

# HATiP

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## In this issue:

### **New laboratory tests to enhance TB diagnosis: Microscopy, LAM and Xpert MTB/RIF used as a drug resistance test; by Theo Smart *page 2***

- This series
- Introduction
- Other possible strategies to reduce GeneXpert costs: microscopy and LAM
- Microscopy
- Alere Determine LAM lateral flow test
- How can LAM be incorporated into diagnostic practice?
- Finding more resistance than expected — could some of it be recently acquired?
- Conclusion

# New laboratory tests to enhance TB diagnosis: Microscopy, LAM and Xpert MTB/RIF used as a drug resistance test

By Theo Smart

## Key points

- This article is the third part of a series on the implications of new diagnostic tests for TB diagnosis in people living with HIV and for TB/HIV programmes.
- Gene Xpert MTB/RIF is a new diagnostic test for TB. It also detects resistance to the TB drug rifampicin. It is being rolled out in a number of countries in order to improve the speed and accuracy of TB diagnosis, and has been shown to reduce the delays between presentation, diagnosis and starting TB treatment substantially.
- However Xpert MTB/RIF is not suitable for use at the point of care in all types of facilities, and for the time being is likely to be unaffordable in many settings.
- Researchers are also looking at improving the accuracy of smear microscopy, which will remain the first-line diagnostic test for M.TB for much of the world for the foreseeable future. LED microscopy is more sensitive than standard microscopy, and new methods of specimen handling may also improve the yield of microscopy.
- Automated smear reading has also been tested; it may be suitable for use in settings where a large number of specimens are being reviewed, but where Xpert MTB/RIF is unaffordable.
- Another test, the Determine TB-LAM assay, looks for a fragment of the M.TB cell wall in urine. The test can be carried out in even the most basic health facility because it is a lateral flow strip test which requires no sample processing, and which can be read easily. It costs around US\$2.50 – 3.50 per test.
- Determine TB-LAM is probably most suitable for use in people living with HIV with low CD4 cell counts, as a test which can positively identify a person as having TB – a ‘rule-in’ test – allowing rapid initiation of TB treatment.
- When its use is combined with smear microscopy, Determine TB-LAM performs nearly as well as Xpert MTB/RIF in HIV-positive people with CD4 counts below 100 – the group of people who need to start TB treatment most rapidly.
- Using LAM and smear microscopy together has the potential to identify the patients in most urgent need of treatment and those patients who are smear-positive and therefore most likely to transmit TB to others. This is especially important information in the healthcare setting, where infection control is critical.
- At the very least, use of the two tests may reduce the number of Xpert MTB/RIF tests that need to be carried out, saving money.
- One study has shown that a positive Determine TB-LAM result, or having a very low CD4 count, is associated with a greatly increased risk of developing TB-IRIS after starting antiretroviral therapy. More work is needed to evaluate how this test could be used as a screening test to predict patients at high risk of TB-IRIS where CD4 counting is not readily available.
- The Xpert MTB/RIF assay is able to identify samples which show evidence of some resistance to rifampicin. It might also give an indication of the bacterial burden of rifampicin-resistant M.TB, but this needs more study.
- This information can be transmitted from the machine running the tests to a central laboratory. Collecting this information might prove useful for evaluating both the success of existing case finding activities and pinpointing ‘hot spots’ where health workers need to be looking especially hard for cases of drug-resistant TB.
- If rifampicin resistance is present it is likely that the patient will need second-line TB treatment, but further drug susceptibility testing is needed to confirm each case identified where MDR-TB prevalence is low. In South Africa, the policy is to provide MDR-TB treatment immediately, altering treatment if further testing shows the TB is drug susceptible.
- There is some evidence to suggest that Xpert MTB/RIF is picking up cases of ‘primary’ MDR-TB infection. Among people living with HIV, the risks of rapid progression from primary infection to severe active TB are great.
- Further tests that can be used at the point of care are still in the pipeline, and likely to emerge in the next few years. Each test is likely to have a different niche, and it is likely that health systems will move towards the use of different combinations of tests according to the type of patient and the type of health facility. This will mean that a wider range of health care workers may need to learn more about the

### **differences between TB tests, and the rationale for various diagnostic algorithms.**

- **The emergence of new tests could also require regular and prompt updates to guidance at the national and international level, and ongoing advocacy to ensure that the new diagnostics become affordable, widely available and used to best effect.**

## **This series**

This edition of HATIP is kindly supported by the Lilly MDR-TB Partnership. This article forms the third in a three-part series on the implications of new laboratory tests for TB diagnosis. The first part, on active case finding, symptom screening and the use of laboratory tests to support active case finding, was published in [January 2012](#). The second, on early experience in the use of the Xpert MTB/RIF assay to improve diagnosis, was published in May 2012.

## **Introduction**

The second part of this series looked at the introduction of the Xpert MTB/RIF test. It identified a number of barriers to the adoption of the test for TB diagnosis in low- and middle-income countries. It may not be cost-effective to use the test in all settings, and there are also some technical challenges that may make the test unsuitable for use in some types of health facility.

This edition looks at new research on ways to reduce the cost of Xpert MTB/RIF use through the adoption of other new tests within the diagnostic algorithm, and also what we can expect to emerge from the diagnostic research pipeline fairly soon.

This edition also looks at the RIF part of the Xpert MTB/RIF assay, and its use in the detection of rifampicin resistance.

## **Other possible strategies to reduce GeneXpert costs: microscopy and LAM**

As a cost-saving strategy, the algorithm suggested by WHO, to first use smear microscopy to identify the smear-positive cases and then perform the Xpert MTB/RIF assay only on the specimens that were negative, did appear to reduce the cost of diagnosis somewhat, according to a study conducted in the Western Cape.<sup>1</sup> The investigators looked at several adjunct tests (smear microscopy, chest-x-ray, and gamma interferon release assays (IGRAs)), given either prior to Xpert MTB/Rif to reduce costs, or afterwards to improve accuracy.

The study found that smear-microscopy followed by Xpert-MTB/RIF (if smear-negative) had the lowest cost-of-diagnosis of any strategy investigated. Adding chest x-ray after microscopy but before Xpert before ruled out TB in 18% of the TB but did not further reduce the cost per TB case diagnosed. The study also found that performing smear microscopy in Xpert-negative TB suspects identified about 21% of the culture-positive cases that Xpert missed. The IGRAs were more or less useless.

However, as the HE2RO study suggested, this approach won't reduce costs by much when using a sensitive symptom screen, because at most, only 10% of TB suspects in that setting were smear-positive – Xpert would still have to be performed for at least 90% of the TB suspects – and depending upon how long the algorithm delays diagnosis (which could vary, based upon whether Xpert is point of care, or based at a separate facility) the costs

related to loss to referrals and delayed treatment might mean it will not be cost saving at all.

