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Diagnosis of tuberculosis and drug resistance: what can new tools bring us?

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SUMMARY

This is an exciting time for tuberculosis (TB) diagnostics. The technology for rapid diagnosis of TB and rifampicin (RMP) resistance in pulmonary sputum smear-positive specimens is well advanced, and assays have high specificity with good sensitivity. Nevertheless, the current sensitivity of TB detection means that these assays still cannot replace the standard diagnostic methods for TB or conventional drug susceptibility testing (DST). In extra-pulmonary specimens, the performance of molecular tools varies and should be considered separately for each specimen type. Evidence for the use of these assays for TB and drug resistance detection in individuals co-infected with TB and the human immunodeficiency virus (HIV) is limited.

As the positive predictive value for RMP resistance reaches ⩾90% only when the prevalence of RMP resistance in new TB patients is >15%, which is rare globally, many cases with such resistance will be false-resistant, emphasising the need for a secondary confirmative test. Similarly, increased (or incorrect) diagnosis of TB may compromise programme effectiveness by increasing the numbers of individuals requiring anti-tuberculosis treatment, unless it is carefully planned.

For the future, 1) assays with greater sensitivity for TB detection are needed; 2) rapid diagnostics for paediatric TB are important, and there is a need for carefully designed studies, including those involving HIV-positive children; 3) more clinical data need to be obtained from longitudinal studies, especially related to the influence of rapid diagnostics on disease outcome; and 4) point-of-care tests using untreated sputum, blood or urine and little or no equipment would be of immeasurable benefit. Although great progress has been made, we are not there yet.

KEY WORDS: line probe assay; Xpert® MTB/RIF; NAAT; microcolony assay; immunoassay

THE WORLD HEALTH Organization (WHO) estimated that there were 8.8 million incident cases of tuberculosis (TB) in 2010, with 1.1 million deaths due to TB among human immunodeficiency virus (HIV) negative people and an additional 0.35 million deaths due to HIV-associated TB.[1] In 2010, there were 5.7 million notifications of new and recurrent cases of TB, equivalent to 65% of the estimated number of incident cases in 2010, reflecting the real difficulties in the diagnosis of active TB. The WHO estimates that about 440 000 multidrug-resistant (MDR-TB) cases (defined as resistance to at least isoniazid [INH] and rifampicin [RMP]) occur every year, of whom 150 000 will die. The fatality rate for extensive drug-resistant (XDR-TB; i.e., MDR-TB plus resistance to any fluoroquinolone [FQ] and amikacin [AMK], capreomycin [CPM] or kanamycin [KM]) is even higher, especially with HIV co-infection. In May 2009, a resolution of the 62nd World Health Assembly (WHA62.15) urged Member states to take action to achieve universal access to diagnosis and treatment of MDR-/XDR-TB by 2015.

Although point-of-care diagnostic tests are under development, accurate diagnosis of both drug-susceptible and drug-resistant TB currently requires some form of laboratory infrastructure. Laboratory facilities, including staff, equipment and infrastructure, were previously neglected, but recent diagnostic developments have offered the hope that this weak link can be improved. Nevertheless, in 2010, eight of the 22 high-burden countries (HBCs) did not meet the benchmark of one microscopy centre per 100 000 population: among the 36 countries in the combined list of 22 HBCs and 27 high MDR-TB burden countries,

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20 had less than the benchmark of one laboratory capable of performing culture and DST per 5 million population, although 16 of the 27 high MDR-TB burden countries had done so.[1]

Despite the excitement caused by the development of an automated diagnostic system for diagnosis of TB and RMP resistance (Xpert® MTB/RIF, Cepheid, Sunnyvale, CA, USA), by June 2011, 6 months after its endorsement by the WHO in December 2010, only 26 of 145 countries eligible to purchase the instruments and sample cartridges at concessional prices had done so, although at least 11 of the 27 high MDR-TB burden countries had successfully adopted the Xpert assay.[2]

This article starts with the premise that accurate diagnosis of active TB is a pre-requisite for any successful TB control programme: at the individual level, a missed case, particularly if sputum smear-positive, remains infectious to others, with the potential risk of the patient dying, while misdiagnosis of non-TB cases unnecessarily exposes the individual to potentially toxic drugs and wastes scarce public health resources. The article focuses on methods that have at least reached a proof-of-principle stage with patient specimens; methods focused on the rapid identification of cultures alone, for example, are not included, nor are interferon gamma release assays (IGRAs), which are primarily tools for identifying latent infection and have been extensively reviewed elsewhere.[3–5] It also excludes non-specimen/laboratory methodology such as novel digital X-ray systems. Existing systematic reviews and meta-analyses on the subject have been included where relevant.[6–9]

MICROSCOPY

Microscopy has been a diagnostic tool for TB for over a century, and thus, ironically, currently the most rapid diagnostic method is also the oldest. Microscopy is also useful in assessing infectivity and cure. Although not particularly sensitive, it is relatively inexpensive, simple to perform using modest equipment, particularly for standard light microscopy (LM), but it requires adequate training and time-consuming internal and external quality control processes to ensure high-quality results. Although not specific (i.e., acid-fast bacilli do not identify the mycobacterial species), in areas where TB prevalence is high and in patients with appropriate symptomology, the positive predictive value (PPV) for TB is likely to be high with sputum smear microscopy.

