TOWARDS LAB-FREE TUBERCULOSIS DIAGNOSIS

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ABBREVIATIONS

AFB	acid-fast bacilli
ARASA	AIDS and Rights Alliance for Southern Africa
CDC	Centers for Disease Control
CRP	C-reactive protein
DNA	deoxyribonucleic acid
EQA	external quality assurance
FDA	US Food and Drug Administration
FIND	Foundation for Innovative New Diagnostics
IGRA	interferon gamma release assay
LAM	lipoarabinomannan
MSF	Médecins Sans Frontières
MTB	mycobacterium tuberculosis
NA	nucleic acid
NALC	N-acetyl-L-cysteine
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
PCR	polymerase chain reaction
PHC	peripheral health centre
POC	point-of-care
QA	quality assurance
R&D	research and development
RFP	request for proposal
RNA	ribonucleic acid
SSM	sputum smear microscopy
TAG	Treatment Action Group
TB	tuberculosis
TBCDRC	Tuberculosis Clinical Diagnostics Research Consortium
TBTC	Tuberculosis Trials Consortium
TDR	Research and Training in Tropical Diseases
trDNA	transrenal DNA
VOC	volatile organic compounds
WHO	World Health Organization

EXECUTIVE SUMMARY

Tuberculosis (TB), fuelled in part by the HIV epidemic, remains a major challenge to public health. Despite the potential for most cases of tuberculosis to be curable, TB causes around two million deaths per annum. These deaths are in part due to late or missed diagnosis. Improving the performance of diagnostics and their availability are key to reducing global morbidity and mortality.

Despite the recognised importance of diagnostics, funding for TB diagnostics remains inadequate. Recent years have seen progress in TB diagnostics with attention focused most recently on the Xpert MTB/RIF system. Despite this progress, there is still a lack of suitable, simple diagnostics for peripheral health centres or community-based diagnosis of TB.

This report provides an overview of the current state of science and research on TB diagnostics, as well as an assessment of resources such as specimen banks available for TB diagnostics research. It details some of the key roadblocks to the development of a simple point-of-care test for TB suitable for peripheral health centres, such as the need for novel biomarkers that can be used to detect TB and can form the basis of test design; the lack of specimen repositories to provide well-characterised samples for the validation of these biomarkers; and the need for technological breakthroughs in sample collection, processing and testing to improve quality of materials collected from the site of disease to enhance diagnostic accuracy.

Several recommendations are provided which advocate for improved research into TB diagnostics. Importantly, test development needs to be better aligned to the clinical decisions that are made in the field. Alternative technologies to target existing biomarkers and the identification of new biomarkers are needed.

The search for new TB biomarkers, critical for the success of novel point-of-care tests, requires increased financial investment and stronger, more focused direction and collaboration from the funders, the test developers and the end-users of the test. Access to new funding mechanisms, research models and sample banks with well-characterised specimens are an important part of this development, as they are critical to identifying and validating biomarkers and developing the much needed point-of-care diagnostic tests.

Without significant changes to the established research and development into TB diagnostics, a true point-of-care test is still far from realisation. However, we believe that if some of these recommendations are implemented, the successful development of a new point-of-care TB test will be one step closer.

1 THE PROBLEM

The unchecked epidemics of tuberculosis (TB) and HIV remain two of the greatest global challenges to medicine and public health. In the absence of an effective vaccine for either condition, the control and ultimate elimination of these diseases still rests on prompt diagnosis and therapeutic intervention to reduce ongoing transmission[1-2]. Whilst HIV diagnosis has been greatly assisted by the development of robust point-of-care (POC) or lab-free diagnostics suitable for field use, the diagnosis of TB remains clinically challenging and logistically difficult in resource-poor settings[3].

The interaction between HIV and TB makes the diagnostic challenge even greater[4-5]. Clinical manifestations of TB differ in HIV-positive individuals, and clinical screening tools perform less well. Traditional diagnostics based on sputum are particularly challenging in HIV-positive patients where the majority have smear-negative disease[6]. The consequences of this shortfall in diagnostics are severe. Most importantly, many individuals with active disease fail to receive treatment, with an attendant increase in mortality and an increase in ongoing disease transmission to family and community members. Developing improved diagnostics to address these challenges will require a substantially larger and targeted coordinated global effort.

1.1 A JOURNEY TO TB DIAGNOSIS

It is estimated that 60 percent of those who require assessment for TB present in local health facilities, where implementing complex diagnostics is not necessarily feasible (Level 3, see Figure 1 and Table 1). Even if TB diagnostics are available, complete diagnosis may require referral to a specialist centre, which may be some distance from the patient's home. Loss to follow-up or defaulting from care is a major problem in healthcare delivery in resource-poor settings[7-9]. With high opportunity costs, the need to travel and the distance to referral centres, the ideal TB diagnostic is therefore a point-of-care test that allows patients to be diagnosed and receive appropriate care within hours of undergoing TB testing[10-11]. It is likely that POC formats will significantly reduce the loss to follow-up, but there are limited data available on the potential health impact of POC tests in resource-poor settings[12-13].

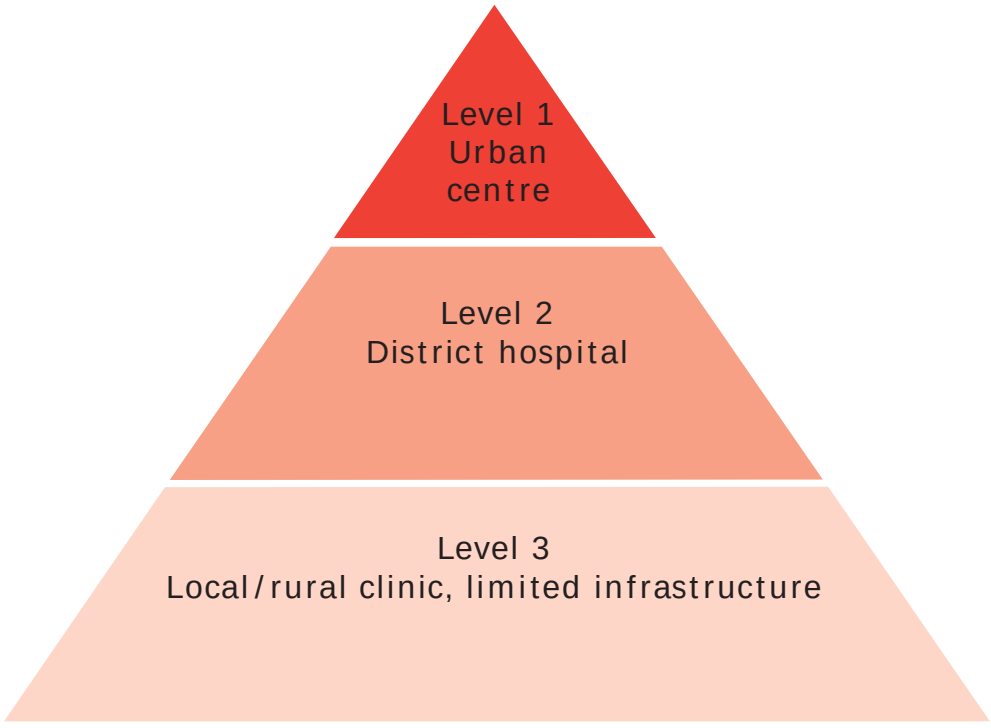

Figure 1: *A categorical classification of facilities where diagnostics may be implemented. These definitions can be applied to low and middle-income countries. The majority of patients in low and middle-income countries are likely first to access healthcare for suspected TB at a Level 3 facility. Community/household diagnostics can be considered separately, but within this report it will be assumed that the requirements are similar to Level 3.*

	LEVEL 1	LEVEL 2	LEVEL 3
Power/AC	Available and reliable	Available	May not be available/reliable
Clean water	Available and reliable	Available	May not be available/reliable
Buildings	Well-equipped laboratories with safety facilities	Smaller laboratory facilities offering less sophisiticated testing and safety facilities	Limited or no laboratory facilities
Staff	Nurses, physicians, trained laboratory staff	Nurses, few physicians, few or variably trained laboratory staff	Nurses, healthcare workers, no/limited laboratory staff
Typical locations	Urban centres, large hospitals, teaching hospitals	District hospitals, urban health centres	Health clinics, usually local

Table 1: *Categorisation of facilities and typical infrastructure available (adapted for use in this report).*

Microscopy and culture methods are the main TB diagnostics used in resource-poor settings. Both require sputum samples which are not always easy to obtain. Sputum is a heterogeneous sample and can be difficult to manipulate, and the excretion of TB bacilli varies by individual. Treatment methods to improve yield, consistency and liquidity, including centrifugation and bleach treatment, have been shown in some studies to improve the sensitivity of smear microscopy and nucleic acid (NA) tests, although results have been varied[14-15]. Sputum samples are usually collected in the field and, if destined for a sample bank, are stored with glass beads to homogenise the sample and allow easier manipulation. Despite the difficulty described above, the majority of TB detected worldwide is pulmonary, explaining the continued use of a sample that is so difficult to collect and manipulate.

The major restrictions for sputum are: 1) not all suspect TB patients are able to produce sufficient quantities of sputum, particularly children; and 2) HIV-positive patients may have reduced numbers of bacilli and so will appear as smear-negative. For these two patient groups, in particular, sputum microscopy is of limited use. In cases of extrapulmonary TB (more common in HIV-positive individuals), a sputum sample will be of no use whatsoever. However, in high TB burden/low HIV prevalence areas, sputum microscopy remains an important diagnostic tool for pulmonary TB.

Recent years have seen significant progress in technologies and products with the potential to advance the diagnosis of active TB. Notable amongst these products is the Xpert MTB/RIF (Cepheid, US) assay which operates on the GeneXpert system and has promising performance in the crucial area of smear-negative/culture-positive disease, and the potential to be used in Level 1 and 2 settings. Its potential for widespread implementation in Level 3 facilities is not yet clear. There are currently no POC TB diagnostic tests which are suitable for use in rural Level 3 health centres. New tests must be POC with rapid results available, to both patient and healthcare worker, in an attempt to ameliorate some of the significant and damaging loss to follow-up in these settings. The focus of this report is to review current progress towards diagnostics which might meet this need and to identify obstacles in test development.

This report is a frank landscape analysis of the status of progress towards a POC TB test. Literature reviews, personal experience and interviews with TB diagnostics researchers in academia and industry are used to assess the state of the art. We will identify the major roadblocks which have so far prevented the successful development of a POC TB test. We will also provide solutions to overcome these hurdles which, if implemented, would reduce the time needed and the barriers to successful development of a POC TB diagnostic test. A second focus of this report is the suitability of specimen resources available to test developers focused on product development, and how they might be improved. This report should be used as an advocacy tool promoting new ways to achieve progress towards a POC TB test.

2 THE STATE OF THE ART IN POINT-OF-CARE TB DIAGNOSTICS

In order to meet Millennium Development Goals, it is clear that new diagnostics suitable for low and middle-income countries are needed. With few financial incentives available, and a low financial return, the diagnostics industry has traditionally failed to engage with these efforts. In the last few years, the need for research and development (R&D) into a POC TB test has been increasingly recognised, and some support has become available, mainly through philanthropic organisations such as the Bill & Melinda Gates Foundation.

However, despite the effort and financial resources that have been invested, POC diagnostics for TB have not materialised. Many of the commercialised serological tests for TB measuring antibody responses to mycobacterium tuberculosis (MTB) (which lend themselves to the POC format) are unsuitable for use due to poor sensitivity and specificity, as described by a comprehensive WHO/TDR report[16]. Recent developments in nucleic acid-based diagnostics with the Cepheid Xpert MTB/RIF assay are very encouraging. Whilst not truly POC, and not ideally suited for Level 3 settings, they present the possibility of creating new TB diagnostics, which could have an enormous impact in the developing world.

The current challenge is manifold: to develop and progress already identified biomarkers into diagnostics with a POC format; to search for new biomarkers specifically for low-cost POC tests; and to search for the holy grail of a 'dipstick' POC TB test. Biomarkers are detectable indicators of a disease or pathology which can be derived from the pathogen itself (e.g. lipid, carbohydrate, protein or nucleic acid) or a molecule which is altered beyond normal ranges in response to the pathogen, for example inflammatory markers in response to MTB infection. Biomarkers such as the rpoB gene in MTB have already been identified as useful pathogen markers, which could be exploited for new diagnostic methods. The challenge remains to deliver these into diagnostics suitable for rural or community-based settings in low and middle-income countries that have little or no laboratory infrastructure and lack highly-trained personnel.

