TOWARDS LAB-FREE TUBERCULOSIS DIAGNOSIS

August 2011

A strategic vision for R&D into point-of-care testing in resource-poor settings

OVERVIEW

Tuberculosis (TB) remains a major challenge to public health globally, particularly in resource-poor settings.

Although most cases of TB are curable, tuberculosis causes around 2 million deaths each year, in part due to late or missed diagnosis. The control and ultimate elimination of this disease, therefore, still rests on prompt diagnosis and therapeutic intervention to reduce ongoing transmission[1,2]. Improving the performance of diagnostics and their availability is therefore key to reducing global morbidity and mortality from TB and thus achieving the Millennium Development Goals.

"Many of the ongoing efforts in R&D could be improved through the adoption of a more focused and strategic approach… Meeting these challenges will undoubtedly require improved funding and commitment from donors."

Despite some progress in recent years, simple diagnostics for community-based diagnosis of TB in resource-poor settings are lacking, and funding remains inadequate. 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 limitations of existing diagnostics result in diagnostic delays that contribute to late or missed diagnoses, with serious consequences for disease progression and TB transmission, thus fueling the epidemic[4,5]. Delayed diagnosis is also often the cause of loss to follow-up or defaulting from care, a major problem in TB health-care delivery[6,7]. It is estimated that 60% of those who require assessment for TB present in local/rural health-care facilities (known as Level 3 health centres), where implementing complex diagnostics is not necessarily feasible[8]. Such facilities may have no electricity, no running water, and limited or no laboratory facilities. The ideal TB diagnostic is therefore a POC test that allows patients to be diagnosed and receive appropriate care within hours of undergoing TB testing.

Médecins Sans Frontières (MSF), the Treatment Action Group (TAG), and the TB/HIV Working Group of the Stop TB Partnership commissioned a report from experts at ArteBio Consulting and the Division of Infectious Diseases, Department of Medicine, Imperial College London, to analyse progress made towards developing a POC TB test for Level 3 health centres in resource-poor settings. The report built on the outcomes of expert meetings held in Cambridge, UK (2008) and Paris, France (2009) organised by Médecins Sans Frontières, TAG, Partners in Health and the AIDS and Rights Alliance for Southern Africa (ARASA). Literature reviews, expert opinion, and key informant interviews with TB diagnostics researchers in academia and industry have been used to assess

the state of the art in TB diagnostics development. This research provides the basis for the strategic vision to expedite progress towards a TB POC test.

The aim of the report is to:

- Provide a frank landscape analysis of the status of progress towards a POC TB test.

- Identify existing roadblocks to novel POC TB test development, providing a strategic vision for research and development (R&D) for the next 5-7 years.

A full version of the report can be downloaded at www.msfaccess.org/content/towards-lab-free-tuberculosis-diagnosis

The report concludes that many of the ongoing efforts in R&D could be improved through the adoption of a more focused and strategic approach, with integrated planning between areas of biological discovery, test development, and specimen repositories. A summary of the report's final proposals for short-term and long-term R&D objectives is presented in Table 1. Meeting these challenges will undoubtedly require increased funding from donors and collaboration among researchers. Indeed, with significant changes, we believe that a POC TB test could be a reality within the next 5 to 7 years. This summary document highlights the advocacy priorities of TAG, MSF, and the Stop TB Partnership's TB/HIV Working Group that are primarily derived from the full report.

Short-term R&D objectives
Develop and progress already identified biomarkers into diagnostics with a POC format
Ensure technological breakthroughs in sample collection and processing to enhance diagnostic accuracy
Improve specimen repositories for the validation of biomarkers to better serve the needs of TB diagnostics R&D
Long-term R&D objectives
Drive the discovery of novel biomarkers for TB diagnosis • Increase collaborative funding efforts • Consider establishing an independently managed model of collaborative research • Prioritise identification of TB biomarkers in samples other than sputum

Table 1: *Making a POC TB test a reality: summary of identified R&D objectives*

POC TB DIAGNOSTICS: CURRENT PROGRESS

Microscopy and culture methods are the main TB diagnostics used in resource-poor settings. Both require sputum samples that are not always easy to collect and manipulate. In addition, a greater number of HIV-positive patients have reduced numbers of bacilli in their sputum and will appear as smear negative. In the last few years, therefore, the need for R&D into a POC TB test that could be employed in resource-poor countries has been increasingly recognised. The ideal POC TB test would diagnose active TB within 3 hours, leading to immediate triage for antibiotic treatment. In recent years financial support has become available for R&D in this area, mainly through the Bill & Melinda Gates Foundation. However, despite significant progress in technologies and products with the potential to advance the diagnosis of active TB, the fact remains that there are currently no POC TB diagnostic tests suitable for use in Level 3 health centres where the majority of TB patients present.