However, the approach might work better if microscopy were significantly more sensitive, or the algorithm used an alternative TB test that was either more sensitive or that didn't delay diagnosis.

## **Microscopy**

It was disappointing to hear that fluorescence microscopy did not increase case detection in the ACTG 5253 study presented by Dr David Katzenstein of Stanford University at the 19th Conference on Retroviruses and Opportunistic Infections in March 2012 ([described in the second part of this series](#)), since according to WHO's [Draft Policy Framework for Implementing New Tuberculosis Diagnostics](#), it is supposed to be about 10% more sensitive – but that document also notes that fluorescence microscopy requires considerable expertise compared to standard microscopy, so the extra sensitivity might only be achievable in the hands of the most skilled technicians.

However, there is a significant effort underway to optimise smear microscopy, which the Optimizing TB Smear Microscopy Subgroup of the STOP TB Partnership's [New Diagnostics Working Group](#) is helping to facilitate and monitor.

## **LED fluorescence microscopy**

The first advance, which is already being introduced into TB labs, is Light Emitting Diode (LED) fluorescent microscopy. Old fluorescence microscopy has several problems – it had to be performed in a dark room, the light bulbs put off an uncomfortable amount of heat, the bulbs had to be changed frequently and had toxic by-products when they were broken. LED lighting addresses all those issues, can be run on batteries (so any remote microscopy site could operate it), and is relatively inexpensive.

After a systematic review and meta-analysis of the available data, WHO reported that in comparison with direct Ziehl-Neelsen (standard) microscopy, LED microscopy was statistically significantly more sensitive by 6% (95% CI, 0.1–13%), with no appreciable loss in specificity; and LED microscopy was 5% (95% CI, 0–11%) more sensitive and 1% (95% CI, -0.7% - 3%) more specific than conventional fluorescence microscopy.<sup>2</sup>

Consequently, WHO strongly recommended that conventional fluorescence microscopy be replaced by LED microscopy and that LED microscopy be phased in as an alternative for conventional light microscopy in both high- and low-volume laboratories. Of course, WHO stressed the switchover should be carefully planned, with adequate training – especially for laboratory staff unfamiliar with fluorescence microscopy techniques – and in-country validation of the performance of LED microscopy, relative to other types of microscopy, with internal quality control and external quality assurance, and monitoring of case detection trends and treatment outcomes post-introduction.

## **Specimen handling**

Other approaches to increase the sensitivity of smear microscopy involve how the sputum specimen is handled. Improved sputum processing involves using bleach, NaOH or other substances to chemically digest, or liquefy the sputum, which eliminates cellular residues, and makes the smear clearer to read – potentially increasing sensitivity by 10-15% according to one study.<sup>3</sup> Subsequently, a number of methods to concentrate the bacilli in the specimen together (which should make them easier to spot), are under evaluation. The most common method, centrifugation, is not

practical in more resource-constrained settings, because most peripheral labs don't have centrifuges. However, a method that does not involve centrifugation is being studied which involves manually concentrating bacilli directly onto the slide by using paramagnetic beads coated with a molecule that selectively binds to mycobacteria in sputum.<sup>4</sup> Others are studying filtering the liquefied sputum to concentrate mycobacteria.<sup>5</sup>

### Automated reading

Finally, another way to improve smear microscopy would be to take the human element out of reading the slides and automate the analysis. For instance, a camera could be put into the microscope and the picture would be analysed by a computer programme. This would free up technician time and allow the process to be standardised. However, while fast and much more sensitive, there appears to be a problem with specificity as Professor Gavin Churchyard of Aurum Institute mentioned in a presentation at the 19th Conference on Retroviruses and Opportunistic Infections in March 2012.<sup>6</sup>

"We've developed a fully automated AFB microscopy system with our colleagues from CSIR, called the TBDx," said Prof Churchyard. "The system automatically loads slides, focuses, captures the images and reads a hundred fields at once using a signature-mapping algorithm to identify and count the number of AFBs present on the slide."

His team evaluated the performance of the TBDx system in a cross-sectional study of pulmonary TB suspects among South African gold miners. Each TB suspect gave one sputum specimen for MGIT culture; and they used anti-MPB64 for organism identification to confirm that that was true TB. Sputum specimens were also taken for microscopy. The slides were auramine stained and read by a research microscopist, and the TBDx system using fluorescence microscopy—almost a thousand specimens of which 27.4% were M.TB culture-positive.

The sensitivity of the TBDx system was 76%, compared to the sensitivity of 52.8% of the research microscopist, but it had many more false positives (the specificity was only 43.5%). This could lead to giving TB treatment to many people without TB.

In order to optimise the performance of the automated system, they used an algorithm which classified all the slides where TBDx hadn't found any acid-fast bacilli (AFBs) as smear-negative, while if there were ten or more AFBs on TBDx, they were classified as smear-positive. For those in between, slides which TBDx classified as in the one to nine AFBs range were referred to the research microscopist, who reviewed the digitised image on the computer screen and if necessary read the original slides to count the AFBs.

Reading the digitised images improved specificity, but not enough. However, when the research microscopist read the original slides which fell in the mid-range of uncertain diagnosis, the performance for both sensitivity and specificity was similar to the human microscopist reading all the slides — but the microscopist's workload was reduced by half.

Prof. Churchyard said that this sort of approach might be beneficial in a setting handling a large volume of tests. It is worth mentioning that WHO recommends that each microscopist should only read up to 20 slides a day (on a standard light microscope — possibly more on LED or fluorescence), because beyond that, their visual acuity suffers and they start making mistakes.<sup>7</sup> So such labour-saving inventions are important.

Yet, it seems a bit disappointing that in the process it also lost all of its added sensitivity — leading one to wonder whether a slightly sharper focus, or one of the sputum handling procedures described

above, might improve accuracy. According to the TB microscopy website, other prototypes are only classifying 'well-shaped' mycobacteria as AFB in order to reduce false positives.

### Alere Determine LAM lateral flow test

It isn't easy to find a test that is as fast and as sensitive as Xpert MTB/Rif. But there could be one candidate that approaches MTB/RIF in sensitivity while being simple enough to use at the point of care in even the most remote low-resourced sites — a recently developed lateral flow version of the LAM urine antigen test.

Liparabinomannan (LAM) is a glycolipid component of the TB outer cell wall. When it was shown to be detectable in urine in certain animal models interest grew in LAM as a potential diagnostic target in human TB. It was an attractive target because it was a direct product of the mycobacteria (rather than an immune response to it — antibodies have not proven to be good diagnostic targets for TB), and because urine is easier to handle, without the infection control concerns that come with handling sputum or blood. Furthermore, urine is suitable for use in inexpensive point-of-care lateral flow strip tests.