The second part of the current challenge is to push for new biomarkers for simple POC diagnostics. It is highly desirable to have a rich pipeline of markers until such time as one (or several in combination) has been characterised and validated. Markers which can be used in a POC format should be prioritised. These biomarkers must be present in sufficient quantities to be detected in a POC system (which generally have a poorer limit of detection than laboratory-based testing). Nucleic acid detection, in particular, will not translate easily into a POC device. The challenges of nucleic acid detection/amplification in a peripheral health centre are not to be underestimated, because the use of small quantities of sample make reproducible nucleic acid extraction more difficult; reagents should not require cold-chain storage; while cross-contamination and the complexity of the nucleic acid extraction procedure are technological hurdles. It is worth noting that there are no successfully marketed genuine POC nucleic acid tests anywhere in the world (for TB or other infectious diseases), due in part to the technical complexity and also to the reduced need for POC tests in high-income countries. The search for a viral load POC test for HIV has not produced a completed commercialised product, despite relatively high investment, and despite viral load being a long-established marker, accepted as both a diagnostic and as a treatment efficacy monitor for HIV.

3 MARKETED SOLUTIONS FOR TB DIAGNOSIS AND THEIR REACH

The next section describes some of the marketed (or close to receive market authorisation) solutions for the diagnosis of TB, with a summary of the authors' assessment of their suitability for low and middle-income countries. This is not an exhaustive list, and other publications and reports have detailed the commercialised diagnostic tests available and their projected impact in Level 3 laboratories/health centres[17-20]. Rather, this report aims to highlight the fact that, despite some recent advances in TB diagnostics, the majority of the commercially available tests remain firmly rooted in Level 1 laboratories, i.e. reference laboratories in large hospitals where a supportive infrastructure, including polymerase chain reaction (PCR) clean rooms and trained personnel, is needed. This is not to say that the following diagnostic tests are not important and useful in a defined setting, but they do have severe accessibility limitations in reaching the populations who most desperately need rapid TB diagnostics.

3.1 DIAGNOSTICS DETECTING PATHOGEN OR HOST RESPONSE

Diagnostics for TB can be split into those which look for the presence of mycobacteria (MTB) and those which look for the host response to the bacterium (usually immune response) to MTB. We have focused here on the diagnosis of disease rather than on the monitoring of treatment efficacy, as there may be different requirements for a marker of treatment success[21]. Following is a list of diagnostics using either host or pathogen detection markers, with a summary of their suitability for low-income countries.

3.2 TB DETECTION TECHNOLOGIES SPLIT BY HEALTH CARE LEVELS

LEVEL 1 HEALTHCARE TEST		
Xpert MTB/RIF assay[22-24]	POSITIVES Excellent sensitivity and specificity in smear-positive sputum. Good sensitivity in smear-negative, culture-positive samples (>70% in single samples, >90% in triplicate samples). Rapid: <2 hours. Potential for Level 2 laboratories: machine footprint small and sample processing is relatively simple. Other sample sources are under investigation.	NEGATIVES Expensive per-test cost (approx $17 and more so if using triplicate samples to obtain high sensitivity in smear-negative sputum). Complex and expensive instrumentation ($17,000). Cannot differentiate between live and dead bacteria. Is mainly focused on sputum as the sample source, although evidence of role in extrapulmonary disease is emerging. Instrument maintenance in low and middle-income countries may be problematic.
BACTEC MGIT series[25-26]	POSITIVES Gold standard liquid culture methodology. High throughput with automated series.	NEGATIVES Expensive per test costs. Complex instrumentation, Trained staff required. Not suitable for Level 2 and 3.
LEVEL 2 HEALTHCARE TEST		
LAMP tests[27-29]	POSITIVES Relatively simple to use. Needs only a limited laboratory structure.	NEGATIVES Mostly in-house assays so far, although commercial release reportedly imminent from Eiken. Problem to read fluorescent results without instrument component; other read-outs needed. Sensitivity and specificity to be improved as well as integration with POC extraction systems.
Urinary LAM tests eg Alere ClearView[30]	POSITIVES Uses non-invasive sample. Relatively easy to perform.	NEGATIVES Requires some supportive lab infrastructure. Sensitivity not promising. Test may be under redevelopment.
LEVEL 3 HEALTHCARE TEST		
Sputum smear microscopy (direct or concentrated)[14-15, 31]	POSITIVES No complex instrumentation needed for direct smear microscopy. Not a complex procedure technologically. Well-established methodology High specificity in most settings.	NEGATIVES Low throughput and need for EQA. Sensitivity very variable (20-80%). Some degree of technical expertise required. Trained staff needed. Fluorescence more expensive (LED costs lower). Infrastructure required, particularly for concentrated samples e.g. centrifugation.

Table 2: *List of currently available case detection TB tests*

3.3 XPERT MTB/RIF ASSAY

The most recently developed solution for TB diagnosis is the Cepheid Xpert MTB/RIF assay that runs on the GeneXpert system. The development of this test represents a step in the right direction for TB diagnostics, as it detects both the pathogen and also mutations associated with drug resistance. A large evaluation study of Xpert MTB/RIF in some 1,500 patients was carried out in five experienced trial sites in 2008-9. The assay detected almost all (97.6%) culture-positive patients (including over 90% of smear-negative patients) when used in triplicate. Using a single sample, the Xpert MTB/RIF test detected more subjects than did LJ culture. Performance was remarkably robust across all sites. Subsequently, over 6,000 patients were tested by Xpert MTB/RIF in demonstration projects at a range of different facility types in six countries. Again, performance remained high, with detection surpassing LJ culture for a single specimen. More than a dozen other studies are completed or ongoing and a number of reports are in print, including for extrapulmonary and paediatric TB. With the improved detection of smear-negative sputum samples, the Xpert MTB/RIF tests represent a further step towards improved TB diagnostics[22-24].

3.3.1 COSTS

Although preferential prices have been negotiated by the Foundation for Innovative New Diagnostics (FIND) for high-burden low and middle-income countries, the instrument and per-test costs are still significant (US$17,000 and US$16.86 respectively). As with all pathogen detection tests, sensitivity in the smear-negative/culture-positive patients is improved by duplicate or triplicate testing, but such an approach clearly increases costs. Preliminary data from a cost-effectiveness study showed the test to be cost-effective even when the fully-loaded cost (including instrument and laboratory staff costs) ranged between US$25.15 and US$33.65[i]. Further data from cost-effectiveness studies will be a welcome addition to make a good case for this test.

3.3.2 SOME SAMPLE PROCESSING REQUIRED

Sample processing is limited to the addition of a single reagent to the sputum cup which sterilises the material, removing the bio-safety requirements usually associated with sputum processing. A minimum of 1ml of sputum is required, aiding the applicability of the assay for those who are unable to produce larger quantities of sputum, such as children or HIV-positive adults.

3.3.3 REACH

A complex instrument with a reasonably high customer capital outlay will have a limited reach in the areas which have the greatest problems with MTB infection. In sub-Saharan Africa the majority of suspected MTB patients will access healthcare at local health centres. In these situations a POC test would be the most suitable and reach the highest number of patients. These health centres often have very limited infrastructure and are not able to maintain and implement real-time PCR-based diagnostics.

The World Health Organization (WHO) has recently endorsed this new diagnostic test and recommended its implementation in district and sub-district laboratories[ii]. The Cepheid test is well suited to urban settings and the use of Xpert MTB/RIF in an advanced laboratory with extensively supportive infrastructure in low-income countries is certainly possible. Level 2 healthcare settings are also likely, due to the relatively small footprint and the limited hands-on technician time necessary. Accessibility for patients is limited by the placement of these tests at Level 2 healthcare settings or higher. Current roll-out of this new test largely aims to place it in Level 2 healthcare facilities, but its performance in these settings under routine programme conditions remains to be fully assessed.

4 BIOMARKER DISCOVERY

The search continues for novel markers to rapidly diagnose and monitor treatment response in active (and latent) TB, and is one of the critical research priorities of TB diagnostics research. There have been recent attempts to review the field of biomarker discovery, notably the TDR/EC report of 2009[32-34], and a further attempt is not made here. Current efforts to identify new diagnostic targets range from those based on pathogen products (whole cells, nucleic acid, protein, lipid, carbohydrates and other small molecules) to molecules of the host immune system (proteins and cells) and both cellular and antibody responses against specific targets.

In the era of "-omics", when it is possible to undertake large-scale searches for biological markers of disease, emerging technologies have been applied and continue to be applied to identify novel markers of disease, particularly from blood and urine, but also from breath analytes. Techniques – including proteomics, transcriptomics, lipomics and metabolomics – are all being applied to the problem, with some projects at a more advanced stage than others. It is not possible to know the full extent of ongoing research activities, given the tendency not to disclose preliminary findings publicly, whether generated in academia, in industry or by non-governmental organisations. However, some critical evaluation of known biomarker discovery approaches is helpful for later discussion of specimen collections and an analysis of which specimen types are needed to support biomarker discovery research.

[i] www.stoptb.org/wg/gli/assets/documents/Mtg3pres/Day%201/session%203/Xpert%20MTB%20RIF%20-%20scenarios%20for%20cost%20effectiveness%20A%20Vassall.pdf
[ii] http://whqlibdoc.who.int/publications/2011/9789241501569_eng.pdf

Proteomic signatures from blood able to diagnose TB have been published, for example the work of Agranoff and colleagues[35-36], and part of the validation process for that work involved the use of specimen banks. Other large projects including proteomic approaches are underway (notably Gates-funded GC6_74, EU-funded FP7 grant, and FIND's work with Antigen Discovery Inc and the Public Health Research Institute to identify TB antigens useful for diagnostics[iii]). It is important to note that, despite these results, no test product has yet emerged based on these findings, and there is little data on the performance of these predominantly host-derived markers in the setting of HIV-positive patients.

Large projects are underway, and are in some cases completed, exploring transcriptomics (the study of the products of gene expression, particularly in the cellular compartment of peripheral blood). Such studies have become possible with the advent of reliable micro-array techniques, and are relevant to the question of whether expression profiles could be potentially used as a diagnostic, particularly in adults without HIV infection. However, evidence to date[37] suggests that diagnostic signatures (discriminating, for example, latent infection from active disease) will require in the region of 100 markers. Whether such profiles can be converted to tests has to be a distant prospect. Perhaps more importantly, whether such host profiles can be used where they are needed most – in the setting of variable degrees of immunosuppression and children – remains to be tested. Nonetheless, appropriate samples collected to allow stabilisation of RNA are not well-represented in specimen banks. However, established protocols exist for their collection which can be undertaken relatively easily.

Metabolic profiling approaches have been applied more recently to the problem of TB diagnostics and are the subject of several ongoing research projects. These are yielding potentially interesting biomarkers for new diagnostics. Whilst there is clear evidence that it is possible to identify pathogen-specific metabolic markers in *in vitro* systems, there are great challenges in being able to identify the same markers *in vivo* and distinguishing them from non-specific host responses. Detailed clinical studies are needed to understand these processes better.

Similarly, approaches targeting lipid identification[38-39] are showing potential in the development of diagnostics, particularly from sputum, where banked specimens have been useful in the evaluation of novel methods, and data is likely to be published soon. However, again there are significant technical challenges in the analysis of lipids in urine which, if overcome, could greatly increase the potential for such markers in developing POC tests.

Putative biomarkers require extensive validation using reference samples which are collected using reproducible and established protocols. Such protocols are not 'future-proof'. Currently, the absence of a single protocol for collection and the potential instability of analytes over time (as highlighted by work focused on transrenal DNA) may mean *de novo* collection of specimens will be preferred for validation in some circumstances[40-41].

Other approaches are being developed and evaluated and this list is not exhaustive. One key issue for specimen collection is that it is currently impossible to predict which protocols could be the most appropriate for specimen collection for all tissue types. All resources collected under pre-defined protocols will therefore be vulnerable to future technical developments.

5 POTENTIAL INTERNATIONAL RESOURCES FOR SPECIMEN REPOSITORIES

It is widely accepted that the early development of novel TB diagnostics is facilitated and accelerated by good specimen repositories available to academic investigators and industrial developers alike. A search for all available bioresources was made through literature review and extensive interviews with active TB researchers in academia and industry (see Appendix 1). Two resources were identified that already exist to address the need for well-characterised samples in TB diagnostics development (WHO/TDR and FIND), and the strength and limitations of these resources are addressed in detail below. In addition, potential new resources for specimen collection are discussed.

