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TUBERCULOSIS DIAGNOSIS AND DRUG SENSITIVITY TESTING

An overview of the current diagnostic pipeline

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1. Introduction

Tuberculosis (TB) remains the leading cause of death from a curable infectious disease[1], despite the availability of short-course therapy that can be both inexpensive and effective.

Clinical management of cases in developing countries is hampered by the lack of a simple and effective diagnostic test. Correct diagnosis of TB is needed to improve treatment, reduce transmission, and control development of drug resistance.

In patients with active pulmonary TB, only an estimated 45% of infections are detected by sputum microscopy[2]. This test, first developed in the 1880s and basically unchanged today, has the advantage of being simple, but is hampered by very low sensitivity: it may only detect half of all cases with active infection. It is also very dependent on the skill of the technician, and a single technician can only process a relatively small number of slides per day[3]. Furthermore, a staggering three million people who present annually with suspected TB may not be properly diagnosed, because their infection (so-called smear-negative disease) cannot be detected by sputum microscopy[4].

There are specific epidemiological factors that present additional challenges to TB diagnosis. HIV infection is thought to be a major contributor to the increase in TB incidence across the world[2]. An estimated 9% of adults globally with newly diagnosed TB are HIV positive, but this rate is 31% in Africa[5]. HIV co-infection with TB presents challenges to effective diagnosis of TB and diagnosis can also be more difficult in children.

The rapid rise of drug-resistant (DR) TB has further complicated TB diagnosis[6]. Tests that measure drug susceptibility are essential to monitor the spread of resistant TB strains, and ensure that patients are given effective treatment. The recent cluster of so-called extensively drug resistant (XDR) TB cases in South Africa were untreatable by any available drugs, and had a devastating mortality rate: 52 of 53 patients died[7].

New diagnostic tests that are simple and robust enough to be used in the field, accurate enough to diagnose all infected individuals, and able to identify drug resistance are desperately needed, and represent an essential complement to new drug development efforts and to effective control and treatment programmes.

Reviewing the TB diagnostics development landscape

In 2005, Médecins Sans Frontières (MSF) treated 17,000 patients for tuberculosis in 94 programmes across 44 countries. This analysis of the TB diagnostics research and development (R&D) pipeline, along with an accompanying report on the TB drug development pipeline[8], stem from the frustration of MSF medical practitioners at the lack of simple, reliable tools for detecting TB at point-of-care, and by the growing need for drug susceptibility testing in MSF and other programmes.

This report aims to review the current status of the development of new diagnostics for TB, and to answer the questions: What can we expect from the TB diagnostics pipeline? and What do we need to do to ensure that effective tools are developed?

The report draws upon a review of the literature, company listings, direct contact with scientists, researchers and companies, and input from MSF doctors and laboratory technicians working in MSF projects around the world. MSF doctors treat TB patients in specialised TB projects, in HIV/TB integrated programmes, in primary health care contexts, and in specialised drug resistant TB settings. This report draws on their experience and frustrations and tries to determine whether the tools that are being developed are likely to respond to the needs identified.

The diagnostic pipeline

This report presents the diagnostics pipeline divided into tests that require culture and those that do not. We have used this division because setting up culture facilities requires a particular investment of resources and expertise, and MSF field experience has shown that this is often more complicated and difficult to realise in the field than is widely appreciated. However, culture remains the gold standard, and is often the most sensitive test for TB. Drug sensitivity testing is dealt with in both sections. We distinguish between tests that are already available and those under development, and describe the stage of development and the potential interest for field application, where possible.

The criteria for field application are based mainly on

feedback from MSF projects, which is both a limit and a strength in such an analysis. We do not claim that tests that are not adapted to MSF settings are therefore not interesting. We limit ourselves to sharing our experience and the result of our ongoing efforts to improve our medical practice.

For any diagnostic test to be useful, it must deliver information that is used to make a medical decision. It is crucial for the TB diagnostic pathway to be viewed in this context – will a given test, when used in the setting in which it is applied, give a result that is interpretable and that will allow a medical decision to be made? Do the resources exist to apply and interpret the test, and can the doctor or health care worker propose something to the patient based on the information obtained?

In some cases it has been hard to find evidence that these aspects have been considered in the design of the test.

Many of the projects to develop TB diagnostics are funded by The Foundation for Innovative New Diagnostics (FIND), supported by the Bill & Melinda Gates Foundation, which has committed US$30 million over five years. This is an important contribution to encouraging the development of new tests. However, it is important that other projects and groups continue to be active in the search for better tools, especially because certain types of project, such as fundamental research, are not part of the FIND strategy.

A major difficulty in preparing the report has been an overall lack of transparency and limited availability and sharing of information. This report is therefore certainly incomplete. We would welcome comments and clarification, and hope that this work will be a starting point for a more open exchange of ideas and strategies to deal with this very complex and challenging public health crisis.

We stress the need for collaboration as the most effective way forward.

The peripheral laboratory setting

In this report, we often talk about use of a test in a peripheral laboratory.
For MSF, this is:

- The most remote point of care at which suspected TB cases are diagnosed and treated.
- Basic laboratory facilities only: usually single room, often lacking electricity and water, understaffed, performing only sputum smear microscopy.
- Level of staff training and quality control can vary widely.
- No facilities for any culture tests (except very few urban facilities which may have rudimentary facilities for culture, and limited training).
- Frequently ill-equipped to handle high workloads.
- May lack any laboratory staff -- just used for sputum collection with testing done at central laboratory

However, the same conditions described above for a peripheral laboratory may be encountered in poorly supported district hospitals or in urban areas. In this report, this term refers to the conditions that exist in the settings where most of our patients are seen.

2. Culture-based Methods

Culture of *Mycobacterium tuberculosis* remains the gold standard for both diagnosis and drug sensitivity testing. This section reviews culture tests currently in use, and newly developed techniques.

Culture-based tests are difficult to implement in the field. They require dedicated facilities and staff, with specific requirements for training, quality assurance, biosafety, and equipment, which can take time and significant local resources to set up. For culture to be reliably implemented, local capacity needs to be supported and developed.

Conventional culture methods using Lowenstein-Jensen (LJ) or 7H11 medium, while cheap and simple, have the major disadvantage of being very slow. LJ cultures take 20 – 56 days for diagnosis and four to six weeks after initial culture for drug sensitivity testing. 7H11 medium slightly accelerates the process, but requires antibiotics in the medium to prevent contamination and a CO2 incubator. Diagnosis with 7H11 medium takes 17 –21 days, DST information is available three to six weeks later.

