TECHNICAL REPORT

ERLN-TB expert opinion on the use of the rapid molecular assays for the diagnosis of tuberculosis and detection of drug resistance

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Annexes 1 and 2 are included in the online report available at www.ecdc.europa.eu/publications

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Contents

Abbreviations iv
Executive summary 1
Foreword 3
1 Background 4
1.1 Current situation 4
1.2 Objectives 5
1.3 Methods 5
2 Background on the rapid molecular assays for *M. tuberculosis* identification and detection of drug-resistance 9
2.1 What are Line Probe Assays and the Cepheid Gene Xpert MTB/RIF? 9
2.2 What are the advantages and disadvantages of rapid molecular methods? 10
2.3 Definitions for accuracy 10
3 Expert opinions and summary of evidence 11
Considerations 11
3.1 Is there a role of LPA/Cepheid Xpert assays for the rapid diagnosis of TB and the detection of drug resistance in individuals suspected of TB? 12
3.2 Is there a role of LPA/Cepheid Xpert assays for the rapid diagnosis of TB and detection of drug resistance in other special groups such as immunocompromised individuals? 25
3.3 Is there a role of LPA/Cepheid Xpert for the rapid diagnosis of TB and detection of drug resistance in contact tracing initiatives? 25
3.4 Is there a role of LPA/Cepheid Xpert for the rapid diagnosis of TB and detection of drug resistance in patients that have initiated anti-TB treatment? 26
4 Future research needs and considerations 27
4.1 Identifying areas for future research 27
4.2 Opinion-based recommendations 27
4.3 Main principles of rapid molecular assays implementation and usage 28
References 29

Annex 1. Assay performance characteristics classified by the type of assay
Annex 2. Extracted data/evidence from selected papers for each individual assay

Abbreviations

Cepheid Xpert	Cepheid Gene Xpert MTB/RIF
CI	Confidence interval
CSF	Cerebral spinal fluid
DST	Drug susceptibility test
ECDC	European Centre for Disease Prevention and Control
ERLN	European Reference Laboratory Network for Tuberculosis
EU	European Union
GT	Genotype MTBDR
GT*plus*	Genotype® MTBDR*plus*
GTsl	Genotype® MTBDR*sl*
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
INH	Isoniazid
inhA	NADH-dependent enoyl ACP reductase gene
INNOLIPA	INNO-LiPA RIF.TB
katG	Catalase-peroxidase gene
LPA	Line probe assay(s)
MDR	Multidrug Resistant
MIRU-VNTR	Mycobacterial interspersed repeat unit - variable number of tandem repeats
NAAT	Nucleic acid amplification test
NPV	Negative predictive value
OR	Odds ratio
PCR	Polymerase chain reaction
PPV	Positive predictive value
RIF	Rifampicin
RFLP	Restriction fragment length polymorphism
RT	Reverse transcriptase
rpoB	*B*-subunit RNA polymerase gene
QC	Quality control
TB	Tuberculosis
WHO	World Health Organization
XDR	Extensively drug-resistant

Executive summary

Multidrug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB) are threats to the elimination of tuberculosis (TB) worldwide. The ability to rapidly and accurately detect *Mycobacterium tuberculosis* and drug resistance in clinical specimens is essential for the appropriate treatment of patients suffering from TB and the prevention of further spread of drug-resistant strains. This is of paramount importance for the control of TB and drug-resistant TB at a national, European Union (EU) and global level. Although TB and drug-resistant TB rates in most EU countries are stable and declining, the expansion of the EU and increasing migration population rates pose challenges to the National TB Programs in the EU. Prevalence of TB is still relatively high in a number of EU Member States (Bulgaria and Romania) and drug-resistant TB remains a serious problem in the Baltic States. Therefore new, highly specific, sensitive, and rapid tools to detect active TB and drug resistance are evidently needed.

Rapid molecular assays for the detection of TB and drug-resistant TB in clinical specimens are molecular assays based on detection of specific nucleotide sequences and/or mutations in the *M. tuberculosis* genome, indicative of the presence of *M. tuberculosis* and/or associated with drug resistance. The commercially available Line-Probe Assays (LPAs); INNO-LiPA Rif.TB (Innogenetics, Zwijndrecht, Belgium), Genotype MTBDR/MTBDR*plus* and Genotype MTBDR*sl* (Hain Lifescience, GmbH, Germany), are based on the targeted amplification (polymerase chain reaction [PCR]) of specific fragments in the *M. tuberculosis* genome followed by hybridization of PCR products to oligonucleotide probes immobilised on membranes. These assays are capable of detecting resistance to rifampicin; rifampicin and isoniazid; or ethambutol, fluoroquinolones and injectable drugs, respectively. The tests are designed for use on both primary respiratory clinical specimens and TB isolates. The Cepheid Gene Xpert MTB/RIF system (Cepheid Xpert Inc., Sunnyvale, CA, USA) is a fully automated real time (RT)-PCR-based assay for the detection of *M. tuberculosis* DNA and mutations associated with drug resistance to rifampicin, directly in clinical specimens.

In September 2008, WHO formally endorsed a policy on the use of the LPAs for the rapid screening of patients at risk of MDR-TB [1], with general guidelines for their implementation. In addition, in December 2010, WHO formally endorsed the Cepheid Gene Xpert MTB/RIF designed for the detection of *M. tuberculosis* DNA and mutations associated with drug resistance to rifampicin in clinical pulmonary specimens.

This current guidance on use of rapid molecular assays for the diagnosis of TB and the detection of drug resistance is based on a systematic review performed in 2011 on the most up-to-date (15/05/2011) evidence extracted from papers published in peer-review journals, existing systematic reviews, official policy documents, and guidelines.

The guidance on the use of these assays on primary clinical specimens should be considered in association with an understanding of the population being assessed i.e. the prevalence of TB and /or MDR-TB in the population.

Expert opinion

The overall opinion of the expert group regarding the use of rapid molecular assays as a stand-alone tool for the diagnosis of TB and detection of drug resistance is as follows:

Based on the evidence reviewed and analysed, rapid molecular assays for TB identification and detection of drug resistance in primary patient specimens should not replace standard diagnostic methods (including clinical, microbiological and radiological assessment) and conventional drug susceptibility testing for the diagnosis of active TB in patients with pulmonary and extrapulmonary TB.

Expert opinion

The overall opinion of the expert group regarding the use of rapid molecular assays to support diagnosis of TB and detection of drug resistance is as follows:

The evidence analysed supports the use of these rapid molecular assays for TB identification and detection of drug resistance, particularly rifampicin drug resistance, as rapid supplements to standard diagnostic methods and conventional drug susceptibility testing in pulmonary smear-positive TB patients, especially to rule out MDR-/XDR-TB. On pulmonary smear-negative and extrapulmonary specimens the performance of the molecular assays varies and should be considered separately for each specific group and specimen type.

Evidence does not support the routine use of the LPA/Cepheid Xpert assays for TB identification and detection of drug resistance in smear-negative pulmonary specimens. Nevertheless, these tools may be used as a rapid supplement to standard diagnostic methods for the diagnosis of TB and drug resistant TB in non-respiratory specimens. There is limited evidence to support the use of these tests for cerebrospinal fluid at this time.

Expert opinion

The overall opinion of the expert group regarding the use of rapid molecular assays for diagnosis of TB and detection of drug resistance in HIV-infected individuals and children is as follows:

At present there is limited evidence on the use of the rapid molecular assays to identify TB and detect drug resistance in TB/HIV-coinfected individuals. There is some evidence to support the use of rapid methods for TB identification and drug resistance detection in HIV/TB coinfected individuals in smear-positive TB when combined with standard methods for diagnosing active TB and conventional drug resistance.

At present there is clear lack of evidence on the use of the rapid molecular assays to identify TB and detect drug resistance in children. However, despite the lack of evidence due to the clinical nature of TB in children, the expert group in principle recommends use of the rapid methods for TB identification and drug resistance detection in children suspected of smear-positive pulmonary TB, both with or without HIV-coinfection when combined with standard methods for diagnosing active TB and conventional drug resistance.

Expert opinion

The opinion of the expert group regarding the use of rapid molecular assays to support diagnosis of resistance to ethambutol and second line, reserve drugs is as follows:

Based on the evidence, the expert group supports the use of the Hain GT MTBDR*sl* assay (GTsl) for early identification of drug resistance to the fluoroquinolones, amikacin, kanamycin and capreomycin on MDR-TB isolates as a laboratory tool in addition to standard drug susceptibility methods. The GTsl should not replace standard diagnostic methods and conventional drug susceptibility testing.

However, based on limited evidence, the GTsl cannot at present be recommended for use directly on primary clinical specimens.

The GTsl assay cannot be recommended for the detection of ethambutol drug resistance.

Implementation of rapid molecular assays (especially LPAs) for the rapid detection of TB, MDR-TB and XDR-TB should take place in laboratories with proven capacity to run molecular tests and where quality control systems have been implemented. Once MDR-TB/XDR-TB is identified there is a need for samples to be cultured so that extended drug-susceptibility testing can be performed for adequate clinical management. Laboratories performing these tests should have proven expertise and infrastructure with adequate internal and external quality procedures in place. The main principles for implementing rapid molecular assays are described in detail in the recently published handbook on TB diagnostic methods[i].

[i] European Centre for Disease Prevention and Control. Mastering the basics of TB control: Development of a handbook on TB diagnostic methods. Stockholm: ECDC; 2011.

Foreword

This document was developed by the European Reference Laboratory Network for TB (ERLN-TB). It presents the opinion of an ERLN-TB appointed expert group on the use of rapid molecular assays for the diagnosis of TB and detection of drug-resistance.