5.1 THE WHO/TDR SPECIMEN AND STRAIN BANKS

The recognition that the absence of well-characterised clinical samples is an obstacle to TB diagnostic development led to the creation of a specimen repository within WHO/TDR in 1999, with the aim of supporting diagnostic test development and evaluation[42]. In its more than 10 years of operation, the bank has distributed more than 10,000 samples. We will focus here on the model adopted by the bank and discuss in detail its strengths and weaknesses.

Initial collection of specimens was parsimonious, based on the ability of existing sites to meet specified criteria with the capacity for clinical phenotyping, adequate laboratory

[iii] www.pnas.org/content/early/2010/07/27/1009080107.abstract

facilities, chest X-ray facilities and facilities for sample export. The sites for specimen collection have evolved in phases, with initial recruitment from sites in South Africa, Uganda and The Gambia, followed by Canada, Spain and Brazil. Following an open application process, further samples were collected from Vietnam, Bangladesh, Kenya and Colombia, with additional HIV-positive samples collected from Zambia.

The bank has collected specimens from adults over 18 years with a persistent cough for more than three weeks. Patients receiving TB treatment in the two months previously are excluded. Those without proven disease are followed for at least two months to establish alternative diagnoses or absence of TB.

For clinical phenotyping, patients are categorised according to both the nature of their diagnosis and their response to antibiotic treatment. Four main clinical groups are included in the archived samples:

1) Smear and culture-positive, with at least two positive smears and two positive cultures.

2) Smear-negative and culture-positive, with >1 negative smear and >=2 positive culture.

3) Smear and culture-negative, but suggestive chest X-ray and response to TB treatment but not broad-spectrum antibiotics.

4) Non-TB (smear and culture-negative) – untreated for TB but response to broad-spectrum antibiotics.

Smear microscopy is based on digested and concentrated sputum. As shown in Appendix 1, Figure 4, the great majority of samples are from individuals in groups 1 and 4 and, given the difficulty in full characterisation of patients, very few are in groups 2 and 3. The data extraction sheet is shown in Appendix 1. No additional information is collected on co-morbidity, concurrent medication or immune status (including CD4 count for those who are HIV-positive). HIV status is available for a majority of the specimens held by the repository.

5.2 SPECIMENS COLLECTED

Collection within the bank is focused on serum, sputum, saliva and urine at the time of enrolment, with no further specimens collected during follow-up. Sputum specimens are concentrated, examined in smears by light microscopy and cultured in both liquid and solid media. Following liquefaction with glass beads and N-acetyl-L-cysteine (NALC), sputa are stored in aliquots of 5 x 0.5 mL. Urine samples are centrifuged and stored without preservatives. Saliva is collected with cotton pads placed on buccal mucosa and centrifuged to leave 5 x 0.5 mL saliva. Twenty mL of un-heparinised blood is drawn, allowed to clot and centrifuged to yield serum stored in 20 x 0.5mLs aliquots. Over time the collection of saliva specimens has been deliberately dropped from some sites, in part due to the difficulties of collecting quality specimens and in part because of relatively low demand for these samples.

5.3 TRANSPORT AND STORAGE OF SPECIMENS

Specimens are collected and aliquoted under locally-approved versions of a master protocol before being transferred to a central biobank repository. In the time that the bank has operated, two facilities have been used. One, in the US, is based in a private organisation which has been the subject of two acquisitions and is now operated by Zeptometix. Although there were no concerns as to the integrity of the samples, the experience led to a parallel arrangement with the Biobanque de Picardie in Luxembourg, which is closely linked to Amiens University and houses the biobank for the hospital.

5.4 MODEL OF ACCESS

Applicants submit their request for samples to the WHO/TDR. Applications are for batches of either 20 or 200 samples and are reviewed by a committee of experts appointed to the advisory group of WHO (made up of seven individuals). Individuals on the committee are asked to make a declaration of conflicts of interest and sign confidentiality agreements. Applicants are not asked to pay for access to samples, but are asked to pay for the handling and shipping of samples from the central repository. Current charges are approximately US$150 for 20 samples, US$250 for 200 samples, with a handling fee of US$85 per shipment.

5.5 USER EXPERIENCE

From 2004 to 2010, 60 percent of applications were approved. The main reasons for rejection were the lack of data supplied by the requester to the bank to explain the work proposed, and the inadequacy of the proposed project to meet the main objectives of the sample bank. The bank was approached by approximately 49 ‘principal investigators’ of whom 53 percent were in the private sector. The majority of requests for samples were for technologies based on immunodiagnosis (see Appendix 1, Figure 7).

There is relatively little information available on the experience of test developers trying to access the bank, and no follow-up to ascertain if the samples had been useful and fulfilled their goal.

A user survey performed by WHO/TDR in 2004 had a very low response rate, and reliable conclusions cannot be drawn from it. However, WHO/TDR provided details of a small number of those who had responded. Whilst wishing to remain anonymous, the users indicated a strong desire for fully characterised samples from confirmed TB patients which were supplied promptly on request. Disclosure of limited data

Anonymous users of the WHO/TDR biobank believe that the provision of samples for confirmation of marker screening and validation is a critically important step in the diagnostic development process.

User feedback suggested that improvements in the access/application process, sample characterisation and timeliness of provision would improve the utility of a sample bank. One user believed that the one-year process for access had ultimately been futile due to the paucity of information provided with the samples

was not deemed problematic, but may not be representative of all current or potential users of the bank.

5.6 OUTPUTS FROM THE SPECIMEN BANK

It is not part of the work of the bank to evaluate the impact it has had on test development. Although there have been some limited efforts internally to collect data, including a limited assessment of end-user experience, no formal evaluation of impact has been made. Cited examples of productive uses of the bank include early evaluation of the performance of the Cepheid Xpert MTB/RIF platform (against strain collection) and the evaluation of a panel of serological diagnostic tests

5.7 COSTS

Costs for the establishment and running of the bank are estimated by WHO/TDR to be approximately US$1m over 10 years, though a detailed breakdown of where these costs lie is not possible, and the reliability of this figure cannot be verified.

5.8 STRENGTHS OF THE MODEL

As the original resource for specimen banking, the WHO/TDR resource has significant experience with the creation and management of a bank, including issues around collection protocols, ethics, sample transportation, storage and access, which have been well characterised. Internal cost estimates published by the group suggest costs of approximately US$1m over 10 years. In light of the costs estimated in preparation for a frozen trial (see below), these costs are at the low end of what might be anticipated for such a resource. Expertise for collection, preparation and sampling is decentralised, providing an opportunity for local training and capacity development. There are established and proven links to professional biobanking facilities allowing for professional quality control, reliable protection of samples and rapid access to samples.

5.9 WEAKNESS OF THE MODEL

General awareness of the WHO/TDR service is low, even within the TB research and development (R&D) field. It is hoped that recent efforts to improve the website, produce papers and speak at meetings will improve access. Researchers have indicated a strong desire for such a service, and demand is expected to increase over time. In common with other resources, there is relatively little data generated from the use of the specimens in the public domain.

Specimens are limited to adults aged 18 and over, thereby excluding paediatric TB cases. This is an important deficiency, also not met by any other resources, as there are potential scientific reasons to believe that a theoretical diagnostic marker might differ both quantitatively and qualitatively between adults and children.

There is some controversy over the inclusion of paediatric samples for a TB bank. Whilst the pathogen biomarkers are not likely to differ between adults and children, high-quality specimens for pathogen-based diagnosis in children are hard to obtain in clinical practice. It is plausible that other specimens (e.g. blood or urine) might play a greater role in paediatric diagnosis than in adults.

Despite the critical need for diagnostic tests for individuals infected with HIV, including those with extrapulmonary TB, the specimen bank is relatively deficient in this area, though recent attempts have been made to increase recruitment of HIV/TB patients from Zambia. In addition, the detail of the clinical definition is also a problem in regard to HIV. By using inclusion criteria based on a prolonged cough, it is likely that, even with an increased number of HIV-positive individuals, pulmonary disease will be overrepresented in relation to extrapulmonary disease, and that non-invasive diagnostic markers (of which urinary M. tuberculosis lipoarabinomannan (LAM) remains the best example) could underperform in such patient groups. In addition, there are relatively few samples from clinical groups 2 and 3 (see paragraph 5.1), which represent the more challenging diagnostic types.

The method of microbiological characterisation is also relevant to the needs of test development. Most programmes in resource-poor settings, where tests are most needed, utilise direct smear microscopy, which has a threshold for detection of approximately 10,000 acid-fast bacilli (AFB)/mL sputum[iv]. However, specimens collected as part of the WHO/TDR bank are characterised using digested/concentrated sputa which have a lower threshold for detection of approximately 1,000 AFB/mL sputum. WHO recommendations that a new test to replace sputum smear should detect >95% smear-positive disease and >50% smear-negative disease do not specify the nature of the smear to be examined. Whilst the nature of these recommendations are a separate issue from those considered here, a sample characterisation for the specimen bank based on direct smear would provide an alternative evaluation of a new test, although potentially lowering the bar for comparison of diagnostic performance. It is possible to envisage a trade-off between diagnostic performance and convenience of a POC device.

[iv] Dr Suman Laal, personal communication.

The mixture of cases and controls from sites of differing population ethnicity, and different circulating pathogens, where some sites contribute more to cases than controls, does open the possibility of bias in the comparisons of different groups. It is difficult to estimate how likely this is to influence the performance of tests. The composition of the group responsible for steering the resource and screening applications for samples comprises several experts in the field. However, there is the potential for other skillsets and experience to complement those already represented to help in the development of a test. For example, there could be greater clinical expertise (particularly in paediatrics), expertise from end-users in primary care settings, and expertise from test developers (both academic and industrial).

There is some evidence to suggest that access to samples is too slow and could impede product development. The lack of a strong contract between applicants and developers means that there is no leverage over test developers to ensure tests are provided at affordable prices where they are most needed. A passive process from the bank regarding product development does not plan for negotiation on access or pricing for products in the developing world.

5.10 WHO/TDR STRAIN BANK

As a related but distinct facility, the WHO/TDR will launch in late 2010 a repository of MTB strains available to the TB R&D community[43]. The aim of this is to collect and curate biological specimens representative of different patterns of drug-resistance in different geographic regions. These samples will be well characterised both genotypically (including VNTR-MIRU and spoligotyping) and phenotypically. Strains will be lyophilised for long-term storage.

5.10.1 STRENGTHS AND WEAKNESSES OF THIS RESOURCE

The strain bank provides a potentially important resource to developers working on pathogen-based detection techniques, and in particular those working on drug susceptibility testing. It is not clear yet how the resource will be administered and access controlled. There is a need for a detailed catalogue of strains representing a full range of possible mutations for the development of sequence-based assays, and it is not yet known how comprehensive this resource will be.

5.11 FIND REFERENCE MATERIALS PROJECT

The specimen repository within FIND has many similar characteristics to the WHO/TDR model, but operates within a different environment. Whilst the FIND resource is a significant component of resources available to test developers, less information is openly available in comparison to the WHO/TDR bank and therefore less detail can be provided here.

The reference materials project is a resource to which test developers can apply for access. Applications are not solicited for use of the specimens *per se*, but rather the use of collected specimens is one part of the process of collaboration between FIND and the test developer which will be agreed through a formal negotiation process.
Two types of specimen collection exist. The first focuses on serum, urine and sputum collected from individuals recruited at one of seven sites in Vietnam, Brazil, South Africa, Peru, Bangladesh, Uganda and Tanzania. Estimates of the numbers of samples collected by site and specimen type by the end of June 2010 are included in Appendix 1. A detailed breakdown of clinical characterisation including HIV status is not available for this report, but all are adults and those recruited in South Africa are comprised mostly of individuals known to be infected with HIV. These reference materials are stored in a centralised facility in Bangkok, Thailand, by an NGO called Health Concept International.

In addition, further samples collected as part of specific projects are kept in local facilities within the countries above, and also in Zimbabwe, Azerbaijan and India, and focus on sputum and urine specimens only. For some companies with well-evolved technologies, the collaboration with FIND has proven attractive. A list of institutions/companies that have some form of approved access is included in Appendix 1.

5.11.1 STRENGTHS AND WEAKNESSES OF THE MODEL

For potential product developers, use of the FIND resource is part of a partnership with an organisation which has experience in working with commercial partners in the development of products, including products that have reached the market. Understandably, in part because of confidentiality agreements, there is limited information on the progress or otherwise of products in development, particularly those that have not progressed. However, a list of organisations/institutions that have accessed the bank is included in Appendix 1.