Some more rapid culture methods have been developed and are commercially available (Section 2.1), most of which are difficult to implement in the field due to the complexity of the technique or the required equipment. There are also some emerging simplified culture techniques that can reduce time to diagnosis or DST that seem more appropriate for use in resource-limited settings (Section 2.2).

The sensitivity of culture is limited by the need to have bacilli present in the sample to be cultured. HIV positive patients and children have difficulty in producing sputum and sputum culture will not detect extrapulmonary (EP) forms of TB. EP TB is very common in HIV positive patients and is rapidly fatal. Even in patients with active pulmonary TB, the bacilli may be protected in lung cavities or not present in a particular sputum sample, or may be lost in the decontamination treatment required to process sputum for mycobacterial culture. All these factors limit the usefulness of the technique.

2.1 New rapid commercial methods for diagnosis and DST

A number of commercial systems are available for culture and DST, some of which may have slight advantages in certain settings. However, none of the tests are easily adaptable to the realities of field projects, given the difficulties in setting up and running culture laboratories.

BACTEC 460-TB®

Company	Becton Dickinson (US).
In the FIND portfolio?	No.
Principle	This system detects the presence of mycobacteria based on their metabolism rather than visible colonial growth. A radioactive marker is present in the tube that is detected by the machine when growth occurs.
Stage of development	No longer commercialised, but still available in some laboratories.
Advantages	Faster than solid media.
Disadvantages	Costs: the machine is expensive, and tests for DST cost Euro 13 for two drugs tested per sample. These combine to make the technology more expensive than the conventional culture method. The machine requires appropriate laboratory infrastructure, including nuclear waste disposal. In addition, the need for radioisotopes, needles, and the cost of equipment limits its use to reference laboratories.
Interest in peripheral laboratory settings?	None. This technique is being phased out because of the difficulty associated with using radioactive reagents.

Rapid liquid TB culture medium: BACTEC MGIT 960® (automated)

Company	Becton Dickinson (US).
In the FIND portfolio?	FIND is involved with Becton Dickinson in a demonstration study for automated MGIT aimed at assessing the cost and feasibility of the implementation phase of BD Bactec system in developing countries.
Principle	Diagnosis through culture of samples (pulmonary and others). The MGIT system is based on a glass tube, recently replaced by a plastic tube, containing 7H9 broth together with a fluorescence quenching-based oxygen sensor. When inoculated with M.Tb, consumption of oxygen produces fluorescence when illuminated by a UV lamp. The fully automated version can incubate up to 960 samples for M.Tb diagnosis through culture of samples (pulmonary and others), and drug sensitivity testing for first-line drugs.
Stage of development	Commercialised.
Advantages	High throughput capacity Automated, standardized reading of samples Speed: diagnosis 7 days for sputum positive, up to 42 days for a negative result DST 8 to 12 days (starting from culture)

Disadvantages	Machine is extremely expensive, priced at Euro 40,000 in Europe, up to Euro 100,000 in Africa. Requires specific training of technical personnel. Liquid media are technically limited as they are prone to contamination. The machine must be maintained, this requires very frequent technical support from the company.
Interest in peripheral laboratory settings?	Very limited due to the price and the dependence of the machine on service. The advantage of this technique is the increased speed of diagnosis. However, 7 days by MGIT corresponds to a sample that would have been positive by smear microscopy, which gives a result in hours. This shows the need for a very thorough analysis of what a test is used for and where before adopting a given technology. This method gives comparable results to the BACTEC 460-TB® radiometric system described above.

Rapid liquid TB culture medium: MGIT (manual)

Company	Becton Dickinson (US).
In the FIND portfolio?	Yes.
Principle	See above for the automated system principle. In the manual system, detection is performed by the technician visually using a hand-held UV lamp.
Stage of development	Field evaluated by MSF.
Advantages	The MGIT machine is not required. More samples can be processed than conventional culture, although fewer than the automated system. Speed: diagnosis 7 days for sputum positive, up to 42 days for a negative result DST 8 to 12 days (starting from culture).
Disadvantages	Tubes are very expensive (Euro 4) “Developing country” price has been negotiated by FIND (no information available). Manual MGIT also requires additional reagents, manipulations and use of a handheld UV lamp. Liquid media are known to have a high percentage of contaminations leading to unreliable results.
Interest in peripheral laboratory settings?	MGIT can only be used in a culture facility, which limits its implementation. However, the handheld reader makes the technique machine independent and cheaper and appears to give acceptable results when implemented in a laboratory that already has experience with culture.

MB/Bact T® system

Company	Organon Teknika (Netherlands).
In the FIND portfolio?	No.
Principle	This technique for diagnosis and DST for first- and second-line drugs is an automated non-radiometric continuous monitoring system with computerized database management. The system is based on detection of CO2 as an indicator of bacterial growth in cultures in a closed and a fully automated system[9,10].
Stage of development	Commercialised.
Advantages	This system can be used for first- and second-line detection of drug resistance[11,12]. System modular (machine and tubes can be bought separately), increasing flexibility of use.
Disadvantages	Relatively slow: diagnosis 17 days (range 7-40), DST 8 to 12 days. Requires an expensive and non-robust machine, complicated and cumbersome.
Interest in peripheral laboratory settings?	Complicated and fragile, it is unlikely to be implemented in peripheral settings.

Versa Trek system (previously called ESP culture system II)

Company	AccuMed International (USA).
In the FIND portfolio?	No.
Principle	This technique for diagnosis and DST for first-line drugs is a fully automated, non-radioactive system, providing continuous monitoring of growth of mycobacteria based on the detection of gases released by the bacteria[13].
Stage of development	Commercialised.
Advantages	
Disadvantages	Cumbersome, not widely used. Relatively slow: diagnosis 14 days (range 14-30), DST 15 days.
Interest in peripheral laboratory settings?	A method for testing susceptibilities of isolates of TB to first-line drugs has been developed[14]. However, the machine does not seem to provide advantages while still being very slow.

E Test

Company	AB Biodisk (Sweden).
In the FIND portfolio?	No.
Principle	The technique is based on determination of drug sensitivity testing using strips containing gradients of impregnated antibiotics. The E Test strip is placed on the surface of the solid culture medium and MICs (minimal inhibitory concentrations, a measure of the susceptibility of a strain to an antibiotic) are determined by interpreting the point at which the ellipse of inhibition crosses the strip.
Stage of development	Commercially available.
Advantages	Simplicity, minimal training required. Shown to be accurate and reproducible[15]. Relatively fast (five to ten days after primary culture).
Disadvantages	Requires high bacterial concentration for inoculum i.e. needs to start from a culture.
Interest in peripheral laboratory settings?	Simple approach that does not require particular training of technical personnel. Requires an initial culture step, which significantly limits its advantages.