Fluorescent microscopy (FM) has clear advantages in that it is more sensitive than LM[10] and has a higher throughput; however, the equipment and bulbs are expensive, the bulbs need monitoring and frequent replacement, the microscopist needs to work in darkness for optimal performance, and even then fluorescent debris may be confused with TB bacteria.[11–13]

The recent development of light emitting diodes (LED), with the appropriate fluorescent light output for FM and low power consumption, has led to the development of simple, robust LED FM microscopes, requiring minimal mains or battery power and no dark room requirement. The WHO has recommended rolling it out as an alternative to LMs in resource-limited settings, based on studies that have shown comparable performance of LM and standard FM systems.[14–21] Minion et al., in a nested case-control study, compared the diagnostic accuracy and time required to read slides with the Zeiss PrimoStar iLED™ (Carl Zeiss MicroImaging, Jena, Germany), LW Scientific Lumin™ (LW Scientific, Lawrenceville, GA, USA) and a conventional fluorescence microscope (Leica DMLS, Leica Microsystems, Milton Keynes, UK) against mycobacterial culture as the reference standard.[22] They found no difference in sensitivity or specificity between the three microscopes. Lumin and conventional FM were equivalent with respect to the time required to read smears, but the Zeiss iLED was significantly more time saving than both of the other techniques.

It is, however, clear that even with LED, there are variations in performance in the different LED systems, and adequate training is required to ensure that overall performance is on a par with that obtained with experienced technicians using standard FM. In a Zambian study, which compared the performance of three LED FMs and standard FM against a culture reference standard following brief training among laboratory technicians in Zambia, LED FM performance was more varied. The Primo Star iLED, FluoLED™ (Olympus, Tokyo, Japan) and standard Olympus BX41TF (Olympus) had comparable sensitivity (respectively 67%, 65% and 65%), but here the performance of Lumin™ was significantly worse (56%, $P < 0.05$). Specificity and inter-operator reliability were low for all microscopes, suggesting that brief training was insufficient in this setting.[16]

Overall, for programmatic purposes, LED FM can reliably replace LM and standard FM systems, but where technicians have little or no experience of standard FM, LED training needs to be more extensive.

RAPID, CULTURE-BASED METHODOLOGY FOR DIAGNOSIS OF ACTIVE TB AND DRUG RESISTANCE

Even in the era of novel rapid molecular assays, conventional culture and DST remain the most sensitive methods for the detection of *Mycobacterium tuberculosis* and the mainstay for DST—including drugs for which there are no molecular tests—and monitoring the efficacy of treatment.[23]

However, traditional solid media culture techniques (commonly Löwenstein-Jensen media, made in-house or commercially), although relatively cheap, are slow. In-house liquid media, such as Kirchner or various

Middlebrook formulations (7H9, 7H10 or 7H11), have been widely used but often lack standardisation.[24] Semi-automated culture systems such as the Bactec 460 radiometric system (BD, Sparks, MD, USA) faced the problem of isotope disposal and have mostly been replaced by non-radiometric automated liquid media systems (ALMS), such as the BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960 (BD), MB/BacT (Biomerieux, Marcy l'Etoile, France) or the ESP Culture System II (AccuMed International, Chicago, IL, USA). These permit significantly faster culture growth (for smear-positive sputum samples within an average of 14 days and for most mycobacteria within 21 days).[25–27] These systems measure the changes in carbon dioxide production or oxygen consumption either fluorimetrically or colorimetrically, with positive growth detected automatically and continuously; best isolation rates are achieved when combining solid and liquid media systems.[24,27,28]

Major studies on culture performance have been reviewed previously,[24] and these have been extended by a small number of large evaluation and demonstration projects demonstrating that the systems perform well in low- and middle-income environments.[25,27]

Conventional (classic) DST methods are based on phenotypic detection of bacterial growth in the presence of antibiotics. DST on solid media is reliable when any core method is used, such as the 1% proportion method, absolute concentration or resistance ratio; however, it is slow, giving an overall turnaround time of 6–12 weeks from specimen culture.[26] The use of ALMS reduces the time to culture to 3–4 weeks. While DST against first-line drugs is considered to be equally reliable on solid and liquid media systems, there is much more controversy around the standardisation of DST for second-line and reserve drugs. The WHO policy of testing for second-line drug susceptibilities favours ALMS for most of these,[29] or no testing where methods produce conflicting results or where there is no clear link between assay results and clinical outcome.

The main drawbacks of modern ALMS are the high associated costs, the need for an uninterrupted supply of commercially manufactured consumables and the higher costs compared with solid media.[30,31] Since 2007, ALMS have been recommended by the WHO for use even in low- and middle-income countries. However, in conditions of restrained resources and high laboratory workload, it would be cost-saving to use ALMS for high-risk patients when the speed of diagnosis is crucial or other methods are insensitive, for example, in HIV-positive (paucibacillary) individuals or most infectious (smear-positive) patients suspected of having MDR-/XDR-TB, to guide individually tailored treatment regimens within the shortest possible time.