In addition to the two existing specimen repositories described above, there are new initiatives which have the potential to make an important contribution to resources for diagnostic development. These are:
1) The development of a ‘frozen trial initiative’.
2) Academic collaborations, notably the TB Clinical Diagnostics Resource Consortium (TBCDRC).

5.12 PROPOSALS FOR THE DEVELOPMENT OF A FROZEN TRIAL INITIATIVE

There are advancing discussions around the development of a ‘frozen trial initiative’, which has been given some impetus by the US Food and Drug Administration (FDA)’s Critical Path Initiative. The key driver to this is that biomarkers are needed to accurately predict treatment response (or “cure”) with a view to assessing shorter treatment regimens and being able

to run shorter (and cheaper) clinical trials. With a number of trials of new drugs anticipated, particularly with products from Tibotec, TB Alliance, and Otsuka, it is hoped that well characterised samples that have undergone detailed and expensive clinical phenotyping will be banked and provide an important resource to validate new biomarkers that can be used as a study endpoint acceptable for regulatory approval.

There are advanced plans from the Global Alliance for TB drug development (which has recently received funding from the FDA[v]) to provide such a resource to the international scientific TB community, from the ongoing ReMox Trial designed to evaluate the substitution of Moxifloxacin into current anti-TB regimens with a view to shortening treatment duration. This project will be carried out in partnership with the CDC TB Trials Consortium (TBTC). The proposed sample collection will focus on a subset of patients and provide sputum (2-3mL), blood (10.5mL) and urine (11.5mL) from 10 sequential patient visits spaced through the course of treatment. Through detailed planning of what is likely to be required for a scheme operating in two countries (South Africa and India), anticipated costs to run the scheme for 10 years are in the region of US$6-7m.

The key benefits of such a process are:

1) Extensive collection of biological samples from well-characterised clinical cases where detailed information will be collected on baseline clinical features, clinical strains, treatment and treatment adherence and, perhaps most importantly, with extensive follow-up to provide hard clinical endpoints ("failure" or relapse).
2) A relatively cost-efficient process for collecting samples, where the infrastructure for staffing and much of the costs of clinical phenotyping will be covered by trial funding.
3) The potential to accelerate regulatory approval of any new diagnostics, drugs and vaccines with the FDA.

There are also limitations to the process:

1) Although many samples can be collected, they will still be precious, particularly as only a small proportion of those collected will be from patients who relapse and fail. Access and use of these samples will therefore need to be carefully controlled.
2) Tension could exist in the priorities for use of samples. Whilst the questions of TB diagnostics and markers of treatment response overlap, they are not necessarily the same. If the rationale for funding is in part based on diagnostic development, this will need to be reflected in the criteria for sample use.
3) The envisaged clinical trials will inevitably need to recruit from resource-poor settings, but it is likely that funding will include public funds from developed countries. Therefore the priorities for how specimens are used and distributed need to be clear from the outset, and it cannot be taken for granted that priorities in, for example, the US will be the same as those in sub-Saharan Africa.
4) Ideally, for validation of markers for diagnosis (as opposed to treatment response), there need to be well-defined control groups, including patients with suspected TB as well as those not proven to have the disease. In a frozen trial setting, this could include recruitment and archiving of those screened before entry to a clinical trial. Such an extension has logistical and financial implications which need to be addressed in the design of the study.

5.13 ACADEMIC RESOURCES POTENTIALLY SUITABLE FOR SPECIMEN COLLECTION

As part of the review of potential resources to the TB test development community, a number of academic investigators and members of collaborative groups were contacted. A list of those contacted is in Appendix 1. Whilst there are number of projects underway that could provide samples suitable for evaluation of novel tests, the quantity of samples that might be available are relatively small and, for the larger projects, it was not felt that sufficient samples would remain once the investigators had finished their planned activities. In addition there are relatively few projects underway whose specific aim is the identification of novel biomarkers for diagnosis and, amongst those that are underway, some are already linked with the resources described above. A further detailed breakdown by project is therefore not included here.

There are a growing number of biobank facilities globally, the scope of which is beyond this report. Although no other resources dedicated to TB were identified, there will be an increasing number of population sample banks where TB specimens will inevitably be part of the sample collection (just one example being the Guangzhou Biobank Cohort Study in China[44]). Whilst it is unlikely that such resources are likely to be a primary resource for test developers, they could prove helpful for validation of non-invasive biomarkers in diverse populations.

One possibility for dedicated sample collection arises with the TBCDRC – newly funded by the US NIH/NIAID[vi] – which addresses the recognised need to evaluate diagnostic methods and diagnostic algorithms rapidly in prospective, well-powered studies, with standardised criteria executed in sites where disease is prevalent and HIV/TB co-infection well-represented. Paediatric studies are currently part of the planned study portfolio. It also has the expressed aim of fostering collaboration between groups. Evaluation proposed within the group includes the evaluation of high-sensitivity, low-specificity tests suitable for rule-out diagnosis. Initial studies are planned to investigate urinary LAM in 100 HIV-positive patients with microbiologically confirmed disease in Kampala, Uganda. With advantages similar to a frozen trial initiative, there is the potential for sample collection as part of that collaboration.

[v] www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm228250.htm
[vi] National Institutes of Health/National Institute of Allergy and Infectious Diseases

6 SAMPLE COLLECTION AND PROCESSING AS PART OF POINT-OF-CARE TEST DEVELOPMENT

The utility of a hypothetical diagnostic in clinical practice based on whole organism detection (or NA detection) will depend on the ability to obtain good quality material from the site of disease, which is often not trivial in the environments where POC diagnostics are most needed. Although the focus of this document is on pathogen and host biomarkers for diagnosis, if the ultimate objective is a diagnosis rather than a diagnostic, the parallel process of developing sample collection and processing methods needs to be borne in mind. Whilst a urine-based system of sample collection is not a significant obstacle, the most important specimen for diagnosis will still be within the lung. This is a crucial limitation in the diagnosis of TB, particularly in the setting of HIV and paediatrics, where patients are often unable to produce a specimen suitable for analysis, and where the generation of a specimen might pose a risk of cross-infection to staff, patients and relatives in close proximity.

6.1 DIFFERENT APPROACHES TO SPECIMEN COLLECTION

One of the key specimens for diagnostic purposes is sputum. For most TB suspects presenting in either peripheral health centres (PHC) or secondary referral centres, there is no dedicated facility for sputum collection. Even in facilities with space for specimen collection, there is no ideal method of sample collection which can be easily implemented. A number of alternative approaches have been or are in the process of evaluation.

a) Nebuliser systems

Method: Inhalation of nebulised fluid (usually hypertonic saline) to generate respiratory specimen which is then expectorated or aspirated mechanically.
Pros: Effective in clinical practice and commonly used in well-resourced settings to generate induced sputum; provides fluid to improve production of specimen.
Cons: Usually requires power supply; infection control risk to those in close proximity; need for consumables, including sterile fluid source.

b) String test

Method: A length of cotton (approx 1m) is coiled into a gelatine capsule and weighted with a metal ball bearing. The string is swallowed and left for a period of time before being removed, cut into sections and placed into saline. It is then transported to a laboratory for further processing, similar to a standard sputum specimen. This utilises the proven benefit of strategies based on gastric sampling, without recourse to the more unpleasant methods of gastric tube insertion.
Pros: Well-tolerated; good yield in HIV-positive patients; able to use in older children; inexpensive.
Cons: Slow (validation for “intragastric downtime” <4 hours not yet available, but pilot data is promising); requires significant downstream processing of specimen.

c) Nasopharyngeal aspiration

Various methods of sample collection have been tried including nasal and laryngeal swabs.
Method: Variable, but involves graduated suction catheter being entered into nostril and then oropharynx, where it stimulates cough reflex and aspiration of secretions.
Pros: Can be performed in children.
Cons: As for nebulisation.

d) The Lung Flute ® (Medical Acoustics, US)

Method: A patient exhales into a mouthpiece and air flows over a long reed, generating a low frequency acoustic wave that travels backwards into the lower airways and improves mucociliary clearance.
Pros: FDA licensed product; rapid; no power/consumable requirements.
Cons: Costs if not reusable; lack of data on efficacy in setting of TB.

All of the options are potential solutions to the difficult problem of obtaining respiratory specimens from patients. However, all have limitations which mean none has been widely adopted in any programmatic setting (though there are examples of local practices where they have been implemented by local champions).

7 RULE-OUT TESTS AND POTENTIAL FOR POINT-OF-CARE DIAGNOSTICS

Whilst the primary objective in point-of-care diagnostics should be the development of a rule-in test for TB, consideration needs to be given to rule-out tests based on existing biomarkers which, whilst they will never be a complete answer, could be delivered within a foreseeable timeframe.

An effective rule-out test for TB would have value in both a primary healthcare setting and at the point of first referral, and could reduce the number of patients requiring either more complex investigations at the point of care (in the theoretical situation that a test existed) or up-referral. Although initially interferon gamma release assays (IGRAs) offered some promise in this area, the negative predictive value of existing assays, particularly in the setting of HIV, has been too low to allow their use in this role[45-47].

A POC rule-out test is likely to be used in conjunction with an algorithm based on symptoms and signs that can be easily recorded by relatively unskilled healthcare workers. While several groups have shown that the performance of clinical algorithms alone in either ruling in or ruling out the diagnosis of TB have limited value, particularly in the setting of HIV, a recent comprehensive systematic meta-analysis of 12 observational studies[48] has found that the absence of any one of the following four symptoms – 1) a current cough, 2) fever 3) night sweats 4) weight loss – was effective in ruling out a diagnosis of TB, with a negative predictive value of 97,7 percent (where TB prevalence among people living with HIV was 5 percent). As a result of these findings WHO now recommends the initiation of Isoniazid preventive therapy to people living with HIV who have had active TB excluded, using this algorithm.

One possibility is that clinical algorithms, to be maximally effective, will need to be combined with a POC test to improve rule-out value. In the absence of such a POC test, the above-mentioned symptom-based algorithm was augmented with abnormal chest x-ray findings, resulting in an insignificantly higher negative predictive factor of 98,7 percent. In addition, chest radiography comes at a high cost, requires infrastructure and training and is not available in most consultations where TB is suspected.

The evaluation of additional tests that could be delivered in a PHC setting is the subject of ongoing studies. Work of note from Wilson and colleagues is exploring decision analysis, where clinical features are combined with C-reactive protein (CRP, a protein raised as part of the acute phase response). Developed POC tests exist for well-resourced settings for such an assay, though none have been developed for a Level 3 setting. Preliminary data suggests that the inclusion of CRP in clinical decision rules might allow TB to be ruled out in approximately 20 percent of TB suspects in the setting of high HIV prevalence. These findings show the potential for such an approach to streamline diagnosis and reduce the need for more expensive diagnostics. However, it also highlights how such approaches, which can enable a treat/not-treat decision for many patients, can only ever be a small part of an overall solution.

8 NEXT STEPS TO DEVELOPING A POINT-OF-CARE TB TEST

There is a desperate need for a rapid POC test for TB which could be employed in resource-poor countries. An ideal POC test rapidly (<2 hours) 'rules in' the diagnosis of active TB, which leads to immediate triage for antibiotic treatment. For such a test to be developed, we believe that more integrated planning is required between areas of biological discovery, test development and activities supporting these, including specimen resource banks. At present the blocks to such a test include:

1) The need for novel biomarkers to form the basis of test design.

2) The lack of specimen repositories aligned with these needs.

3) The need for technological breakthroughs in sample collection, processing and testing.

8.1 ACTIVITIES TO DRIVE THE DEVELOPMENT OF NEW POC DIAGNOSTIC TESTS

There are several deficiencies to be addressed in order to increase the chances of a POC test being developed in the next five to seven years. The response can be broadly categorised into the following areas:

1. Develop defined (and realistic) end-user requirements/ product profile, and focus funding on strategies best suited to meet them, i.e.:

• Allocate funding to solutions that can be delivered within a relatively short timeframe, even if they are partial solutions to a POC TB test.

• Drive biomarker discovery in a targeted way, employing focused funding strategies.

• Advance diagnostic development for existing biomarkers.

2. Improve open resources for diagnostics developers and, in particular, improve specimen banks to validate any emerging biomarkers and diagnostic tests.