MB redox®

Company	Biotest (Germany).
In the FIND portfolio?	No.
Principle	This system is based on the reduction of a tetrazolium salt indicator in liquid medium[16]. MB Redox® allows an easy macroscopic visualisation of the bacterial growth. The tetrazolium salt indicator forms red to violet particles when reduced by the growth of the mycobacteria.
Stage of development	Commercially available.
Advantages	Simplified 96 well plate format, does not require specialised machine (can be read by eye or simple spectrophotometer).
Disadvantages	Cannot be used to measure DST on initially positive samples as dye is toxic; visualisation is not easy; very few reports available; relatively slow (16 days for a smear positive sample, 3 weeks for a negative result).
Interest in peripheral laboratory settings?	Interest would seem to be limited as better-performing alternatives exist.

In addition to the commercialised systems above, the following test is being developed by FIND

Solid culture indicator medium: TK medium

Company	Salubris (Turkey).
In the FIND portfolio?	Yes (since July 2004).
Principle	The test uses a newly developed culture medium that changes colour depending on the bacteria growing on it. The medium is initially red; if TB is present it turns yellow. If it becomes green, the sample is contaminated with non-TB bacteria. The colour changes are visible earlier than with the usual solid medium (Lowenstein Jensen), and so reduce the time to an answer by about half (from roughly 6 weeks to about 3 weeks).
Stage of development	The medium is under evaluation, but no details are available. No pricing information available.
Advantages	Ease of use and rapid results. The test is suitable for co-infected patients and children as any kind of sample can be cultured. Another advantage is that there is no need to open the tube to obtain a result.
Disadvantages	Full TB culture facilities are required.
Interest in peripheral laboratory settings?	When the test becomes available it will likely allow simpler identification of TB infection (by colour change) in a shorter time. We do not have any information about how successful the development is and when a product might be available.

2.1.1 Phage-based tests

Phage-based tests require limited culture facilities and promise rapid results (~2 days). However, MSF field evaluations shown that it is very hard to implement in non-culture facilities in the field, even in relatively well-supported urban settings.

Dedicated areas are required, careful control of access to the rooms is needed to reduce contamination, and even seemingly simple requirements, like a stable power supply and a functioning biosafety hood, are very difficult and often enormously expensive to ensure.

Metanalyses comparing phage based tests to culture in field settings have shown that in most cases they are no more informative that smear microscopy (see box below).

Phage-based tests

Companies	Biotec (UK and South Africa) for *FASTPlaque TB test* and *FastPlaqueTB-RIF™*, as well as in-house systems.
In the FIND portfolio?	Yes, for Biotec (since May 2004).
Principle	Diagnostic (FASTPlaque TB test) and rifampicin DST (FastPlaque TB-RIF™). Phage tests are based on the ability of viable *M. Tuberculosis* to support the replication of an infecting mycobacteriophage (a virus that infects mycobacteria). Plaques of lysed cells in a lawn culture of mycobacteria are counted[17].
Stage of development	An evaluation study of the *Rif phage test* version is being conducted in Peru by FIND. FASTPlaque is also being evaluated in Kenya in an MSF project. Preliminary results show that the feasibility of technique is not optimal for a peripheral laboratory. Final results will be available shortly.
Advantages	Speed (2-3 days). For in-house systems: relatively cheap.
Disadvantages	Phage-based tests are technically complex to perform, requiring a well-functioning bacteriology laboratory, a strict incubation protocol and well-trained technicians. They are very labour intensive and some studies also report a high rate of contamination, making the test and its results both difficult to perform and to interpret. FASTPlaque cannot be used for children or HIV-positive patients as it needs sputum.
Interest in peripheral laboratory settings?	Three days for a negative result is very interesting. However, this is only helpful if the test is reliable and technically feasible. The consensus of a number of published studies is that, given all these constraints, phage methods are less reliable than smear microscopy, even if smears are poorly done. Given the enormous relative cost and complexity of phage methods, they are looking less useful than originally hoped[18].

Luciferase reporter phages

Company	Sequella Inc. (USA).
In the FIND portfolio?	No.
Principle	The technique is used for DST. This recombinant phage (phage which incorporated the gene for luciferase) can express the luciferase gene when infecting a mycobacterium. In the presence of luciferin substrate, infected bacteria emit light that can be detected with a luminometer or by photosensitive film in a Polaroid film box called the “Bronx Box”.
Stage of development	Under development, not commercially available.
Advantages	Rapid result (2 days post culture).
Disadvantages	To date, only limited reports of clinical application are available. The Bronx box is not easy to manipulate. Conditions of use not clearly defined; needs repeated testing. Results obtained 40 hours post-culture.
Interest in peripheral laboratory settings?	Very limited due to technical constraints in using the system; requires an initial culture step, which further limits its usefulness.

2.2 New rapid noncommercial methods for diagnosis and DST

There are also some tests that have not been commercialised that are of potential interest.

MODS

Company	Not commercialised, freely useable technique.
In the FIND portfolio?	No.
Principle	This method is performed in liquid medium (7H9) with or without drug incorporated in the medium[19,20].
Stage of development	A number of field evaluations have been published.
Advantages	Quicker than solid culture, may be quicker than MGIT as volumes are smaller. Fairly cheap, non-commercial, adaptable technique.
Disadvantages	MODS is a delicate method that requires very experienced personnel. As the test is performed in liquid medium, and needs to be handled often, it is more of a biosafety risk for laboratory staff. The test requires an inverted microscope, which is very seldom available or useful in field labs.
Interest in peripheral laboratory settings?	Some reports claim very good results[21], but when evaluated by two independent experts for implementation in MSF field projects, both agreed that it is not easy to use and requires a well trained and experience technician, which limits the settings in which it could be introduced.

Nitrate reductase assay

Company	Not commercialised, freely useable technique.
In the FIND portfolio?	No.
Principle	Used for DST of first-line drugs, the test is based on the ability of *M. Tuberculosis* to reduce nitrate to nitrite by using the nitrate reductase enzyme[22,23].
Stage of development	Can be used from culture, needs to be validated for sputum or other clinical samples.
Advantages	Performed on solid media. Only minimal training and no special equipment are required. Relatively rapid results (10 days). Readout of results is visual, not necessary to open the tubes therefore safer.

Disadvantages	Only for DST for first-line drugs. Low throughput.
Interest in peripheral laboratory settings?	Easy to implement in resource-limited settings. Further validation needed for use from patient samples.