"A point-of-care test for tuberculosis is a desperately needed advance. The introduction of such a simple test would undoubtedly save millions of lives….."

Assessment of marketed solutions

Table 2 (overleaf) highlights some of the TB diagnostics that are being marketed or are close to receiving market authorisation. The table presents an assessment of their suitability for resource-poor countries. Although this is not an exhaustive list, the report concludes that despite some recent advances in TB diagnostics, the majority of tests that are commercially available (or near to being commercially available) remain firmly routed in Level 1 or Level 2 facilities - i.e, large hospitals with well equipped reference laboratories or district hospital/urban health clinics with moderately equipped laboratories characterised by a supportive infrastructure, and where trained personnel are available. Despite being important advances, the tools currently approved to diagnose TB have a limited reach among populations most in need of rapid TB diagnostics. In addition, it is worth noting that among all the currently available commercialised TB diagnostic tests, the only ones that lend themselves to the POC format are the serological tests measuring antibody response to mycobacterium tuberculosis (MTB), which are unsuitable for use due to poor sensitivity and specificity[9].

Xpert MTB/RIF assay

Recent developments in nucleic-acid-based diagnostics with the GeneXpert system and Xpert MTB/RIF assay (Cepheid), which uses sputum as the sample, are very encouraging. The development of this test represents a step in the right direction for TB diagnostics, because it detects both the pathogen and also mutations associated with drug resistance. Studies have shown that performance is remarkably robust[10-12], with detection surpassing Lowenstein-Jensen culture for a single specimen. More than a dozen other studies are completed or ongoing and a number of reports have now been published, including for extrapulmonary and paediatric TB.

The World Health Organization (WHO) has recently endorsed this new diagnostic test and recommended its implementation in Level 2 health centres - i.e, district level hospitals/urban health clinics with power, clean water, small laboratories, and some or variably trained staff (2). Xpert MTB/RIF is well suited to urban settings and its use in an advanced laboratory with extensively supportive infrastructure in resource-poor countries is certainly possible. On the basis of WHO recommendations, current roll-out of this new test largely aims to place it in Level 2 health centres. However, Xpert MTB/RIF's test performance in these settings under routine programme conditions remains to be fully assessed. So whilst not truly POC, and not suited for Level 3 health centres, Xpert MTB/RIF certainly paves the way for the creation of new TB diagnostics.

Defining the ideal POC TB test and its characteristics

During the expert meeting in Paris, France, in 2009, experts came together to develop and define the test specifications and end-user requirements for a potential new POC TB test (http://www.msfaccess.org/TB_POC_Parismeeting/). This work provided information about the key characteristics that a POC TB test should meet in order to respond to medical needs in resource-poor settings. It also enabled experts to

Level of health centre	Positives	Negatives
LEVEL 1 BACTEC MGIT series	• Gold standard liquid culture methodology. • High throughput with automated series.	• Expensive per test costs. • Complex instrumentation. • Trained staff required. • Not suitable for Level 2 and 3.
LEVEL 2 Cepheid Xpert MTB/RIF assay	• 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: within 3 hours. • Other sample sources are under investigation	• Currently being rolled out in Level 2 laboratories but feasibility in routine programme settings yet to be fully assessed • Expensive per-test cost (approx US$17 and more so if using triplicate samples to obtain high sensitivity in smear-negative sputum). • Complex and expensive instrumentation (US$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 problematic in low and middle-income countries.
LAMP tests	• Relatively simple to use. • Needs only a limited laboratory structure.	• Mostly in-house assays so far, although commercial release reportedly imminent from Eiken. • Problem to read fluorescent results without instrument component. • Sensitivity and specificity to be improved as well as integration with POC extraction systems.
Urinary LAM tests - e.g. Alere ClearView	• Uses non-invasive sample. • Relatively easy to perform.	• Requires some supportive laboratory infrastructure. • Sensitivity not promising. • Test may be under redevelopment.
LEVEL 3 Sputum smear microscopy (direct or concentrated)	• No complex instrumentation needed for direct smear microscopy. • Not a technically complex procedure. • Well-established methodology. • High specificity in most settings.	• Low throughput and need for External Quality Assurance (EQA). • Sensitivity very variable (20-80%). • Technical expertise required. • Trained staff needed. • Fluorescence more expensive. • Infrastructure required, particularly for concentrated samples - e.g, centrifugation.