The main document consists of the following core sections:

- Background (including brief analysis of the current situation, objectives and details on evidence analysed and how the document was prepared).
- Background information on rapid molecular assays for the diagnosis of TB and detection of drug-resistance.
- Evidence-based expert opinions on frequently asked questions, preceded by the expert groups' considerations and followed by summary of evidence presented at the expert group meeting
- Future needs and considerations, where recommendations in regard to future studies, opinion-based recommendations and considerations in regard to implementation of the tools are summarised.
- Annexes 1 and 2: Annex 1 contains tables summarising assay performance characteristics classified by the type of assay. Annex 2 contains tables and computations summarising extracted evidence classified by each frequently asked question.

The field of rapid molecular diagnostic assays for tuberculosis is a rapidly developing field. Given the heterogeneous TB epidemiology and setting in the EU/EEA, ECDC and the ERLN-TB identified the need to provide evidence-based support to Member States on the utility of these assays in the EU/EEA-context. This document presents a basis for EU/EEA Member States when they consider the use of the rapid molecular assays in their TB control programmes within their TB diagnostic schemes and services. Given the rapid development within the area of molecular diagnostic assays, ECDC together with the ERLN-TB will look to update the current document regularly in order to ensure available guidance in this area of TB diagnosis.

1 Background

1.1 Current situation

Multi-drug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) are threats to the elimination of tuberculosis (TB) worldwide. The ability to rapidly and accurately detect drug resistance in *Mycobacterium tuberculosis* clinical specimens is essential for appropriate treatment to be initiated in patients suffering from TB and for the prevention of further spread of drug-resistant strains. This is of paramount importance for TB control and control of drug-resistant TB at a national, European Union (EU) and global level. New, highly specific, sensitive, and rapid tools to detect active TB and drug resistance are evidently needed.

Nucleic Acid Amplification Technologies (NAAT) based on amplification of specific fragments of nucleic acids (usually followed by hybridization to specific probes to ensure specificity) offer a rapid alternative to conventional bacteriological methods. Of these, Line-Probe Assays (LPAs) and the recently developed automated nucleic acid amplification technology for simultaneous and rapid detection of tuberculosis and rifampicin resistance (Cepheid Gene Xpert MTB/RIF) are most advanced and capable of simultaneous detection of *M. tuberculosis* Complex and resistance to rifampicin (RIF; widely recognized as a marker of MDR-TB) [1]. Line-probe assays can identify resistance to some other TB drugs. The current guidance document will therefore concentrate specifically on commercial LPAs and the Cepheid Gene Xpert MTB/RIF.

In September 2008, the World Health Organization (WHO) formally endorsed a policy on the use of the LPAs for the rapid screening of patients at risk of MDR-TB, with general guidelines for their implementation[1]. The policy was based on the opinion of an expert group who assembled and assessed the existing scientific evidence. It recommends the use of commercial LPAs (to ensure reliability and reproducibility of results) for the detection of TB and drug resistance in *M. tuberculosis* isolates and smear-positive sputum specimens.

There are currently three main LPAs for the rapid detection of RIF-resistance and MDR/XDR-TB available on the market, all also detecting *M. tuberculosis*: INNO-LiPA Rif.TB (INNOLIPA; Innogenetics, Zwijndrecht, Belgium), Genotype MTBDR/MTBDR*plus* (GT/GT*plus*) and Genotype MTBDR*sl* (GTsl;Hain Lifescience, GmbH, Germany). INNOLIPA detects only RIF-resistance, GT/GT*plus* detect both RIF and isoniazid (INH) resistance, and the GTsl detects resistance to fluoroquinolones, injectable second line drugs and ethambutol. These tests are designed for use on both *M. tuberculosis* isolates and primary respiratory specimens (although not all have full regulatory approval for all uses, e.g. in children and other specific groups and regulations in different countries vary).

In addition, in December 2010, WHO also endorsed the fully automated real-time (RT)-PCR based NAAT assay for the detection of *M. tuberculosis* DNA and mutations associated with resistance to RIF for use directly on primary respiratory specimens (Cepheid Gene Xpert MTB/RIF system, Cepheid Xpert Inc., Sunnyvale, CA, USA)[2]. Hereon forward, for the simplicity of terminology / wording this assay will be referred to as Cepheid Xpert.

Once a new tool for TB is developed and readily available, whether it is a diagnostic method, new drug or new vaccine, a key challenge is to ensure its rapid and optimal adoption, introduction and implementation in a country's National TB Control Program (NTP) and/or Health Care system [3].

Although TB and drug-resistant TB rates in most EU countries are stable and declining, expansion of the EU and increasing population migration rates pose challenges to the NTPs in the EU. Prevalence of TB is still relatively high in a number of EU Member States (Bulgaria and Romania) and drug-resistant TB remains a serious problem in the Baltic States [4]. Several EU Member States have already implemented the LPAs and are planning to integrate Cepheid Xpert into their NTPs. In addition, these assays are currently being used for the rapid diagnosis of suspected MDR-TB patients. As stated in the WHO endorsement policy for the commercial LPAs, there is a need to support countries in introducing these assays at the country level in a manner adapted to the country's TB, MDR-TB, XDR-TB epidemiological and resource situation [1].

ECDC therefore aimed to develop a guidance document, in the format of 'frequently asked questions', on the operational aspects to consider when implementing the LPAs and Cepheid Gene Xpert MTB/RIF system (hereon denoted as Cepheid Xpert) at a country level. Within this guidance the aim is to bring into consideration the different TB epidemiological and clinical situations within the EU based on the scientific evidence available, and to assure optimal information for all EU Member States requesting such guidance. The guidance is offered against an understanding that many EU countries do not have a formal national TB control programme.

1.2 Objectives

The aim of this guidance document is to present the most recent scientific evidence and ERLN-TB expert group opinion on the use of LPA/Cepheid Xpert assays for the diagnosis of tuberculosis and detection of drug-resistance. It presents several aspects to consider when implementing rapid molecular assays, including the accuracy of the assays, their application within different patient groups and/or TB incidence settings, and future research needs in order to provide the Member States with support when considering the introduction of rapid molecular assays in NTPs and/or tuberculosis control strategies. This document should provide a strong evidence base, complement global policies and be tailored to the needs and capacities of the EU.

1.3 Methods

1.3.1 Development of evidence-based ERLN-TB opinion at EU level

An evidence-package consisting of the two key systematic reviews on the subject (see 1.3.5), other reviews, meta-analyses and scientific papers reporting on the performance of the commercial LPA/Cepheid Xpert assays, was assembled by a core writing group identified by, and consisting of ERLN-TB experts [5–8]. The evidence covered the time period from December 2004 to May 2011 (INNOLIPA), from April 2008 to May 2011 (Hain GT assays); for Cepheid Xpert all publications meeting the selection criteria were included. Full description of the search strategy, publication selection criteria, collection, consideration and presentation of evidence is given in section 1.3.5.

A guidance document to present the evidence was then drafted (the current document): the extracted and reviewed evidence was collected and structured in a frequently asked question format by the writing group. An ERLN-TB expert group comprising experts in TB and related areas, including biomedical statistics, convened and considered the presented evidence and expressed their expert opinion (May 2011, London). Their expert opinion is presented in Section 3 of the current report, preceding the presented evidence.

Core writing group composition:

- Co-ordinator Professor Francis Drobniewski (UK)
- Deputy co-ordinator Mr Ulf Dahle (Norway)
- Writer/rapporteur Dr Dimitrios Papaventsis (Greece)
- Writer/rapporteur Dr Didi Bang (Denmark)
- Project Scientist Dr Vladyslav Nikolayevskyy (UK)

ERLN-TB appointed expert group composition:

- Dr Daniela Cirillo (Italy)
- Dr Yanina Balabanova (Germany and UK)
- Dr Susan Liebeshchwitz (UK)
- Mr Andre Charlett (UK)
- Dr Girts Skenders (Latvia)
- Dr Vera Katalinic-Jankovich (Croatia)

1.3.2. Molecular methods considered

For the current guidance, commercial molecular methods for rapid TB identification and detection of drug resistance to first- and second-line drugs were considered.

Since the performance of in-house molecular assays has not been adequately validated and are not recommended for clinical use in patients and have also not been endorsed by WHO, only commercially available rapid molecular assays were included in the current document [1,5]. These are:

A. Line Probe Assays (LPA) for rapid identification of *M. tuberculosis* and detection of drug resistance. Only WHO-endorsed methods are included as follows:

Assay 1: LPA for the identification of *M. tuberculosis* and the detection of rifampicin-resistance

InnoLiPA RIF.TB Kit (INNOLIPA) – Innogenetics, Ghent, Belgium

Assay 2: LPA for the identification of *M. tuberculosis* and the detection of resistance to rifampicin and/or isoniazid

GenoType® MTBDR and MTBDR*plus* (GT / GT*plus*) - Hain Lifescience, Nehren, Germany

Assay 3: LPA for the identification of *M. tuberculosis* and the detection of resistance to fluoroquinolones, injectable drugs and ethambutol

GenoType® MTBDR*sl* (GTsl) - Hain Lifescience, Nehren, Germany

B. Other assays:

Assay 1: Automated nucleic acid amplification technology for simultaneous and rapid detection of tuberculosis and rifampicin resistance

Cepheid Gene Xpert MTB/RIF (Cepheid Xpert)- Cepheid Xpert Inc., Sunnyvale, CA, USA

1.3.3 Frequently Asked Questions

The ERLN-TB core writing and expert group (see 1.3.1) identified the following frequently asked questions :