Thin Layer Agar (microcolony detection)

Company	Not commercialised, freely useable technique.
In the FIND portfolio?	No.
Principle	For diagnosis of *M. Tuberculosis*, plates with a thin layer of 7H11 agar medium are incubated and examined microscopically on alternate days for the first two weeks and less frequently thereafter. Microcolonies of *M. Tuberculosis* can be detected in less than 7 days[24,25]. The technique has only recently been developed for DST as well (results not published), but looks promising.
Stage of development	Validated for diagnosis. Under evaluation for DST use. More validation needed.
Advantages	Test can be done with a standard light microscope. It uses solid media, which is safer than liquid media. No specific equipment is required. The results are obtained in half the time of conventional culture. Diagnosis 5-10 days; DST 10-15 days. Simpler to manage large numbers of samples than for manual liquid culture.
Disadvantages	Requires a CO2 incubator (but could possibly be done in a candle jar, a simplified, non-machine dependent equivalent) Not as fast as liquid culture.
Interest in peripheral laboratory settings?	Cheap, requires no specific equipment, uses solid media. May give an acceptable time to result. An interesting track to pursue as it may be a good compromise between pragmatic constraints and the need for rapid results.

"Resa" Colorimetric method

Company	Not commercialised, freely useable technique.
In the FIND portfolio?	No.
Principle	Colorimetric methods for DST of first- and second-line drugs; based on the ability of live bacteria to reduce a coloured indicator upon growth producing a change of colour. *M. Tuberculosis* exposed in vitro to different antibiotics will reduce the indicator only if it is resistant to these drugs[26,27]. The test is also known as the resazurin assay.
Stage of development	Validated in large field studies.
Advantages	The test is performed in a small volume of liquid media. It is easy to implement in resource-limited settings that have culture facilities (already implemented in Latin America, Madagascar, Benin and Rwanda). Only minimal training, and no special equipment required. Rapid results in 8-10 days (especially for DST). The test is done in 96-well dish, and is field-adapted. Results determined visually by a colour change of the medium.
Disadvantages	Only for DST (requires prior identification of M.Tb from culture) Euro 1.7 for two drugs per sample.
Interest in peripheral laboratory settings?	Not as robust as thin layer, but has been shown to be feasible and practical in laboratories with some culture capacity. The exact cutoffs for determining DST levels need to be defined by the international community before this can be included in a medical decision.

3. Non-Culture Methods

A number of strategies to detect and report the presence of *M. Tuberculosis* have been developed. Serology (detection of antibodies) has not produced any reliable, informative tests despite decades of work (Sect 3.1). Detection of antigens is a more promising approach (Sect 3.2), as it detects the presence of the organism and thus may be able to diagnose active infection.

The use of nucleic acid amplification (NAA) tests (Sect 3.3) in non-specialised laboratories is technically challenging. These tests have been shown to be highly specific, but sensitivity, if starting from patient samples, is low and highly variable, and is difficult to assess[28]. These tests can also be used from primary culture. Although this improves the sensitivity, the technique is then very slow. For this reason, we have decided to include NAA tests in the non-culture section of this report, in order to focus on the tests' use on direct clinical samples. Here we also look at some PCR based techniques are being validated for use on patient samples for rapid detection of rifampicin/isoniazid resistance. There are also some tests being developed that detect immunological responses (interferon gamma assays). These tests are rather expensive and complicated to perform, and still need to be validated in endemic areas, and their interpretation is not clear. We discuss these tests and some projects that are at early stages of research in Sect. 3.4.

3.1 Techniques using antibody detection

In 2005, WHO/TDR performed an evaluation of commercially available rapid diagnostic tests (RDTs). Twenty-seven manufacturers were invited to submit their products for evaluation, but of the 19 who agreed (Table 1), only six provided information on the antigen used. All tests detect antibodies in serum. Test samples came from the TDR specimen bank.

Table 1. Manufacturers and tests reviewed in WHO/TDR study of RDTs.

1.	ABP Diagnostics Focus Sure Check TB
2.	Advanced Diagnostics Tuberculosis Rapid Test
3.	American Bionostica Rapid Test for TB
4.	Ameritek dBest One Step TB Test
5.	BioMedical Products Corp TB Rapid Screen Test
6.	Chembio TB Stat-Pak II
7.	CTK Biotech TB Antibody onsite Rapid Screening Test Kit
8.	Hema Diagnostic Rapid 1-2-3 TB Test
9.	Millenium Biotechnology Immuno-Sure TB Plus
10.	Minerva Biotech V Scan
11.	Mossman Associates MycoDot
12.	Pacific Biotech Bioline TB
13.	Premier Medical Corporation First Response Rapid TB
14.	Princeton BioMeditech BioSign M.tuberculosis
15.	Silanes TB-Instantest
16.	Span Diagnostics TB Spot ver. 2.0
17.	Standard Diagnostics SD Rapid TB
18.	UniMED International Inc. FirstSign MTB Card Test
19.	Veda Lab TB Rapid Test

The WHO study found that TB rapid diagnostic tests currently available on the market vary widely in performance, with some products showing a high lot-to-lot and reader-to-reader variability. At less than 80%, the specificity was poor in the majority of products when tested in TB suspected cases from endemic settings. Those tests with a better specificity (over 90%) had poor sensitivity, detecting fewer than 40% of TB patients. The tests performed even worse in HIV co-infected samples. The conclusion of the study was that none of the assays perform well enough even to replace microscopy[29].

Based on this and other information, it seems that antibody detection is unlikely to be a good strategy for the development of a reliable diagnostic test for TB. It is important to note that the tests named in the above table are only those that agreed to participate in the study: absence from the list does not imply that the test works. We found no convincing evidence supporting the use of any existing antibody detection tests.

3.2 Techniques using antigen detection

Several tests using antigen detection are currently commercialised or under development.

LAM urine test

Company	Chemogen (USA).
In the FIND portfolio?	Yes (since April 2005). A "letter of intent" has been signed, according to FIND information.
Principle	The test detects lipoarabinomannan (LAM) in urine as a surrogate marker for TB infection. LAM is a component of the TB bacterial cell wall. The test exists in Elisa and simplified 'tube' format. Clinical trials to develop a dipstick format are ongoing. The simplified tube formatis apparently robust and does not need cold chain. The use of the test needs to be evaluated within a careful choice of algorithms, in order to determine whether it will help guide clinical decision making. The tube format requires at least three hours, several manipulations, a supply of distilled water and some amount of training. It also requires reading the result in a machine, but apparently a portable format has been developed. However, a dipstick format would be very welcome.
Stage of development	The results of a preliminary study conducted in July 2003 in Tanzania on 242 suspect TB patients and 220 US healthy persons have been published[30]. In a separate project, the Swedish Institute for Infectious Disease Control is also working with industrial partners to develop a urine dipstick detecting LAM.
Advantages	The test may be suitable for children, co-infected patients, and extra-pulmonary patients. A dipstick would be extremely useful as a high-throughput point-of-care test. It holds potential for monitoring of treatment as LAM is in theory quickly eliminated. A urine sample is a good non-invasive approach. Possibly, there will be no need to treat the urine sample prior to testing. This is currently a limiting step in many field tests.