All culture-based systems require biosafety category 3 laboratory facilities and extensive training of personnel, particularly when liquid culture systems are used. Furthermore, it is imperative that all isolates grown on culture media be speciated at least to the level of *M. tuberculosis* complex or non-tuberculous mycobacteria;[25] to obtain optimal benefit from the rapidity of the ALMS and to ultimately reduce the overall turn-around time, rapid identification methods need to be used.[24]

Significant effort has been invested into further development of simple, alternative, non-commercial phenotypic methods such as the nitrate reductase assay (NRA), thin-layer agar (TLA) colour test (Color Test), the microscopic observation drug susceptibility assay (MODS), the colorimetric redox indicator (CRI) method and phage-based assays, most of which can be set up directly on specimens.[8,26,30,32] These methods can detect *M. tuberculosis* and resistance to INH and RMP. While MODS, NRA and CRI have been endorsed by the WHO, current evidence was considered to be insufficient for recommending the use of TLA or phage-based assays.[30]

The main advantages of these methods are that they utilise inexpensive and mainly generic laboratory materials, and most require limited staff training. Each has advantages and limitations: for example, the NRA has a moderate rate of indeterminate results and is unable to detect nitrate-reductase negative *M. tuberculosis,* although this is rare,[26,30] while the CRI can only be used on cultures.

MODS is an extensively validated method that has almost perfect agreement with conventional DST for INH, RMP and MDR-TB (100%, 97% and 99%). The results are available within a median of 7 days; the method is cheap, non-commercial and works well on all types of primary specimens as well as on isolates. On-line support for the method and video instructions are available using free Internet access (http://www.modsperu.org/). However, it requires relatively long, detailed staff training.[8,22,26,33–35]

A recent costing exercise showed that NRA and MODS are the most cost-effective technologies permitting testing of the largest number of patients,[26] and they therefore present a feasible alternative to commercial ALMS for DST in resource-constrained settings. However, the time to detection of drug resistance is not necessarily faster when using these tests, and the range of drugs that can be tested using the tests is currently limited. Conventional culture and DST are therefore still required in all settings.[30] The WHO currently recommends these assays to be used as an interim solution for resource-constrained settings.[30] Individual laboratories should decide on the most suitable algorithms to employ appropriate combinations of microbiological and molecular methods.

M. tuberculosis microcolony detection on TLA has been used for a long time.[36–38] Although it has been evaluated on primary specimens against other culture

methods, such as MGIT and LJ media, with good results,[22,39–40] there is still insufficient evidence for its endorsement globally.[30] It is an inexpensive, non-commercial technique, which only requires a 37°C incubator and a light microscope. TLA recently demonstrated a good performance of the MDR-/XDR-TB colour test for the identification of *M. tuberculosis* complex and detection of resistance to INH, RMP and ciprofloxacin in cultures.[41] Further validation studies directly using primary specimens are underway.

Although commercial systems have an advantage over in-house methods in terms of standardisation, internal and external quality assurance are essential for correct implementation of testing at every laboratory, irrespective of the methods used.[26]

NUCLEIC ACID AMPLIFICATION TEST APPROACHES: BENEFITS AND DISADVANTAGES

Nucleic acid amplification tests (NAATs) are molecular tests that are based on the highly specific detection of fragments of nucleic acids (DNA and/or RNA) of mycobacteria, followed by amplification of target sequences indicating the presence of *M. tuberculosis* complex (or subspecies) in the test species.

NAATs are characterised by their high sensitivity (theoretically they can detect as little as one copy of nucleic acid per specimen); however, in reality the sensitivity can be significantly compromised by the presence of inhibitors in the test material and loss of nucleic acid during specimen preparation. Specificity is normally very high, and can be improved further by the inclusion of hybridisation steps (hybridisation of polymerase chain reaction [PCR] products to specific immobilised probes); the latter also allows the use of NAATs for the detection of resistance to anti-tuberculosis drugs.

The main advantage of molecular methods is their speed compared to culture: they detect mutations in genes associated with resistance to anti-tuberculosis drugs and can reduce the time to detection of drug resistance to 1–2 days, while conventional DST methods are relatively slow and results are normally available in weeks or months, as described earlier. Handling is easier, as molecular methods require high biosafety conditions only initially; specimen processing and DNA extraction renders them non-infectious, allowing for further analysis potentially outside high containment.

The principal disadvantages of molecular methods of DST are that they are unable to determine the proportion of drug-resistant bacteria and may thus have difficulties in detecting strains with heteroresistance, i.e., mixed wild-type and mutant strains, or the levels of conventional drug resistance.[42] They may also detect silent mutations that do not lead to phenotypic DST drug resistance, producing false-resistant results,

Table 1 Commercially available NAA assays for tuberculosis detection in clinical specimens*