- Is there a role for LPA/Cepheid Xpert assays for the rapid diagnosis of TB and the detection of drug resistance in individuals suspected of TB?
  - Can the LPA/Cepheid Xpert assays be used for the rapid diagnosis of TB and detection of drug resistance in smear positive pulmonary TB specimens in adults?
  - Can the LPA/Cepheid Xpert assays be used for the rapid diagnosis of TB and detection of drug resistance in smear positive pulmonary TB specimens in HIV-positive adults?
  - Can the LPA/Cepheid Xpert assays be used for the rapid diagnosis of TB and detection of drug resistance in smear positive pulmonary TB specimens in children (both HIV-negative and HIV-positive)?
  - Can the LPA/Cepheid Xpert assays be used for the rapid diagnosis of TB in smear negative pulmonary TB specimens in adults?
  - Can the LPA/Cepheid Xpert assays be used for the rapid diagnosis of TB and detection of drug resistance in smear negative pulmonary TB specimens in HIV-positive adults?
  - Can the LPA/Cepheid Xpert assays be used for the rapid diagnosis of TB and detection of drug resistance in smear negative pulmonary TB specimens in children (both HIV-negative and HIV-positive)?
  - Can the LPA/Cepheid Xpert assays be used for the rapid diagnosis of extrapulmonary TB in adults (both HIV-negative and HIV-positive)?
  - Can the LPA/Cepheid Xpert assays be used for the rapid diagnosis of extrapulmonary TB and detection of drug resistance in children (both HIV-negative and HIV-positive)?
  - Can the LPA assays be used for the rapid diagnosis of isoniazid-resistant TB?
  - Can the LPA assays be used for the rapid diagnosis of ethambutol-resistant TB and resistance to other reserve drugs?
- Is there a role for LPA/Cepheid Xpert assays for the rapid diagnosis of TB and detection of drug resistance in other special groups such as immunocompromised individuals?
- Is there a role for LPA/Cepheid Xpert for the rapid diagnosis of TB and detection of drug resistance in contact tracing initiatives?

- Is there a role for LPA/Cepheid Xpert for the rapid diagnosis of TB and detection of drug resistance in patients that have initiated anti-TB treatment?

1.3.4 Collection of the evidence

Data from scientific papers, systematic reviews and meta-analyses reporting on the performance of commercial LPA/Cepheid Xpert assays were used to assess the evidence base on the performance characteristics of these assays and are summarised below.

1.3.5 Study search strategy

Baseline information: Key publications and process of evidence extraction

The following two key publications (systematic reviews performed by WHO-appointed expert groups and presented to the WHO Strategic and Technical Advisory Group for TB; STAG-TB) regarding LPAs were initially identified and used as the basis for the analyses regarding both LPAs and the Cepheid Xpert. The search strategy (including keywords, databases, and templates for tables) used in the current assessment was similar to that adopted in the two listed systematic reviews to ensure consistency in the evidence collection, assessment and presentation as well as to avoid necessity of re-assessment of studies already included in the existing reviews [6,7]:

- Morgan, et al. (2005). A commercial line probe assay for the rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*: a systematic review and meta-analysis. BMC Infect Dis 5: 62.
- Ling, et al. (2008). GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. Eur Respir J. 32:1165–74.

A description follows below of the study search strategy; as mentioned, it followed the methods used by the two above-listed systematic reviews.

Published methods for systematic reviews and meta-analyses of diagnostic tests to evaluate pooled and individual test accuracy and performance characteristics were followed [6,7]. Titles and/or abstracts of all citations found were screened independently by two reviewers using predefined inclusion criteria, with consensus on articles warranting full text review. Discrepancies were reconciled by consensus.

The following QUADAS criteria were used to assess study quality [9]:

- Study design (i.e. cross-sectional vs. case-control)
- Prospective enrolment of consecutive patients or random recruitment
- Comparison with an appropriate reference standard
- Blind and independent comparison of the index test with a reference standard
- Verification (partial or complete) of the index test results by reference standards

Statistical analysis was conducted using MedCalc v.11.0 and GraphPad Prism software. Sensitivity, specificity, PPV and NPV with 95% confidence intervals, were used as primary measures of diagnostic accuracy, using conventional culture and drug susceptibility test (DST) results as the reference standard. These values were considered by the expertgGroup as evidence when providing experts' opinion.

Databases used

- PubMed, Embase, Biosis, Web of Science, Google Scholar (>December 2004 for INNOLIPA)
- PubMed, Embase, Biosis (>April 2008 for GT/GTplus)
- Cepheid Xpert and GTsl: PubMed, Google Scholar, BIOSIS, EMBASE
- Reference lists from included papers

Keywords and search terms used for each assay

- InnoLiPA RIF.TB Kit: "Tuberculosis", "Mycobacterium tuberculosis", "Tuberculosis, Multidrug-Resistant", "Drug Resistance", "Drug Resistance, Bacterial", "rifampicin", "Rifampicin", "mutation", "mutant", "rpob", "rpob gene", "line probe", "line probe assay", "LiPA", and "INNO-LiPA"
- GenoType® MTBDR and GenoType® MTBDRplus: "tuberculosis", "Mycobacterium tuberculosis", "Hain LifeScience", "line probe assay", "GenoType MTBDR" and "molecular diagnostic techniques".
- GenoType® MTBDRsl: "tuberculosis", "Mycobacterium tuberculosis", "Hain LifeScience", "line probe assay" and "GenoType MTBDRsl".
- Cepheid Gene Xpert MTB/RIF: "tuberculosis", "Mycobacterium tuberculosis", "Cepheid Xpert", "Real-Time PCR" ''Gene Xpert MTB/RIF" and "molecular beacons".

Study selection criteria

The following criteria for selection of publications (similar to those used by Morgan et al. 2005 and Ling et al. 2008) were used [6,7]:

- Language of publication: English
- Comparison of LPA or Cepheid Xpert with reference standards for diagnosis of TB and detection of drug resistance (conventional DST).
- Evaluation of a minimum of tested samples to avoid potential selection bias in small studies (>10 sensitive, >10 resistant, >20 total) and to provide sufficient information on accuracy (sensitivity, specificity, PPV, NPV, kappa).
- When more than one comparison was made, comparisons were considered separately (rifampicin, isoniazid, fluoroquinolones, second line injectable drugs and ethambutol).
- Inclusion of available studies submitted or awaiting publication (cut-off date: 15 May 2011)

Templates

Based on the two key publications and following the format presented in key publications (Morgan et al., 2005, and Ling et al., 2008) template tables were constructed to extract the evidence and present the data in a format consistent with the previous systematic reviews and meta-analysis [6,7].

Presentation of the evidence

Study characteristics for all the included papers are presented in Annex 1 and the detailed extracted data/evidence is presented in Annex 2 [10–41].

2 Background on the rapid molecular assays for *M. tuberculosis* identification and detection of drug-resistance

2.1 What are Line Probe Assays and the Cepheid Gene Xpert MTB/RIF?

Line-probe assays are generally based on the amplification of gene fragments specific for *M. tuberculosis* and/or associated with drug-resistance, followed by the hybridization to specific probes immobilised on membranes. These assays are the most advanced compared to other NAAT since they are capable of simultaneous detection of species of the *M. tuberculosis* complex and drug resistance-conferring mutations. They can also be used both directly on clinical specimens (sputum etc) and on bacterial cultures.

The Cepheid Xpert assay is a Real-Time (RT)- PCR based assay capable of simultaneous detection of *M. tuberculosis* and resistance to RIF directly on clinical specimens (sputum etc).

INNO-LiPA Rif.TB

The commercially available INNO-LiPA Rif. TB kit (INNOLIPA; Innogenetics, Zwijndrecht, Belgium) is an LPA able to identify the *M. tuberculosis* complex and simultaneously detect genetic mutations in the region of the *rpo*B gene associated with RIF resistance. The oligonucleotide probe array contains 10 oligonucleotide probes (one specific for the *M. tuberculosis* complex, five overlapping wild-type "S" probes, and four "R" probes for detecting specific mutations associated with resistance) immobilised on nitrocellulose paper strips.

The INNOLIPA is performed by extracting DNA from cultures or directly from clinical samples and, using PCR, amplifying the RIF-resistance determining region of the *rpo*B gene. Biotinylated PCR products are then hybridized with the immobilised probes, and results are determined by colorimetric development. The *M. tuberculosis* isolate is considered RIF-susceptible if all of the wild-type S probes give a positive signal and all of the R probes react negatively. RIF-resistance is indicated by the absence of one or more of the wild-type S probes. When RIF-resistance is due to one of the four most frequently observed mutations, a positive reaction is obtained with one of the four R probes.

Genotype MTBDR, Genotype MTBDR*plus* and Genotype MTBDR*sl*

The GenoType MTBDR*plus* (GT*plus*) test allows the detection of the *M. tuberculosis* complex and the simultaneous detection of resistance to RIF and/or INH by the detection of resistance-conferring mutations in the *rpoB* and *katG/inhA* (high/low level isoniazid resistance) genes, respectively. The original version, the GenoTypeMTBDR is no longer commercially available.

The GenoType MTBDR*sl* (GTsl) detects *M. tuberculosis* and the simultaneous detection of resistance to fluoroquinolones (e.g. ofloxacin and moxifloxacin) and/or aminoglycosides/cyclic peptides (injectable antibiotics such as capreomycin, viomycin/kanamycin, amikacin) and/or ethambutol. This is through the detection of mutations in the relevant genes associated with resistance to these drugs. The GT*plus* and GT*sl* are validated for Mycobacterial DNA specimens extracted from both positive smear-positive pulmonary specimens and on *M. tuberculosis* cultures. According to manufacturer's recommendations, these tests should not be used to detect *Mycobacteria* directly from smear-negative material, unless the laboratory independently validates their use.

The GT assays include three steps: DNA extraction, multiplex PCR amplification, and reverse hybridisation. The GT assay has an additional advantage over the INNOLIPA as it can detect both RIF-and INH-resistance. All three assays are based on the same principles described above for the INNOLIPA.