Table 2: *Currently available case detection TB tests by health-care level Level of health centre*

prioritise the critical specifications such a test would need, and identified areas for intense R&D. Outcomes of the expert meeting are summarised in the appendices (Table 3).

The report provides an analysis of the end-user requirements agreed upon during the Paris meeting, and includes a further assessment of the risk of failure to develop a POC TB test within the next 5 years meeting identified test specifications. The risk assessment takes into account current funding levels and effort and progress so far towards POC TB tests. The full table summarising the risk-assessment analysis and the authors' proposed solutions to ameliorate risks is presented in the appendices (Table 4).

POC TB TEST DEVELOPMENT: A STRATEGIC VISION FOR R&D FOR THE NEXT 5-7 YEARS

The report highlights that without a significant change in strategy and funding the world could be a decade away from developing a POC TB test for resource-poor countries, and probably longer. With almost 2 million deaths per year attributable to a treatable disease, we cannot wait for 20 million more deaths before its realisation. An alternative strategy is urgently needed to improve the current situation.

A POC TB test could be developed within the next 5-7 years with some significant changes to strategy and funding. Experts identified numerous existing roadblocks to the development of a POC TB test suitable for Level 3 health centres in resource-poor settings. This has enabled us to define both short-term and long-term objectives for the R&D community, which are described below.

"Developing improved diagnostics to address these challenges will require a substantially larger and targeted coordinated global effort."

SHORT-TERM OBJECTIVES IN R&D

■ Develop already identified biomarkers into diagnostics with a POC format

Biomarkers such as the rpoB gene in MTB have already been identified as useful pathogen markers, which could be exploited for new diagnostic methods. At present nucleic-acid-amplification-based tests targeting the rpoB gene remain arguably the most appealing biomarker for diagnostics development; no other promising biomarkers have emerged so far. Their appeal is based on the fact that: (a) There is DNA sequence within rpoB that is specific to MTB; (b) Within a small region of 81bp in the rpoB there are a number of well- characterised mutations which can confer resistance to rifampicin; and (c) Rifampicin-resistance remains an important surrogate marker for multidrug-resistant tuberculosis. Detection methods based on MTB rpoB can therefore identify both the pathogen and likely multi-drug resistance. Commercial detection systems based on MTB rpoB are available, but so far detection is only possible through nucleic-acid-based tests so none is feasible for use in a Level 3 health centre.

The development of the nucleic-acid-amplification-based Xpert MTB/RIF assay has changed expectations of what we should be able to expect from an MTB rpoB targeted approach. A system with these performance characteristics, but that could meet the end-user specifications for a POC TB test would be game changing. The challenge remains to ensure these

advances develop into diagnostics suitable for Level 3 health centres in resource-poor countries. Many activities are underway for the development of POC TB tests that can detect nucleic acid; however, most are geared towards use in resource-rich countries. Of the handful of activities that are focused on resource-poor settings, at present these developments are either at a very early developmental stage or have unrealistic formats. A complete overview of the current state of play is presented in the full report.

"The challenge remains to ensure these advances develop into diagnostics suitable for Level 3 health centres in resource-poor countries."

The next step should be to encourage and incentivise developers to establish POC tests based on the existing biomarker rpoB. This could yield high rewards providing the management of the project is suitably established. Strong industry-style project management approaches have shown that they can deliver results. Key factors for creating this framework are as follows:

- Establish contractual collaborative frameworks for intellectual property sharing, and putting in place appropriate policies to ensure that the principle of delinkage is applied and that the cost of the final product is not linked to the development costs.
- Set out very clearly a set of end-user specifications that are technologically feasible.
- Put in place a small and involved management team to oversee progress and drive the project forward.
- Establish clear milestones with indicators for achievement agreed by both the management team and the developers.
- Take decisions to discontinue funding if progress is not forthcoming, thereby saving funds that can be reinvested back into successful projects.

