	480
MB/HO	22-jul-03	2*480 tests LC Faststart DNA Master Hybridization (2 239 272)	960
MB/HO	03-sep-03	1*480 tests LC Faststart DNA Master Hybridization (2 239 272)	480
MB/HO	03-okt-03	1*480 tests LC Faststart DNA Master Hybridization (2 239 272)	480
MB/HO	09-dec-03	5*400 tests Taq DNA Polymerase 500 U, Cloned (10 342 020)	2 000
MB/HO	23-jun-03	5*400 tests Taq DNA Polymerase 500 U, Cloned (10 342 020)	2 000
MB/HO	01-sep-03	3*400 tests Taq DNA Polymerase 500 U, Cloned (10 342 020)	1 200
MB/HO	02-okt-03	5*400 tests Taq DNA Polymerase 500 U, Cloned (10 342 020)	2 000
MB/HO	14-nov-03	1*50 tests Faststart High Fidelity PCR system 50 reactions (3 553 426)	50
MB/HO	13-jan-04	1*100 QIAGEN OneStep RT-PCR Kit (QI 210 212)	100
MB/HO	09-sep-03	1*100 QIAGEN OneStep RT-PCR Kit (QI 210 212)	100
MB/HO	06-mei-03	2*400 tests PCR Pack (Nucleotides & TAQ 3x500 U) (10 884 021)	800
MB/HO	01-apr-03	1*400 tests PCR Pack (Nucleotides & TAQ 3x500 U) (10 884 021)	400
12		PCR assays achievable from the Taq DNA polymerase bought	14 450

Depart.	Date	Invoice's descriptor	Tests
MB	29-apr-03	1*96 tests RTG RT PCR Beads 0,2 ml tubes (27-9259-01)	96
MB	15-okt-03	1*200 tests TaqMan Universal PCR Master Mix, No AmpErase UNG (4 324 018)	200
MB	15-okt-03	1*200 tests TaqMan One-Step RT-PCR Master Mix Reagents Kit (4 309 169)	200
MB	12-jan-04	3*200 tests TaqMan Universal PCR Master Mix, MNL (4 304 437)	600
MB	09-jan-04	4*200 tests SYBR Green PCR Master Mix (4 309 155)	800
MB	12-jan-04	2*200 tests SYBR Green PCR Master Mix (4 309 155)	400
MB	09-mei-03	1*160 tests Super Taq Polymerase 200 U (BRN-119 002)	160
MB	28-jan-04	5*80 tests GoTaq DNA Polymerase 100 U (M3001)	400
MB	07-mei-03	12*200 tests Ampli Taq Polymerase 250 U (10 Feb 03)	2 400
MB	07-mei-03	4*200 tests Ampli Taq Polymerase 250 U (14 Feb 03)	800
MB	08-mei-03	4*200 tests Ampli Taq Polymerase 250 U (14 Feb 03)	800
MB	08-mei-03	4*200 tests Ampli Taq Polymerase 250 U (5 Mar 03)	800
MB	08-mei-03	4*200 tests Ampli Taq Polymerase 250 U (21 Mar 03)	800
MB	08-mei-03	4*200 tests Ampli Taq Polymerase 250 U (2 Apr 03)	800
MB	04-jul-03	4*200 tests Ampli Taq Polymerase 250 U (16 Apr 03)	800
MB	04-jul-03	4*200 tests Ampli Taq Polymerase 250 U (22 Apr 03)	800
MB	04-jul-03	8*200 tests Ampli Taq Polymerase 250 U (6 May 03)	1 600
MB	07-jul-03	4*200 tests Ampli Taq Polymerase 250 U (12 May 03)	800
MB	07-jul-03	8*200 tests Ampli Taq Polymerase 250 U (21 May 03)	1 600
MB	08-jul-03	4*200 tests Ampli Taq Polymerase 250 U (12 Jun 03)	800
MB	08-jul-03	4*200 tests Ampli Taq Polymerase 250 U (17 Jun 03)	800
MB	19-jan-04	8*200 tests Ampli Taq Polymerase 250 U (18 Nov 03)	1 600
MB	19-jan-04	4*200 tests Ampli Taq Polymerase 250 U (18 Nov 03)	800
MB	19-jan-04	4*200 tests Ampli Taq Polymerase 250 U (24 Nov 03)	800
MB	19-jan-04	16*200 tests Ampli Taq Polymerase 250 U (20 Nov 03)	3 200
MB	21-jan-04	4*200 tests Ampli Taq Polymerase 250 U (26 Nov 03)	800
MB	21-jan-04	4*200 tests Ampli Taq Polymerase 250 U (10 Dec 03)	800
MB	21-jan-04	4*200 tests Ampli Taq Polymerase 250 U (18 Dec 03)	800
MB	21-jan-04	8*200 tests Ampli Taq Polymerase 250 U (20 Nov 03)	1 600
13		PCR assays achievable from the Taq DNA polymerase bought	26 856