Assay	Manufacturer	Method	Material	Sensitivity % (95%CI)†	Specificity % (95%CI)†	PLR†	NLR†	Reference
Amplified MTD	Gen-Probe Inc, San Diego, CA, USA	Transcription-mediated amplification of RNA	DNA from decontaminated sputum	86.0 (74.2–93.7)	99.3 (96.3–100.0)	57.6 (25.5–129.9)	0.1 (0.07–0.22)	7, 52
COBAS® TaqMan® MTB Test	Roche Molecular Diagnostics, Pleasanton, CA, USA	RT-PCR	DNA from decontaminated sputum	91.5 (86.9–96.1) 79.1	98.7 (98.0–99.4) 98.2	—	—	47, 48
artus® *M. tuberculosis* PCR	Qiagen, Hilden, Germany	RT-PCR	DNA from decontaminated sputum	97.8 (93.6–95.5)	85.1 (75.8–91.8)	6.54 (4.0–10.8)	0.03 (0.01–0.08)	49
Loopamp® Tuberculosis Complex Detection Reagent Kit	Eiken Chemical, Tokyo, Japan	LAMP	Untreated sputum	88.2 (81.4–92.7)	—	—	—	50
Amplicor MTB	Roche Molecular Diagnostics	PCR amplification of 16S RNA	DNA from decontaminated sputum	—	—	26.04 (17.04–39.80)	0.15 (0.11–0.22)	7
Cobas Amplicor	Roche Molecular Diagnostics	PCR amplification of 16S RNA	DNA from decontaminated sputum	—	—	58.59 (37.77–90.86)	0.17 (0.13–0.22)	7
LCx	Abbott Laboratories, Abbott Park, IL, USA	Ligase chain reaction amplification of 38kDa protein	DNA from decontaminated sputum	88.9 (82.5–96.3)	96.8 (95.1–98.5)	26.91 (17.21–42.09)	0.16 (0.12–0.20)	7, 53
BD Probe Tec Direct	BD, Sparks, MD, USA	Strand displacement amplification of IS*6110* and 16S RNA	DNA from decontaminated sputum	77.5 (72.0–83.0)	98.0 (97.1–98.9)	20.11 (10.42–38.82)	0.06 (0.04–0.10)	7, 51

*Sales of many of these commercial assays have now been discontinued.
†Overall performance characteristics for both smear-positive and smear-negative specimens.
NAA = nucleic acid amplification; CI = confidence interval; PLR = positive likelihood ratio; NLR = negative likelihood ratio; RT-PCR = real-time PCR; LAMP = loop-mediated isothermal amplification; PCR = polymerase chain reaction.

and not all mutations are covered by the commercial assays; their performance may therefore vary in different geographical settings. Rapid molecular methods cannot therefore currently replace conventional DST, but can serve as a rapid supplement to conventional DST.[42]

Commercially available NAAT for TB diagnosis only (i.e., not resistance)

PCR is arguably the most common format of NAAT; other methodologies include ligase chain reaction, strain displacement amplification, loop-mediated isothermal amplification (LAMP) and transcription-mediated amplification.[7,43] More recently, real-time (RT) PCR technologies based on fluorescent-probe detection or melting-curve analysis have been developed.[44–46]

The performance characteristics for commercial NAATs in respiratory specimens (Table 1) are highly variable, with sensitivity lower and more inconsistent than specificity.[7,43,44,47–51]

LINE-PROBE ASSAYS AND XPERT MTB/RIF: TB DIAGNOSIS

Three main LPAs for the rapid diagnosis of TB and/or rapid detection of RMP resistance and MDR-/XDR-TB are currently available on the market: INNO-LiPA Rif.TB (Innogenetics, Zwijndrecht, Belgium), GenoType® MTBDR/MTBDR*plus* and GenoType® MTBDR*sl* (both Hain Lifescience, Nehren, Germany). These assays are based on the targeted amplification (PCR) of specific fragments of the *M. tuberculosis* genome, followed by hybridisation of PCR products to oligonucleotide probes immobilised on membranes. INNO-LiPA Rif.TB detects only RMP resistance, GenoType MTBDR/MTBDR*plus* detects both RMP and INH resistance, and GenoType MTBDR*sl* detects resistance to FQs, injectable second-line drugs and ethambutol (EMB). These tests are designed for use on both *M. tuberculosis* isolates and primary respiratory specimens. Xpert® MTB/RIF (Cepheid Inc, Sunnyvale, CA, USA) is a fully automated RT-PCR-based assay for the detection of TB bacteria and resistance to RMP in direct clinical specimens.

Two excellent reviews have summarised the performance of these assays,[6,54] showing good sensitivity for TB diagnosis. The pooled sensitivity and specificity for recent studies are given in Table 2.[44,45,55–64] For TB diagnosis in sputum smear-positive samples, these studies showed pooled sensitivities ranging from 93% to 98% and pooled specificities of 83% to 99%. One large national study conducted by Seoudi et al. over a decade,[62] which was included in the pooled analysis, examined 7836 consecutive patient samples using INNO-LiPA Rif.TB and the reference microbiological technique (conventional liquid and solid media culture with rapid molecular identification and culture-based drug resistance testing). For all sputum specimens (n = 3382), the sensitivity, specificity, PPV, negative predictive value (NPV), and accuracy for *M. tuberculosis* complex detection compared to reference microbiology were respectively 93.4%, 85.6%, 92.7%, 86.9% and 90.7%; the equivalent values for smear-positive sputum specimens (n = 2606) were 94.7%, 80.9%, 93.9%, 83.3% and 91.3%.