Disadvantages	In its current form as an Elisa test, it is not suitable for the peripheral laboratory setting, as it will require skilled staff, electricity supply, cold chain and specific equipment. The tube format does not seem to completely address these problems, but a dipstick, if it were to become available, will be a significant improvement. Chemogen recommends boiling and centrifuging the urine, which is technically difficult in many peripheral settings.
Interest in peripheral laboratory settings?	A urine dipstick that performs well and that eliminates the problems associated with an Elisa test will be very useful in many peripheral and pointof-care situations. More data and further evaluations are needed.

Antigen-based detection test

Company	Proteome Systems (Australia).
In the FIND portfolio?	Yes (since June 2005)-joint project on "reagent discovery science"
Principle	*Note: the only information we have is from the company's website.* Proteome Systems are using high throughput protein assays to identify antigens that are specific to *M. Tuberculosis* and that could potentially be used to develop a rapid test and diagnose active TB and infection, or to monitor treatment efficacy. They will examine all types of biological fluids such as sputum, saliva, plasma or whole blood.
Stage of development	From what we know, the development is still at the research stage. According to an initial press release, a rapid test was expected to be finalized by the end of 2005. Proteome owns patents on four in vivo expressed TB proteins, method of preparing sputum for 2 D gel analysis and an improved diagnostic testing apparatus for multiple sample testing. How this will be translated into a test adapted for the peripheral level is not clear.
Advantages	Potentially interesting because antigen detection avoids some of the problems associated with antibody detection. But there is no information about the preliminary results on characterized samples, nor on the technical background nor on performances of their format. We also do not have information about the initial specifications of the test: did the company look for molecules present in endemic populations or naïve groups, latent versus active disease, pulmonary or extra-pulmonary, etc. This will affect the range of possible uses for the test, and is important information in determining whether the needs are being sufficiently addressed.
Disadvantages	Information on the test is currently severely lacking. It appears there may be a need for a reader or some kind of machine to perform the test.
Interest in peripheral laboratory settings?	Insufficient information available.

Flow-through filter tests

Companies	Companies ANDA (France) for *patho-tb* and BioMed (India) for *diagnos-tb.*
In the FIND portfolio?	ANDA to be evaluated with FIND.
Principle	Both tests rely on detection of *M. Tuberculosis* in sputum or body fluids with a polyclonal antibody, using a flow-through device.
Stage of development	The ANDA test is currently under field evaluation. No information on laboratory or field evaluation is available about the BioMed test. Assessment of both tests in reference laboratory contexts, followed by further field evaluation, would be useful.
Advantages	BioMed's *diagnos-tb* does not require boiling or centrifuging and seems to be easy to perform. The tests may increase the throughput of laboratories that have too many samples to perform microscopy well. It may also increase sensitivity over microscopy.
Disadvantages	For ANDA's *patho-tb*, sample preparation requires decontamination and boiling, and several manipulations including centrifugation. For BioMed, insufficient information is available concerning the performance of the test, although the company claims it is sensitive to 3000 bacteria/ml.
Interest in peripheral laboratory settings?	Sample preparation requirements mean that ANDA's test could not be implemented in very peripheral settings. However, in high throughput laboratories or where microscopy is unreliable, it may increase the detection rate or reduce the workload for microscopy.

3.3 Nucleic Acid Amplification (NAA)

NAA techniques require strong laboratory capacities, good quality control procedures, and remain relatively expensive. In recent years, some improvements have been made, such as isothermal amplification steps, the inclusion of internal amplification controls to ensure that inhibitors (resulting in false negatives) are not present, the design of single-tube reactions to reduce contamination and the development of detection by emitted light or by dipstick.

The use of NAA techniques remains technically challenging. Despite being usually highly specific, NAA tests have lower (and greatly variable) sensitivity. A positive NAA test is considered good evidence of infection but a negative result is not informative enough. Use of NAA tests has not been recommended for sputum negative patients. As these tests cannot distinguish live from dead bacteria, they cannot be used for patients receiving treatment. One study considered that current NAA tests cannot replace microscopy or culture, and should be used only in conjunction with these tests and clinical data[31,32]. While some NAA assays reported seem to work quite well (sometimes sensitivities near 90% were reported), there is very wide variability, even from very resource-rich laboratories, making their use in the field uncertain. Their difficulty of use and quality control issues combined with the variability and lack of sensitivity in sputum negative and extrapulmonary TB do not support their use[33].

The following table shows existing PCR techniques for diagnosis of *M. Tuberculosis*.

Name of test	Company	Principle	Advantages	Disadvantages	Interest for peripheral lab settings?
Amplified Mycobacterium Tuberculosis Direct Test	Gen-Probe Inc. (USA)	Isothermal NAA. Gen-Probe Amplified™ MTD Test detects *M. Tuberculosis rRNA*. The test is specific for M.tb complex. Is an isothermal TMA (transcription-mediated amplification) test in which the target is the mycobacterial *16SrRNA*. The entire process is performed at 42ºC.	Highly specific. Very rapid (turnaround time of 2.5 hours) The first test to be FDA approved for smearpositive respiratory specimens.	Expensive; Requires highly skilled personnel; Requires dedicated pre- and post- PCR rooms to avoid contamination.	Unlikely to be usable in peripheral settings due to technical constraints of NAA tests
AMPLICOR® MTB tests	Roche Diagnostic Systems (USA)	PCR test targeting the 16SrRNA gene. The process can be automatically performed on the Roche COBAS AMPLICOR machine.	Very rapid (turnaround time of 6 to 7 hours) The FDA have approved this method for testing smear positive respiratory specimens.	As above	As above

Name of test	Company	Principle	Advantages	Disadvantages	Interest for peripheral lab settings?
BD ProbeTec ET® assay	Becton Dickinson Biosciences (USA)	Isothermal NAA. Isothermal SDA (strand displacement amplification) process in which target sequences of *IS6110* and 16S-rRNA gene are co-amplified. Simultaneous amplification and detection is performed at a single temperature in the ProbeTec instrument.	Very rapid (turnaround time of 3.5 to 4 hours)	As above	As above
In-house PCR	N/A	"Home brew" NAA assays. Most protocols use the repeat insertion sequence IS6110 as a target for amplification. This sequence is specific to the *M. Tuberculosis* complex and is present in many copies in the *M. Tuberculosis* genome.	Often cheaper than commercial kits	Same limitations as for other NAA techniques. Lack of standardisation may make it difficult to compare between different centres or studies. Each test needs its own validation studies.	As above
Real-time PCR	N/A	Real-time PCR N/A Real-time PCR techniques have also been introduced for rapid detection of rifampicin resistance. Different probes have been used like the TaqMan probe, fluorescence resonance energy transfer (FRET) probes, molecular beacons and bioprobes. Real-time PCR was initially applied to M. tuberculosis strains but more recently it has been successfully applied directly in clinical samples[34,35].	May have less problems with cross contamination as the detection takes place in a closed system, no need for separate detection room.	Expensive equipment and reagents. Requires skilled personnel	Prohibitively expensive, but even if it were less expensive, the technology and sample preparation complexity make it almost impossible to use in peripheral laboratories.