Depart.	Date	Invoice's descriptor	Tests
AP	07-jul-03	5*80 tests Taq DNA polymerase 100 U in buffer B (M 1661)	400
HO	02-jan-03	5*80 tests Taq DNA polymerase 100 U in buffer B (M 1661)	400
AP	19-aug-03	2*400 tests Taq DNA polymerase 500 U in buffer B (M 1665)	800
AP	27-okt-03	4*400 tests Taq DNA polymerase 500 U in buffer B (M 1665)	1 600
AP	30-sep-03	1*800 tests HotStarTaq Polymerase 1,000 U (QI 203 205)	800
HO	06-jan-03	1*800 tests HotStarTaq Polymerase 1,000 U (QI 203 205)	800
AP	19-nov-03	1*50 tests Faststart High Fidelity PCR system (3 553 426)	50
AP	05-dec-03	2*200 tests Faststart High Fidelity PCR system (3 553 400)	200
MB/HO	06-feb-03	1*200 tests TaqMan PCR Master MIX, MNL (4 304 437)	200
MB/HO	12-feb-03	4*200 tests TaqMan PCR Master MIX, MNL (4 304 437)	800
MB/HO	02-mei-03	6*200 tests TaqMan PCR Master MIX, MNL (4 304 437)	1 200
MB/HO	15-aug-03	5*200 tests TaqMan PCR Master MIX, MNL (4 304 437)	1 000
MB/HO	23-okt-03	6*200 tests TaqMan PCR Master MIX, MNL (4 304 437)	1 200
MB/HO	28-okt-03	5*200 tests TaqMan PCR Master MIX, MNL (4 304 437)	1 000
MB/HO	16-jan-04	8*200 tests TaqMan PCR Master MIX, MNL (4 304 437)	1 600
MB/HO	13-feb-03	4*800 tests Taq DNA Polymerase (4x250 U) (1 418 432)	3 200
MB/HO	13-mrt-03	5*800 tests Taq DNA Polymerase (4x250 U) (1 418 432)	4 000
MB/HO	24-mrt-03	10*800 tests Taq DNA Polymerase (4x250 U) (1 418 432)	8 000
MB/HO	04-jun-03	3*800 tests Taq DNA Polymerase (4x250 U) (1 418 432)	2 400
MB/HO	05-dec-03	3*800 tests Taq DNA Polymerase (4x250 U) (1 418 432)	2 400
MB/HO	13-jan-04	10*100 tests Platinum QRT-PCR Thermoscript one step (11 731 015)	1000
MB/HO	02-feb-04	10*200 tests AmpliTaq Gold 250 U & gold buffer & MgCl2 (4 311 806)	2 000
MB/HO	07-jul-03	5*200 tests AmpliTaq Gold 250 U & gold buffer & MgCl2 (4 311 806)	1 000
MB/HO	28-okt-03	2*200 tests SYBR GREEN PCR Master Mix (4 309 155)	400
14		PCR assays achievable from the Taq DNA polymerase bought	36 450

Depart.	Date	Invoice's descriptor	Tests
MB/HO	09-apr-03	3*800 tests AmpliTaq Gold 1000 U Buffer II (N8 080 247)	2 400
MB/HO	09-okt-03	2*800 tests AmpliTaq Gold 1000 U Buffer II (N8 080 247)	1 600
MB/HO	04-dec-03	1*800 tests AmpliTaq Gold 1000 U Buffer II (N8 080 247)	800
MB/HO	19-dec-03	3*800 tests AmpliTaq Gold 1000 U Buffer II (N8 080 247)	2 400
MB/HO	12-mrt-03	6*200 tests FG, TaqMan PCR Master Mix, MNL (4 304 437)	1 200
MB/HO	09-mei-03	1*200 tests FG, TaqMan PCR Master Mix, MNL (4 304 437)	200
MB/HO	30-mei-03	5*200 tests FG, TaqMan PCR Master Mix, MNL (4 304 437)	1 000
MB/HO	01-apr-03	1*2,000 tests TaqMan Universal PCR Master Mix 10-pack (4 305 719)	2 000
MB/HO	19-jun-03	1*2,000 tests TaqMan Universal PCR Master Mix 10-pack (4 305 719)	2 000
MB/HO	22-jul-03	1*2,000 tests TaqMan Universal PCR Master Mix 10-pack (4 305 719)	2 000
MB/HO	04-sep-03	1*2,000 tests TaqMan Universal PCR Master Mix 10-pack (4 305 719)	2 000
MB/HO	09-okt-03	1*2,000 tests TaqMan Universal PCR Master Mix 10-pack (4 305 719)	2 000
MB/HO	10-dec-03	1*2,000 tests TaqMan Universal PCR Master Mix 10-pack (4 305 719)	2 000
MB/HO	04-dec-03	1*200 tests FG SYBR GREEN PCR Master Mix (4 309 155)	200
MB/HO	16-jul-03	4*200 tests FG SYBR GREEN PCR Master Mix (4 309 155)	800
MB/HO	22-jul-03	3*200 tests TaqMan One-Step RT-PCR Master Mix (4 309 169)	600
HO	18-feb-03	4*800 tests HotStarTaq Polymerase 1000 U (QI 203 205)	3 200
HO	01-apr-03	2*200 tests Taq DNA Polymerase 250 U (QI 201 203)	400
HO	19-jun-03	4*800 tests HotStarTaq Polymerase 1000 U (QI 203 205)	3 200
HO	11-nov-03	4*800 tests HotStarTaq Polymerase 1000 U (QI 203 205)	3 200
15		PCR assays achievable from the Taq DNA polymerase bought	33 200