Sensitivity and specificity of the Xpert MTB/RIF assay for TB identification in HIV-positive individuals

Rapidity of detection is of great benefit, as HIV-infected individuals are at a higher risk of reactivation of latent TB infection. Conversely, immunosuppression, such as that due to HIV infection, may lead to a lower excreted bacillary load, making the diagnosis of active TB by sputum microscopy or culture more difficult.[65] The specificity of the assays would not be significantly different, but sensitivity is the critical issue. Two studies have described the performance of the Xpert MTB/RIF assay in patients co-infected with HIV, with good performance in active smear-positive pulmonary TB cases.[45,66] In the study by Theron et al., the sensitivity and specificity of Xpert MTB/RIF in patients with HIV infection was stratified on the basis of CD4 T-cell counts, giving a sensitivity compared to smear microscopy in patients with $\geqslant$200, and $<$200 CD4 T-cells/ml of respectively 76% and 65%. Specificity was respectively 97% and 93%. For HIV patients with active TB, the assay performed with equivalent sensitivity, regardless of CD4 threshold. Further studies on CD4 T-cell stratification in patients with TB-HIV co-infection are needed.

Table 2 Sensitivity and specificity of rapid molecular assays for TB detection in microbiologically culture-confirmed pulmonary TB clinical specimens*

Methodology	AFB status	Pooled sensitivity % (95%CI)	Pooled specificity % (95%CI)	Studies *n*	Subjects with determinate results *n*
INNO-LiPA Rif.TB, Innogenetics, Gent, Belgium	Smear-positive	93 (92–94)	83 (81–85)	4	4481
	Smear-negative	65 (58–71)	96 (94–97)	2	1442
Xpert® MTB/RIF, Cepheid Inc, Sunnyvale, CA, USA	Smear-positive	98 (98–99)	99 (99–99)	7	4986
	Smear-negative	75 (72–78)	99 (99–99)	7	4466

*Data extracted from references 44, 45, 55–64.
TB = tuberculosis; AFB = acid-fast bacilli; CI = confidence interval.

Table 3 Diagnosis of extra-pulmonary tuberculosis*

Methodology	AFB status	Pooled sensitivity % (95%CI)	Pooled specificity % (95%CI)	Studies *n*	Subjects with determinate results *n*
INNO-LiPA Rif.TB	All	68 (65–71)	94 (93–94)	4	5286
Xpert® MTB/RIF	All	63 (49–75)	96 (91–99)	4	177

* Data extracted from references 56, 58, 59, 62–64, 70 and 71.
AFB = acid-fast bacilli; CI = confidence interval.

Using LPA and Xpert MTB/RIF in diagnosing TB in children

Children, particularly infants aged <2 years, are at increased risk of infection and of developing active TB. The exact number of cases of childhood TB occurring each year is unknown, not least because the diagnosis of TB in children is challenging. At least 10–15% of TB cases globally and up to 25% of those in high TB burden countries occur in children. Paediatric TB thus represents a significant clinical and public health problem.[67,68]

Most of the evidence to support the use of these assays has come from studies in adults extrapolated to children. Symptoms may be non-specific, and sputum samples are difficult to obtain from children; only 10–15% of active TB cases in children are diagnosed by smear. Many children are therefore diagnosed using gastric lavage culture or treated on clinical grounds alone. Aside from the difficulty of obtaining samples, research on paediatric TB diagnosis has been neglected due to the poor performance of microbiological culture (the accepted reference standard in adults), the lower frequency of cavitary presentation of the disease and the perception that conducting TB research in children is too difficult.

Recent attempts co-ordinated by the US National Institutes of Health have proposed a consensus clinical case definition for research on intrathoracic TB in childhood and attempted to establish methodological issues that should be considered when conducting research on TB diagnostics in children.[68,69]

DIAGNOSING TB IN EXTRA-PULMONARY SPECIMENS

For extra-pulmonary specimens, the pooled sensitivity of INNO-LiPA and Xpert MTB/RIF for TB identification was somewhat lower, at 63–68% (Table 3). In one study (n = 10), the sensitivity of the MTBDR*plus* assay for TB identification was 91%.[72] The pooled specificity of INNO-LiPA and Xpert MTB/RIF for extra-pulmonary specimens was respectively 94% (95% confidence interval [CI] 93–94) and 96% (95%CI 91–99).

A large study of consecutive extra-pulmonary clinical specimens (n = 1476, from 1068 patients), including both paediatric (n = 494) and adult samples, has recently been published using the Xpert MTB/RIF assay.[73] Compared to a reference standard consisting of a combination of culture and clinical diagnosis of TB, an overall sensitivity and specificity of respectively 81.3% and 99.8% for the Xpert assay was obtained. These assays appear to have a role in screening selected extra-pulmonary specimens in addition to microbiological culture.

USE OF ASSAYS TO DIAGNOSE ISONIAZID RESISTANCE

The MTBDR*plus* assay is a commercial assay that can detect INH resistance in addition to RMP resistance. The assay detects both high (≥0.4 mg/l) and low level (≤0.1 mg/l) resistance by detecting mutations in the *kat*G and *inh*A genes, respectively.

Overall, in seven studies, the pooled sensitivity of the MTBDR*plus* assay for the detection of INH resistance was 77% (95%CI 69–83%), while the pooled specificity was 99% (95%CI 97–100%).[74–80] The GenoType MTBDR*plus* assay may be a useful rapid tool to detect INH drug resistance when used as a supplement to gold standard conventional INH DST; however, its sensitivity (77%) makes it unsuitable as a rule-out test for INH-resistant TB.