This type of funding model has been shown to be successful for driving R&D in diagnostics for use in resource-poor settings. Whilst not a panacea, this model could be replicated for POC TB tests and be used to drive the search for a suitable POC test for rpoB. Other incentive structures that separate the costs of R&D 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 generated renewed interest in the field of TB diagnostics[13].

■ Ensure technological breakthroughs in sample collection and processing to enhance diagnostic accuracy

Given the nature of the disease, respiratory samples will remain an important sample for the diagnosis of TB and the parallel process of improving respiratory sample collection and processing methods needs to be considered. A number of alternative approaches are currently in the process of evaluation. Nebuliser systems, for example, have been developed whereby inhalation of nebulised fluid (usually hypertonic saline) is used to generate respiratory specimen that is then expectorated or aspirated mechanically. Although this is effective in clinical practice and commonly used in well-resourced settings to induce sputum, the nebuliser system is not suitable for Level 3 health centres as it usually requires a power supply, provides an infection-control risk to those in close proximity, and requires consumables, including a sterile fluid source.

Indeed, all of the options currently in the development pipeline have limitations and none has yet been widely adopted in any resource-poor setting. The parallel process of developing sample collection and processing methods – alongside a POC TB test – therefore needs to be considered with urgency. One way forward would be for the funding agencies and donor community to issue Request For Proposals (RFPs) for new sputum collection methods and processing, to incentivise developers to explore options for Level 3 health centres. Importantly, it is critical for researchers to focus on developing new ways for sputum collection and processing in HIV-positive individuals and children.

■ Improve specimen repositories for the validation of biomarkers to better serve the needs of TB diagnostic R&D

Specimen repositories with well-characterised specimens, available to academic investigators and industrial developers, are an important component in the development of any potential POC TB test because they are critical to identifying and validating biomarkers as well as for early evaluations of new diagnostic tests. Diagnostic test developers must be able to screen potential markers in a set of well-defined samples (not

restricted to sputum and serum) from TB and non-TB patients. An independent source of these TB samples is important for several reasons. The first is that most small companies and academic units will not have sufficient funds to establish a complete specimen repository, and the lack of a set of validation samples should not be a barrier to POC diagnostic development. Secondly, the independence of the specimen repository will make the validation process more rigorous for the TB research community.

"We found that the specimen repositories currently available are not adequate and improvements are urgently needed."

An overview of specimen repository resources globally can be found in the full report. The specimen repositories currently available were not considered adequate by the authors, with improvements urgently needed. In particular, these repositories and their samples need to be better defined. Of the several repositories 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 and the geography of the samples (endemic versus non-endemic areas and inclusion of sufficient samples with HIV co-infection). The greater investment required to address such shortfalls is likely to be beyond the financial and logistical limits of small biotech companies and academic research units.

There is increasing consensus that specimen repositories 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. However, these resources, if based solely around trial participants, are likely to be deficient in important areas such as lacking adequate controls and paediatric specimens. 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 will likely be more effective to adapt and improve existing resources initially; however, 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 that could better meet the needs of POC TB test development community. Importantly, it provides an open access resource for developers.

The report recommends the following to improve the WHO/TDR specimen bank:

- There is a need to change the clinical phenotyping categories currently used in favour of categories that would better reflect the clinical scenarios in which a test is needed. Detailed characterisation of HIV-positive specimens (in particular a patient's immune status and medication received) would be useful and specimens could be collected as matched cohorts (from HIV-positive and HIV-negative patients) from the same clinical settings and that reflect the setting for which tests are needed (i.e, rural health posts rather than tertiary referral centres).

- A paediatric resource is needed; ideally, the WHO/TDR specimen bank should be expanded in order to house the pediatric specimens. This would be challenging and expensive, but represents a crucial gap in current efforts. Clinical characterisation should be based on significant follow-up of children to provide a reliable reference standard of diagnosis. The categorisation of samples should be aligned with the new consensus on definitions of diagnostic categories for childhood TB[14].

- Ensure the collection of a broader range of biological specimens. The decision about the type of specimens to collect is a complex one and depends on a number of factors, including cost, utility, stability when frozen, and the likelihood of a particular method/protocol. The main additions needed at this stage are ribonucleic acid (RNA) extracted from blood, and both blood and urine suitable for analysis of metabolites. RNA protocols are

well established. Protocols for metabolites are more variable for urine where specimen is easier to collect. The best protocols will have to be chosen to reflect current discovery efforts. DNA (human) is unlikely to form the basis of a future test. However, its collection and storage is cheap and straightforward, so it should be included in new efforts because it may prove useful for future analysis in conjunction with other samples.