Depart.	Date	Invoice's descriptor	Tests
MB	20-feb-03	3*200 TaqMan Universal PCR Master Mix, MNL (4 304 437)	600
MB	18-mrt-03	3*200 TaqMan Universal PCR Master Mix, MNL (4 304 437)	600
MB	22-apr-03	4*200 TaqMan Universal PCR Master Mix, MNL (4 304 437)	800
MB	21-mei-03	2*200 TaqMan Universal PCR Master Mix, MNL (4 304 437)	400
MB	17-jun-03	CN 4*200 TaqMan Universal PCR Master Mix, MNL (4 304 437)	-800
MB	17-jun-03	4*200 TaqMan Universal PCR Master Mix, MNL (4 304 437)	800
MB	26-aug-03	3*200 TaqMan Universal PCR Master Mix, MNL (4 304 437)	600
MB	10-okt-03	4*200 TaqMan Universal PCR Master Mix, MNL (4 304 437)	800
MB	01-dec-03	2*200 TaqMan Universal PCR Master Mix, MNL (4 304 437)	400
MB	07-jan-04	4*200 TaqMan Universal PCR Master Mix, MNL (4 304 437)	800
MB	06-mrt-03	3*400 tests Taq DNA Polymerase 500 U in Storage Buffer A (M1865)	1 200
MB	22-apr-03	4*400 tests Taq DNA Polymerase 500 U in Storage Buffer A (M1865)	1 600
MB	08-aug-03	3*400 tests Taq DNA Polymerase 500 U in Storage Buffer A (M1865)	1 200
MB	26-aug-03	3*400 tests Taq DNA Polymerase 500 U in Storage Buffer A (M1865)	1 200
MB	10-okt-03	3*400 tests Taq DNA Polymerase 500 U in Storage Buffer A (M1865)	1 200
MB	13-okt-03	3*400 tests Taq DNA Polymerase 500 U in Storage Buffer A (M1865)	1 200
MB	21-mei-03	1*4,000 tests FastStar DNA polymerase 20*250 U (2 032 953)	4 000
MB	26-feb-03	1*200 QuantiTech Probe RT-PCR Kit (QI 204 443)	200
MB	17-apr-03	2*200 QuantiTech Probe RT-PCR Kit (QI 204 443)	400
MB	05-mei-03	2*200 QuantiTech Probe RT-PCR Kit (QI 204 443)	400
MB	23-jun-03	2*200 QuantiTech Probe RT-PCR Kit (QI 204 443)	400
MB	28-jul-03	2*200 QuantiTech Probe RT-PCR Kit (QI 204 443)	400
MB	13-okt-03	1*200 QuantiTech Probe RT-PCR Kit (QI 204 443)	200
MB	23-okt-03	1*200 QuantiTech Probe RT-PCR Kit (QI 204 443)	200
MB	25-nov-03	2*200 QuantiTech Probe RT-PCR Kit (QI 204 443)	400
MB	08-jan-04	2*200 QuantiTech Probe RT-PCR Kit (QI 204 443)	400
HO	08-jul-03	1*800 tests AmpliTaq Gold DNA Polymerase 1000 U, Gold Buffer & Mg (4 311 816)	800
HO	04-jun-03	1*200 tests AmpliTaq Gold DNA Polymerase 250 U, Gold Buffer & Mg (4 311 806)	200
HO	28-jul-03	1*800 tests AmpliTaq Gold DNA Polymerase 1000 U, Gold Buffer & Mg (4 311 816)	800
HO	29-jul-03	1*800 tests AmpliTaq Gold DNA Polymerase 1000 U, Gold Buffer & Mg (4 311 816)	800
HO	10-sep-03	3*800 tests AmpliTaq Gold DNA Polymerase 1000 U, Gold Buffer & Mg (4 311 816)	2 400
HO	28-nov-03	3*800 tests AmpliTaq Gold DNA Polymerase 1000 U, Gold Buffer & Mg (4 311 816)	2 400
HO	22-jan-04	3*800 tests AmpliTaq Gold DNA Polymerase 1000 U, Gold Buffer & Mg (4 311 816)	2 400
HO	06-okt-03	4*300 tests RT Mix 2x (Q-PCR mastermix) (RT-QP2X-03)	1 200
HO	20-nov-03	4*300 tests RT Mix 2x (Q-PCR mastermix) (RT-QP2X-03)	1 200
HO	16-dec-03	2*300 tests RT Mix 2x (Q-PCR mastermix) (RT-QP2X-03)	600
HO	13-jan-04	2*300 tests RT Mix 2x (Q-PCR mastermix) (RT-QP2X-03)	600
HO	05-mrt-03	4*4,000 tests FastStar DNA polymerase 20*250 U (2 032 953)	16 000
HO	25-sep-03	1*4,000 tests FastStar DNA polymerase 20*250 U (2 032 953)	4 000
HO	13-okt-03	1*4,000 tests Taq DNA Polymerase 20*250 U (1 435 094)	4 000
16		PCR assays achievable from the Taq DNA polymerase bought	57 000