8.2 FOR POC TB DIAGNOSTICS WHERE THE OUTCOME OF THE TESTING IS TREATMENT INITIATION

In 2008 and 2009, Médecins Sans Frontières (MSF), the Treatment Action Group (TAG), Partners in Health (PIH), and the AIDS and Rights Alliance for Southern Africa (ARASA) together organised two expert meetings in Cambridge and Paris to discuss TB diagnostics and how to drive the development of a POC test[19]. At the 2009 Paris meeting, the following test criteria or end-user requirements were put forward as potential test characteristics (see Table 3, page 20). This strong consensus has been a major step forward in defining the ideal POC TB test and its characteristics.

A good diagnostic development will begin with a set of end-user specifications. When test developers set specifications for a new test, they pass customer needs and wishes through the filter of technological and developmental possibilities and the constraints identified. In the development of any product, there are likely to be some constraints and risks, technological or otherwise, that will have an influence on the final product's design and performance. Some of these constraints and risks can be managed by careful implementation of strategies to reduce or manage risk. For test developers, the customer needs (see Figure 2) must be brought together with potential scientific solutions, identifying the constraints along the way. From this merging of the ideal product wishlist and the developers' expertise and technological know-how, a set of test specifications will emerge. Using the 2009 ideal POC TB test specifications, we have assessed the likelihood of achieving these specifications in the next five years, given the state of the art and the technological constraints. We have applied a simple qualitative risk matrix to each of the specifications listed and quantified the risk of failure and its impact on each specification. This analysis will allow prioritisation of critical specifications and identify areas for intense R&D. We have offered potential solutions to mitigate some of the risks. One caveat from this assessment is that whilst we have endeavoured to assess the state of the art, not all information is openly available and so this cannot be a complete assessment. The introduction of a disruptive technology or marker, or a significant increase in funding support, will necessitate a reassessment of the risks and constraints. However, all projects should undergo regular risk reassessment during the project lifetime.

The risk analysis was performed using standard criteria for risk and impact on each specification, and represents the authors' assumptions, given the available information of the state of the art. We have assessed the risk of failure to develop a POC TB test in the next five years, given current funding levels and effort, the technological state of the art and progress so far towards POC TB tests.

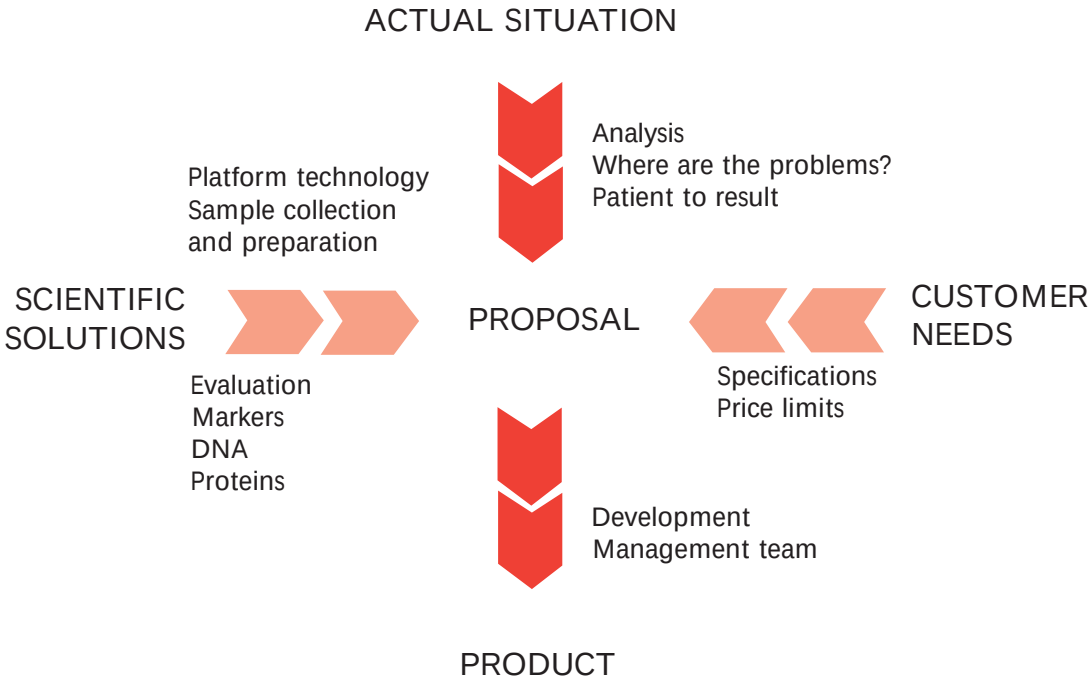

Figure 2: *Schematic of some of the key processes in the drive for a POC TB test. The bringing together of the scientific solutions with the customer needs should result in a proposal for a target product profile which is needed to start the development process.*

	MINIMUM REQUIRED VALUE	FIVE-YEAR RISK	SOLUTION	ACTION
SENSITIVITY (IN ADULT PULMONARY TB)	95% for smear-positive, culture-positive patients. 60-80% for smear-negative, culture-positive patients.	Moderate-to-high risk of failure to achieve. *There is likely to be a technological constraint here which explains our rating of moderate-to-high risk of failure.* The Cepheid system, which is PCR- based, using sputum as the sample, currently achieves 72% sensitivity with a single test in smear-negative patients. This 72% sensitivity is achieved only with the benefit of a complex, high-level laboratory instrument from the Cepheid GeneXpert system. POC tests will be unlikely to meet this target as POC tests usually have lower sensitivity than lab-based equivalents. ***This complex DNA amplification technology is unlikely to be POC and affordable for low and middle-income countries in the next five years.***	Much greater investment is needed to deliver a POC DNA amplification/detection technology for the developing world in the next 5-10 years. This is a high-risk strategy but would bring very high rewards if successful. The risks can be ameliorated by prioritising the strategies for biomarker discovery. Host biomarkers alone appear the least promising (particularly in HIV co-infection). Pathogen biomarkers are the lowest risk strategy and conceptually more sound. These should be prioritised for POC test development as: proteins/lipids/ macromolecules, NA detection and volatile organic compounds (VOC) tests.	Push investment in pathogen biomarkers, prioritising non-NA based markers first. This can be achieved by advocating for priority funding to release specific RFPs for non-NA based pathogen biomarkers only. Develop interactions with industrial partners for marker screening tools: link RFP to industrial partners in a Product Development Partnership model. Explore incentive mechanisms to drive R&D towards identifying and validating novel biomarkers/ biosignatures. Developments must factor in the inherent difficulties of NA extraction and detection in peripheral health centres/Level 3 facilities.
SPECIFICITY	95% compared to culture.	Moderate-to-high risk of failure. A technological constraint here resulting in the moderate risk of failure to achieve. Whilst 95% specificity may be achievable with NA-based technologies, they are unlikely to be POC in the next five years.	There are few bacterial POC diagnostics available, and such high specificity in a POC test for low-income countries is unlikely (bacterial-specific PCR will achieve this kind of specificity, but requires extensive infrastructure).	Risks would be mitigated if a new, unique pathogen marker for TB was identified which could be detected easily. Linked to the drive for biomarker discovery and the strategy of focus on pathogen biomarkers.

	MINIMUM REQUIRED VALUE	FIVE-YEAR RISK	SOLUTION	ACTION
TIME TO RESULT	3 hours.	Low risk of failure.	Current testing (although not POC) can be 2-3 hours, but simple POC test should be quicker to perform. Current lateral flow assays for other diseases are <60 minutes. If POC DNA tests were available, there is no reason to expect they would take more than 2 hours if truly POC.	Continue to highlight <3 hour testing time in the development process and RFPs.
THROUGHPUT	20 tests/staff member/day.	Low risk of failure.	Samples should be able to be batched and therefore 20 tests/staff member/day is quite achievable.	Take into consideration the ability to batch test during investment phase.
SPECIMEN TYPE	Urine, oral, breath, venous blood, sputum.	Low-to-medium risk of failure.	Urine would represent an ideal sample type: non-invasive and in high quantity. However, detectable protein in urine is often dependent upon kidney function (overwhelming kidneys' re-absorptive capacity) so protein marker must be present in sufficient quantities. Urine is at a different concentration during the day and so can be relatively heterogeneous sample type. This will be important if operating close to the limit of detection of assays. In low-income country settings, urine concentration will not be possible. Test would probably require very low limit of detection. Not useful to identify biomarkers which are only detectable in the (*cont.*)	Prioritise urine (with non- protein based markers) and blood, but focus on new ways for sputum collection and processing in HIV-positive individuals and children. VOCs and oral samples should be given lower priority.

MINIMUM REQUIRED VALUE	FIVE-YEAR RISK	SOLUTION	ACTION
		laboratory: must ensure that the POC test limit of detection is appropriate (could be particularly important for cytokine biomarkers). Oral swab samples are interesting for the same reasons as urine, but chances of finding marker in an oral sample may be low. Breath/VOCs. Technological constraints currently. Rely on complex instrumentation. Whilst possible, highly unlikely to develop into a POC test in the next 5 years. Venous blood: capillary blood would be most useful in the settings described and represents a highly available sample type. Most POC and diagnostic tests use blood as the sample type. Is likely to be more suitable for a host marker (with the caveats described above). Sputum: difficult sample type to work with as evidenced by the sensitivity of smear microscopy. However, if collection processes better (particularly in HIV-positive individuals and children), and sputum treatment addressed, sputum could be a medium priority for the diagnosis of pulmonary TB.	Issue RFP for new sputum collection methods and processing.

	MINIMUM REQUIRED VALUE	FIVE-YEAR RISK	SOLUTION	ACTION
SAMPLE PREPARATION	Three steps maximum. Biosafety level 1. No need for pipetting. No time-sensitive processes.	Multiple risks from low to high. Three steps is maximum likely achievable for sample preparation. Biosafety level 1 may not be achievable so easily. Time-insensitive processes are unlikely in POC testing so moderate-to-high risk to achieve.	Technological blocks to improved sample preparation should be linked with sample collection, and potential solutions are feasible.	Link RFP for sample collection with sample processing.
READOUT	Easy for 'yes' and 'no' answers. Readable for 1 hour.	Low risk of failure.		
WASTE DISPOSAL	Simple burning or sharps: no glass.	Low risk of failure.	Should be achievable to avoid glass.	
CONTROLS	Positive control in test kit. QA simpler and easier than with SSM.	Low risk of failure.	Provision of low-cost QA material achievable if biomarker identified.	
STORAGE/SHELF LIFE	Shelflife 24 months, including reagents. Stable at 30 and higher for shorter periods of time. Stable in high humidity.	Low-to-medium risk of failure.	Dependent upon test format (dried reagents more stable). No technological reason why 12-18 months not achievable.	
INSTRUMENTATION	If instrument, no maintenance. Works in tropical conditions. Acceptable replacement costs. Fits in backpack. Shock-resistant.	Multiple risks: medium-to-high risk of failure.	Technological constraints. If instrument-based POC test is developed, the development of a non-maintenance instrument is at very high risk of failure. Instruments inevitably break down, and repair and replacement will be necessary. The business model for free replacement of broken or damaged instruments needs to be very carefully examined. These costs will inevitably be folded into the per-test price. If DNA detector or similar, outlay costs will be significant and manufacturer unlikely to (*cont.*)	Prioritise non-instrument methods first, followed by the instruments in order of complexity and read-out. Instruments with software least desirable.

	MINIMUM REQUIRED VALUE	FIVE-YEAR RISK	SOLUTION	ACTION
			provide maintenance without high costs. Nor is replacement likely if >US$5,000 without significant increases in the per- test price.	
POWER REQUIRED	Can work on battery.	Low-to-medium risk.	The number of tests to be carried out per battery charge should be part of the test specifications.	If a battery is required, it should not be removable from the instrument.
TRAINING	1 day maximum. Used by any healthcare worker.	Low risk.	If the POC test is simple with <3 steps, then this is achievable.	
COSTS	<US$10 per test.	Medium risk.	DNA detection methods will need higher cost prices in all probability. Lateral flow assays with a biomarker(s) are achievable in <US$10.	Economy of scale benefits are clear in diagnostics, but need to carefully incentivise industry to be involved in large-scale manufacture. Low cost possible with lateral flow but unlikely with NA detection. Important to focus on pricing structures for developing world.

Table 3: *Review of end-user specifications filtered by risks and constraints.*

The points above need to be addressed or incorporated into any new POC TB test development. It is important to address the end-user requirements for a test, versus the reality of what can be achieved given the current state of technology and levels of funding. A careful evaluation of these assumptions will inform any test development.