Cepheid Gene Xpert MTB/RIF

Recent advances in RT-PCR technology have led to the development of the first automated, sputum processing, and real-time-based molecular beacon assay; the Cepheid Gene Xpert MTB/RIF assay (Cepheid Xpert; Cepheid Xpert Inc., Sunnyvale, CA, USA). This assay allows the simultaneous detection of the *M. tuberculosis* and RIF resistance-conferring mutations, directly on sputum samples, using ultra-sensitive hemi-nested PCR in a closed cartridge system. No information on particular genes affected and/or mutations identified is included in the report generated by this system.

2.2 What are the advantages and disadvantages of rapid molecular methods?

The main advantage of the molecular assays is speed: they can identify *M. tuberculosis* and detect mutations in genes associated with resistance to anti-TB drugs, reducing the time for drug resistance detection to one to two days. This compares to conventional culture and DST methods that are slow and final DST results are normally only available within four to six weeks (solid media) and one to two weeks (liquid media) once growth of the pure culture is available [42]. The handling of samples is eased as molecular methods only require high biosafety conditions at the initial steps; specimen processing (decontamination) and DNA extraction renders the samples non-infectious allowing further analysis to be performed using NAAT laboratory facilities outside Containment 3 level laboratories. Furthermore, molecular methods may provide specification of the *M. tuberculosis* complex species as well as specific mutation information not obtainable with the conventional DST methods. The detection of exact RIF mutations may for example indicate whether RIF-resistant *M. tuberculosis* strains are susceptible to rifabutin [42].

There are a number of disadvantages with the molecular methods that must be considered and kept in mind. Compared to the gold standard, conventional microbiological culture and DST assays, the molecular methods are unable to determine the proportion of drug-resistant bacteria present in the sample. Thus, molecular methods may have difficulties in detecting strains with heteroresistance i.e. mixed wild-type and mutant strains or the levels of conventional drug resistance [42]. Molecular methods may further detect silent mutations that do not confer phenotypic drug-resistance, therefore presenting false resistant results. As not all resistance-conferring mutations are covered by the commercial assays, the performance of the molecular assays may vary in different geographical settings with a high prevalence of specific *M. tuberculosis* resistance genotypes; with the exception of RIF, only a proportion of resistance-conferring mutations are known for specific anti-TB drugs. In general, this means that in many cases, rapid molecular methods cannot replace conventional DST, but rather serve as a rapid screening method and/or supplement to conventional culture and DST [42].

Direct and indirect costs of LPAs and the Cepheid Xpert

The authors of the current systematic review were unable to obtain actual costs of the assays from all the national centres, but are aware of complex individual arrangements that determine the final price obtained, especially where intermediate distributors are involved. Detailed actual cost data is needed together with usage data, to perform cost-benefit analyses of these assays. Further analysis of cost-benefit was not possible in the current document, but would be of importance in deciding local, regional and national use.

2.3 Definitions for accuracy

Below follow the definitions used for accuracy when performing the systematic reviews.

Sensitivity

Sensitivity measures the ability of a test to correctly identify individuals who have a certain disease. In the context of rapid molecular assays for the detection of *M. tuberculosis*, sensitivity denotes the proportion of individuals with known TB who test positive when rapid molecular assays are used, i.e. the ability of the molecular assays to correctly identify individuals with TB and classify them as test-positive.

With regard to the detection of drug resistance, sensitivity denotes the proportion of individuals with known drug-resistant TB who test positive when rapid molecular assays are used, i.e. the ability of the molecular assays to correctly identify individuals with drug-resistant TB and classify them as test-positive. Resistance to RIF is widely recognised as a surrogate marker of MDR-TB (since monoresistance to RIF is considered to be uncommon).

Specificity

Specificity measures the ability of a test to correctly identify individuals who do not have the disease under investigation. In the context of the rapid molecular assays to detect TB, specificity denotes the proportion of individuals known not to have TB and who test negative when the assay is used, i.e. the ability of molecular assays to correctly diagnose individuals who do not have TB and classify them as test-negative.

In the context of the rapid molecular assays to detect drug resistance, specificity denotes the proportion of individuals known not to have drug resistance and who test negative when the assay is used, i.e. the ability of rapid molecular assays to correctly diagnose individuals who do not have drug-resistant TB and classify them as test-negative.

3 Expert opinions and summary of evidence

For each question on the applicability of rapid molecular assays for TB identification and detection of drug resistance, the core writing group extracted and presented the evidence (see 1.3 for details) which was then considered by the ERLN-TB expert group members. The expert group expressed its opinion. The evidence was complemented with other published systematic reviews and meta-analyses [6–8].

Unless specified, both LPAs and Cepheid Xpert are collectively referred as to 'rapid molecular assays' throughout the document.

Considerations

- Following international standards, active TB is diagnosed by evaluating a patient's medical history, physical examination, radiography, and identifying *M. tuberculosis* bacteria using microbiologic and molecular diagnostic methods (smear microscopy, *M. tuberculosis* culture and nucleic acid amplification) [43,44].
- MDR-TB poses a global health problem of great concern. Emergence of the more recently defined XDR-TB is an urgent call to improve standards of care and treatment outcomes for these patients across Europe.
- In some instances, the diagnosis of drug-resistant TB is difficult and time-consuming, slowed down by the time it takes for a culture to become positive, and the turnover time for DST which may take approximately four to six weeks [45]. Pure growth of bacteria is required before conventional DST can be performed. Conventional DST results may be inconclusive due to poor growth or contamination with other microorganisms.
- New useful, sensitive, and rapid molecular tools to detect TB and drug resistance within one to two days have become available. Such rapid new assays for the direct detection of *M. tuberculosis* combined with drug resistance detection may complement conventional TB diagnosis and drug resistance detection.
- Molecular LPA and the RT-PCR-based assay (Cepheid Xpert MTB/RIF) have been developed for detecting TB and drug resistance. They can be performed on cultured TB isolates or directly on pretreated primary specimens. The molecular assays detect DNA and cannot differentiate between viable or dead bacteria.
- Some assays (INNOLIPA and Cepheid Xpert) have been designed to detect TB and resistance to RIF only, while others (GT*plus*) are able to detect both RIF-and INH-resistance in primary specimens and cultures. This is why direct comparison of assays was not always possible (see below). Rifampicin drug-resistance has previously been shown to be a good surrogate marker of MDR-TB [46]; however recent pan-European data indicate that a significant proportion of RIF-resistant strains are not MDR. Monoresistance to RIF, however, also poses treatment difficulties.
- In March 2008, WHO and UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases convened an expert group that reviewed the evidence and recommendations on the 'Use of the Molecular line probe assays for rapid screening of patients at risk of MDR-TB in low-income and middle-income countries' and a policy statement was endorsed [1]. The policy included information on laboratory infrastructure, human resources requirements, published literature on laboratory validation studies, and field demonstrations studies of programmatic implementation. In 2008, STAG-TB endorsed the WHO policy statement on use of line-probe assays for rapid screening of patients at risk of MDR-TB [1].
- In September 2010, WHO convened an expert group that reviewed the evidence and recommendations on the use of the automated Real-Time NAAT for rapid and simultaneous detection of TB and RIF resistance (Cepheid Xpert) and a policy statement was developed. In 2010, STAG-TB endorsed the findings of the expert group [2].
- These policies opened up for the global use of the commercial LPAs and the Cepheid Xpert system for use directly on smear-positive specimens or on cultured material under relevant biosafety levels (as appropriate). However, these policies have not previously been adapted to high and medium income settings that exist in many countries of the European region.

3.1 Is there a role of LPA/Cepheid Xpert assays for the rapid diagnosis of TB and the detection of drug resistance in individuals suspected of TB?

Expert opinion

Based on the evidence, rapid molecular assays for TB identification and detection of drug resistance should not replace the standard diagnostic methods (including microbiology, molecular tests, and clinical and radiological assessment) and conventional drug susceptibility testing for diagnosing active TB in pulmonary and extrapulmonary TB patients.

Evidence supports the use of the rapid molecular assays for TB identification and detection of drug resistance particularly rifampicin drug-resistance as rapid supplements to standard diagnostic methods and conventional drug susceptibility testing in pulmonary smear-positive TB patients.

Evidence

General

The current systematic review assessed the accuracy of rapid molecular assays for TB identification and detection of drug resistance in pulmonary and extrapulmonary samples. The assessment included studies with specific data on sensitivity and specificity. Indeterminate results were excluded before sensitivity and specificity were calculated.

Detection of rifampicin drug resistance with rapid molecular assays

Rifampicin drug resistance has previously been shown to be a good surrogate marker of MDR-TB [46]. The following evidence presents the accuracy of the LPA/Cepheid Xpert assays in detection of RIF- resistance directly in primary pulmonary and extrapulmonary clinical specimens, regardless of smear grade.

As listed in Table 1, the pooled sensitivity (95% CI) for TB detection in primary clinical specimens for INNOLIPA, Cepheid Xpert and GT*plus* was 85% (84–86%) and 91% (90–92%), respectively.

Table 1. Sensitivity of the rapid molecular assays for the detection of TB in primary clinical specimens*

	Pooled sensitivity (%)	95% CI	Number of studies	Total number of subjects with determinate results
INNOLIPA	85	84–86	7	14 372
Cepheid Xpert	91	90–92	11	7 377

**Annex 1 and Annex 2*

Based on Table 1, the authors of the current systematic review concluded that the pooled sensitivity of the rapid molecular assays to detect TB was high (>85%) and support their use as rapid tests for TB detection as a supplement to the gold standard conventional culture. For the GT*plus* assay, only limited data was available since primary samples were not analysed separately in many studies [17–20,23,25].

As listed in Table 2, the pooled sensitivity (95% CI) for RIF-resistance detection directly on primary clinical specimens for INNOLIPA, GT*plus*, and Cepheid Xpert was: 93% (89–96%), 96% (94–97%), and 98% (97–99%), respectively.