- Ensure greater flexibility to change. The specimen repository should be able to respond rapidly to advances in biomarker discovery and to adapt accordingly. As part of this process, any new resource should include an advisory group that includes representatives from industry, academia (particularly biological discovery), and technology to allow emerging trends to be spotted.

- The resource must take a broader view on the development of new resources that could be helpful to diagnostic developers. The development of the Xpert MTB/RIF assay, for example, was aided by existing specimen repositories, 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-tuberculosis mycobacteria). As part of the service offered to developers by specimen repositories, the target time to process applications could be reduced to a suggested maximum of 3 months from application to specimen delivery.

- Ensure 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 specimen repository and independent monitoring of its function.

LONG-TERM OBJECTIVES IN R&D

Drive the discovery of novel biomarkers for TB diagnosis

The search continues for novel biomarkers to rapidly diagnose and monitor treatment response in active and latent TB[15,16,17], and represents one of the critical research priorities in TB diagnostic research. The aim of the R&D community must be to have a rich pipeline of markers until such time as one (or several in combination) has been characterised and validated. Markers that can be used in a POC format should be prioritised. An overview of the current state of play for novel biomarker discovery is available in the full report.

Figure 1 presents an overview of the process of biomarker discovery to eventual diagnostic product release. This is a 7-year process of validation and test development, post-discovery, assuming a very generous budget investment. None of the large pharmaceutical companies, diagnostics companies, or academics interviewed for this report believed that a novel

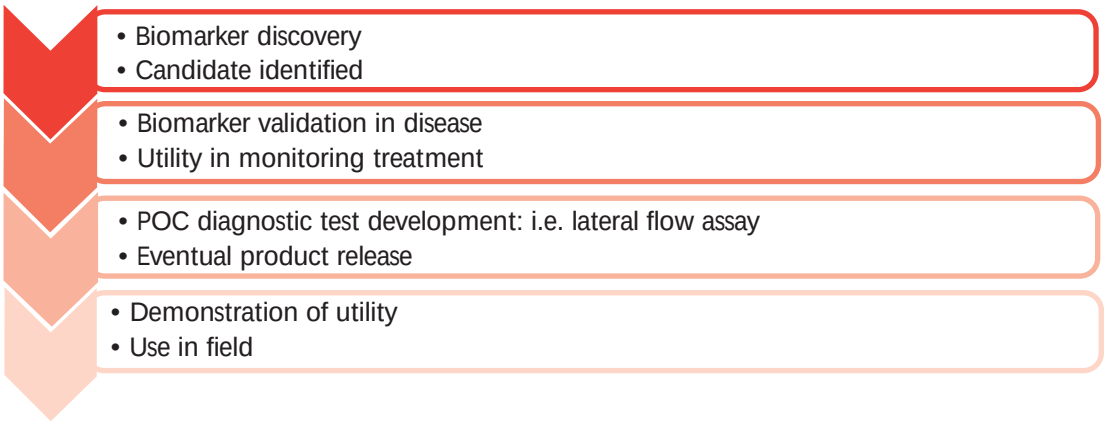

Figure 1: *Simplified schematic of the process of biomarker discovery to eventual diagnostic product release. Assuming very generous budget investment, this process could be shortened, but is still unlikely to be completed in less than 7 years. This assumes the selection of the biomarker at Year 0.*

biomarker useful for POC TB testing is likely to emerge in the next 3-5 years. This is a bleak assessment of the state of biomarker discovery in TB diagnostics and so it is vital for TB R&D funders and researchers to take steps now to reduce this time delay.

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. Biomarker discovery can be usefully divided into approaches identifying host-derived markers and those targeting markers from the pathogen. The search for host biomarkers, whilst offering some promise, is the highest-risk strategy.

"The discovery of novel biomarkers must be driven in a more targeted way, employing a more focused funding strategy."

To speed up this process of discovery, the R&D community must prioritise the discovery of pathogen-based biomarkers. Discovery should be focused on non-nucleic-acid pathogen markers as the first priority, closely followed by nucleic-acid pathogen markers. However, given the risks of failure, alternative biomarkers derived from pathogens need to be found and a focus on pathogen-based detection should not mean the complete exclusion of host biomarkers.