8.3 IMPROVE BIOMARKER DISCOVERY

It is difficult to obtain a fully comprehensive overview of ongoing biomarker discovery work, as those in the field may be understandably reluctant to publicly disclose biomarker data until they have passed validation and registered for patents. However, none of the large pharmaceutical companies, diagnostics companies or academics working in TB who were contacted by the authors of this report believed that a novel biomarker(s) useful for POC testing is likely to emerge in the next three to five years. Whilst this is a sobering thought, we believe that it is vital to take steps now to reduce this time delay.

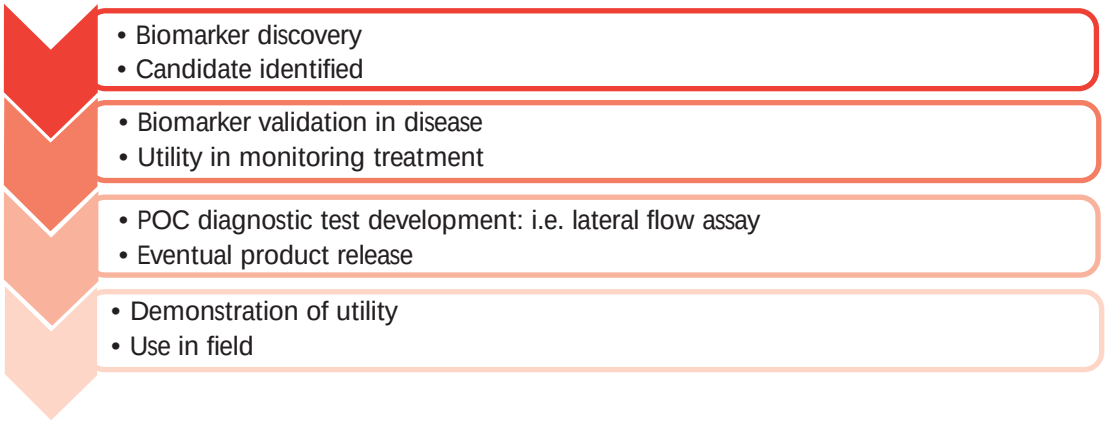

Figure 3: *Simplified schematic of the process of biomarker discovery to eventual diagnostic product release. Assuming very generous budget investment, this process could be shortened, but is still unlikely to be completed in less than seven years, and this assumes the selection of the biomarker at Time 0.*

Even if a novel candidate biomarker were identified tomorrow, validation and test development would likely last around seven years (see Figure 3); this also assumes enormous resources are ploughed into the diagnostic development. This is a bleak assessment of the state of biomarker discovery in TB diagnostics, and the authors of this report have not uncovered any additional evidence to the contrary.

8.4 EXISTING BIOMARKERS

The search for novel biomarkers is an essential yet high-risk strategy, albeit with the potential to yield high rewards. To increase the likelihood of success, the strategy needs to be refined and focused and some of the risks mitigated without damaging the high reward. Biomarker discovery can be usefully divided into approaches identifying host-derived markers and those targeting markers from the pathogen. The search for host biomarkers or a 'signature' of biomarkers, whilst offering some promise, is the highest-risk strategy and is achievable only in the long term, particularly for HIV-positive individuals. Immune-compromised individuals, in particular, may not produce a comparable host biomarker(s), potentially excluding some of the most urgent unmet diagnostic needs for TB. The priority should lie with pathogen detection.

At present, DNA-based detection targeting the rpoB gene remains arguably the most appealing biomarker for diagnostics development, based on the fact that:

1) There is DNA sequence within rpoB that is specific to mycobacterium tuberculosis.
2) Within a small region of 81bp in the rpoB there are a number of well- characterised mutations which can confer resistance to rifampicin.
3) Rifampicin-resistance remains an important surrogate marker for multidrug-resistant tuberculosis.

Detection methods based on MTB rpoB can therefore identify both pathogen and likely multidrug-resistance. Commercial systems based on the MTB rpoB gene are available as discussed above, but none is approaching a feasible use in a rural peripheral health centre outside a well-supported clinic. The development of the Cepheid Xpert MTB/RIF assay discussed above has changed expectations of what we should be able to expect from an MTB rpoB-targeted approach, and a system with the performance characteristics of that platform that could meet the end-user specifications outlined above would be game-changing.

RpoB is not without its limitations in a clinical setting (for example, it does not detect isoniazid-resistance, the most common form of mono-resistance in many settings). More importantly for POC testing, a detection system has yet to be developed for nucleic acids of any kind that can meet the necessary requirement. There are many activities underway for the development of POC tests which can detect nucleic acid. Most are geared towards use in the developed world, but some are focused on resource-poor settings. At present

these developments are either at a very early stage or have unrealistic formats. We have speculated that NA detection methods in a POC format are a high-risk/high-reward strategy and, without a significant change in investment or strategy, will remain so. However, we believe that if the development process for nucleic acid detection were focused, many of the risks could be ameliorated. It is well established that rpoB is a good TB marker and therefore has a head start on new biomarkers, which must demonstrate a strong correlation before advancing to the diagnostic development stage.

However, given the risks of failure, alternative biomarkers derived from pathogens need to be found. A focus on pathogen-based detection should not mean the complete exclusion of host biomarkers. Novel methods of gene expression profiling have had some success in differentiating active tuberculosis from latent tuberculosis from healthy controls in the absence of HIV. However, whether such profiling will be able to help in the setting of HIV remains challenging, given the greater variability in pathogens and immune activation that might be present. Transforming these profiles into a POC test will be a long-term strategy.

A new strategy which will bring together diagnostic developers with interest in a Level 3 clinic/lab-suitable POC test may yield results. Incentive mechanisms such as milestone prizes also hold promise in increasing R&D in this area. However, funders must be made aware that this is still a relatively high-risk strategy, albeit with the potential for high rewards. Such strategies will only attract organisations with access to large R&D budgets and as such will exclude many smaller businesses and academic institutions.

8.5 NEW FUNDING MECHANISMS FOR BIOMARKER DISCOVERY

a) COLLABORATION

The biomarker search is also responsible for another effect on the TB academic and research community: a lack of collaboration between many involved in the field. Researchers (from both industry and academia) release only limited information on which biomarkers have been tested and failed, and in which systems/screening methods. If the challenge of TB diagnostics is to be met and overcome, researchers must collaborate in order to bring about this change. There must be a drive to increase collaboration and openness between researchers. As well as advocating for increased collaboration, future funding should depend upon this openness in a concerted effort by major funders.

b) NEW FUNDING MECHANISMS AND MODELS

A different style of funding is needed to drive R&D in TB diagnostics for resource-poor settings. Given the current incentive structures, industry is unlikely to devote serious amounts of time and money to delivering diagnostics which are aimed primarily at low-income countries. This does not mean to say that companies have no interest, but rather that low-profit/low-return investments are not given high-priority status in the product pipeline. Although a POC TB test would be useful for the developed world, the number of TB-positive individuals is relatively low, and so business models need to take this into account when deciding to invest in developing TB diagnostics. For example, the numbers of TB diagnoses in the UK was 8,600 in 2008 (and only half of these were culture-positive). This has implications for the developers, but also for the purchasers of TB diagnostic tests. Clinical diagnostic laboratories in the developed world are unlikely to invest in purchasing a complex and expensive instrument which would have little use outside one or two major cities. Light usage pushes up the 'per-test price', as prices are usually negotiated around procurement agreements with guaranteed ordering sizes.

However, there will not be a POC TB diagnostic without the input of diagnostic companies, and so it is important to incentivise their involvement. One way to do this would be to use charitable funds to pay for the R&D and, in return, the industrial partners could obtain a small profit from the sale of the test in low and middle-income countries. Other incentive structures that separate the costs of research and development from the price of the innovation, and therefore allow for a reorientation of industry priorities, should also be explored. Alternative incentive mechanisms, such as prizes, have recently received interest, also in the field of TB diagnostics[49-50].

Strong industry-style project management approaches have shown that they can deliver results. Key factors for creating this framework are:

1) Establishing contractual collaborative frameworks for intellectual property sharing.

2) Setting out very clearly a set of end-user specifications which are technologically feasible.

3) Putting in place a small, deeply-involved management team to oversee progress and drive the project forward.

4) Establishing clear milestones with indicators for achievement agreed by both the management team and the developers.

5) Taking decisions to discontinue funding if progress is not visible, thereby saving funds which can be ploughed back into successful projects.

This type of funding model has been shown to be successful for driving R&D for resource-poor settings. Whilst not a panacea, this model could be replicated for POC TB tests, either wholly or in part. As mentioned above, this mechanism could be used to drive the search for a suitable POC test for rpoB.

A new funding mechanism must be brought forward to foster this collaborative environment, which will increase the chances of success. The current funding streams may not be driving discovery in an efficient way and may not take advantage of the screening methods that can be harnessed by industry. Many of the ongoing multi-partner discovery efforts are, in fact, closed and there are very few major collaborations with the specific aim for biomarkers for diagnostics.

Recently, the Bill and Melinda Gates Foundation launched a US$12m grant programme specifically focused on the validation of diagnostics biomarkers: Biomarkers for the Diagnosis of Tuberculosis, as part of the Grand Challenges in Global Health initiative[51]. Of particular note is the fact that the aim of this programme is to validate biomarkers able to diagnose active TB, and to distinguish active TB from non-TB cases, as this will be essential for the clinical utility of a test. Moreover, the priority given by the programme to samples other than sputum is very valuable. The Gates Foundation grant programme will support research efforts looking at both host and pathogen-derived biomarkers and at combinations of multiple biomarkers from either or both classes. Although this programme has certainly the potential to positively influence the landscape of TB diagnostic biomarkers, this initiative alone will not be sufficient to close the knowledge gap in this research field, and more efforts need to be focused in this area to ensure fast progress.

Besides funding, an independently-managed, collaborative system – ideally separated from current diagnostic development mechanisms – is also needed to drive biomarker discovery to uncover potential candidate markers. This new mechanism should prioritise pathogen-based discovery. This structure should ensure that:

1) Due to the size of the challenge, funders work together and coordinate funding efforts for biomarkers. This will avoid unnecessary duplication and redundancy and will promote collaboration.

2) An independent management is established to oversee the biomarker discovery, negotiating milestones and deliverables as part of the funding agreement.

3) Collaboration with a defined amount of biomarker target disclosure is established. (This can be determined, but full disclosure is optimal. Intellectual property management must be carefully negotiated.)

4) There is only a limited amount of overlap in the biomarkers of each funding recipient. This will ensure a reasonable spread of pathogen-based targets without significant duplication and redundancy.

5) Different screening tools are contained within the collaborative effort so that good biomarker candidates can be searched for and verified using alternative methods.

6) There is a structure in place to provide independent validation of any biomarkers discovered.

7) There is a mechanism for the termination of funding/contract cancellation when biomarkers are proven not to be useful in the collaborative effort.

Key activities to drive development of new POC diagnostic tests

- Drive collaboration between researchers in biomarker discovery.
- Ensure new biomarker funding is linked to an agreement to collaborate and disclose.
- Establish independent management model of collaborative research.
- Establish industry-style management models around rpoB.
- Drive new biomarker discovery with new funding mechanisms.
- Prioritise pathogen-based markers (and encourage funders to do the same).
- Remember that new biomarkers will need 5+ years for discovery and validation.
- Ensure collaboration is tied to receipt of funding by innovative contractual mechanisms.
- Create partnerships with industry to take advantage of high-throughput screening methodology.
- Develop funding mechanism to drive product development.

8.6 SAMPLE BANKS, VALIDATION AND OPEN ACCESS RESOURCES

The effort for biomarker discovery is usually undertaken by academic institutions and private companies and, although limited dedicated funding for TB diagnostic biomarkers discovery has been allocated so far, there are some ongoing efforts in this area. The validation of potential biomarkers represents a crucial gap that must be addressed, as it requires much more investment and is likely to be beyond the financial and logistical limits of small biotech companies and academic research units. The validation stage of biomarker discovery is critical: to be useful, the biomarker must be detectable in the great majority of the samples (whichever sample source that may be) from active TB patients with and without HIV. For example, a new biomarker that is only present in 50 percent of active TB patients will not be useful on its own for further development. Developers must be able to screen potential markers in a set of well-defined samples from TB and non-TB patients.