USE OF ASSAYS TO DIAGNOSE RIFAMPICIN RESISTANCE AND MDR-TB

RMP resistance can be a good surrogate marker of MDR-TB.[81] Most rapid commercial assays utilise the principle that MDR-TB is highly likely if RMP resistance is detected. Table 4 shows the accuracy of LPA assays in detecting RMP resistance directly

Table 4 Performance of rapid molecular assays in detecting rifampicin resistance compared with equivalent microbiologically detected rifampicin resistance in culture-confirmed cases*

Methodology	Pooled sensitivity % (95%CI)	Pooled specificity % (95%CI)	Studies *n*	Subjects with determinate results *n*
INNO-LiPA Rif.TB	93 (89–96)	99 (99–100)	6	3794
GenoType MTBDR*plus*	97 (92–99)	98 (95–99)	7	438
Xpert® MTB/RIF	98 (97–99)	99 (98–99)	7	2831

*Data extracted from references 44, 45, 55, 56, 58, 60–62, 64, 71, 74–80, 82 and 83.
CI = confidence interval.

Table 5 Sensitivity and specificity of the rapid molecular assays in the direct detection of rifampicin resistance in DST culture-confirmed clinical tuberculosis specimens*

Method	AFB status	Pooled sensitivity % (95%CI)	Pooled specificity % (95%CI)	Studies *n*	Subjects with determinate results *n*
INNO-LiPA Rif.TB	Smear-positive	94 (88–97)	100 (99–100)	4	2644
INNO-LiPA Rif.TB	Smear-negative	96 (77–99)	100 (97–100)	2	136

*Data extracted from references 60–63.
DST = drug susceptibility testing; AFB = acid-fast bacilli; CI = confidence interval.

in both clinical pulmonary and extra-pulmonary specimens, regardless of smear positivity, in several studies.[44,45,55,56,58,60–62,64,71,74–80,82,83] The pooled sensitivity of RMP resistance detection in patient specimens for the INNO-LiPA, MTBDR*plus* and Xpert MTB/RIF assays was respectively 93% (95%CI 89–96), 97% (95%CI 92–99) and 98% (95%CI 97–99), for the studies indicated. The pooled specificity of INNO-LiPA, MTBDR*plus* and Xpert was respectively 99% (95%CI 99–100), 98% (95%CI 95–99) and 99% (95%CI 98–99). Sensitivity is approximately proportional to smear status, i.e., the more bacteria per ml, the greater the probability of detection. Table 5 shows the sensitivity and specificity for RMP resistance detection using the INNO-LiPA Rif. TB assay divided by smear status.

In the study by Seoudi et al., the sensitivity, specificity, PPV, NPV and accuracy for detection of RMP resistance in all sputum samples (n = 1667) were respectively 92.1%, 99.3%, 89.4%, 99.5% and 98.9%;[62] the corresponding values for smear-positive sputum specimens (n = 1477) were respectively 93.3%, 99.3%, 87.5%, 99.6% and 99%. INNO-LiPA saved respectively 25.3 and 32.2 days for TB diagnosis and RMP resistance in smear-positive samples.

The high pooled sensitivity and specificity in detecting RMP resistance means that these assays are very useful in diagnosing RMP resistance directly in primary specimens, depending on the type of specimen tested and the bacterial load in the sample, i.e., in samples with a very low bacterial load there will be no amplification to establish TB identification initially and therefore no identification of RMP resistance either. As the PPV for RMP resistance only reaches ⩾90% when the prevalence of RMP resistance is >15%, which is globally rare among new TB cases, a substantial proportion of such resistance could represent false resistance. Confirmation of molecular findings using a second molecular assay or conventional DST is therefore essential.

USE OF ASSAYS TO ANALYSE MDR-TB DIRECTLY

The MTBDR*plus* assay identifies resistance to RMP and INH directly within the same assay. In two studies, the pooled sensitivity of the MTBDR*plus* assay for the detection of MDR-TB was 92% (95%CI 75–99) and the pooled specificity was 99% (95%CI 96–100) in comparison to microbiological DST of culture-confirmed smear-positive pulmonary specimens.[79,80] This level of performance would support their use as rapid rule-out screening tests for MDR-TB in smear-positive sputum specimens, supplementing subsequent conventional DST for MDR-TB detection. This was broadly the conclusion reached in several studies.[72,76,79,80,82,84]

USE OF MOLECULAR ASSAYS FOR THE RAPID DIAGNOSIS OF SECOND-LINE DRUG RESISTANCE

The MTBDR*sl* assay is the only rapid molecular assay that simultaneously identifies TB and detects resistance to the second-line drugs FQs, AMK, KM and CPM, and also mutations for EMB. The assay thus allows the detection of extensively drug-resistant TB (XDR-TB).

The pooled sensitivity of MTBDR*sl* in the detection of FQs, AMK, KM and CPM, drawn from between two and five studies, was respectively 85% (95%CI 78–91), 90% (95%CI 81–96), 83% (95%CI 59–96) and 87% (95%CI 77–94) when applied to cultured isolates (Table 6).[85–89] The pooled specificity of MTBDR*sl* for the detection of FQs, AMK, KM and CPM was respectively 100% (95%CI 97–100), 100% (95%CI 98–100), 100% (95%CI 96–100), and 99% (95%CI 96–100). In a blinded multicentre study of the performance of the MTBDR*sl* assay

Table 6 Sensitivity of the MTBDR*sl* assay in the detection of resistance to fluoroquinolones, amikacin, kanamycin, capreomycin and ethambutol in DST culture-confirmed primary specimens and isolates*