Centre	Date	Invoice's descriptor	Tests
MB	04-feb-03	1*4,000 tests AmpliTaq DNA Polymerase 5x1,000 units, Buffer II (N8 080 156)	4 000
MB	08-apr-03	1*4,000 tests AmpliTaq DNA Polymerase 5x1,000 units, Buffer II (N8 080 156)	4 000
MB	03-jun-03	1*4,000 tests AmpliTaq DNA Polymerase 5x1,000 units, Buffer II (N8 080 156)	4 000
MB	05-aug-03	1*4,000 tests AmpliTaq DNA Polymerase 5x1,000 units, Buffer II (N8 080 156)	4 000
MB	07-okt-03	1*4,000 tests AmpliTaq DNA Polymerase 5x1,000 units, Buffer II (N8 080 156)	4 000
HO	13-jun-03	1*800 tests AmpliTaq Gold DNA Polymerase 1,000 units Buffer II (N8 080 247)	
HO	07-nov-03	1*800 tests AmpliTaq Gold DNA Polymerase 1,000 units Buffer II (N8 080 247)	
MB	04-feb-03	1*4,000 tests AmpliTaq Gold DNA Polymerase 5x1000 units Buffer II (N8 080 249)	4 000
MB	08-apr-03	1*4,000 tests AmpliTaq Gold DNA Polymerase 5x1000 units Buffer II (N8 080 249)	4 000
MB	03-jun-03	1*4,000 tests AmpliTaq Gold DNA Polymerase 5x1000 units Buffer II (N8 080 249)	4 000
MB	05-aug-03	1*4,000 tests AmpliTaq Gold DNA Polymerase 5x1000 units Buffer II (N8 080 249)	4 000
MB	07-okt-03	1*4,000 tests AmpliTaq Gold DNA Polymerase 5x1000 units Buffer II (N8 080 249)	4 000
MB	03-dec-03	1*4,000 tests AmpliTaq Gold DNA Polymerase 5x1000 units Buffer II (N8 080 249)	4 000
MB	05-jan-04	1*4,000 tests AmpliTaq Gold DNA Polymerase 5x1000 units Buffer II (N8 080 249)	4 000
MB	28-apr-03	10*100 tests GeneAmp RNA PCR Core (N8 080 143)	1 000
MB	31-jul-03	10*100 tests GeneAmp RNA PCR Core (N8 080 143)	1 000
MB	07-nov-03	10*100 tests GeneAmp RNA PCR Core (N8 080 143)	1 000
MB	22-jan-04	10*100 tests GeneAmp RNA PCR Core (N8 080 143)	1 000
HO	06-mrt-03	1*100 tests AmpFLSTR SGM Plus PCR Amplification (4 307133)	100
HO	30-jul-03	1*100 tests AmpFLSTR SGM Plus PCR Amplification (4 307133)	100
HO	03-okt-03	1*100 tests AmpFLSTR SGM Plus PCR Amplification (4 307133)	100
MB	02-apr-03	1*1,200 tests AMPLITAQ Gold 6x250 U + Gold Buffer (4 311 814)	1 200
AP	28-feb-03	1*2,000 tests TaqMan Universal PCR Master Mix (4 318 157)	2 000
HO	04-feb-03	2*500 tests Platinum Taq DNA Polymerase (10 966 034)	1 000
HO	23-mei-03	2*500 tests Platinum Taq DNA Polymerase (10 966 034)	1 000
HO	22-aug-03	2*500 tests Platinum Taq DNA Polymerase (10 966 034)	1 000
HO	02-okt-03	2*500 tests Platinum Taq DNA Polymerase (10 966 034)	1 000
HO	01-dec-03	4*500 tests Platinum Taq DNA Polymerase (10 966 034)	2 000
HO	20-mrt-03	1*500 tests Platinum QPCR Supermix-UDG (11 730 025)	500
MB	01-apr-03	2*500 tests Platinum QPCR Supermix-UDG (11 730 025)	1 000
HO	12-jun-03	1*500 tests Platinum QPCR Supermix-UDG (11 730 025)	500
MB	02-jul-03	2*500 tests Platinum QPCR Supermix-UDG (11 730 025)	1 000
MB	28-okt-03	2*500 tests Platinum QPCR Supermix-UDG (11 730 025)	1 000
MB	19-mrt-03	2*800 tests Taq I 1,000 U/100 µl (15 218 019)	1 600
MB	12-sep-03	2*800 tests Taq I 1,000 U/100 µl (15 218 019)	1 600
MB	12-mei-03	2*800 tests HotStarTaq Polymerase 1,000 U (QI 203 205)	1 600
MB	27-okt-03	1*800 tests HotStarTaq Polymerase 1,000 U (QI 203 205)	800
HO	07-mei-03	1*800 tests HotStarTaq Master Mix 1,000 U (QI 203 445)	800
HO	21-jul-03	2*800 tests HotStarTaq Master Mix 1,000 U (QI 203 445)	1 600
17*		PCR assays achievable from the Taq DNA polymerase bought	75 100