NAM/HATIP has written about LAM several times over the years — initially excited about great results from a study in Tanzania — but then two studies elsewhere in sub-Saharan Africa could not reproduce the results. These evaluated an ELISA version of the test for detection of LAM in urine — that assay is a direct sandwich immunoassay that uses polyclonal antibodies. However, while the ELISA didn't look as useful in the general population of people with TB, in adults living with advanced HIV disease the sensitivity of Determine TB-LAM for culture-positive TB was approximately 56%, with specificity of 91-95%.<sup>7</sup> This means that it is an interesting rule-in test for TB. But to be cost-effective, specimens had to be batched and run on the ELISA test, which delayed diagnosis. This is not the case with the new lateral flow version, which is being marketed as a point-of-care test that can be performed on the urine sample of any HIV positive TB suspect who needs it, with results that should be available about 30 minutes to an hour after the urine is applied to the test. It costs around US \$2.50 - \$3.50 per test.

Dr Jonny Peter of the University of Cape Town presented some preliminary data on the new test at the South African AIDS conference last June.<sup>8</sup> The hand-held test has a urine loading platform on one side, where a 60µl urine specimen is applied. Unlike the ELISA, no urine sample processing is required. A spot of urine is placed on to the base of the strip; the detection system uses polyclonal antibodies and colloidal gold; and the reporting scale is semi-quantitative. No band is a negative result and any bands present are then graded in intensity from 1+ to 5+. But as per the manufacturers recommendations, the presence of any band is read as a positive test.

To evaluate the assay, 335 hospitalised people with suspected TB/HIV coinfection, and 88 non-TB control cases (also HIV-positive) were recruited at four secondary hospitals in Cape Town. 87% of the participants were HIV-infected, with a median interquartile CD4 cell count range of 115 (54-243). 146 were culture-positive TB cases. Smear microscopy in this study was 51% sensitive, indicating just how sensitive this test can be in some circumstances.

As for the performance of Determine TB-LAM using the manufacturer's cut-point of grade 1, inter-observer agreement was moderate ( $\kappa=0.51$ ), the sensitivity was 68% (74% in CD4 cell counts of 200 or below), but the specificity was on the low side at 90%. However, using a cut-point of grade 2 for rapid 'rule-in',



inter-observer agreement was good ( $\kappa=0.89$ ), the sensitivity was 50% (59% in  $CD4 \leq 200$  cells) and the specificity was 97%.

“As for its clinical utility, we can say that point-of-care LAM is a rapid, inexpensive ‘rule-in’ of TB in hospitalized, HIV-coinfected patients for commencement of early TB treatment,” said Dr Peter.

In addition, the sensitivity of point-of-care LAM in smear-negative, culture-positive TB was 45%. When the Determine LAM test was combined with smear microscopy (which seemed unusually sensitive in this study), the combined sensitivity was 70% for culture-positive TB.

Dr Peter cautioned that LAM should not be seen as a replacement for microbiological TB diagnosis, and DST where possible — but as has already been mentioned, culture and DST are not available everywhere.

### Determine TB-LAM compared with Xpert MTB/RIF

A second study, conducted by Dr Stephen Lawn and colleagues at the Desmond Tutu HIV Centre, enrolled 602 adults referred for TB screening at a community-based ART clinic in Gugulethu township near Cape Town.<sup>9</sup> Sputum samples were obtained for fluorescence microscopy, automated liquid culture and Xpert MTB/RIF assays, and urine samples for the Clearview TB-ELISA and the Determine TB-LAM test.

Of those enrolled, 542 subjects provided one or more sputum samples and 94 of the patients had culture-positive tuberculosis (prevalence 17.4%, 95% CI 14.2–20.8). 516 patients had complete results available for all the tests (including 85 of the culture-positive patients). These were relatively advanced patients, with a median  $CD4$  cell count of 160.

It is possible that the reference scale card was modified since Dr Peter’s study, but in this study, any result above negative was treated as a positive result. The inter-reader agreement was very high between the two readers ( $\kappa=0.97$ ) — although it was the policy in this study that if there was too great a discrepancy between the readers, they would wait another 25–35 minutes and come back to read the test results again, so this may have increased agreement at the end of the day — and between the test strips and TB-ELISA ( $\kappa=0.84$ ).

Lawn et al stratified the results for Determine TB-LAM by  $CD4$  count, and noted that test was far more sensitive for TB among those with the lowest  $CD4$  cell counts — the very group of patients for whom a rapid diagnosis is most urgent — 66.7% positive (95% CI 41.0–86.7) below 50 cells, 51.7% (32.5–70.6) at  $<100$  cells, and 39.0% (26.5–52.6) at  $<200$  cells; specificity was greater than 98% for all strata.

Similar to the finding in the Peter study, combining the Determine TB-LAM with smear microscopy increased sensitivity to 72.2% (95% CI 46.5–90.3) at  $CD4$  counts less than 50 cells, 65.5% (45.7–82.1) at less than 100 cells, and 52.5% (39.1–65.7) at less than 200 cells. The gain in sensitivity achieved by combining the methods was more pronounced in patients with higher  $CD4$  cell counts.

Then the researchers compared these results to the sensitivity and specificity of the Xpert MTB/RIF when testing a single sputum sample. When all the  $CD4$  counts were included, Xpert was more sensitive than Determine TB-LAM test strips and microscopy combined. But in the patients with less than 100 and less than 50  $CD4$  cells, the sensitivity of Determine TB-LAM and smear microscopy combined did not differ significantly from that of the Xpert MTB/RIF assay in patients from those strata. Assuming that further research shows that the test works as well in other African settings, the Determine TB-LAM may provide an affordable

alternative for those settings that can’t afford the capital outlays for GeneXpert equipment, particularly when combined with microscopy.

***Determine TB-LAM may provide an affordable alternative for those settings that can’t afford the capital outlays for GeneXpert equipment, particularly when combined with microscopy.***

In addition, there was a small increase in sensitivity when results from both Xpert MTB/RIF and Determine TB-LAM were combined — and the authors noted that combining the two might be an alternative solution to the expensive strategy of performing two GeneXperts to diagnose smear-negative TB in PLHIV.

The authors noted that the negative predictive value for Determine TB-LAM was not high enough to rule out TB when there is a negative result, and wrote: “The assay should be restricted for use as a test for tuberculosis in patients with advanced immunodeficiency. These are the patients in whom tuberculosis diagnosis is so challenging such that, in the absence of suitable diagnostic assays, empirical treatment has been suggested as a strategy to reduce the high mortality of patients with very low  $CD4$  cell counts in settings with the highest disease burden. However, with the development of this simple point-of-care assay, such a strategy might prove unnecessary. Studies of the use of this assay and its effects on clinical outcomes in such patient groups are now needed.”