Methodology	Pooled sensitivity % (95%CI)	Pooled specificity % (95%CI)	Studies *n*	Subjects with determinate results n
Fluoroquinolones	85 (78–91)	100 (97–100)	5	356
Amikacin	90 (81–96)	100 (98–100)	3	246
Kanamycin	83 (59–96)	100 (96–100)	2	114
Capreomycin	87 (77–94)	99 (96–100)	3	246
Ethambutol	60 (52–68)	98 (94–100)	3	280

*Data extracted from-references 85–89.
DST = drug susceptibility testing; CI = confidence interval.

compared to ALMS using MGIT 960 in eastern Europe, sensitivity in the detection of resistance to FQs, EMB, AMK and CPM varied between 77.3% and 92.3%; it was much lower for KM (42.7%). Test specificity was >82% for all drugs.[90]

The generally high specificity (>99%) of the MTBDR*sl* assay makes it suitable for the rapid screening of cultured isolates for XDR-TB. Two small studies have assessed the direct application of MTBDR*sl* in respectively 64 and 59 primary specimens, with promising results;[89,91] for example, the detection of resistance to second-line injectable drugs (SLIDs; AMK, KM, CPM) showed a specificity of 89.1% (95%CI 77.0–95.3) and a PPV of 58.3% (95%CI 32.0–80.7). Sensitivity in the detection of FQ resistance was 100% (95%CI 64.6–100), whereas for SLID and EMB it was respectively 89.1% (95%CI 77.0–95.3) and 86.1% (95%CI 71.3–93.9).[91] Further large studies of its performance in the direct analysis of primary specimens are needed.

IMMUNOASSAYS FOR RAPID DETECTION OF ACTIVE TB

Detection of antibodies

Although the detection of antibodies against *M. tuberculosis* in the blood is a relatively simple and cost-effective method, recent meta-analyses and systematic reviews concluded that commercial serological tests provided inconsistent results.[92,93] As the overall test performance and data quality of these assays were poor, the WHO currently recommends against their use for the diagnosis of pulmonary and extra-pulmonary TB.[92] However, there has been some initial evidence that a serological approach may be significantly improved using the results of proteome analysis results.[94]

Detection of antigens

Lipoarabinomannan (LAM) was identified as a promising target for antigen detection for TB diagnosis due to its temperature stability; it could be detected in urine, which is normally easier to collect and safer to handle than sputum. LAM-based assays are currently being developed by a number of commercial companies, and preliminary results indicate their potential applicability in the rapid diagnosis of TB by detecting LAM in a variety of body fluids, including urine.[95–97] LAM-based assays are included in the WHO TB diagnosis re-tooling programme,[98] and form a part of a Foundation for Innovative New Diagnostics (FIND) funded TB project.

ASSAYS BEING DEVELOPED/EVALUATED

Transrenal DNA detection provides a challenging new target for molecular TB diagnosis from an accessible and abundant sample.[99] No commercial assays are currently available, largely due to the difficulties in the development of TB detection/read-out assays.

With a putative PPV of 100% and NPV of at least 99.9%, combined high-resolution melting (HRM) curve analysis using a closed-tube RT-PCR is potentially an ideal screening method for screening large specimen numbers in any TB laboratory.[46,100] New amplification methodologies and refinements of 'molecular beacon' approaches, such as linear-after-the-exponential PCR, offer future improvements, particularly in drug resistance analysis.[101]

CONCLUSIONS AND FURTHER WORK

This is an exciting time for new TB diagnostics. This is in part a reflection of the funding and application of good science, a clear understanding of unmet needs, a commercial sector that is considering new approaches to a global market, and the complexity of and limited progress in new drug and vaccine development, which has encouraged more academic and industrial partners to participate in diagnostic development.

Overall, the technology for the diagnosis of TB and RMP resistance in pulmonary specimens is well advanced, with high specificity and increasingly high sensitivity, and one may recommend the use of molecular methods, including LPAs and Xpert MTB/RIF, for TB case detection and the detection of RMP and INH resistance in pulmonary sputum smear-positive specimens. Rapid, high-specificity molecular assays for TB identification and drug resistance cannot replace the standard diagnostic methods, such as microbiology, clinical and radiological assessments, and conventional DST for active TB in pulmonary (particularly sputum smear-negative) and extra-pulmonary TB specimens. Implementation of all of these tools in routine laboratory practice requires the implementation of appropriate quality assurance systems.

In the case of extra-pulmonary specimens, the performance of molecular tools varies and should be considered separately for each specific specimen type. Evidence for the use of these assays to identify TB and detect drug resistance in TB-HIV co-infected individuals is limited; however, it is reasonable to use these in smear-positive (and probably smear-negative) patients if combined with standard methods for diagnosing active TB and conventional drug resistance.

Given the importance of better diagnosis of paediatric TB, there is a need for carefully designed studies among children, including HIV-positive children. Clinical data obtained from longitudinal studies, particularly data related to the influence of rapid diagnostics on disease outcome, are lacking. There also remains a need to increase the sensitivity of TB detection among all patients, but especially among immunocompromised patients and children.