*Data from centre 17 were received late and were therefore not included in the analysis

Centre	Date	Invoice's descriptor	Tests
AP	31-jan-03	1*200 reactions Red'y Star Mix (PK-0073-02R)	200
AP	31-mrt-03	1*200 reactions Red'y Star Mix (PK-0073-02R)	200
AP	30-apr-03	5*200 reactions Red'y Star Mix (PK-0073-02R)	1 000
AP	23-jun-03	5*200 reactions Red'y Star Mix (PK-0073-02R)	1 000
AP	11-sep-03	5*200 reactions Red'y Star Mix (PK-0073-02R)	1 000
AP	22-mei-03	1*500 U Hot GoldStar DNA Polymerase (ME-0073-05)	500
AP		PCR assays achievable from the Taq DNA polymerase bought	3 900

Appendix 6: List of EQA programs for molecular tests

EQA organizations and tests covered.

Scheme	Language	Program or project	Fee or not	Cost	Parameters	Group	Website/ contact person
European Molecular Genetics Quality Network (EMQN) (European Community)	English	Started as EU project; from 2002 Program financed by participants	Fee	150 € per scheme	Different schemes (vary annually): e.g. Retinoblastoma, Fragile-X syndrome, Duchenne Muscular Dystrophy, DNA sequencing, Breast cancer, Hereditary Haemochromatosis, Prader Willi / Angelman syndromes, Friedreich Ataxia, Charcot-Marie-Tooth disease, Huntington disease, Hereditary non-polyposis colon cancer, Spinocerebellar ataxia's, Phenylketonuria, Y-Chromosome microdeletion	Genetics	www.emqn.org stefan.vermeulen@UGent.be
European Leukemia Network (European Community)	English	Part of 6th European Framework Project	Varies	Varies by program	CML, AML, CLL, ALL, MDS, CMPD, diagnostics, cytogenetics, MRD, gene profiling, stem cell transplantation	Haematology	http://www.leukemia-net.org/elnet.htm Ruediger.Hehlmann@med3.ma.uni-heidelberg.de gabriele.lalla@med3.ma.uni-heidelberg.de
DGKL (Germany)	German	Program	Fee	I/2005: 1 set 40 €, 2 sets 55 €, 3 sets 70 € (analytes not specified)	In 2004: Factor V Leiden, Factor II 20210, Factor XIII V34L, MTHFR, GP IIIa L33P, HPE, aI-proteinase-inhibitor, ApoE, ApoB100, ACE, TPMT,	Haematology, Genetics	www.dgkl-rfb.de