Table 2. Sensitivity of the rapid molecular assays for the detection of rifampicin-resistance in primary clinical specimens*

	Pooled sensitivity (%)	95% CI	Number of studies	Total number of subjects with determinate results
INNOLIPA	93	89–96	6	3 794
GT*plus*	96	94–97	8	1 579
Cepheid Xpert	98	97–99	7	2 831

**Annex 1 and Annex 2*

Based on Table 2, the authors of the current systematic review concluded that the pooled sensitivity of rapid molecular assays to detect RIF-resistance in primary clinical specimens was high (>93%) and support their use as rapid tests to detect RIF -resistant TB as a supplement to the gold standard conventional culture DST.

As listed in Table 3, the pooled specificity for excluding TB in primary specimens for INNOLIPA and Cepheid Xpert was 95% (94–95%) and 98% (98–99%), respectively.

Table 3. Specificity of the rapid molecular assays for exclusion of TB in primary clinical specimens*

	Pooled specificity (%)	95% CI	Number of studies	Total number of subjects with determinate results
INNOLIPA	95	94–95	7	14 372
Cepheid Xpert	98	98–99	11	7 377

**Annex 1 and Annex 2*

Based on Table 3, the rapid molecular assays have a high value for excluding TB in primary specimens, and the assays can be used as a useful supplement to the gold standard conventional culture. For the GTplus assay, primary samples were not analysed separately and specificity data is not available.

The available evidence and accuracy was found to depend on the type of specimen analysed (smear-positive, smear-negative, pulmonary, extrapulmonary with or without HIV-coinfection i.e. where the amount of bacilli and so DNA available varied; please refer to the below frequently asked questions for detailed results on the specific specimens and/or patient groups of interest).

As listed in Table 4, the pooled specificity (95% CI) of the INNOLIPA, GT*plus*, and Cepheid Xpert for excluding RIF-resistance was: 99% (99–100%), 92% (90–94%), and 99% (98–99%), respectively.

Table 4. Specificity of the rapid molecular assays for the exclusion of rifampicin resistance directly in primary clinical specimens*

	Pooled specificity (%)	95% CI	Number of studies	Total number of subjects with determinate results
INNOLIPA	99	99–100	6	3 794
GT*plus*	92	90–94	8	1 579
Cepheid Xpert	99	98–99	7	2 831

**Annex 1 and Annex 2*

Based on Table 4, the high pooled specificity for the exclusion of RIF-resistant TB (>92%) implies that a high proportion of individuals who do not have RIF-resistant TB would test negative, were the rapid molecular assays to be used to diagnose RIF- resistant TB. The authors of the current systematic review concluded that the high specificity of the rapid molecular assays indicated the high value of the assays in the diagnosis of RIF-resistant TB, directly in primary specimens depending on the type of specimen tested (smear-positive, smear negative, pulmonary or extrapulmonary specimen).

Based on the above systematic analysis, the rapid molecular assays have a high value for diagnosing RIF- resistant TB, and the assays can be used as a useful supplement to the gold standard conventional DST to primarily rule-out rifampicin resistant TB. The authors of the current systematic review further conclude that the high accuracy of rapid molecular assays in detecting RIF-resistance may indicate that the assays may be valuable tools in detecting MDR-TB (since monoresistance to RIF is uncommon). The available evidence and accuracy was found to depend on the type of specimen analysed (smear-positive, smear-negative, pulmonary, extrapulmonary with or without HIV-coinfection i.e. where the amount of bacilli and so DNA available varied).

3.1.1 Can the LPA/Cepheid Xpert assays be used for the rapid diagnosis of TB and detection of drug resistance in smear positive pulmonary TB specimens in adults?

Expert opinion

Evidence supports the use of the LPA/Cepheid Xpert assays for TB identification and detection of drug resistance in smear-positive pulmonary TB specimens as rapid rule-out tests for rifampicin drug-resistant TB in supplement to the gold standard conventional DST.

Evidence

General

The current systematic review assessed the accuracy of the rapid molecular assays for TB identification and detection of drug resistance and showed that these assays have been extensively used in adults and have shown high sensitivities and specificities. The authors of the current review find that they may be recommended for use in smear positive pulmonary adults in addition to the gold standard methods [6–8,10,12,13,16,17,19,23,31,32,36–39,41].

Sensitivity for TB detection

As listed in Table 5, the pooled sensitivity (95% CI) of INNOLIPA and Cepheid Xpert was 93% (92–94%) and 98% (98–99%), respectively.

Table 5. Sensitivity of the rapid molecular assays for TB identification directly on smear positive pulmonary specimens*

	Pooled sensitivity (%)	95% CI	Number of studies	Total number of subjects with determinate results
INNOLIPA	93	92–94	4	4 481
Cepheid Xpert	98	98–99	7	4 986

**Annex 1 and Annex 2*

The pooled sensitivity for the INNOLIPA and Cepheid Xpert was ≥93%. Based on this analysis, the authors of the current systematic review concluded that the pooled sensitivity of the INNOLIPA and the Cepheid Xpert for the identification of TB in smear-positive pulmonary specimens was high and support their use in the rapid detection of TB directly on primary pulmonary specimens. For the GT*plus* assay, pulmonary smear-positive samples were not analysed separately. However, evidence supports the use of the GT*plus* assays in smear-positive specimens as the performance was found to be high in several studies [17–20,23,25].

Specificity for TB detection

As listed in Table 6, the pooled specificity (95% CI) of INNOLIPA, and Cepheid Xpert was 83% (81–85%), and 99% (99–99%), respectively.

Table 6. Specificity of rapid molecular assays for the exclusion of TB in smear positive pulmonary specimens*

	Pooled specificity (%)	95% CI	Number of studies	Total number of subjects with determinate results
INNOLIPA	83	81–85	4	4 481
Cepheid Xpert	99	99–99	7	4 986

**Annex 1 and Annex 2*

The pooled specificity for the INNOLIPA and Cepheid Xpert was ≥83%. Based on the analysis, the authors of the current systematic review concluded that the pooled specificity of the INNOLIPA and the Cepheid Xpert for excluding the presence of TB in smear-positive pulmonary specimens was sufficiently high and support their use in the rapid exclusion of TB directly on such samples. For the GT*plus* assay, the specificity for smear positive samples was not analysed separately, however the authors of the current review predict similar findings to the other rapid molecular assays with regards to specificity.

Likewise, the evidence below supports the use of the rapid LPA/Cepheid Xpert tests for the detection of MDR-TB directly in smear-positive pulmonary specimens, as a supplement to the gold standard DST.

As listed in Table 7, the pooled sensitivity (95% CI) of the GT*plus* assay for the detection of MDR-TB was: 92% (89–98%).

Table 7. Sensitivity of the rapid molecular assays for the detection of MDR-TB directly on smear positive pulmonary specimens*

	Pooled sensitivity (%)	95% CI	Number of studies	Total number of subjects with determinate results
GT*plus*	95	89-98	3	405

**Annex 1 and Annex 2*

Based on the analysis, the authors of the current systematic review concluded that the sensitivity of GT*plus* assay was sufficiently high for the detection of MDR-TB, and support its use as rapid test to detect MDR-TB in supplement to the gold standard conventional DST methods. For the Cepheid Xpert assay, only one of the studies included analysis of MDR-TB detection and found a sensitivity of 98% for the detection of MDR-TB in pulmonary specimens regardless of smear grade (n=200) [38]. In the rest of the Cepheid Xpert and INNOLIPA studies included in the analysis, data was insufficient or they did not analyse the detection of RIF resistance/MDR-TB separately.

As listed in Table 8, the pooled specificity (95% CI) of the GT*plus* assay for the exclusion of MDR-TB was: 99% (96–100%).

Table 8. Specificity of the rapid molecular assays for the exclusion of MDR-TB directly on smear positive pulmonary specimens*

	Pooled specificity (%)	95% CI	Number of studies	Total number of subjects with determinate results
GT*plus*	98	95–99	3	405

**Annex 1 and Annex 2*

The high specificity >99% of the GT*plus* assay implies that a high proportion of individuals who do not have MDR-TB resistance would test negative with the GT*plus* assay. The authors for the current systematic review concluded that the high specificity of the GT*plus* assay indicates the high value of the assay in the ruling out MDR-TB directly in smear-positive pulmonary specimens. Even though for INNOLIPA and Cepheid Xpert studies, data was insufficient or they did not specifically analyse MDR-TB detection, given the high RIF sensitivities and specificities found in section 3, the authors of the current review predict that for MDR-TB detection with these assays, similar findings would be found.

3.1.2 Can the LPA/Cepheid Xpert assays be used for the rapid diagnosis of TB and detection of drug resistance in smear positive pulmonary TB specimens in HIV-positive adults?

Considerations

More studies addressing the accuracy of rapid molecular assays in the diagnosis of active TB and the detection of drug resistance in HIV-positive pulmonary smear-positive and smear-negative patients are urgently needed to allow for the analysis of especially the LPA and Cepheid Xpert assays accuracy in this sub-group of patients.

Expert opinion

At present there is limited evidence on the use of the rapid molecular assays to identify TB and detect drug resistance in TB/HIV-coinfected individuals who tend to present with paucibacillary disease. However, there is some evidence to support the use of the rapid methods for TB identification and drug resistance detection in HIV infected individuals suspected of TB when combined with standard methods for diagnosing active TB and conventional drug resistance given the clinical importance of early diagnosis.

Evidence

HIV-infected individuals are at a higher risk of reactivating a latent TB infection and progressing faster to TB after infection. Immunosuppression due to HIV-infection may lead to a lower bacillary load, making the diagnosis of active TB by sputum microscopy more difficult [47]. Immunosuppressed HIV-infected individuals require highly active antiretroviral therapy (HAART) and the presence of drug-resistant TB in these patients further complicates treatment. Therefore, it is essential that new diagnostic tools which rapidly detect drug resistance in this subgroup of patients are developed and evaluated.