In addition, studies are urgently needed to reproduce these findings in other settings and to determine whether the test can be used in isolation for reliable rapid tuberculosis diagnosis in this population, or whether the results have to be confirmed with another lab test before they can be acted on clinically (to inform the decision to treat).

At the 2011 World Lung Health conference in Lille, only two oral presentations made any significant mention of the LAM lateral flow test. During the New Diagnostics Working Group Annual Meeting, Professor Ruth McNerney of the London School of Hygiene & Tropical Medicine gave a talk on the prospects of a true point-of-care TB test in the near future, and described the Determine TB LAM test as having many of the qualities of a point-of-care test, except perhaps its narrow applicability to people with advanced HIV disease alone — and the fact that “it is a ‘rule-in’ test — not a ‘rule-out’ test.”<sup>10</sup>

The other presentation was from a Cambodian study evaluating Xpert MTB/Rif and LAM in 829 children, 121 of whom had culture-proven TB. Xpert was negative in all the induced sputum and stool samples but was able to detect TB in some gastric aspirates.<sup>11</sup> LAM on the other hand was positive in 12 of the children with culture-positive TB (10%) but was positive on 28 cases that did not have TB (specificity 84%). “Urine LAM showed poor test characteristics,” the researchers concluded.

Other than that, only two posters reported on LAM, but not the lateral flow test. One poster described a systematic review on diagnostic accuracy of mycobacterial antigen detection tests for pulmonary and extrapulmonary TB.<sup>12</sup> This mostly concerned LAM, since it is the only TB antigen to have been investigated in a large number of studies, and included a meta-analysis of studies (involving a number of test formats, 60% of which used urine as the specimen tested) published up to August 2010: “Compared with HIV-uninfected patients [4 studies, sensitivity 14% (95% CI 4–38), specificity 97% (95% CI 86–100)], urinary LAM detection in

HIV-infected patients (5 studies) yielded higher sensitivity for TB [47% (95% CI 26–68)] and similar specificity [96% (95% CI 81–100).] Meanwhile the ability to diagnose extrapulmonary TB by detecting antigens in cerebrospinal fluid (CSF), serum, biopsy, pleural fluid, urine, and lymph node aspirates has been extremely variable. “No current test is sufficiently accurate for active TB diagnosis. Considering that such tests can be translated into rapid and inexpensive point-of-care tests, research to improve performance of these tests is imperative,” the authors wrote.

### LAM as a predictor for TB-IRIS

The other poster involved the Clearview LAM ELISA in people with advanced HIV disease — but asked a rather novel question: is LAM detectability in people living with HIV who are on treatment for active TB disease associated with the development of TB Immune Reconstitution Inflammatory Syndrome (TB-IRIS) when they go onto antiretroviral therapy — and could it be used to predict it?<sup>13</sup>

The study was a prospective observational cohort at Mulago Hospital, in Kampala, Uganda, which was followed for about 11 months. Cases were defined as those patients who developed TB IRIS during the first 3 months of HAART (the definition from the International Network for the Study of HIV-associated IRIS (INSHI)), while controls were patients who remained TB IRIS-free during the follow up period.

There were 26 IRIS cases and 66 controls included in the analysis. The median time to development of IRIS was 14 days. 81% of the cases had a positive LAM test compared to 48% among the controls. In the univariate analysis a positive urinary LAM test pre-HAART had an odds ratio of 4.5 [95% CI:1.5–13.3],  $P = 0.007$  while a baseline CD4 cell T-cell count < 50 cells had an odds ratio of 21 [95% CI:2.6–168.8],  $P = 0.004$  for an increased risk of TB IRIS. But only the baseline CD4 cell count was predictive of IRIS ( $P < 0.001$ ).

Overall, LAM ELISA’s sensitivity for an IRIS diagnosis was 80.8% (60.6–93.4) with a low specificity of 51.6% (95%CI: 38.7–64.2). The researchers concluded that, “a positive LAM test was associated with TB IRIS. In the absence of CD4 T-cell count, LAM detection in a point-of-care format could be useful to detect patients at high risk of TB IRIS. Further testing with lateral flow point-of-care format is warranted.”

In other words, it sounds as though a point-of-care LAM test could be a surrogate marker test for having a very low CD4 cell count in people with HIV/TB.

The low specificity for IRIS isn’t that surprising because all of these people had advanced HIV/TB. However, the idea that LAM antigen is more likely to be detected by the test because it is more likely to be shed in the most advanced patients or those with the poorest immune control is interesting because it might also be shed in greater quantities in the patients most at risk — and the Determine TB LAM might be able to distinguish between a lot of antigen versus just a little with its cut points of 1-5. By using a higher cut-off point, it might indeed be possible to identify those at higher risk and exclude those who are not. It is worth further study.

But it must be said that, for such a huge conference on tuberculosis, there was noticeably little attention given to LAM, which could be for two reasons. Either the hardcore TB experts don’t trust it, and think it won’t really pan out in future studies (which is quite possible), or they are disinterested because it is just for patients with the most advanced HIV disease — and think it probably won’t make a very important contribution to TB control. If that is the case, HIV programme people and activists may have to take the lead in making sure that Determine TB LAM is adequately

evaluated and if warranted, integrated into the clinical management algorithms for TB/HIV.

### LAM in HIV-positive populations

Indeed, there appeared to be more interest in the test on the final day of an HIV conference, CROI, where Dr Susan Dorman presented the interim results of a multicentre study of the lateral flow test for diagnosis of TB in adults with HIV infection.<sup>14</sup>

The main study objectives were to estimate the sensitivity and specificity of the Determine LAM lateral flow assay in adult HIV-positive TB suspects; and to compare the performance characteristics of the lateral flow point-of-care test versus that of the existing in-laboratory, more complicated, ELISA version. They are also trying to determine operating characteristics of the LAM lateral flow assay (test failures, between-reader variability), and whether there are any clinical characteristics associated with a positive LAM test.

The study was mainly cross-sectional with limited longitudinal follow-up in the two settings, each of which included an in-patient as well as an out-patient setting: Mulago Hospital (the same team as the TB-IRIS study) and nearby outpatient clinics in Kampala, Uganda, and at GF Jooste Hospital in Cape Town and two outpatient clinics in Khayelitsha township.