The discovery of novel biomarkers must be driven in a more targeted way, employing a more focused funding strategy. The report recommends the following next steps:

- Increase collaborative funding efforts
- Consider establishing an independently managed model of collaborative research
- Prioritise identification of TB biomarkers in samples other than sputum
- Increase collaborative funding efforts

The biomarker search has been so far characterised by a lack of collaboration within the TB research community. Researchers release only limited information detailing which biomarkers have been tested and whether they have failed, and in which systems/screening methods. If the challenge of TB diagnostics is to be met and overcome, there must be a drive to increase collaboration and openness between researchers to avoid wasteful duplication of work. Future funding should depend upon this openness.

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 the delivery of diagnostics aimed primarily at resource-poor countries. This does not mean 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 resource-rich countries, the number of individuals with TB is relatively low, and so business models need to take this into account when deciding whether to invest in TB diagnostics. This has implications for the developers, but also for the purchasers of TB diagnostic tests. Clinical diagnostic laboratories in resource-rich countries are unlikely to invest in purchasing a complex and expensive instrument that 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.

"A different style of funding is needed to drive R&D in TB diagnostics for resource-poor settings."

There will not be a POC TB diagnostic without the input of diagnostic companies, and so it is important to incentivise their involvement. This must be done in a way that allows for the separation of the costs of R&D from the price of the innovation, so that once developed the diagnostic test is accessible and affordable in resource-poor countries.

A new funding mechanism must be set up to foster this collaborative environment and to increase its chances of success. The current funding streams are not 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 closed, and there are very

few major collaborations that aim to develop biomarkers for diagnostics. Recently, the Bill & Melinda Gates Foundation launched a US$12million grant programme specifically focused on the validation of diagnostics biomarkers, as part of the Grand Challenges in Global Health initiative. Although this programme certainly has 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 faster progress.

"A new funding mechanism must be set up to foster this collaborative environment and to increase its chances of success. The current funding streams are not driving discovery in an efficient way"

Consider establishing an independently managed model of collaborative research

Besides funding, an independently managed, collaborative system – ideally separated from current diagnostic development mechanisms – is also needed to drive biomarker discovery. This structure should ensure that:

- Funders work together and coordinate funding efforts for biomarkers. This will avoid unnecessary duplication and redundancy and will promote collaboration.
- An independent management system is established to oversee the biomarker discovery, negotiating milestones and deliverables as part of the funding agreement.
- 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.
- There is only a limited amount of overlap in the biomarkers being studied by each funding recipient. This will ensure a reasonable spread of pathogen-based targets without significant duplication and redundancy.
- Different screening tools are contained within the collaborative effort so that good biomarker candidates can be searched for and verified using alternative methods.
- There is a structure in place to provide independent validation of any biomarkers discovered.
- There is a mechanism for the termination of funding/contract cancellation when biomarkers are proven not to be useful in the collaborative effort.
- Ideally, the biomarker discovery programme should be steered by a manager with experience in R&D in this field, but without his/her own scientific or intellectual property interests or projects in the programme. Such a manager must have authority to decide when projects should be stopped based on clear and established criteria. The manager should report to a small Board that should ideally be selected to avoid possible conflicts of interest.

Prioritise identification of TB biomarkers in samples other than sputum

Although sputum remains one of the key specimens for TB diagnosis, the collection of good quality samples adequate for proper diagnosis is difficult. This represents a particularly important limitation in the setting of HIV and paediatrics, in which patients are often unable to produce sputum specimens suitable for analysis or their sputum contains low levels of TB[18]. The sensitivity of sputum smear microscopy in routine clinical practice varies between 35% and 80% in HIV-negative patients, but drops to as low as 20% among HIV-infected and is even lower (typically less than 10%) in children. Techniques have been developed to allow generation of a specimen from these patients; however these methods pose a risk of cross-infection to staff and require techniques that are rarely available in resource-poor settings.

"The research community should prioritise the identification of TB biomarkers in urine and blood."

For these reasons novel diagnostics using samples other than sputum, for example urine and blood, could have a major impact, especially if a POC format could be developed. A urine-based system of sample collection is a possibility. Urine would be an ideal sample as it is non-invasive and available in high quantity. However, detectable protein in urine is often dependent upon kidney function so the protein marker must be present in sufficient quantities. For these reasons, the research community should prioritise the identification of TB biomarkers in urine (ideally non-protein based markers) and blood.