An independent source of these validation samples is important for several reasons. The first is that most small companies and academic units will not have sufficient funds to establish a full sample bank, and the lack of a set of validation samples should not be a barrier to POC diagnostic development. Secondly, the independence of the sample bank will make the validation process more rigorous for the TB research community. However, before all of this can be established, the sample bank and its samples need to be defined. Of the several banks in existence now (some open access and others not), the sample collection process is not standardised across banks, the phenotypes of the samples are not all fully characterised, and the sample types and volumes are not all clearly defined. This means that developers may arrive at different validation results depending on the samples' tests, the geography of the samples (endemic versus non-endemic areas and inclusion of sufficient samples with HIV co-infection).

There is an increasing consensus that specimen bank facilities are an important component in the development of any potential POC test, though it is also the case that specimen banks require significant resources and will not themselves lead to a new test. The enthusiasm for specimen banking is reflected in efforts to archive samples from clinical trials as described above. However, these resources, if based solely around trial participants, are likely to be deficient in important areas, including adequate controls and paediatric specimens, which means money allocated to such resources should not be considered as a significant contribution to the effort to develop a POC test.

One key question is whether there should be a new entity set up to provide samples for diagnostic developers. The answer to this depends in part on the extent to which existing facilities can adapt and evolve. It is the view of the authors of this report that it will be more effective to adapt and improve existing resources initially, but if that is unsuccessful or unduly slow, a separate entity should be created. The well-established WHO/TDR specimen bank, whilst deficient in important areas, offers the basis of a model which could better meet the need of POC test development. Other important issues include the ethics of samples being collected and removed to other countries, and the return of benefit to the countries which provide extensive samples for banks.

Specific recommendations for improving the resource include:

a) A change to clinical phenotyping categories used to reflect the clinical scenarios where a test is needed:

1) The use of direct smear microscopy in characterisation. If specimens were categorised by direct smear, this would address the relative deficiency in this category (category 2, smear-negative, culture-positive) in sample collection. It would also address concerns about the potential of current specimens to usefully address the performance of any new test.

2) More detailed characterisation of HIV-positive specimens, in particular patients' immune status (CD4) and medication may be useful. To be most useful, specimens could be collected as matched cohorts from the same clinical settings in clinical arenas that reflect the setting for which tests are needed (i.e. rural health posts through preference, rather than tertiary referral centres).

3) The creation of a paediatric resource. This would be challenging and expensive, but is a crucial deficiency in current efforts. Such a resource could exist as a separate entity, but ideally would be housed within one single resource. Clinical characterisation would have to be based on significant follow-up of children to provide a gold standard of diagnosis. In the process, new consensus diagnostic definitions might need to be agreed. Specimens would be precious and their use less freely available than those from adults, but simple criteria could be applied to ensure appropriate use (for example, validation of markers identified from dedicated paediatric studies, or already validated in adult studies). Blood and urine would be the key specimens to collect, as specific pathogen-based tests are likely to be best evaluated in adults and will be limited by specimen availability in children.

b) A broader range of biological specimens to be collected:

The decision about which types of specimens to collect is a complex one and depends on a number of factors, including cost, likelihood of utility, stability when frozen, and the likelihood of a particular method/protocol. The main additions that seem necessary at this stage appear to be ribonucleic acid (RNA) extracted from blood, and both blood and urine suitable for analysis of metabolites. RNA protocols are well established, and one example is provided in Appendix 1. Protocols for metabolites are more variable for urine where specimen is easier to collect. The two best protocols would have to be chosen to reflect current discovery efforts. DNA (human) is unlikely to be the basis of a future test, but its collection and storage is cheap and straightforward and it should therefore be included in new efforts, as it may prove useful for future analysis in conjunction with other samples.

c) Flexibility to change:

As discussed above, there is diverse activity in biomarker discovery, and trying to predict the future is foolish. However, it should be possible to create a resource which could respond rapidly to advances and adapt. As part of this process, an advisory group for a resource should include representatives of industry, academia (particularly biological discovery) and technology to allow emerging trends to be spotted and evolve the resource appropriately.

d) A broader view on resources helpful to diagnostic developers:
The development of the most recent addition to the diagnostic armoury, the Xpert MTB/RIF assay, was helped by existing banks, but also required other resources that proved invaluable. Two examples of this were an expression library, representing all described rpoB mutations to allow validation of an rpoB-based assay, and libraries of other pathogens that could influence test specificity (particularly non-tuberculous mycobacteria). As part of this service to developers, the target time to process applications could be reduced to a suggested maximum of three months from application to specimen delivery.

e) Value for money:
An improved resource would be a great benefit to the R&D community, but would require in the region of US$5m. For such money to be justified, there would need to be clear monitoring of the performance of the bank and a continuous critical view of its utility.

8.7 SUPPORTIVE PROJECTS AND PARTIAL ANSWERS

Without a significant change in funding and strategy, the co-primary goals of a useful POC test for an existing biomarker or a new biomarker for a POC test are years away. We have estimated in this report that, given the current situation, the world is at least seven to ten years away from realising a POC test for low-income countries, and possibly longer. With almost two million deaths per year attributable to a treatable disease, we cannot wait for 20 million more deaths before the realisation of the holy grail of a lateral flow or dipstick TB test. The impact of a POC TB test cannot be fully examined until the test is developed, but it clearly needs to be considered in order to support funding for additional POC TB test development[17, 52-53]. Clearly the high-risk but potentially high-yield biomarker discovery must continue and, in the meantime, we must put in place an alternative strategy to improve the current situation.

9 SUMMARY AND CONCLUSIONS

A POC test for TB is a desperately needed advance. The introduction of such a simple test would undoubtedly save millions of lives, and go some way to halting the rise of TB in resource-limited settings. Current funding into TB diagnostics is inadequate, and many of the ongoing efforts could be improved by a focused and strategic approach. The TB community must become more collaborative and cohesive, and funders must develop a unifying strategy to pick off targets on the way to the development of a POC test. There are many groups working on, for example, biomarker discovery, mostly in isolation from one another; only by a shift in the funding mechanisms can these be brought together synergistically. The new Cepheid Xpert MTB/RIF is an encouraging step in the right direction, but it is far from point-of-care and is not yet game-changing in resource-poor settings. However, its performance in well-resourced settings is likely to be good and will likely be improved by natural market forces. This does mean that the gap between well-resourced settings and resource-poor ones is wider than ever, and that new products receiving financial support from public sector or charitable sources need to be explicitly focused on Level 2 or 3 facilities.

Biomarker discovery must form an important path towards the goal of a POC TB test. However, the authors believe that this discovery should be focused on non-nucleic acid pathogen markers as the first priority, closely followed by nucleic acid pathogen markers. This should be established with a new funding mechanism(s) to ensure collaboration and openness to avoid wasteful duplication. This is a high-risk but high-reward strategy, and some of the activities in this document highlight some steps that could be taken which reduce some of these risks. Current biomarker discovery is unclear, and any organisations with potentially useful markers need to be funded and supported. For example, concentrating developers on establishing POC tests for rpoB could yield high rewards, providing the management of the project is suitably established.

Accessible well-characterised sample banks are a critical resource for the development of a TB POC diagnostic. Potential markers will need to be validated by a well-characterised set of samples. The only openly available sample bank is that of the WHO/TDR. Paediatric samples are under-represented and should be included if possible. The sample bank effort should be supported and strengthened to allow continued and dynamic provision of samples to any developers with good candidate markers. Failure to do so now will lead to a future block in the diagnostic development pathway.

Strong leadership among developers is critical to strengthen TB diagnostics efforts and it has been clear of late that this is emerging. This will be particularly important for developers when deciding on the target product profile of the POC TB test; shaping end-user specifications with technological possibilities will be challenging. The increased involvement of industry will accomplish some of this process.

New R&D models for organising how development efforts for TB POC diagnostics are carried out will increase the chances of success and quicken progress to a truly POC TB test. We need advocacy for diagnostics, biomarkers and sample banks across many stakeholders to speed this lengthy and involved process. If even some of the recommendations in this document are implemented, we believe that it will be possible to reduce the time needed to introduce a POC test, preventing millions of new infections and deaths.

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Dr Anne Bendt
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The researchers who wished to remain anonymous.

APPENDIX 1

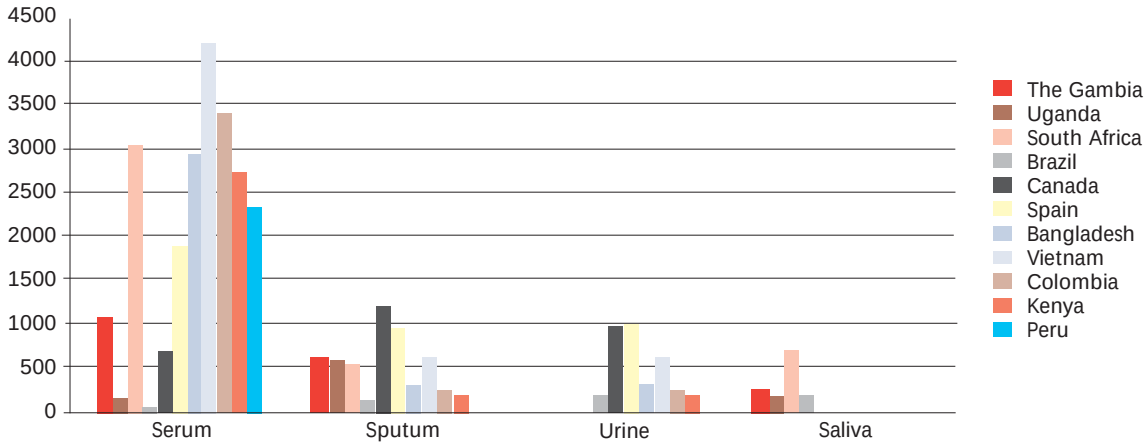

Figure 4 *Nature of specimens collected by site 2000-9*

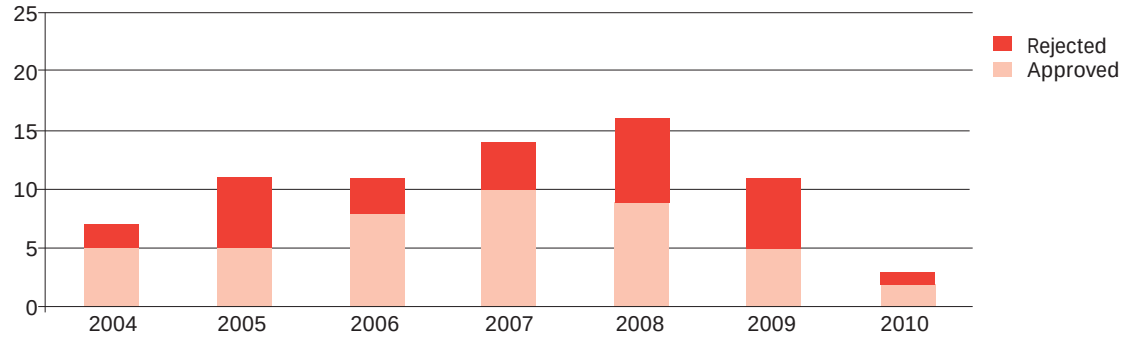

Figure 5 *Applications to bank for specimens 2004-9*

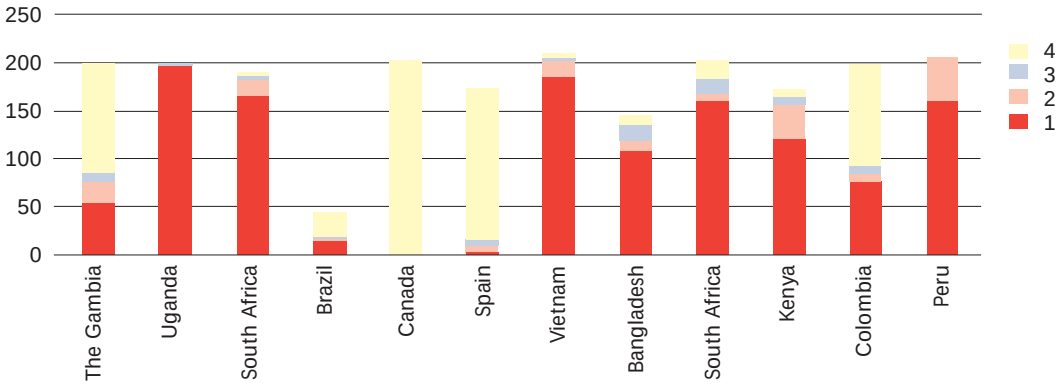

Figure 6 *Categorisation of patients by clinical site*

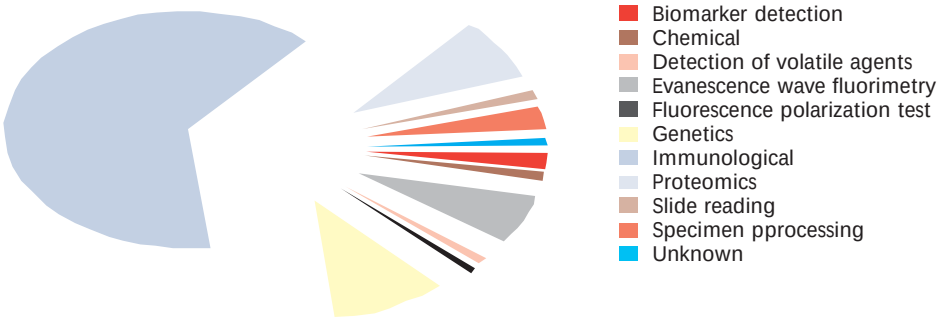

Figure 7 *Nature of requests to WHO/TDR biobank*

Country of Origin	Patients total	Patients sputum	Patients serum	Patients urine	Aliquots sputum	Aliquots serum	Aliquots urine
Vietnam	2,052	1,033	2,137	1,033	9,226	27,012	4,752
Brazil	243	0	243	0	0	243	0
South Africa	363	363	363	363	1,788	7,032	1,731
Peru	320	130	153	308	2,846	991	1,421
Bangladesh	100	64	85	100	126	1,308	444
Uganda	255	255	255	0	255	255	0
Tanzania	185	0	185	0	0	185	0
Totals	3,518	1,845	3,421	1,804	14,241	37,026	8,348