					Cyp2D6, UGT1A1		
OEQUAST (Austria)	German	Program	Fee	(prices 2004): APC: 124 € FVLeiden: 99 € Other: 1 set: 80 € 2 sets: 110 € 3 sets: 140 €	Refers to DGKC; also Factor V Leiden, APC resistance	Haematology, Genetics	http://62.99.208.26/oequasta
BVDH (Berufsverband Deutscher Humangenetiker e.V) (Germany)	German	Program	Fee	Per scheme (= parameter): 40 € for members; 60 € for non- members	DMD / BMD, HNPCC, Huntington-disease, Haemochromatosis, CMT / HMSN, Prader-Willi / Angelman, CF, Myotonic Dystrophy, SCA, FraX, Friedreich Ataxia (FRDA), AZF/DAZ, AGS, Men2a/Men2b, Interphase-FISH cytogenetics	Haematology, Genetics	http://www.bvmedgen.de/welcome.html
MODHEM (MOlecular Diagnostics for HEmatological Malignancies) (Netherlands)	English	Program			Hemato-onco, e.g. autumn 2004: AML1- ETO, BCR-ABL, CBFB- MYH11, PML-RARA (fusion transcripts), and FLT-3	Haematology	www.modhem.nl webmaster@modhem.nl
INSTAND (Germany)	German/English	Program	Fee	Varying between 133 € and 148 € depending on parameter	HIV, HBV, HCV, HSV,-1, HSV-2, CMV, VZV, ParvoB19, Influenza A en B, Adenovirus, Enter virus, HPV, Mycobacteria, N. gonorrhoeae, C. trachomatis, B. pertussis, H. pylori, EHEC/STEC, B. burgdorferi, L. pneumophila, S. enterica,	Microbiology, Genetics	www.instand-ev.de

					Listeria sp., Factor V Leiden, HFE, UDPG, AAT, ApoE, Copper Binding Protein, Prothrombin 20210, MTHFR		
SZU (Czech republic)							http://www.szu.cz/English/english.htm
QCMD (European Community)	English	Started as EU project; from 2002 Program financed by participants	Fee	295 € per panel (= parameter)	HSV-1, HSV-2, VZV, Enterovirus, Toxoplasma gondii, C. trachomatis, N. gonorrhoeae, HIV-1 (RNA), HIV-1 (DNA), HIV-drug-resistance, HBV, HCV, HCV genotyping, CMV, EBV, M. tuberculosis, RSV, L. pneumophila,	Microbiology	www.qcmd.org
SKML (Netherlands)	Dutch	Program	Fee	405 € (= whole package of 2 * 6 samples per year)	The QA Molecular biology consists of: - Analytical part aiming at the diagnostics; e.g. Determination of Factor V mutation Leiden, Factor II mutation, HLA-B27 and HCC- mutation – Technical part; in which a “subdivision” of the technique of analysis is tested separately. – Theoretical part, consisting of theoretical questions	Genetics, General	http://www.skzl.nl/
Labquality (Finland)	Finnish/English	Program	Fee		Forensic Mock Case, Fragile-X DNA test, Lactose malabsorption DNA test, Molecular	Microbiology, genetics, general	www.labquality.com

					cytogenetics, Standard cytogenetics, C. trachomatis, N. gonorrhoeae, HBV, HCV, HIV confirmatory tests (?)		
CAP (USA)	English	Program	Fee	Varying between 218 \$ and 1311 \$ depending on parameter + 70 \$ for each product sent outside USA	C. trachomatis, N. gonorrhoeae, HIV-RNA, HIV genotyping, HCV qualitative, HCV viral load, HCV genotyping, B. pertussis, B. parapertussis, CMV, Enterovirus, HBV, HSV, HPV, Mec A detection, Molecular typing of bacterial isolates, M. tuberculosis, chromosome abnormality, karyotype nomenclature designation, modal chromosome number, sex chromosome designation, Fish for neoplastic disorder, Fish for congenital abnormality, Fish for HER2 gene amplification, Fish for urothelial carcinoma surveillance, acylcarnitine (qual and quant), amino acids (quant and qual), mucopolysaccharides (qual), organic acids (qual and quant), specified metabolic disorder, BRCA1/2, cystic fibrosis,	Microbiology, genetics, pathology	www.cap.org