The eventual target sample size is 1000 participants with HIV who are suspected to have active TB (at last one symptom from the WHO TB symptom screen). After enrolment participants underwent a brief interview; two sputa were collected for smear, MGIT, mycobacterial culture, and LJ solid culture. All participants had blood drawn for mycobacterial blood culture and CD4 cell counts, had a chest x-ray and urine was obtained for the point-of-care lateral flow testing as well as batched for near-term ELISA testing. During the study a subset of participants, namely those who had any positive LAM test at enrolment, but no positive culture for TB, underwent an in-person follow-up two months later at which point the baseline investigations were repeated. Everyone else had a record review at two months.

For the purpose of the interim analysis, Dr Dorman presented only the results at baseline — so anybody with a positive mycobacterial culture for TB was defined as a TB case — all others were not TB cases.

561 participants were enrolled as of the time of this analysis and 409 were eligible for analysis, strictly based on their date of enrolment. About 60% were women; largely young adults in their 30s and 40s. Approximately one-third were on ART at enrolment. There was a significant difference between Uganda and South Africa — Ugandan patients had more advanced HIV disease, with a median CD4 cell count of 78 and approximately three-quarters were enrolled in hospital, whereas in South Africa, the median CD4 cell count was 234, and fewer than half were enrolled in hospital.

Approximately one-third had a TB culture of sputum and/or blood that was positive. Strikingly in Uganda 16% of participants had a blood culture that was positive for TB. The sensitivity of sputum smear microscopy among TB cases was about 43%. Dr Dorman displayed the distribution of LAM lateral flow results by band intensity (cut point): 68% of tests had no band; 12% of tests had a band of intensity 1+; and 21% of tests had a band intensity greater than 1+.

But just like Dr Peter’s study, this study found problems when any band was considered positive. The sensitivity was about 62%, but the specificity was about 78%. However, if one were to use more stringent criteria for positivity and increase the cut point to 2+ , the sensitivity decreased to about 45%, and specificity increased to just

over 90%. If one were to increase the cut point further, the sensitivity falls off fairly abruptly without much gain in specificity.

### Results of conventional mycobacteriology among analysis-eligible participants

	<b>OVERALL N = 409 N (%)</b>	<b>UGANDA N = 211 N (%)</b>	<b>CAPE TOWN N = 198 N (%)</b>
MTB in sputum and/or blood ("TB case")	<b>125 (31%)</b>	80 (38%)	45 (23%)
MTB in sputum	<b>121 (30%)</b>	78 (37%)	43 (22%)
MTB in blood	<b>43 (11%)</b>	33 (16%)	10 (5%)
Sputum smear+ among TB cases	<b>54/125 (43%)</b>	35/80 (44%)	19/45 (42%)

Dr Dorman said that in subsequent analyses, if a positivity cut point of 2+ was used and a 1+ band was considered negative, the performance of the lateral flow point-of-care assay was strikingly similar to that of the existing ELISA laboratory assay.

Just like Dr Lawn's study, they were able to show that lateral flow assay performance varied by CD4 count.

"The sensitivity of the assay is strikingly higher in those with lower CD4 counts than in those with higher CD4 counts. In terms of specificity – the specificity fell off a little bit as the CD4 count was reduced. I think there are some reasons for this, that I hope we will be able to discern once a clinical definition of TB is brought into the picture in the final analysis," she said.

Lateral flow sensitivity in TB patients was similarly high if their TB was cultured from blood cultures or their CD4 cells were low. The sensitivity was 79% in those with mycobacteraemia, ( $p < 0.001$ ) and 24% in patients without mycobacteraemia. ( $p < 0.001$ ), while the sensitivity was 66.7% in those with  $CD4 < 100$  ( $p < 0.001$ ), and 20.3% among those with  $CD4 > 100$  ( $p < 0.001$ ).

The last patient completed follow-up in February. All cultures were expected to be mature sometime in April, and analysis should be completed by June 2012.

Dr Dorman believes that the study has shown that using a cut-off of cut-point of 2+ may optimise sensitivity and specificity, which seems to be supported by the fact that when using that cut point, it has accuracy similar to the ELISA format.

She also suspects the loss of specificity at the lowest CD4 cell counts is due to a greater frequency of culture-negative TB in very immune-deficient individuals.

"A planned incorporation of a clinical TB definition may result in higher observed LAM test specificities if cultures were 'falsely negative', as expected in some participants," she said. This may be added by documenting some clinical outcomes associated with LAM detection at low CD4 cell strata. She noted that the mortality was strikingly high, especially in Uganda, and that they plan to analyse the association between LAM results and mortality, before offering some provisional conclusions.

"This POC test performed best in the subset of patients that are most challenging to diagnose, and have the highest mortality," she said. Dr Dorman added that she thinks proof of principle has been established for LAM detection, and that a substantial research and development effort should go into refining how LAM is detected – in

other words, could some of the problems with sensitivity and specificity be resolved by developing a better test for it?

Finally, she said she believes that clinical trials are warranted in looking at how use of the lateral flow test in clinical management will affect patient outcomes, "especially in ill hospitalised patients with low CD4 counts and in pre-ART settings – as Steve Lawn and his group have nicely explored," she said.

## How can LAM be incorporated into diagnostic practice?

### Is LAM a marker for poor prognosis?

Dr Lawn would probably agree. In another recently-published paper, Lawn et al make a very strong case that LAM identifies those patients with the poorest prognosis – patients who need to go on TB treatment immediately and who may not have the time to wait for other lab tests.<sup>15</sup> They point out nothing else can fill this need right now in low resourced settings with a high burden of HIV-related TB, not even Xpert MTB/Rif.

"The cost and infrastructure requirements [for Xpert] are currently prohibitive in many poor countries and simple, low-cost alternatives are needed," Lawn et al wrote. "In South Africa, Xpert MTB/RIF is being implemented nationally, but only within centralized laboratories rather than at the district and sub-district levels and this will inevitably result in ongoing delays in diagnosis. For example, although use of Xpert MTB/RIF for screening for TB in patients pre-ART in a South African township clinic increased case finding by 45% compared to sputum smear microscopy, delays associated with results reaching the clinic and with subsequent patient recall were substantial. As a result, some patients died before starting TB treatment."

A point-of-care test might have prevented some or all of these deaths because diagnosing a patient's tuberculosis during the clinic visit makes it possible to start treatment immediately – they don't get lost to follow-up.

Moreover, Lawn et al theorised that, while the LAM detection assays may not be the most sensitive tests, they are sensitive in an important patient group. Since LAM becomes more sensitive in people with lower CD4 cell counts, it may be picking up those with worst types of TB disease – potentially with the highest mycobacterial loads, more disseminated disease, and a poorer prognosis.