Table 9. Sensitivity of the Cepheid Xpert assay for TB identification directly on smear positive pulmonary specimens of HIV co-infected individuals [35,38]

	Sensitivity (%)	95% CI	Total number of subjects with determinate results
Boehme [38]	98	92–99	86
Theron [35]	70	55–80	130

Performance characteristics of rapid molecular assays in HIV-infected individuals were specifically addressed in two studies only [35,38]. These studies (Table 9) found good evidence for the assay's application in primary samples of smear-positive pulmonary TB/HIV co-infected individuals with active disease [35,38]. Based on these findings, the authors of the current systematic review concluded that the Cepheid Xpert assay can be recommended for use in smear-positive pulmonary TB/HIV co-infected individuals as a supplement to existing gold standard methods. The authors further highlight though, that there is a need for further research into the rapid molecular assays for TB identification and detection of drug resistance in HIV-infected individuals suspected of TB with differing levels of immunosupression.

In the study by Theron et al. the accuracy (sensitivity and specificity) of the Cepheid Xpert in patients with HIV infection was determined for different strata defined by CD4 T-cell counts [35].

As listed in Table 10, the sensitivity for the Cepheid Xpert compared to smear microscopy in patients with >200, and <200 CD4 T-cells/ml was 76%, and 65%, respectively. Specificity was 97% and 93%, respectively.

Table 10. Sensitivity and specificity of the Cepheid Xpert for the diagnosis of active TB in patients with HIV infection, stratified by CD4 T-cell count [35]

CD4 count (cells/ml)	Sensitivity (%)	Specificity (%)	Total number of subjects
≥200	76	97	57
<200	65	93	66

The authors of the current systematic review concluded that for HIV patients with active TB and ≥ 200 CD4 T cells/ml or <200 CD4 T cells/ml, Cepheid Xpert demonstrated the same sensitivity as compared to smear (no statistical difference, p=0.32) and that the Cepheid Xpert sensitivity was not affected by CD4 T-cell count. However, more studies on CD4 T-cell stratification in patients with TB HIV-coinfection are warranted.

3.1.3 Can the LPA/Cepheid Xpert assays be used for the rapid diagnosis of TB and detection of drug resistance in smear positive pulmonary TB specimens in children (both HIV-negative and HIV-positive)?

Considerations

- Future studies on the use of the rapid molecular assays need be conducted in children with smear-positive and smear-negative pulmonary TB, both with or without HIV-coinfection, given the importance of rapid TB diagnosis and drug resistance detection for these subgroups.
- Despite the lack of evidence, the expert group concluded that there is value in recommending the use of the rapid assays for TB identification and detection of drug resistance in children suspected of smear-positive pulmonary TB, as it is predicted that the accuracy of the LPA/Cepheid Xpert tests would be similar to those of adults with smear-positive pulmonary TB and would, in principle, be of clinical value in children.
- The expert group also concluded that there is value in recommending the use of the rapid assays for TB identification and detection of drug resistance in children suffering from smear-positive pulmonary TB/HIV co-infection, as it is predicted that the accuracy of the LPA/Cepheid Xpert tests would be similar to those of adults with smear-positive pulmonary TB, and there is in principle clinical value of performing the tests in these children. There is an urgent need to evaluate the rapid molecular assays in children with suspected TB/HIV co-infection.

Expert opinion

At present there is limited evidence on the use of rapid molecular assays to identify TB and detect drug resistance in children. However, despite the lack of evidence due to the clinical nature of TB in children, the expert group in principle recommends the additional use of rapid methods for TB identification and drug resistance detection in children suspected of smear-positive pulmonary TB, both with or without HIV-coinfection when combined with standard methods for diagnosing active TB and conventional drug resistance.

Evidence

General

Children, particularly infants under two years of age, are at an increased risk of infection and developing active TB, which may further be in disseminated form. The diagnosis of TB in children is challenging as symptoms may be non-specific. Furthermore, sputum samples are difficult to obtain from children, and only 10-15% of active TB cases in children are diagnosed by smear microscopy. Many children are therefore diagnosed by culture of gastric lavage or treated on clinical grounds alone. As children are vulnerable and often have more severe TB disease from fewer bacteria, obtaining a rapid DST result is essential. There is currently no evidence to support the use of rapid molecular assays for TB identification and detection of drug resistance directly in specimens from children.

HIV-infected children

As with adults, HIV co-infection with active TB disease is very complicated to diagnose and treat in children. At present there is no evidence on the use of the rapid molecular assays to identify TB and detect drug resistance in children infected with HIV and with a positive smear result.

3.1.4 Can the LPA/Cepheid Xpert assays be used for the rapid diagnosis of TB in smear negative pulmonary TB specimens in adults?

Expert opinion

Evidence does not support the routine use of the LPA/Cepheid Xpert assays to identify TB in individuals suspected of smear-negative pulmonary TB as a stand-alone tool.

Evidence

Sensitivity for TB detection

As listed in Table 11, the pooled sensitivity (95% CI) of INNOLIPA and Cepheid Xpert was 65% (58–71%) and 75% (72–78%), respectively.

Table 11. Sensitivity of the rapid molecular assays for the detection of TB directly on smear negative pulmonary specimens*

	Pooled sensitivity (%)	95% CI	Number of studies	Total number of subjects with determinate results
INNOLIPA	65	58–71	2	1 442
Cepheid Xpert	75	72–78	7	4 466

**Annex 1 and Annex 2*

The pooled sensitivity of the INNOLIPA and Cepheid Xpert were low and the authors of the current systematic review concluded that these assays cannot be recommended as rapid tests for detection of TB in individuals suspected of pulmonary smear-negative TB. For GT*plus* the authors predict that similar sensitivity would be found.

Specificity for TB detection

As listed in Table 12, the pooled specificity (95% CI) of INNOLIPA and Cepheid Xpert was 96% (94–97%) and 99% (99-99%) respectively.

Table 12 Specificity of rapid molecular assays for the exclusion of TB directly on smear negative pulmonary specimens*

	Pooled specificity (%)	95% CI	Number of studies	Total number of subjects with determinate results
INNOLIPA	96	94–97	2	1 442
Cepheid Xpert	99	99–99	7	4 466

**Annex 1 and Annex 2*

The pooled specificity of the INNOLIPA and Cepheid Xpert were sufficiently high (>96%) and the authors of the current systematic review concluded that these assays could be used as a rapid supplement to standard diagnostic methods to correctly rule out individuals who do not have the disease.

3.1.5 Can the LPA/Cepheid Xpert assays be used for the rapid diagnosis of TB and detection of drug resistance in smear negative pulmonary TB specimens in HIV-positive adults?

Considerations

More studies addressing the rapid molecular assays accuracy in the diagnosis of active pulmonary TB smear-negative HIV-positive individuals are needed to allow for the analysis of especially the LPA and Cepheid Xpert assays accuracy in this sub-group.

Expert opinion

At present there is limited evidence on the use of the rapid molecular assays to identify TB and detect drug resistance in smear-negative TB/HIV-coinfected individuals. There is some evidence to support the use of the rapid methods for TB identification and drug resistance detection in HIV-infected individuals suspected of smear-negative pulmonary TB when combined with standard methods for diagnosing active TB and conventional drug resistance.

Evidence

HIV-infected adult patients

TB in HIV-coinfected individuals with smear-negative pulmonary TB is difficult to diagnose and relies on TB culture for a definitive diagnosis. Patients with smear-negative pulmonary TB and HIV-coinfection may be immunosuppressed due to HIV-infection resulting in a lower bacillary load, making the diagnosis of active TB more difficult [47].

The treatment with HAART for severe HIV and the presence of drug-resistant TB may further complicate the choice of TB treatment. Therefore, it is essential that new diagnostic tools which rapidly detect drug resistance in this subgroup of patients become available.

Table 13. Sensitivity of the Cepheid Xpert assay for TB identification directly on smear negative pulmonary specimens of HIV co-infected individuals [35,38]

	Sensitivity (%)	95% CI	Total number of subjects with determinate results
Boehme [38]	72	63–79	124
Theron [35]	47	29–67	130

Two studies, Theron et al. and Boehme et al. (Table 13) have tested the Cepheid Xpert in individuals with TB/HIV co-infection and found some evidence for its use in smear-negative TB/HIV co-infected individuals with active disease [35,38]. Based on these findings, and taking into consideration that TB and drug resistance is difficult to diagnose in this subgroup, the authors of the current systematic review concluded that the Cepheid Xpert assay can be recommended for use in smear-negative TB/HIV co-infected individuals. The authors further believe that the other LPAs may have similar performance as the Cepheid Xpert and may also be used for individuals suspected of pulmonary smear-negative TB/HIV co-infected individuals as a supplementary tool. However, there is an urgent need for further research into the use of the rapid molecular assays for TB identification and detection of drug resistance in TB/HIV-coinfected individuals.

3.1.6 Can the LPA/Cepheid Xpert assays be used for the rapid diagnosis of TB and detection of drug resistance in smear negative pulmonary TB specimens in children (both HIV-negative and HIV-positive)?

Considerations

- Future studies on the use of the rapid molecular assays need be conducted in children with smear-negative pulmonary TB, both with or without HIV-coinfection, given the importance of rapid TB diagnosis and drug resistance detection of these subgroups.
- Despite the lack of evidence, the expert group concluded that there is value in recommending the use of the rapid assays for TB identification and detection of drug resistance in children suspected of smear-negative pulmonary TB, as there is in principle clinical value of running these tests in children.
- The expert group recommends the use of the rapid assays for TB identification and detection of drug resistance in children suspected of smear-negative pulmonary TB/HIV co-infection, as there is in principle clinical value of performing the tests in children. There is an urgent need for further studies on TB/HIV co-infection in children.