					DMD/Becker, Factor V Leiden, Fragile X, Friedreich ataxia, haemochromatosis, haemoglobin S/C, Huntingdon disease, MTHFR, MEN2, myotonic dystrophy, Prader-Willy/Angelman Syndrome, Prothrombin, RhD, spinal muscular atrophy, spinocerebellar ataxia		
EQA-CF (European Community)	English	EU Project, ongoing since 1996	No fee for labs;	Fee only for private companies (administration costs)	<i>CFTR</i> mutations	Genetics	http://www.cfnetwork.be/ Els.Dequeker@med.kuleuven.ac.be
UK neqas (United Kingdom)	English	Program	Fee	For virological parameters varying from 393 € to 649 €	HCV qual, HCV quant, HCV genotyping, HIV RNA quantification, molecular detection of viruses in CSF, HBV DNA quantification, Factor V Leiden, HLA-DRB1 and HLA-DQB1 typing using DNA, phenotyping for HLA-A, B Cw DR and DQ by DNA techniques, HFE gene mutations, DNA analysis for carrier detection, diagnosis and presymptomatic testing using linkage analysis and mutation detection, Prenatal and postnatal chromosome analysis from amniotic fluid,	Microbiology, Genetics, Haematology, Pathology	www.ukneqas.org.uk

					chorionic villi, blood and solid tissue cultures. Analysis of bone marrow and blood samples to detect clonal chromosome changes in leukaemia etc.		
ENIVD (European Network for Diagnostics of "Imported" Viral Diseases) (European Community)	English	Program	No fee		Exotic viruses (e.g. Poxvirus,...)	Microbiology	www.enivd.de SchaedlerR@rki.de
OECD (Organization for Economic Cooperation and Development) (International Organization)	English	Project	?		survey on molecular genetic testing	Genetics	www.oecd.org
PROGRAMMA LEONARDO (Italy)	Italian	Program	Fee		Only for Italian labs Methodological program	General	www.abanalitica.it
ICS-CISEL (Italy)							www.cismel.it
ISS (Istituto Superiore di Sanità) (Italy)	Italian	Italian National Project on Standardisation and QA of Genetic Tests; only public laboratories (performing	Voluntary basis		cytogenetics and molecular genetics	Genetics	http://www.iss.it/taruscio@iss.it

		cytogenetics and/or molecular genetics)					
OMCL (Official Medicines Control Laboratories) (European Community)	English	Program	No fee (?)		Varies annually: List of PTS planned for 2005 Biological Studies - PTS061 HCV- NAT(Nukleinsäure- Amplifikations-Techniken) : First quarter 2005 - PTS064 B19-NAT: Second quarter 2005 - PTS079 PKA (Prekallikrein activator) in albumin: Third quarter 2005 - PTS078 IPV-D-antigen assay: Fourth quarter 2005		http://www.pheur.org/index.php3
AABB (American Association of Blood Banks) (USA)	English						http://www.aabb.org
ESG-MRD-ALL (European Organisation)	English	Project of the Study Group for Minimal Residual Disease in ALL	?		Research of MRD in ALL	Haematology	
DEKS (Denmark)	Danish	Program	Fee	Cfr. Prices of other organizations	Participation through DEKS to programs of QCMD, UK NEQAS and Labquality; also programs for molecular biology and	Microbiology, Genetics, Haematology, Pathology	www.deks.dk

					genetics		
EQUALIS (Sweden)	Swedish	Program	Fee	Varying between 2750 Swedish Crown and 5200 Swedish Crown depending on parameter	ACE I/D genotyping, APoE, Factor II 20210G- A, Factor V 1691G-A, Haemochromatosis (HFE), MTHFR, C. trachomatis, C. pneumoniae, M. pneumoniae, Parvovirus B19, + programs from UK NEQAS, DEKS, Labquality and QCMD	Microbiology, Genetics, Haematology, Pathology	http://www.equalis.se/
ENVA (European Network for the Virological Evaluation of International Trials for New Anti-HIV Therapies) (Europe)							r.schuurman@lab.azu.nl
CGMS (Clinical Molecular Genetics Society) (UK)	English	Program	?		Genetic tests	Genetics	www.cmgs.org
AACTG (The Adult AIDS Clinical Trials Group) Program is called Virology Quality Assessment Program (VQA)	English	Program	?		HIV DNA, HIV Genotypic Resistance, and HIV RNA	Microbiology	http://aactg.s-3.com/INDEX.HTM http://aactg.s-3.com/vqaproftest.htm