To test this hypothesis, they analysed data from consecutive PLHIV who had come to the clinic in Gugulethu township to join the ART programme, but were then recruited to participate in a TB diagnostics study. These were all screened for TB using a symptom screen (including WHO's 4-symptom screen), sputum smear microscopy, culture, Xpert and chest-x-rays, and then their cases were managed according to these results. Urine specimens were also collected at baseline and stored. Once TB had been diagnosed (culture confirmed), urine specimens from the individuals with culture confirmed TB were assessed with the Determine TB-LAM – if the patient had less than 200 CD4 cells. Then the baseline characteristics and clinical outcomes of those who tested LAM-positive were compared to those who were LAM-negative for M.TB.

Out of several hundred patients who had enrolled, 325 had CD4 cell counts below 200; out of these, 59 people had culture-positive TB, 18.2% (95%CI, 14.1–22.8).

The Determine TB results showed that 23 (39.0%) culture-confirmed TB cases had urine that tested LAM-positive, and 36 (61.0%) had urine that tested LAM-negative.



And just as the investigators had suspected, compared to LAM-negative cases, LAM-positive cases were in much worse health generally and had more advanced immunodeficiency. They were three times more likely to have a cough lasting two or more weeks, and lower body mass indices. Their blood-work was off as well, with substantially lower haemoglobin concentrations and higher neutrophil counts. LAM-positive individuals had a median CD4 cell count almost three times lower than the LAM-negative people and had substantially higher viral loads. In a multivariate analysis, there were strong associations between testing LAM-positive and low haemoglobin, low body mass index, and low blood CD4 cell count, and there was a somewhat weaker association with higher blood neutrophil counts.

Looking at the other lab tests, LAM was more sensitive than smear microscopy which picked up 30% of the culture-positive TB cases in this population. Xpert was almost twice as sensitive as LAM, picking up 62.7% of the culture-positive TB cases with one cartridge and 76.3% with two.

Notably, the turnaround time in getting lab results back for either smear microscopy or Xpert MTB/Rif was a median of 4 days. The median time to getting culture results back (liquid culture) was 18 days for smear-positive TB, and 21 days for smear-negative TB.

Some of the other lab-related findings run counter to the image of Determine TB-LAM diagnosing the 'hard to diagnose cases' because LAM-positive cases were actually more likely to be smear-positive cases of TB with a higher mycobacterial burden that led to faster culture growth. About 21 (91.3%) of the LAM-positive TB cases were also positive on the Xpert MTB/Rif assay.

As for patient outcomes, a smaller proportion of LAM positive people remained alive and retained in the programme. There were five deaths that occurred within the first 30 days of the programme, these were found to all be LAM positive.

In contrast, the LAM negative cases were discovered to have a relatively good prognosis, even though their TB diagnosis took up to three times longer (sometimes because they were smear negative and had to wait for culture results – and yet they appeared to have had milder disease).

So there are clearly a subset of cases among people living with HIV and TB with a worse prognosis who might benefit from a point of care test, even one of less than optimal sensitivity.

"It was striking that despite the availability of high sensitivity rapid molecular testing for TB in a centralised laboratory service, delays or failure to start TB treatment were common," wrote Lawn et al, and the point-of-care LAM test might indeed quickly identify the patient group most in need of immediate treatment.

"Had this assay been used in the clinic during initial screening visit, approximately 40% of the sickest patients might have been able to start TB treatment immediately. The chances of survival of those who died without TB treatment may thereby have been improved," Lawn et al wrote.

### Combination algorithms

Lawn et al concluded with a consideration of how the lateral flow LAM assay would have to be combined with other TB diagnostics since it is not sensitive enough to be a stand-alone test. Combining it with smear microscopy might be attractive because the two tests together have a sensitivity close to one Xpert MTB/Rif test (at least in people living with HIV and less than 200 CD4 cells). But on top of that, LAM would identify the patients with poor prognosis, while microscopy would identify smear-positive cases, who are the most infectious.

They suggested that LAM might help in the interpretation of chest x-rays for TB diagnosis.

Finally, if there are adequate resources to roll out Xpert at laboratories, use of LAM at the point of care could diagnose the sickest patients most in need of treatment, and laboratory Xpert would identify the majority of the rest. As for cases that LAM and Xpert both miss, the data from Lawn et al suggest, that "such patients have good prognosis and low TB transmission risk, allowing time for repeat screening."

Of course, it would be good to have some further data to support what Lawn et al found in their settings, on whether LAM positivity and negatively are so consistently predictive of good or bad outcomes, and whether these combinations of algorithms would indeed work as well as Lawn et al suppose.

Of note, during her question and answer session, Dr Dorman had a somewhat different take on which populations LAM was better at detecting.

"Smear performs least well in individuals with the most advanced immunosuppression. When combined in an algorithm with LAM and there is either a positive smear or a positive LAM test, you get an additive increase in sensitivity beyond either test. So for example smear had a sensitivity of about 43%; LAM of about 40% or so; when combined the sensitivity was about 65% or so," she said.

This was in response to a question from the audience as to whether the POC LAM combined with microscopy could be an alternative to Xpert, for countries that can't afford to scale up Xpert at the level where it can play much of a role in clinical management. Furthermore, it may not just be about expense of the Xpert as operational utility. Given the challenges of keeping GeneXpert equipment cool and consistently powered, smear microscopy and a lateral flow test may simply be more robust and work better in some remote low resourced settings.

"I'm wondering if we'll find that the LAM and the Cepheid aren't complimentary, in the sense that those patients with extra-pulmonary disease – or disseminated disease – are much more likely to be LAM-positive. And in many cases it seems they may in fact not have much pulmonary disease, or certainly not many mycobacteria in their sputum. So, [it may be that] the two technologies that we are discussing here are in fact complimentary rather than competitive – and that we need to find a way to look for one or the other in some order that precludes the duplicate use of both," said Dr David Katzenstein of Stanford University during the discussion at CROI.

Dr Dorman agreed. "Xpert was not a component of our study – but we did store the sputum. So we're going back and doing Xperts on all of these sputum to really try to hone in on the question of: if you have two tests that are either point-of-care, or quasi point-of-care, what proportion of TB patients can you diagnose, using these two tests?" she said.

However, even if the tests do overlap somewhat, at least in PLHIV with CD4 cell counts below 200, performing a LAM as a point-of-care test could moderately reduce the number of Xperts that need to be carried out, and it wouldn't add delay to diagnosis and treatment in the same way that performing smear microscopy before Xpert could.

The only drawback is that one wouldn't have the benefit of the rifampicin resistance assay in the people diagnosed by the point-of-care test.