Expert opinion

At present there is limited evidence on the use of the rapid molecular assays to identify TB and detect drug resistance in children. However, despite the lack of evidence due to the clinical nature of TB in children, the expert group in principle recommends the use of the rapid methods for TB identification and drug resistance detection in children suspected of smear-negative pulmonary TB, both with or without HIV-coinfection, when combined with standard methods for diagnosing active TB and conventional drug resistance.

Evidence

General

Children, particularly infants are at an increased risk of infection and developing active TB. The diagnosis of TB in children is challenging as symptoms may be non-specific. Furthermore, sputum samples are difficult to obtain from children. The majority of children who have smear-negative pulmonary TB are diagnosed by culture of gastric lavage or treated on clinical grounds alone. As children are vulnerable and often have more severe TB disease from fewer bacteria TB, obtaining a rapid DST result is of essence. There is at the present time no evidence to support the use of the rapid molecular assays for TB identification and detection of drug resistance directly in specimens from children.

HIV-infected children

Active TB disease is very complicated to diagnose and treat in children with HIV co-infection. At present there is no evidence on the use of the rapid molecular assays to identify TB and detect drug resistance in children with smear-negative pulmonary TB.

3.1.7 Can the LPA/Cepheid Xpert assays be used for the rapid diagnosis of extrapulmonary TB in adults (both HIV-negative and HIV-positive)?

Expert opinion

Although less sensitive than for pulmonary TB, evidence supports the use of the LPA/Cepheid Xpert assays for TB identification in smear-positive extrapulmonary TB specimens as rapid rule-out tests in supplement to the gold standard conventional culture and DST.

There is not enough evidence to recommend using assays on cerebrospinal fluid.

Evidence

Sensitivity for TB detection

As listed in Table 14, the pooled sensitivity (95% CI) of INNOLIPA and Cepheid Xpert for extrapulmonary specimens was: 68% (65–71%) and 63% (49–75%), respectively.

Table 14. Sensitivity of the rapid molecular assays for the detection of TB directly on primary extrapulmonary clinical specimens*

	Pooled sensitivity (%)	95% CI	Number of studies	Total number of subjects with determinate results
INNOLIPA	68	65–71	4	5 286
Cepheid Xpert	63	49–75	4	177

**Annex 1 and Annex 2*

In the extrapulmonary specimens, the pooled sensitivity for INNOLIPA and Cepheid Xpert for TB identification was somewhat lower at 63–68%. For the GT*plus* assay only one study analysed the sensitivity of TB identification and found a sensitivity of 91% (n=10)[19].

Specificity for TB detection

As listed in Table 15, the pooled specificity (95% CI) of INNOLIPA and Cepheid Xpert for extrapulmonary specimens was: 94% (93–94%) and 96% (91–99%) respectively.

Table 15. Specificity of rapid molecular assays for the exclusion of TB directly on primary extrapulmonary clinical specimens*

	Pooled specificity (%)	95% CI	Number of studies	Total number of subjects with determinate results
INNOLIPA	94	93–94	4	5 286
Cepheid Xpert	96	91–99	4	177

**Annex 1 and Annex 2*

The high specificity (>94%) of the INNOLIPA and Cepheid Xpert assays implies that a high proportion of individuals who do not have active TB would test negative were rapid molecular assays to be used to diagnose active TB when applied to extrapulmonary specimens. The GT*plus* studies included in the analysis did not analyse identification of tuberculosis separately for extrapulmonary specimens.

Based on the evidence presented, the authors of the current systematic review concluded that the accuracy (sensitivity and specificity) of the rapid molecular assays in extrapulmonary specimens for TB identification was lower than for pulmonary specimens. However, rapid molecular assays may still be valuable as a rapid supplement in the identification of TB in certain situations. For instance, the evidence supports the use of the LPA/Cepheid Xpert directly in smear-positive extrapulmonary specimens, however not in smear-negative extrapulmonary specimens [12,16,19,32,36,41]. The LPA/Cepheid Xpert assays can be used for diagnosis directly on clinical extrapulmonary specimens such as biopsy material. Sensitivity of the assay, however, is suboptimal for cerebrospinal fluid.

3.1.8 Can the LPA/Cepheid Xpert assays be used for the rapid diagnosis of extrapulmonary TB and detection of drug resistance in children (both HIV-negative and HIV-positive)?

Considerations

- Future studies on the use of the rapid molecular assays need to be conducted in children with smear-positive and smear-negative extrapulmonary TB, both with or without HIV-coinfection, given the importance of rapid TB diagnosis and drug resistance detection for these subgroups.
- Children, particularly infants, are at an increased risk of infection and developing active TB. The diagnosis of extrapulmonary TB in children is challenging as symptoms may be non-specific. As children are vulnerable and often have more severe TB disease from fewer TB bacteria, obtaining a rapid DST result is essential. There is at present no evidence to support the use of the rapid molecular assays for TB identification and detection of drug resistance directly in extrapulmonary specimens from children. However, despite the lack of evidence, the expert group concluded that there is value in recommending the use of the rapid assays for TB identification and detection of drug resistance in children suspected of smear-positive or smear-negative extrapulmonary TB as there is in principle clinical value of running these tests in children.

Expert opinion

At present there is very limited evidence on the use of the rapid molecular assays to identify TB and detect drug resistance in children. However, despite the lack of evidence due to the clinical nature of TB in children the expert group in principle recommends the use of the rapid methods for TB identification and drug resistance detection in children suspected of with smear-positive or smear-negative extrapulmonary TB, both with or without HIV-coinfection when combined with standard methods for diagnosing active TB and conventional drug resistance.

Evidence

HIV-infected children

As with adults with an HIV co-infection, active extrapulmonary TB disease is very complicated to diagnose and treat in children. At present there is no evidence on the use of the rapid molecular assays to identify TB and detect drug resistance in children with extrapulmonary TB. However, authors of the current review concluded that there is value in recommending the use of the rapid assays for TB identification and detection of drug resistance in children suspected of smear-positive and smear-negative extrapulmonary TB/HIV co-infection, as there is in principle clinical value of performing the tests in these children. There is an urgent need for further studies on TB/HIV co-infection in children.

3.1.9 Can the LPA assays be used for the rapid diagnosis of isoniazid-resistant TB?

Expert opinion

Based on the evidence, the expert group concludes that the pooled sensitivity of the GT*plus* assay is not sufficiently high to support the use as a rule-out test for isoniazid-resistant TB. In some situations, the GT*plus* assay may be a useful rapid tool to detect isoniazid drug resistance when used as a supplement to the gold standard conventional isoniazid DST, such as in areas where isoniazid drug-resistant TB is prevalent, and for contact tracing of isoniazid drug-resistant cases.

Evidence

The GT*plus* assay is the only rapid molecular assay that, in addition to the detection of RIF-resistance also detects INH-resistance both at high (≥0.4 mg/l) and low levels (≤0.1mg/l) by detecting mutations in the genes *katG* and *inhA*, respectively.

As listed in Table 16, the pooled sensitivity (95% CI) of the GT*plus* in the detection of INH-resistance in smear positive pulmonary and extrapulmonary specimens was: 77% (69–83%).

Table 16. Sensitivity of the GTplus for the detection of isoniazid resistance directly on primary smear-positive clinical specimens (both pulmonary and extrapulmonary)*

	Pooled sensitivity (%)	95% CI	Number of studies	Total number of subjects with determinate results
GT*plus*	77	69–83	7	441

**Annex 1 and Annex 2*

Based on the evidence, the authors of the current systematic review concluded that the pooled sensitivity of the GT*plus* (77%) was not sufficiently high to support its use as test to detect INH-resistant TB. However, the GT*plus* assay may be a useful rapid tool to detect INH-resistance when used as a supplement to the gold standard conventional INH DST.

As listed in Table 17, the pooled specificity (95% CI) of the GT*plus* for the exclusion of INH-resistance in smear-positive pulmonary and extrapulmonary specimens was: 99% (97–100%).

Table 17. Specificity of the GTplus for the exclusion of isoniazid resistance on primary smear-positive clinical specimens (both pulmonary and extrapulmonary)*

	Pooled specificity (%)	95% CI	Number of studies	Total number of subjects with determinate results
GT*plus*	99	97–100	7	441

**Annex 1 and Annex 2*

The high specificity (>99%) implies that a high proportion of individuals who do not have INH-resistant TB would test negative with the GT*plus* assay. The authors for the current systematic review concluded that the high specificity of the GT*plus* assay indicates the high value of the assay in the diagnosis of INH-resistant TB directly in clinical specimens. However, the effect of identified INH-resistant TB on treatment outcome still remains to be determined [42].

The authors for the current systematic review further concluded that these results suggest that the GT*plus* assay represents a valuable rapid supplemental tool for the diagnosis of INH-resistant TB in individuals in areas with high INH-resistance levels and contact tracing of INH-resistance cases.

3.1.10 Can the LPA assays be used for the rapid diagnosis of ethambutol-resistant TB and resistance to other reserve drugs?

Expert opinion

There is enough evidence to support the use of GTsl for early identification of drug resistance to the fluoroquinolones, amikacin, kanamycin and capreomycin on MDR-TB isolates as a laboratory tool, supplementary to existing phenotypical (culture-based) methods.

Based on limited evidence, the GTsl cannot at present be recommended to be used directly on primary clinical specimens.

The GTsl assay cannot be recommended for the detection of ethambutol drug-resistance.

Evidence

Sensitivity for detection of drug resistance to fluoroquinolones, amikacin, kanamycin, capreomycin and ethambutol

The GTsl assay is the only rapid molecular assay that simultaneously identifies TB and detects drug resistance for the reserve drugs: the fluoroquinolones, amikacin, kanamycin and capreomycin, and additionally also mutations for the first-line drug ethambutol. Thus the assay allows for detection of XDR-TB.