Figure 8 *Estimates for numbers and nature of specimens collected within the FIND reference material project by the end of June 2010*

Institution/Company
Forsyth Institute, Boston, US
Naturwissenschaftliches und Medizinisches Institut an der Universität Tübingen, Germany
National University of Singapore
Swedish Institute for Infectious Disease Control, Sweden
GENOVAC, Germany
Response Biomedical Corporation, Canada
Nanogen, US
Colorado State University, US
Public Health Research Institute, US
Chimera Biotec GmbH, Germany
NYU School of Medicine, New York,US
Eiken Chemical Co, Japan
Emerging Bacterial Pathogens Unit, San Raffaele Scientific Institute, Italy
Center for Emerging Pathogens, UMDNJ, US
Future Diagnostics, US
Cepheid, US
Nationales Referenzlabor für Mykobakterien, Borstel, Germany
ANDA Biologicals, France

Table 4 *Institutions given approval for use of FIND reference materials*

REFERENCES

1. WHO. Global tuberculosis control: a short update to the 2009 report. 2009.
2. WHO. Global tuberculosis control: epidemiology, strategy, financing. 2009.
3. WHO. The Stop TB strategy. 2006.
4. Harries AD, Rony Z, Elizabeth LC, et al. The HIV-associated tuberculosis epidemic – when will we act? Lancet. 2010;375(9729):1906-1919.
5. Harries AD, Zachariah R, Corbett EL, et al. The HIV-associated tuberculosis epidemic —when will we act? Lancet. May 29 2010;375(9729):1906-1919.
6. Perkins Mark D, Cunningham J. Facing the Crisis: Improving the Diagnosis of Tuberculosis in the HIV Era. The Journal of Infectious Diseases. 2007;196(S1):S15-S27.
7. Losina E, Bassett IV, Giddy J, et al. The "ART" of Linkage: Pre-Treatment Loss to Care after HIV Diagnosis at Two PEPFAR Sites in Durban, South Africa. PLoS One. 2010;5(3):e9538.
8. Lessells RJ, Mutevedzi PC, Cooke GS, Newell M-L. Retention in HIV Care for Individuals Not Yet Eligible for Antiretroviral Therapy: Rural KwaZulu-Natal, South Africa. Journal of Acquired Immune Deficiency Syndromes. 2011;56(3):79-86.
9. Tayler-Smith K, Zachariah R, Massaquoi M, et al. Unacceptable attrition among WHO stages 1 and 2 patients in a hospital-based setting in rural Malawi: can we retain such patients within the general health system? Trans R Soc Trop Med Hyg. May 2010;104(5):313-319.
10. Aledort JE, Ronald A, Rafael ME, et al. Reducing the burden of sexually transmitted infections in resource-limited settings: the role of improved diagnostics. Nature. 2006.
11. Keeler E, Perkins MD, Small P, et al. Reducing the global burden of tuberculosis: the contribution of improved diagnostics. Nature. 2006.
12. Urdea M, Penny LA, Olmsted SS, et al. Requirements for high impact diagnostics in the developing world. Nature. 2006.
13. Yager P, Domingo GJ, Gerdes J. Point-of-care diagnostics for global health. Annu Rev Biomed Eng. 2008;10:107-144.
14. Cattamanchi A, Davis JL, Pai M, Huang L, Hopewell PC, Steingart KR. Does Bleach Processing Increase the Accuracy of Sputum Smear Microscopy for Diagnosing Pulmonary Tuberculosis? J. Clin. Microbiol. July 1, 2010 2010;48(7):2433-2439.
15. Steingart KR, Ng V, Henry M, et al. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis. Oct 2006;6(10):664-674.
16. WHO. Laboratory-based evaluation of 19 commercially available rapid diagnostic tests for tuberculosis. 2008.
17. Pai M, Minion J, Steingart K, Ramsay A. New and improved tuberculosis diagnostics: evidence, policy, practice, and impact. Curr Opin Pulm Med. May 2010;16(3):271-284.
18. Pai M, Minion J, Sohn H, Zwerling A, Perkins MD. Novel and Improved Technologies for Tuberculosis Diagnosis: Progress and Challenges. Clinics in chest medicine. 2009;30(4):701-716.
19. Lemaire JF, Casenghi M. New diagnostics for tuberculosis: fulfilling patient needs first. J Int AIDS Soc. 2010;13:40.
20. Nyendak MR, Lewinsohn DA, Lewinsohn DM. New diagnostic methods for tuberculosis. Current Opinion in Infectious Diseases. . 2009;22(2):174-182.
21. WHO. Treatment of Tuberculosis: guidelines for national programmes. 2009.
22. Helb D, Jones M, Story E, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol. Jan 2010;48(1):229-237.
23. Boehme CC, Nabeta P, Hillemann D, et al. Rapid Molecular Detection of Tuberculosis and Rifampin Resistance. New England Journal of Medicine. 2010;0(0).
24. Blakemore R, Story E, Helb D, et al. Evaluation of the analytical performance of the Xpert MTB/RIF assay. J Clin Microbiol. Jul 2010;48(7):2495-2501.
25. Martin A, Fissette K, Varaine F, Portaels F, Palomino JC. Thin layer agar compared to BACTEC MGIT 960 for early detection of Mycobacterium tuberculosis. Journal of Microbiological Methods. 2009;78(1):107-108.
26. Aono A, Azuma Y, Mitarai S, Ogata H. Rapid prediction of BACTEC MGIT 960 culture results by COBAS Amplicor Mycobacterium polymerase chain reaction detection. Diagnostic Microbiology and Infectious Disease. 2009;64(1):27-30.
27. Adhikari BR, Pandey BD, Ghimire P, et al. Loop-mediated isothermal amplification (LAMP) for the direct detection of human pulmonary infections with environmental (nontuberculosis) mycobacteria. Jpn J Infect Dis. May 2009;62(3):212-214.
28. Boehme CC, Nabeta P, Henostroza G, et al. Operational feasibility of using loop-mediated isothermal amplification for diagnosis of pulmonary tuberculosis in microscopy centers of developing countries. J Clin Microbiol. Jun 2007;45(6):1936-1940.
29. Iwamoto T, Sonobe T, Hayashi K. Loop-mediated isothermal amplification for direct detection of Mycobacterium tuberculosis complex, M. avium, and M. intracellulare in sputum samples. J Clin Microbiol. Jun 2003;41(6):2616-2622.
30. Dheda K, Davids V, Lenders L, et al. Clinical utility of a commercial LAM-ELISA assay for TB diagnosis in HIV-infected patients using urine and sputum samples. PLoS One. 2010;5(3):e9848.
31. Steingart KR, Henry M, Ng V, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis. Sep 2006;6(9):570-581.
32. Wallis RS, Pai M, Menzies D, et al. Biomarkers and

diagnostics for tuberculosis: progress, needs, and translation into practice. Lancet. May 29 2010;375(9729):1920-1937.
33. Walzl G, Ronacher K, Djoba Siawaya JF, Dockrell HM. Biomarkers for TB treatment response: challenges and future strategies. J Infect. Aug 2008;57(2):103-109.
34. WHO. Joint TDR/EC expert consultation on biomarkers in tuberculosis: report of the joint TDR/EC expert consultation to evaluate the potential roles of biomarkers in the management of HIV infected and HIV uninfected patients with tuberculosis. 2008.
35. Sadiq ST, Agranoff D. Pooling serum samples may lead to loss of potential biomarkers in SELDI-ToF MS proteomic profiling. Proteome Sci. 2008;6:16.
36. Agranoff D, Fernandez-Reyes D, Papadopoulos MC, et al. Identification of diagnostic markers for tuberculosis by proteomic fingerprinting of serum. Lancet. Sep 16 2006;368(9540):1012-1021.
37. Berry MP, Graham CM, McNab FW, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature. Aug 19 2010;466(7309):973-977.
38. Marques MA, Neves-Ferreira AG, da Silveira EK, et al. Deciphering the proteomic profile of Mycobacterium leprae cell envelope. Proteomics. Jun 2008;8(12):2477-2491.
39. Mahrous EA, Lee RB, Lee RE. A rapid approach to lipid profiling of mycobacteria using 2D HSQC NMR maps. J Lipid Res. Feb 2008;49(2):455-463.
40. Pooran A, Booth H, Miller RF, et al. Different screening strategies (single or dual) for the diagnosis of suspected latent tuberculosis: a cost effectiveness analysis. BMC Pulm Med. 2010;10:7.
41. Cannas A, Kalunga G, Green C, et al. Implications of storing urinary DNA from different populations for molecular analyses. PLoS One. 2009;4(9):e6985.
42. Nathanson CM, Cuevas LE, Cunningham J, et al. The TDR Tuberculosis Specimen Bank: a resource for diagnostic test developers. Int J Tuberc Lung Dis. Nov 2010;14(11):1461-1467.
43. Nathanson CM, Cuevas L E, Cunningham J, et al. The TDR Tuberculosis Specimen Bank: a resource for diagnostic test developers. Int J Tuberc Lung Dis 2010; 14 (In press). . 2010.
44. Lam K-bH, Jiang CQ, Jordan RE, et al. Prior TB, Smoking, and Airflow Obstruction. Chest. March 1, 2010 2010;137(3):593-600.
45. Raby E, Moyo M, Devendra A, et al. The effects of HIV on the sensitivity of a whole blood IFN-gamma release assay in Zambian adults with active tuberculosis. PLoS One. 2008;3(6):e2489.
46. Stout JE, Menzies D. Predicting tuberculosis: does the IGRA tell the tale? Am J Respir Crit Care Med. May 15 2008;177(10):1055-1057.
47. Mandalakas AM, Hesseling AC, Chegou NN, et al. High level of discordant IGRA results in HIV-infected adults and children. Int J Tuberc Lung Dis. Apr 2008;12(4):417-423.
48. Getahun H, Kittikraisak W, Heilig CM , et al. Development of a Standardized Screening Rule for Tuberculosis in People Living with HIV in Resource-Constrained Settings: Individual Participant Data Meta-analysis of Observational Studies. PLoS Medicine, 2011, 8(1): e1000391.
49. Wilson P, Palriwala A. Prizes for Global Health Technologies: An Assessment with a Case Study on TB Diagnostics. 2010.
50. Proposal by Bangladesh, Barbados Bolivia, and Suriname submitted to WHO: Prize Fund for Development of Low-Cost Rapid Diagnostic Test for Tuberculosis. 2009.
51. Grand Challenges in Global Health: Biomarkers. Accessed 1st April 2011.
52. Ramsay A, Al-Agbhari N, Scherchand J, et al. Direct patient costs associated with tuberculosis diagnosis in Yemen and Nepal. Int J Tuberc Lung Dis. Feb 2010;14(2):165-170.
53. Ramsay A, Steingart KR, Pai M. Assessing the impact of new diagnostics on tuberculosis control. Int J Tuberc Lung Dis. Dec 2010;14(12):1506-1507.

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