## Finding more resistance than expected — could some of it be recently acquired?

As noted in the [second edition in this series](#), resistance, by most accounts, is being detected much more frequently than in the past. Findings from the pilot phase of the roll-out involving 244,024 tests, indicated that 17.3% were positive for M.TB and 6.8% of these were rifampicin resistant, indicating a likelihood of MDR-TB.

Those numbers are exact, and one advantage of the Xpert MTB/Rif system that is being implemented at the peripheral laboratories in South Africa is that all the equipment is linked to the laboratory information system. According to Professor Lesley Scott, speaking at CROI, that may make the test a very useful tool for surveillance purposes.

The Xpert MTB/Rif test contains species-specific primers that allow the test to amplify targeted segments of M.TB's DNA in the *rpoB*-gene region, which is usually highly conserved (non-variable) in the organism — unless there is rifampicin resistance.<sup>16</sup>

- If the test results show that all five targeted segments (amplicons) are present, then it has detected drug-susceptible TB.
- If fewer signals show up, but at least two, it means the M.TB is present, but that some of that gene region has mutated to confer resistance to rifampicin.
- Only one signal is either an invalid or negative result, because even the most drug-resistant TB can't mutate substantially in this region.
- A sixth probe, designed to target an unrelated bacilli, is included in the Xpert MTB/Rif test to serve as a control — in other words, in order to distinguish between a negative versus an invalid test result.
- All of this information is reported in real time to the laboratory information system.

"Using this data, we explored whether the frequency of the five probes across the 81 base pair *rpoB*-gene region can be used to indicate mutation profile; and secondly, whether the cycle threshold can be used to indicate population disease burden," said Professor Scott. A higher cycle threshold is thought to indicate a higher bacterial burden and its presence would suggest a need to intensify case-finding efforts, since these patients are likely to be particularly infectious, and a need to shorten the interval between presentation and treatment — the diagnostic delay. The supply of real-time data to the NHLS will allow any interventions in response to these signals to be monitored.

Prof. Scott and colleagues used data from the pilot phase of the programme from tests conducted in the Western Cape, KwaZulu Natal and Gauteng between March and April 2011 (more than 20,000 tests). They found significant differences in cycle threshold between some regions, namely Gauteng and the KZN Prince Mshiyeni, KwaMashu site. However, the tests did not show any significant variation in probe frequency (resistance detected) across any of the geographic sites, but the test cannot provide specific information about which mutations are being picked up, and some mutations may not be as clinically relevant — in other words, they may not confer as much rifampicin resistance as another mutation in the same area.

This is one possible explanation for why not all of the cases of resistance being detected by the Xpert MTB/Rif prove to be resistant when further drug susceptibility testing (DST) is performed. How often this occurs is a subject of some debate. According to a

prospective study by Lawn et al, including sputum specimens from 468 patients in Gugulethu, DST confirmed that Xpert correctly identified all four cases of resistance in the cohort (equaling 100% sensitivity for resistance) — but it also incorrectly identified resistance in three cases where there was no resistance (a specificity for resistance of 94.1%). That means that a positive result for Rif resistance in this study only had a positive predictive value of 57% — which would not be a very good guide for clinical management.<sup>17</sup>

However, in Lille, Professor Scott told HATIP that the programmatic data show that only 20% of the positive Rif results are false. This is why the South African programme has chosen to go ahead and initiate second-line regimens in people testing positive for Rif resistance, and to modify treatment only when DST after the fact shows that the patient actually has drug-susceptible TB.

Of note, at the session on diagnostics at the South African AIDS conference last June, one TB doctor in the audience suggested that the programme should give both first- and second-line treatment together to these patients and then remove whichever regimen is unnecessary after DST confirmation — because first-line treatment is only one more fixed-dose combination pill per day, and is a far better treatment for first-line TB, and would reduce the unnecessary exposure of drug-susceptible M.TB to a possibly sub-optimal second-line anti-TB regimen. A point, which, from our perspective at least, seemed to make a lot of sense — especially because there could be subtle regional variations in the M.TB that people are infected with, leading to more false positives in some areas than others.

Indeed, regional variations in specificity were detected in the pivotal demonstration studies/study by Boehme et al, with sub-optimal specificities seen in the settings with a lower prevalence of MDR-TB.<sup>18, 19</sup> This included India (MDR prevalence 6.7%, RIF test PPV of 73.43%), South Africa (MDR-TB prevalence 5.3%, RIF test PPV of 84.84% and Uganda (MDR prevalence 4.4%, RIF test PPV of 71.89%), compared to settings such as Azerbaijan, Peru and the Philippines, where MDR-TB is much more prevalent and the PPVs were around 95%. This led to the recommendation to confirm resistance with further DST in low MDR-TB prevalence settings.

A regional variation like this, possibly due to the increased frequency of certain drug-resistant M.TB strains (of which there are many) is one of the variations that an Xpert-based surveillance system, like that proposed by Prof. Scott, could possibly pick up. It would be much more difficult to reliably compile that sort of information using any diagnostic algorithm, or combination of tests, which does not include automatic reporting of Xpert MTB/Rif results back to NHLS.

Even so, Professor Scott noted some further limitations.

"Any changes in the assay definition files — the versions of the cartridges — will affect the long-term analysis. And you need to take that into account," she said. Indeed, Cepheid has made and is making ongoing refinements to the cartridges fairly regularly.<sup>20</sup> Some of these refinements are specifically to enhance Rif resistance specificity by modifying the test probes. For instance, at the New Diagnostics Working Group meeting in Lille, Dr Boehme described how Cepheid is modifying probe B to make it less sensitive to temperature fluctuations, because it wasn't binding to and amplifying wild type M.TB when the test was operating above optimum temperature — and thus generating more false positives for Rif resistance. Other changes in fluidics were made to eliminate

the 5011 errors, (signal loss detection) that was happening in about 6.9% of the runs with the G3 stage of the Xpert MTB/Rif cartridge.

Each of these changes would have to be taken into consideration when using programmatic test data for surveillance, according to Professor Scott. Finally, she concluded that “expanding regional scope and monitoring timeframe is then needed to determine this as a surveillance value. But the data are there, and lots of it.”

### Could some resistance represent cases of primary MDR-TB infection?

But the false positives for rifampicin resistance are not so common as to explain why, at least initially, rifampicin resistance was at least three times more common in the pilot phase of the roll-out than prior to the rollout. However, one must remember that there is probably some selection bias (contact tracing of MDR-TB has been more aggressive than for drug-susceptible TB), and also much of that resistance is being detected much earlier in the course of the illness — since Xpert MTB/Rif is capable of detecting TB smear-negative disease, and TB that is only slightly or not yet symptomatic. In other words, cases that would have been previously detected over a span of several months or a year are instead being picked up all at once because of the combination of active case finding (intensified case finding in PLHIV, and home contact tracing) and this fast and more sensitive diagnostic.