As listed in Table 18, the pooled sensitivity (95% CI) of the GTsl for the detection of fluoroquinolones, amikacin, kanamycin and capreomycin in clinical isolates were: 85% (78–91%), 90% (81–96%), 83% (59–96) and 87% (77–94%), respectively.

Table 18. Sensitivity of the GTsl for the detection of drug resistance to fluoroquinolones, amikacin, kanamycin, capreomycin and ethambutol on clinical isolates*

	Pooled sensitivity (%)	95% CI	Number of studies	Total number of subjects with determinate results
Fluoroquinolones	85	78–91	5	297
Amikacin	90	81–96	3	246
Kanamycin	83	59–96	2	114
Capreomycin	87	77–94	3	246
Ethambutol	60	52–68	3	280

**Annex 1 and Annex 2*

Based on the analysis, the authors of the current systematic review concluded that the sensitivity of GTsl assay was high for the detection of resistance to fluoroquinolones, amikacin, kanamycin and capreomycin on clinical isolates. Only one study applied the GTsl assay directly on primary clinical specimens, albeit on only a few. Further evidence is required on the direct use on primary specimens [28]. For ethambutol, the pooled sensitivity (95% CI) of the GTsl assay was unacceptably low at 60% (52–68), and the authors therefore concluded that GTsl assay cannot be recommended to be used for ethambutol detection.

Specificity for detection of drug resistance to fluoroquinolones, amikacin, kanamycin, capreomycin and ethambutol

As listed in Table 19, the pooled specificity (95% CI) of GTsl for the exclusion of resistance to fluoroquinolones, amikacin, kanamycin and capreomycin was: 99% (97–100%), 100% (98–100%), 100% (96–100%), and 99% (96–100), respectively.

Table 19. Specificity of the GTsl for the exclusion of drug resistance to fluoroquinolones, amikacin, kanamycin, capreomycin and ethambutol on clinical isolates*

	Pooled specificity (%)	95% CI	Number of studies	Total number of subjects with determinate results
Fluoroquinolones	99	97–100	5	297
Amikacin	100	98–100	3	246
Kanamycin	100	96–100	2	114
Capreomycin	99	96–100	3	246
Ethambutol	98	94–100	3	280

**Annex 1 and Annex 2*

The high specificity >99% of the GTsl assay implies that a high proportion of MDR-TB individuals who do not have resistance to the reserve drugs would test negative. The authors of the current systematic review concluded that the high specificity of the GTsl assay indicates the high value of the assay in the ruling out resistance to reserve drugs in MDR-TB isolates. However, it must be noted that mutations in the *gyrB*, *eis* and some other genes are not covered by the assay and therefore the GTsl can only be recommended as a supplement to conventional second-line DST.

One study further assessed the application of the GTsl directly in 64 primary specimens and although promising, further studies are required before recommendations can be made on the use of the GTsl assay directly in clinical specimens [28]. The GTsl assay may prove to be a potential rapid tool to detect XDR-TB in the future.

3.2 Is there a role of LPA/Cepheid Xpert assays for the rapid diagnosis of TB and detection of drug resistance in other special groups such as immunocompromised individuals?

Immunocompromised individuals represent a heterogeneous group which includes patients receiving immunosuppressive treatment and patients with immunodeficiency disorders, such as chronic kidney diseases, HIV (addressed above), genetic or acquired immune defects, immunosuppression associated with other infections, and malignancies. There is currently no evidence on the use of the rapid molecular assays for TB identification and detection of drug resistance in other special groups with immunocompromised conditions (except for HIV infected); as for example among persons on immunosuppressive drugs, cancer patients, or in other situations such as testing of healthcare workers with TB, and in patients with severe TB disease residing in intensive care units. There is a need for further studies into the use of the rapid assays in all these fields.

3.3 Is there a role of LPA/Cepheid Xpert for the rapid diagnosis of TB and detection of drug resistance in contact tracing initiatives?

Contact tracing initiatives (on TB patients and/or individuals showing symptoms of TB) represent a potential for the use of rapid molecular assays for TB identification and detections of drug resistance. There is at present no evidence to support the use of the rapid molecular assays for contact tracing. However, from a theoretical point of view, finding the presence of different mutation patterns in relation to the golden standard bacterial subtype MIRU-VNTR and also the previous standard bacterial subtype RFLP may be used to in contact tracing initiatives [42]. The rapid assays for TB identification and detection of drug resistance may be of valuable use in close contacts to drug-resistant cases. More research into the use of the rapid LPA/Cepheid Xpert assays for contact tracing initiatives is required.

3.4 Is there a role of LPA/Cepheid Xpert for the rapid diagnosis of TB and detection of drug resistance in patients that have initiated anti-TB treatment?

There is currently no evidence for the use of the rapid molecular assays for TB identification and detection of drug resistance in patients that have initiated anti-TB treatment. The potential of monitoring patients with rapid molecular assays for TB identification and detection of drug resistance is unknown, especially in patients that are at risk of developing drug resistance due to treatment default (interrupted treatment for ≥2 months), treatment failure (a sputum smear or culture-positive case at ≥5 months or later during treatment), transfer out (a patient that left the area to another reporting and recording unit), non-adherence, malabsorption of drugs and other concurrent disease related problems. There is therefore a need for further studies into the use of the assays in these fields.

4 Future research needs and considerations

4.1 Identifying areas for future research

Evidence presented in the current document and experts' opinions demonstrate that rapid molecular assays have a diagnostic value in TB identification and detection of drug resistance, particularly in adult patients suspected of smear-positive pulmonary TB (including those HIV-positive) and for the diagnosis of some forms of extrapulmonary TB. These methods, however, should not replace standard diagnostic methods (including clinical, microbiological and radiological assessment) and conventional drug susceptibility testing for diagnosing active TB and drug resistance (rifampicin and isoniazid) in individuals suspected of pulmonary and extrapulmonary TB.

Molecular tests may be used as a laboratory tool for the detection of resistance to selected reserve drugs (eg FQ, AG/CP,) in cultures and for ruling out XDR-TB.

While there is generally enough evidence to recommend using molecular methods for TB case detection and detection of RIF and INH resistance in pulmonary smear-positive specimens in adults, the diagnostic value of these tools in case detection and MDR/XDR-TB screening in specific groups (children, immunocompromised individuals, healthcare workers) still remains unclear largely due to a lack of available data.

Based on the evidence provided, the expert group has therefore identified areas for future research that are likely to provide the evidence needed to assess the diagnostic value of the rapid molecular tools.

- Rapid diagnosis of TB and drug-resistant TB is especially important in children; however there are only a few studies where rapid molecular tools were evaluated in children. Given the importance of validation of available tools and implementation of molecular tools in TB case detection and MDR/XDR-TB screening in children, including HIV-positive children, the expert group highlights the importance of conducting research in this particular area.
- There is currently enough evidence to recommend using rapid molecular tools for some types of extrapulmonary TB and detection of drug resistance. There is insufficient evidence for TB meningitis using cerebral spinal fluid (CSF) clinical specimens. The expert group would like to highlight the importance of conducting more research and validation/assessing diagnostic value of these tools when used on CSF clinical specimens.
- Although rapid molecular tools (GTsl) can be used as a laboratory tool for detection of resistance to selected reserve drugs (eg fluoroquinolones, aminoglycosides/capreomycin) in cultures and to rule out XDR-TB, there is currently not enough evidence to recommend using rapid molecular tools for the detection of resistance to the fluoroquinolones, injectable drugs and ethambutol on direct clinical specimens. Given the importance of XDR-TB screening, especially in areas with a high prevalence of MDR-TB, the expert group would like to highlight the importance of conducting more research in this area.
- In existing publications there is a clear lack of clinical data obtained from longitudinal studies, especially relating to disease outcome analysis and how using rapid molecular tools affects disease outcome. The expert group therefore considers conducting longitudinal studies with analysis of outcomes as one of the priority areas for future research.
- It is currently widely accepted that resistance to RIF serves as a good indicator of multi drug resistance. Across the EU, the proportion of RIF-monoresistant strains in 2005–2009 varied between 0.45% and 0.69% and proportion of polyresistant (but not MDR) RIF resistant strains varied between 0.53% and 0.74%, indicating that RIF resistance could be used as a valid predictor of MDR-TB. However, significant proportions of INH-monoresistant strains in EU countries (varying between 5.82 and 6.35) along with relatively low treatment success rates in patients infected with INH-monoresistant strains (63.9% and 66.9% in 2007 and 2008 respectively) highlights the necessity of implementation of molecular tools capable of the detection of resistance to both RIF and INH.

4.2 Opinion-based recommendations

Although there was not enough statistically significant evidence to recommend using these rapid molecular tools in all populations and situations, the expert group believes that LPA/Cepheid Xpert systems should be considered for implementation given the importance of rapid detection of TB and drug resistance TB:

- In smear-negative HIV-negative adults in high-MDR-TB risk groups
- There is in principle clinical value in using these tests for diagnosis in children.

4.3 Main principles of rapid molecular assays implementation and usage

Implementation of the rapid molecular assays, especially LPAs, for rapid detection of TB, MDR-TB and XDR-TB should take place in laboratories with proven capability to run molecular tests and where quality control (QC) systems have been implemented. The main principles of rapid molecular assays implementation and usage include:

- availability of appropriate laboratory infrastructure and equipment meeting biosafety standards; DNA extraction for the LPAs should be performed within Cat 3 facilities
- prevention of amplicon contamination leading to false-positive results by separation of PCR zones and strict adherence to protocols and standard operating procedures
- laboratory staff should be trained to conduct each specific molecular assay
- appropriate maintenance/calibration of assays, laboratory facilities and equipment by authorised companies
- availability of standard operation procedures and mechanisms for their regular update and document control
- availability of internal and external QC systems to maintain and improve quality.

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