Proficiency Testing Program (Worldwide)							
API (American Proficiency Institute) (USA)	English	Program	?		C. trachomatis, N. gonorrhoeae	Microbiology	http://www.api-pt.com/
MPEP (Model Performance Evaluation Program) of the CDC (USA)	English	Program	Fee		HIV RNA (only US labs and selected labs from other countries, e.g. ref centres)	Microbiology	http://www.phppo.cdc.gov/mpep/
DigitalPT (Australia)	English	Program	Fee	Varying between 500 Australian dollar and 1495 Australian dollar depending on parameter	HIV-I resistance, HIV viral load, HCV RNA, HCV genotyping, CMV, HAV, HBV, Parvovirus B19, HSV (US, Canadian and Australian labs)	Microbiology	www.digitalpt.com info@accutest.org sales@digitalpt.com
Viral Quality Control (VQC) (Netherlands)	English	Program	Fee	Catalogue with prices for 2005 will be published in 1st quarter of 2005	HCV, HIV, HBV, CMV, HAV, HSV, B19, C. trachomatis	Microbiology	http://www.vqc.nl
NRL (The National Serology Reference Laboratory) (Australia)	English	Program	free of charge or provided at a competitive rate		HIV, HCV, HTLV	Microbiology	www.nrl.gov.au
QMPLS - Quality Management	English	Program	?		“routine” genetics	Genetics	www.qmpls.org

Program - Laboratory Services (Canada)							
Qualicont (Hungary)	Hungarian	Program	Fee		PCR techniques, Factor V Leiden, haemochromatosis HFE, prothrombin G2010A, MTHFR	Genetics	http://www.tiszanet.hu/qualicont/
RCPA (Australia)	English	Program	Fee		C. trachomatis, N. gonorrhoeae, B. pertussis, CMV, enterovirus, HSV, M. avium complex, mecA (MRSA), M. tuberculosis, VZV, Factor V Leiden, haemochromatosis Cys282Tyr, haemochromatosis His63Asp, prothrombin G2010A, MTHFR, bcr-abl for CML, bcr-abl for ALL, bcl-1, bcl-2, T cell receptor gene, Immunoglobulin heavy chain gene, Chimerism, thalassaemia beta, thalassaemia alpha	Microbiology, Genetics, Haematology	http://www.rcpaqapa.netcore.com.au/
Biologie prospective (France)	French	Program	Fee		C. trachomatis	Microbiology	www.biologie-prospective.org
Equal (European Community)	English	Project	No fee		three different programmes on External Quality Assessment in Molecular Methods that are independent of the analyte: EQUAL-qual for	General	www.equal.unifi.it

					monitoring of performance of qualitative PCR based assays EQUAL-quant for monitoring performance of the 5'-nuclease quantitative PCR based assay EQUAL-seq for monitoring sequencing based assays		
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KCE reports

1. Efficacité et rentabilité des thérapies de sevrage tabagique. D/2004/10.273/2.
2. Etude relative aux coûts potentiels liés à une éventuelle modification des règles du droit de la responsabilité médicale (Phase I). D/2004/10.273/4.
3. Utilisation des antibiotiques en milieu hospitalier dans le cas de la pyélonéphrite aiguë. D/2004/10.273/6.
4. Leucoréduction. Une mesure envisageable dans le cadre de la politique nationale de sécurité des transfusions sanguines. D/2004/10.273/8.
5. Evaluation des risques préopératoires. D/2004/10.273/10.
6. Validation du rapport de la Commission d'examen du sous financement des hôpitaux. D/2004/10.273/12.
7. Recommandation nationale relative aux soins prénatals: Une base pour un itinéraire clinique de suivi de grossesses. D/2004/10.273/14.
8. Systèmes de financement des médicaments hospitaliers: étude descriptive de certains pays européens et du Canada. D/2004/10.273/16.
9. Feedback: évaluation de l'impact et des barrières à l'implémentation – Rapport de recherche: partie I. D/2005/10.273/02.
10. Le coût des prothèses dentaires. D/2005/10.273/04.
11. Dépistage du cancer du sein. D/2005/10.273/06.
12. Etude d'une méthode de financement alternative pour le sang et les dérivés sanguins labiles dans les hôpitaux. D/2005/10.273/08.
13. Traitement endovasculaire de la sténose carotidienne. D/2005/10.273/10.
14. Variations des pratiques médicales hospitalières en cas d'infarctus aigu du myocarde en Belgique. D/2005/10.273/12.
15. Evolution des dépenses de santé. D/2005/10.273/14.
16. Etude relative aux coûts potentiels liés à une éventuelle modification des règles du droit de la responsabilité médicale. Phase II : développement d'un modèle actuariel et premières estimations. D/2005/10.273/16.
17. Evaluation des montants de référence. D/2005/10.273/18.
18. Utilisation des itinéraires cliniques et guides de bonne pratique afin de déterminer de manière prospective les honoraires des médecins hospitaliers: plus facile à dire qu'à faire.. D/2005/10.273/20.
19. Evaluation de l'impact d'une contribution personnelle forfaitaire sur le recours au service d'urgences. D/2005/10.273/22.
20. HTA Diagnostic Moléculaire en Belgique. D/2005/10.273/24, D/2005/10.273/26.

Renseignements

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