CONCLUSIONS

A POC test for TB is a desperately needed advance. The introduction of such a simple test will undoubtedly contribute to reducing substantial mortality by ensuring many more patients receive life-saving treatment, and contribute substantially to reducing TB cases in resource-poor settings. Current funding for TB diagnostics R&D is inadequate at US$41million, with the Global Plan to Stop TB: 2011-2015 projecting an annual funding requirement of US$340million[19,20]. The development of improved diagnostics will require a substantially larger and targeted coordinated global effort, with greater funding and commitment from donors. Many of the ongoing efforts could be improved by a focused and strategic approach, with available funding better targeted to projects that can be delivered within a relatively short timeframe, even if they are only partial solutions to a POC TB test. Strong leadership among developers will be vital to strengthening TB diagnostics efforts. It is critical that POC TB test development is better aligned to the clinical decisions being made in Level 3 health centres in resource-poor settings, where the majority of TB patients present.

"Strong leadership among developers will be vital to strengthening TB diagnostics efforts."

In summary, the report highlights both short-term and long-term R&D objectives that could ensure a POC TB test becomes a reality within the next 5-7 years.

Short-term objectives

- Develop already identified biomarkers into diagnostics with a POC format
- Ensure technological breakthroughs in sample collection and processing methods to enhance diagnostic accuracy
- Improve specimen repositories for the validation of biomarkers to better serve the needs of TB diagnostics R&D

Long-term objectives

- Drive the discovery of novel biomarkers for TB diagnosis
- Increase collaborative funding efforts
- Consider establishing an independent management model of collaborative research
- Prioritise the identification of TB biomarkers in samples other than sputum

Without significant change to the established R&D agenda for TB diagnostics, a truly POC test is still far from realisation. We cannot wait a decade or more for a POC TB test that can be used in resource-poor settings. If just some of these recommendations are implemented, we believe that the successful development of a new POC TB test will be one step closer.

"It is critical that POC TB test development is better aligned to the clinical decisions being made in Level 3 health centres in resource-poor settings, where the majority of TB patients present."

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APPENDICES

Table 3: Minimum specifications for a POC TB diagnostic test

Test specification	Minimum required value
Medical decision	Treatment initiation
Sensitivity - Adults *[for pulmonary TB only; regardless of HIV status]*	Pulmonary TB: - 95% for smear positive, culture positive - 60%-80% for smear negative, culture positive *[Detection of extrapulmonary TB being a preferred but not minimal requirement]*
Sensitivity - Children *[including extrapulmonary TB; regardless of HIV status]*	- 80% compared to culture of any specimen and - 60% of probable TB (noting problem of lack of a gold standard)
Specificity - adults	- 95% compared to culture
Specificity - children	- 90% for culture-negative probable TB (noting problem of lack of a gold standard) - 95% compared to culture
Time to results	3 hours max. (patient must receive results the same day) [Desirable would be <15min]
Throughput	20 tests/day minimum, by 1 laboratory technician
Specimen type	Adults: urine, oral, breath, venous blood, sputum *[Desired: NON sputum-based sample type and use of finger prick instead of venous blood]* Children: urine, oral, capillary blood (finger/heel prick)
Sample preparation	- 3 steps maximum - Safe: biosafety level 1 - Ability to use approximate volumes (i.e., no need for precise pipetting) - Preparation that is not highly time sensitive
Number of samples	One sample per test
Readout	- Easy-to-read, unambiguous, simple "yes", "no", or "invalid" answer - Readable for at least 1 hour
Waste disposal	- Simple burning or sharps disposal; no glass component - Environmentally acceptable disposal
Controls	- Positive control included in test kit - Quality control simpler and easier than with sputum smear microscopy
Reagents	- All reagents in self-contained kit - Kit contains sample collection device and water (if needed)
Storage/stability required	- Shelf life of 24 months, including reagents - Stable at 30°C, and at higher temperatures for shorter time periods - Stable in high humidity environments
Instrumentation	- If instrument needed, no maintenance required - Instrument works in tropical conditions - Acceptable replacement cost - Fits in backpack - Shock resistant
Power requirement	Can work on battery
Training	- 1 day maximum training time - Can be performed by any health worker
Cost	<US$10 per test after scale-up

Table 4: Review of end-user specifications filtered by risks and constraints

	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.

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.	VOCs and oral samples should be given lower priority.
		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. Quality Assurance (QA) simpler and easier than with sputum smear microscopy.	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.

Footnotes *+RFP: Request for Proposals.*

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