Those wishing to introduce assays for RMP resistance/MDR-TB in laboratories and programmes need

to understand the relationship between the PPV of the assay and disease prevalence. As the WHO indicated in its careful consideration of the problems faced by global and programmatic implementation of these assays,[2,23] the PPV for RMP resistance reaches ⩾90% only if the prevalence of RMP resistance is >15%, which is globally rare among new TB cases. A substantial proportion of such resistance could represent false resistance, entailing the need for a secondary confirmative test (another molecular test or culture-based DST). Unless it is planned for, increased diagnosis (or incorrect over-diagnosis) of TB may also compromise the effectiveness of public health programmes by increasing the numbers of individuals placed on antituberculosis treatment, although TB diagnosis itself, unlike drug resistance, is not a laboratory-defined condition and clinicians are more likely to identify false TB cases than false resistance.

Clearly, point-of-care tests using untreated sputum, blood or urine and little or no equipment would be of immeasurable benefit in the future. Great progress has been made but we are not there as yet.

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RÉSUMÉ

Nous vivons une période excitante en matière de diagnostic de la tuberculose (TB). La technologie du diagnostic rapide de la TB et de la résistance à la rifampicine (RMP) dans les échantillons de crachats d'origine pulmonaire et à frottis positifs a bien progressé et les essais montrent une haute spécificité et une bonne sensibilité ; néanmoins, la sensibilité actuelle pour la détection de la TB indique que ces tests ne peuvent pas encore remplacer les méthodes standard de diagnostic de la TB ni les tests conventionnels de sensibilité à l'égard des médicaments (DST). Pour les échantillons extrapulmonaires, la performance des outils moléculaires varie et doit être considérée séparément pour chaque type d'échantillon. De la même manière, on ne dispose encore que d'une évidence limitée en ce qui concerne l'utilisation de ces tests pour la détection de la TB et la résistance aux médicaments chez les individus co-infectés par la TB et le virus de l'immunodéficience humaine (VIH).

Comme la valeur prédictive positive de ces tests pour la résistance à la RMP n'atteint que 90% ou davantage lorsque la prévalence de la résistance à la RMP dans les nouveaux cas de TB est supérieure à 15% (ce qui est rare au niveau mondial), un grand nombre de cas classés comme résistants peuvent être faussement résistants, ce qui fait ressortir la nécessité d'un test secondaire de confirmation. De la même manière, un diagnostic abusif (ou incorrect) de TB peut compromettre l'efficience des programmes (sauf s'ils sont planifiés dans ce but) en augmentant le nombre d'individus exigeant un traitement de la TB.

A l'avenir, 1) des tests comportant une sensibilité meilleure pour la détection de la TB s'imposent ; 2) des techniques rapides de diagnostic de la TB pédiatrique sont importantes et il est nécessaire que des études soigneusement élaborées incluent aussi les enfants séropositifs pour le VIH ; 3) un plus grand nombre de données cliniques provenant d'études longitudinales s'imposent particulièrement en rapport avec l'influence des techniques de diagnostic rapide sur les résultats du traitement ; et 4) des tests sur les sites de soins utilisant des crachats, du sang ou de l'urine non traités et ne demandant qu'un équipement minime ou aucun équipement représenteraient un bénéfice incommensurable. Quoique de grands progrès aient été obtenus, nous n'y sommes pas encore.

RESUMEN

El presente es un período interesante con respecto al diagnóstico de la tuberculosis (TB). Han avanzado considerablemente las técnicas rápidas de diagnóstico de la enfermedad y de la resistencia a rifampicina (RMP) a partir de muestras de esputo con baciloscopia positiva y las pruebas ofrecen alta especificidad y buena sensibilidad. Sin embargo, la sensibilidad actual de la detección de la TB indica que estas pruebas todavía no pueden remplazar los métodos diagnósticos corrientes (ni las pruebas ordinarias de sensibilidad a los medicamentos). En cuanto a las muestras extrapulmonares, el rendimiento de las pruebas moleculares es variable y se deben considerar separadamente en función del tipo de muestras. De igual manera, existen pocos datos científicos sobre el uso de estas pruebas en la detección de la TB y de la resistencia a los medicamentos en personas coinfectadas por el virus de la inmunodeficiencia humana (VIH) y la TB.

Dado que el valor pronóstico de un resultado positivo de resistencia a RMP solo alcanza un 90% o más, cuando la prevalencia de resistencia en los casos nuevos de TB es superior al 15% (lo cual es poco frecuente, en general), muchos de los casos así definidos serán falsos casos de resistencia y surge la necesidad de una segunda prueba de confirmación. De igual manera, el diagnóstico de más casos de TB (o el diagnóstico incorrecto de casos) puede constituir un obstáculo a la eficacia del programa, a menos que se haya previsto, pues se aumenta el número de personas que precisan tratamiento antituberculoso.

En el futuro: 1) se precisarán pruebas más sensibles de detección de la TB; 2) serán muy importantes los medios diagnósticos rápidos en los niños y se precisarán estudios diseñados cuidadosamente, que incluyan niños positivos frente al VIH; 3) se necesitarían más datos clínicos obtenidos a partir de estudios longitudinales, en especial en materia de repercusión de los diagnósticos rápidos sobre los desenlaces clínicos; y 4) sería de gran utilidad contar en el consultorio con pruebas que utilicen muestras no tratadas de esputo, sangre u orina y que precisen escasos materiales o ningún equipo especial. Aunque se han realizado grandes progresos, queda mucho por hacer.