A further PCR test for the diagnosis of *M. Tuberculosis* is under development:

Simplified NAT test, TB-LAMP test

Company	Eiken Chemical Co Ltd (Japan).
In the FIND portfolio?	Yes (since July 2005).
Principle	LAMP (loop mediated isothermal amplification) is a method to amplify TB DNA directly from clinical samples. A positive result is signalled by a colour reaction visible to the naked eye. The format under development is an adaptation of an existing technology. The technique requires sample preparation after the decontamination process and a special extraction device, then isothermal amplification and detection of a fluorescence signal, leading to a qualitative result. Apparently no cross-reaction with M. avium. A more direct method using sputum without decontamination of samples gives less specific results on negative samples, and a higher incidence of false positive results.
Stage of development	The test is currently in development, it is being evaluated in comparison to culture. The sensitivity claimed is ten copies of TB DNA, which is comparable to culture. This however needs to be determined, as does the correlation with clinical stage. A prototype is apparently being evaluated in Peru and Tanzania with good preliminary results compared to classical PCR. The development of a second generation test is scheduled but when MSF met with the company, they were unclear on where the test was to be used or what the optimal requirements would be. The estimated price would be US$ 5 per test. (Information based on a meeting with Eiken representatives and Martine Guillerm, MSF).
Advantages	This is a simplified NAT test, suitable in theory for the monitoring of treatment. Results are supposed to be obtained in two hours. Sample preparation apparently has been simplified. We have no recent information about the stage of development or whether the format is feasible.
Disadvantages	The feasibility of using the test at the district hospital level is of concern, and the test may be unsuitable for use at the periphery due to the complexity of the process. Certain specific equipment is needed. Reagents currently require cold storage. Heat stability studies are on going. Data on cross contamination and test performance are needed.
Interest in peripheral laboratory settings?	Greater information on non-pulmonary samples or potential use in children is needed. If this test can be made sufficiently simple, it could be interesting, given its high sensitivity. The procedure would require staff with PCR training however, and so its use may be limited for the periphery. The technical platform is being investigated by FIND for implementation in other diseases. However, many of the same technical concerns and reservations remain.

Two other tests have been commercialised for drug sensitivity testing:

INNO-LiPa assays

Company	Innogenetics (Belgium).
In the FIND portfolio?	No.
Principle	Two Inno-LiPa assays are commercialised. The first is for tuberculosis diagnosis (INNO-LiPA Mycobacteria Assay), the second for detection of rifampicin resistance (INNO-LiPA Rif TB Assay). The LiPA assay is based on the hybridization of amplified DNA (mycobacterial 16S-23S rRNA spacer region) from cultured strains or clinical samples to 10 probes covering the core region of the rpoB gene of M.Tb, immobilized on a nitrocellulose strip[36,37].
Stage of development	Commercialised. Transfer of technology is being explored.
Advantages	It is possible to use INNO-LiPA Rif TB Assay to confirm TB infection and to detect resistance to rifampicin at the same time. The test is fairly robust, and easy to use in a routine PCR laboratory.
Disadvantages	The test is extremely expensive, at Euro 40 a sample. It also lacks an internal control, and data on its sensitivity are contradictory. Sensitivity is lower from sputum or other patient samples. Tests not easy to perform in the very large numbers likely be needed if routine resistance testing is adopted.
Interest in peripheral laboratory settings?	Although the INNO-LiPA Rif TB Assay offers resistance information in 48 hours, PCR is in general not suitable for peripheral use. The limited sensitivity from sputum make its use limited.

GenoType Assays

Company	Hain Lifesciences (Germany).
In the FIND portfolio?	Yes (since October 2006).
Principle	Two GenoTypes are commercialised. The first is for tuberculosis diagnosis (GenoType Mycobacteria Assay), the second for detection of rifampicin and isoniazid resistance (GenoType MTBDR Assay). Isolation is commonly done by PCR amplification of the 16S-23S ribosomal DNA spacer region followed by hybridization of the biotinylated amplified DNA products with 16 specific oligonucleotide probes. The specific probes are immobilized as parallel lines on a membrane strip.

	The GenoType MTBDR detects resistance to isoniazid and rifampicin in culture samples, based on the detection of the most common mutations in the katG and *rpoB* genes respectively[38].
Stage of development	Commercialised.
Advantages	It is possible to use Genotype MTBDR Assay to confirm TB infection and detect resistance to rifampicin and isoniazid at the same time.
Disadvantages	The tests are not validated yet on direct clinical samples and sensitivity is not known. Test is only for use on smear positive samples. Tests not easy to perform in the very large numbers likely be needed if routine resistance testing is adopted.
Interest in peripheral laboratory settings?	Although Genotype MTBDR Assay would enable resistance testing, it comes with the usual caveats about PCR and its unsuitability for peripheral settings. Use from direct sputum remains to be determined. Only useful for smear positive patients, therefore does not cover most patients (e.g. HIV co-infected). May not be able to process large numbers of samples.

Additional nucleic acid amplification techniques include:

PCR sequencing

Company	N/A.
In the FIND portfolio?	No.
Principle	Specific *M. Tuberculosis* genetic material is amplified and sequenced, allowing the DNA to be "read". This is the gold standard and the most widely used method for defining genetic resistance for drug sensitivity testing. It has been commonly used for characterizing mutations in the *rpoB* gene in rifampicin-resistant strains and to detect mutations responsible for other anti-tuberculosis drugs[39,40,41]. Most protocols include the repeat insertion sequence IS6110 as a target for amplification. This sequence is specific of *M. Tuberculosis* complex and is present in many copies in the *M. Tuberculosis* genome.
Stage of development	The technique is the gold standard for monitoring resistance.
Advantages	PCR sequencing gives specific strain and mutation information.
Disadvantages	Using the technique requires sequencing capacity and sophisticated laboratory technology. PCR sequencing detects only some mutations, and only gives a "theoretical" result (as opposed to culture, which gives a "functional" result).
Interest in peripheral laboratory settings?	The technique may be needed in epidemiology or surveillance programmes, but is not suitable for use in routine patient care.