In some cases, Xpert MTB/Rif could be picking up recent infections and cases of primary infection that are in the process of becoming latent infections. This would be the case for any of the sensitive nucleic acid amplification tests (NAAT), which only look for genetic evidence of M.TB and cannot distinguish between a live and dead microbe.

Another study presented at CROI, using the Hain line probe assay Genotype MTBDR*plus*, for expanded testing on all smear and culture-positive cases from 25 public health clinics in the Northern Cape Province, seems to confirm this.<sup>21</sup> When compared to the standard method of DST, targeting conventional DST to cases considered to be at high risk of MDR-TB (mostly retreatment cases), there was little difference in the prevalence of resistance among retreatment cases: 6.2% (95% CI 3.5-8.8%) on MGIT DST, compared to 6.6% (3.8 – 9.4%) on the line probe assay.

Among the new cases of TB there was a dramatic difference however. MGIT DST had only picked up a prevalence of 0.7% (0.01 – 1.2%) of MDR-TB in new TB cases — the line probe picked up MDR-TB in 3.7% (2.4 – 5.0%).

Since most cases of TB are new cases, “We found that after implementation the bulk of MDR was among new cases, compared to retreatment cases,” said Dr Colleen Hanrahan, who presented the poster on behalf of Dr Dorman and other colleagues at the NHLS.

Dr Hanrahan said there was a possibility that a proportion of the new cases represented primary transmission—new or recent infections rather than reactivation disease. Among PLHIV, the risks are great that primary infection will simply launch directly into active disease, with only a brief period of latent infection, so treating those cases right away is probably a safe bet. Among HIV-negative cases, it is not so clear — cases may go into latency and never become active — but the approach thus far has been to assume the cases are active MDR-TB, and to treat anyway. Given the cost of second-line treatment to the public health system, and the aggravation and side-effects that second-line treatment brings to the patient, it may make sense to determine what proportion of these cases actually are active disease, and whether it is possible

to distinguish between a case bound for latency, and a case of active disease.

Since NAAT testing led to a substantial increase in the burden of MDR-TB being detected, there are consequences for the health system.

“In terms of time to treatment initiation, there was a modest decrease in time to initiating MDR treatment after implementation – it still took about two months to start patients on treatment [after receiving the test results],” she said. In fact, there was a trend towards an increase in time from when the results were back at the clinic, until MDR treatment was initiated after implementation.

Because MDR-TB was suddenly being detected sooner and in many more patients than previously, there was a bottleneck getting people onto treatment — such as in Kwazulu-Natal, where second-line TB treatment requires a period of hospitalisation. Nevertheless, the time from presentation to initiation of appropriate second-line treatment was reduced.

However Hanrahan presented data suggesting that initially at least, there was a clinical benefit from the approach, with higher rates of culture conversion by the eighth month of treatment from 27% (95% CI 10-44%) before the study, to 52% (95% CI 38-66%) after the trial.

Also, “we found that expanded testing using the line-probe assay was cost-effective,” said Dr Colleen Hanrahan.

Key Cost-Effectiveness Findings			
	MGIT	MTBDR direct	MTBDR on culture
Cost/test	US \$33.40	US \$39.08	US \$43.86
ICER FOR “Test all patients using MTBDR direct” vs “Test high risk using MGIT”:		US \$262/DALY (\$0/DALY; \$1.322/DALY)	

As MDR-TB prevalence increases, cost-effectiveness increases, even if sample numbers or batches decrease. As test specificity decreases, cost-effectiveness decreases.

As one member of the audience remarked, the approach of performing line probes on *all* the cases would be unnecessary in areas where Xpert MTB/Rif has already been rolled out, since if rifampicin resistance is a proxy for MDR-TB, line probes would only be needed to confirm that the Rif resistance was indeed MDR-TB, or to assess resistance in culture-positive, Xpert-negative cases of TB. Nevertheless, the study suggests that, in settings where there is a high enough prevalence of MDR-TB, but where Xpert MTB/Rif cannot be implemented, it might be prudent to include MTBDR *plus* as part of the alternative diagnostic algorithm.

## Conclusion

A final take-home message from the experience of implementing Xpert MTB/Rif in South Africa may serve as a cautionary note to programme managers and implementers considering introducing Xpert MTB/Rif in their setting: the South African National Health Laboratory Services is one of the most sophisticated anywhere, and has some of the most brilliant and experienced laboratory specialists in the world working for it — and thus far, they have only installed Xpert MTB/Rif at selected sites in the country. The performance may vary and new challenges could be encountered at some of the more remote laboratory facilities in the country.

The efforts by the Union and WHO to monitor implementation carefully across a range of settings will be crucial for programmes

considering how best to support active case finding locally. For instance, does it make sense for health systems to purchase GeneXpert systems gradually in a phased implementation, or for the HIV programmes in those countries to purchase them specifically for better case detection in PLHIV?

Their decisions could hinge on the potential point-of-care TB tests coming up in the pipeline, some of which Professor McNerney described at the 2011 World Lung Health conference in Lille. The ideal point-of-care test should not require a cold chain for storage of reagents, nor require a constant source of electricity, specialist training, supervision or maintenance. Some of these technologies appear to be a long way off, while others such as LAMP (Loop-mediated isothermal amplification) are in relatively advanced stages of testing. Isothermal amplification methods are faster, and are believed to be more tolerant of dirty samples than PCR. However, most only work at elevated temperatures (~65°C) and require a cold chain for reagents — and not every technology can be multiplexed or have an internal control.

There may be some low-cost solutions to some of these problems. For instance PATH has been trying to develop an instrument-free heat source that is being tested with LAMP.

Several other interesting technologies are in development and may turn out to be breakthroughs. However, the best hope may rest with tests that aren't all that different from GeneXpert, for instance Truelab, a real time PCR platform from The Tulip Group being developed by Bigtec Labs.

The improvements are primarily in cost and portability. TrueLab's instrument is a battery-powered, handheld device used with a 'TruePrep' sample prep platform, and the test is performed using 'TrueNat' chips (DNA chips). These types of DNA testing platforms have been developed for other purposes such as testing water quality and environmental contamination.

TrueLab has a TB test currently in development. It takes less than an hour to get a result including sample prep, and according to Professor McNerney, the company was expected to move from prototype to manufacture towards the end of last year.

The instruments costs around \$6000 and the TrueNAT chips cost approximately \$10. However, DNA chip technology is scaleable in much the same way as computer chips, and with demand, that price could drop considerably.

So if activist demands do not get Cepheid to reduce their costs, perhaps a little competition will.

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