PCR directly from AFB slides

Company	N/A.
In the FIND portfolio?	No.
Principle	The basic technique consists of conducting PCR directly on samples washed off sputum slides. Different methods have been used[42,43,44].
Stage of development	Early research stage.
Advantages	The technique may be more sensitive than microscopy alone. It may also be a way to ship samples to reference laboratories for testing.
Disadvantages	The technique is not well validated. There is a high risk of contamination, in addition to all the associated difficulties of PCR.
Interest in peripheral laboratory settings?	Unlikely to have advantages over other techniques for peripheral laboratories, but may be a way to ship samples for processing at a reference laboratory. The technique needs more validation before it can be considered.

3.4 Other non-culture techniques

T-cell based tests

Companies	Cellestis (Australia/USA) for *Quantiferon*, and Oxford Immunotec (UK) for *TB Elispot.*
In the FIND portfolio?	FIND to fund evaluation study of Quantiferon.
Principle	These were touted as a replacement to Mantoux test (tuberculin skin testing). Blood is taken from a patient, and placed in an incubator for 12 hours in sterile culture with the tuberculin antigen. The tests measure release of a protein that is associated with immune activation. If a patient has previously mounted an immune response to TB, they will have cells in their blood that respond to the presence of the tuberculin antigen. These "primed" cells then produce the immune response measured in the test.
Stage of development	Several versions of the tests have been released, including some as a kit with all the required reagents. These tests are being validated in children and HIV+ patients.
Advantages	24 hour result; requires blood therefore can be used on patients unable to produce sputum, and may detect extra-pulmonary form.

Disadvantages	Blood must be incubated within 12 hours of the draw. A result is known after 24 hours, which means that the patient needs to come back to the clinic to be treated, which is less desirable. The tests require complex and sensitive laboratory manipulations. The Elisa format tests require precise pipetting, reagents require storage at 4 - 8°C and an electricity supply. There are also individual variations in the immune response that can make the results difficult to interpret. Current versions are not simple, robust diagnostic tests and many technical and medical questions remain associated with its use.
Interest in peripheral laboratory settings?	Currently, very limited. The tests do not give a positive diagnosis in itself - a positive result is suggestive of previous TB exposure, but is not necessarily informative about current disease status. The tests alone are not enough for a diagnosis (or exclusion) of TB and the interpretation of test results is not clearly defined. The relationship between detection of latent and active disease has been questioned. More information and investigation are needed.

Transdermal patch test for active TB

Company	Sequella (USA).
In the FIND portfolio?	No - the test was removed from FIND's portfolio in 2005.
Principle	This was touted as a replacement to Mantoux test, both to allow distinction between active and latent infection and to allow monitoring of treatment success. The test is a transdermal patch containing a TB protein, to be applied on the forearm. It leaves a red spot after a few hours if the patient has been exposed to TB.
Stage of development	An evaluation study was conducted in South Africa showed 65% sensitivity and 96% specificity.
Advantages	Test delivered at point-of-care. Non-invasive technique that requires no sample collection or handling. The company claims the test detects active TB only.
Disadvantages	Signal is not clearly visible on dark skin. Its use is unclear in patients in high prevalence regions. A follow-up visit is required to read the result. More evaluation is needed as not enough data are available yet.
Interest in peripheral laboratory settings?	The non-invasive method is a plus. More evaluation is needed, especially in HIV+ patients. The need for a follow-up visit to read the results on the patient's arm may limit its usefulness. The true performance of the test is not yet known.

DNA Microarrays

Companies	Not commercialised, research tool.
In the FIND portfolio?	No.
Principle	This proposed new molecular method for detecting drug resistance in M.Tb is based on the hybridization of DNA obtained from clinical samples to oligonucleotides immobilised on a solid support, such as miniaturized glass slides. Microarrays have been mainly used to detect resistance to rifampicin[45].
Stage of development	The technique is used in some research laboratories but it is unclear whether it will be developed further. Some groups are looking at hand-held "chip" versions as point-of-care tools.
Advantages	Rapid (~2 hrs); can be used to answer specific research questions.
Disadvantages	Expensive; beyond reach of most clinical mycobacteriology laboratories anywhere in the world. Technology complex and not adapted to routine use.
Interest in peripheral laboratory settings?	Not suitable for use in the periphery. The technology may however be developed into a point-of-care format, which may be useable in some settings, although it will likely always remain dependent on a particular machine or technology.

Non-NAA based DNA detection

Companies	Various companies/researchers identified.
In the FIND portfolio?	No.
Principle	Various methods exist to detect DNA sequences without amplification. For example, a probe binds to the specific target, thereby uncovering the catalytic site buried in the probe molecule. The exposed catalyst acts to change the colour of the buffer.
Stage of development	Still in early stages of development, although very little information available
Advantages	The techniques do not need NAA and therefore may be simpler to perform and more robust, as well as being less prone to contamination problems.
Disadvantages	Sample will nevertheless need preparation (DNA extraction and/or purification and concentration); sensitivity of detection may be difficult to achieve.
Interest in peripheral laboratory settings?	Depends on how the technology is developed, but a potentially simplified detection can be imagined.

Breath detection methods

Companies	Various companies/researchers identified.
In the FIND portfolio?	One UK-based group in FIND portfolio, other groups are also active.
Principle	Detection of volatile organic compounds in the breath of TB patients relative to controls.
Stage of development	Currently at the research stage. Many attempts have been made but the technology is not very specific yet. The Royal Tropical Institute in Amsterdam (KIT), in partnership with academic laboratories, are developing an electronic nose[46], as are some companies.
Advantages	Non-invasive technique. Possibly no reagents or consumables. High throughput possible.
Disadvantages	May detect only pulmonary form; Technically difficult to develop a breath test that is both specific and sensitive; Will remain "machine dependent".
Interest in peripheral laboratory settings?	No field prototypes are available yet. The non-invasive nature and the ability to screen large numbers of patients would make such a test interesting, but the technology seems to be a long way from being developed, despite some recent promising results[47,48].

One final technique warrants mention. The gel microdrop DST assay method encapsulates single cells in an agarose matrix and measures growth in the presence of antibiotics by flow cytometry[49].

Although in its current format this method would not be practical for use in the peripheral laboratory setting, its use might be developed. Further investigations would be necessary.

4. Conclusions and Recommendations

This overview of the research and development pipeline for tuberculosis diagnostics and drug sensitivity testing gives a mixed picture. On the one hand, the pipeline is unquestionably active, with a number of different tracks being explored, culture or otherwise, and some improvements have been made. However, if it is now possible to increase throughput, or obtain results faster than can be achieved with traditional culture methods, these improvements often come at the expense of sensitivity and simplicity.

In part this is due to the complexity of the problem. However, it is also revealing of a certain number of shortcomings in the way R&D is conducted for TB diagnostics today:

4.1 Diagnostic development must be based on an assessment of medical needs

4.1.1 Diagnostic development must start with the field specifications

The development of tests should be based on medical needs at the field level, and aim to answer specific questions raised by field practitioners. A critical pre-requisite for the tuberculosis diagnostic pipeline is for there to be consensus from the medical community on the main gaps that are to be addressed by any newly developed tool. Precise medical specifications must be explored, and defined, in order to guide scientists and the industry. Contact and collaboration between clinical and scientific communities must be reinforced: the medical end-users of the tests must feed into the test development pathway.

4.1.2 Diagnostic development must include a discussion of the medical algorithm within which the test is to be used

It is not useful to develop a test with no prior consideration of the medical context in which it will be used. Developers must consider how a test will be interpreted by practitioners, what information the test can give and how that information will be used to make a medical decision.

For example, knowing what mutation a patient has to a particular antibiotic will not help the doctor choose a treatment - it is more important to know the DST profile. A test that gives a result in one week may be too long for a patient that has suspected XDR TB but acceptable in a patient that is HIV negative. A test to be used in a remote health post with no doctor will have different requirements than a test to determine whether it is safe to put an HIV positive person on INH prophylaxis. A test to determine latent TB will be of limited use if there are no facilities or structures to deliver drugs or no agreement on whether latent TB should be treated.

4.2 Specifications must be defined

4.2.1 One size does not fit all

No one test will fit all the gaps. Armed with precise specifications and medical parameters, scientists and industry can aim to address specific TB patient populations that today are desperately neglected. These include children, TB/HIV co-infected patients, and patients infected with drug-resistant strains. Specific tests and strategies are likely to be needed for diagnosis of TB, especially in high HIV prevalence areas, in settings with high drug resistance prevalence, and detection of treatment failure. These considerations should inform and direct any priority-setting exercise to identify urgent needs.

4.3 The tests should be designed to be used where the patients are

Today, a significant number of tests suitable for reference laboratories or for district hospitals are being developed, although the majority of patients in urgent need of diagnosis are in the most remote or unsupported settings.

Given the number of people at risk, methods will need to be rapid, high throughput and inexpensive. To simplify the already complex process of patient management, and given the lack of human resources in many settings, the techniques should be point of care and rapid, so that patients are not required to return to the clinic.

A point of care tool to reach the widest patient pool is important, but so is how to integrate these tests into patient management if there is limited health infrastructure, resources and trained staff.

4.3.1 There is insufficient knowledge about local conditions

Very few of the companies approached for this analysis were well-briefed about the working conditions, including human, financial and technical resources, in peripheral settings. There is a need to distribute information about the reality of field conditions. Technical specifications of the new tests to be developed must be defined and made very clear, and must form the starting point of research and development.

4.3.2 High-tech strategies should not be force-fit to low-tech settings

It is tempting to take existing or nearly finalised technologies developed for highly equipped, resource-rich settings, and implement them in developing countries. However, we have too many examples of how this is not an appropriate approach. For example, focusing R&D efforts on adapting an Elisa format for peripheral laboratories or on developing an integrated nucleic acid amplification test ignores local needs and conditions, and will not deliver a diagnostic tool usable where it is most needed. A more useful approach would be to develop an integrated strategy that uses available tools in suitable supported settings in the interim, with active research into improving the options based on the approaches discussed above.

4.3.3 Simplification is needed in the interim

As part of a short- to medium-term strategy, existing technologies must be simplified, and alternative uses for them devised. Some tools, such as thin layer culture or manual MGIT, may be ready for immediate field implementation in specific settings provided they are introduced with a specific algorithm prepared in collaboration with clinicians. Despite the serious limitations of current diagnostics, their use should be optimized. In many settings this is not done.

4.4 Fundamental research questions must be addressed

TB diagnosis is very complex, largely because we do not really understand the disease and much of its fundamental biology. Some of these unanswered fundamental questions include how M.Tb hides from the immune system, the relationship between infection and disease, the type of antibody response, the bacterial load in different body sites. The underlying knowledge base can only be strengthened through a comprehensive strategy that prioritizes understanding the disease. It is not enough to "wait and see" whether existing tools can be adapted to resource-poor settings, or to hope for the "magic bullet" before addressing these questions. If a concerted effort is not put into fundamental research and into missing areas of knowledge, existing gaps will remain unchanged, and appropriate tools will continue to lack.

4.4.1 Public leadership is required in priority setting

There is a huge resource gap in TB R&D, and in particular in TB diagnostics, that cannot be filled only by the private sector. Governments and public agencies must take a leadership role in setting priorities and providing the necessary resources in order to ensure that appropriate tools are developed. Creation of a new inter-governmental working group on R&D as mandated by the World Health Assembly in 2006 could provide the necessary framework for action.

Scientists, doctors, public health specialists, laboratory technicians, and FIND should share information and resources, and work together to develop an integrated strategy. This should include groups from areas of expertise other than tuberculosis, who may provide innovative solutions to long standing difficulties (for example for ways to improve the sensitivity of rapid diagnostic tests).

4.4.2 Independent evaluation of new tools must be performed

There is a need for appropriate, high quality, independent evaluation studies of tests. To avoid any possible conflicts of interest, the bodies evaluating the test should be separate from the test developers or sponsors.

4.5 Access and pricing issues must be clarified

If interesting tools are priced beyond the reach of developing country health systems, access barriers will not be resolved. Crucial questions remain unanswered here. The Foundation for Innovative New Diagnostics, for example, negotiates rights with companies to provide the tests "at affordable prices" to the public sector in developing countries. Who defines what is affordable? What of pricing issues for tests developed outside of the FIND portfolio? Will companies provide tools at affordable levels? What about countries in which the public sector is weak and patients are obliged to go to the private sector for care?

Conclusion

We repeat our call for greater transparency, public leadership in priority setting, funding for R&D for TB diagnostics, and sharing of resources and information in a unified struggle against this terrible illness. In addition, field needs should guide product development. While the situation is bleak in terms of a “magic bullet” to be implemented in the field today, there is more research, funding and interest internationally than ever before in developing appropriate diagnostic tools. In the immediate term, we can make better use of existing tools. At the same time, we should invest resources in the development of alternatives.

We hope that some of the recommendations proposed here will contribute to the development of improved diagnostic tools.

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