The progression of TB diagnosis in the HIV era: from microscopes to molecules and back to the bedside

South Africa suffers from dual epidemics of TB and HIV.

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South Africa (SA) has half a million new cases of TB annually with two out of three of these cases HIV-infected, making it the epicentre of the dual epidemics of TB and HIV.[1] TB remains the leading cause of death in persons with HIV infection,[2] while multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB continue to increase nationally. Traditional TB diagnostic tools such as smear microscopy and chest X-ray (CXR) perform sub-optimally in HIV co-infected patients,[2] meaning techniques such as culture, which have a time-to-result of up to 6 weeks, have become increasingly relied upon, thereby creating a diagnostic delay and increased morbidity and transmission. With the high HIV prevalence in SA, there is thus a major need for rapid and effective TB diagnostic tests, including the diagnosis of drug resistance. The search for rapid, effective and cheap point-of-care TB diagnostic tools remains ongoing, but a number of exciting new molecular tools offer hope. This review article will outline some of these developments relevant to the diagnosis of active TB in high HIV prevalence resource-limited settings. It will highlight the transition to a rapid molecular test (GeneXpert (GX) MTB/RIF) as the frontline TB diagnostic, the continued need for mycobacterial culture and other available tools both for diagnosis and drug susceptibility testing (DST), and the exciting progress towards developing novel point-of-care TB diagnostics (illustrated in Fig. 1). Finally, we emphasise the need for continued clinical skill to enhance and individualise TB management.

From microscopy to molecules for the frontline diagnosis of TB

TB, with its wide range of clinical presentations, its ability to cause both latent and active disease, and slow growth, is diagnostically challenging. Traditional diagnostic modalities such as smear microscopy, CXR and mycobacterial culture performed adequately prior to the onset of the HIV epidemic.[3] Ziehl-Neelsen staining for acid-fast bacilli using light microscopy offered a sensitivity of up to 80% in HIV-uninfected individuals, excellent specificity and a cost of <US$3/test.[3] However, HIV co-infection has increased the incidence of extrapulmonary, disseminated and smear-negative pulmonary disease.[2] Smears are negative more often because cavities are less common – the organism load in tissues is higher than in individuals who are not infected with HIV. The sensitivity of routine smear microscopy has dropped to as low as 20%,[3] CXRs may be normal in the presence of active TB[4] and mycobacterial culture results may only be available after patients have died or have been lost to follow-up. Nevertheless, in the majority of resource-limited settings smear microscopy continues to be the frontline TB diagnostic tool. In SA, despite improvements in smear processing and staining techniques, only 41% of the total notified TB cases for 2009 were smear-positive.

TB remains the leading cause of death in persons with HIV infection.

Nucleic acid amplification tests (NAATs), usually using the polymerase chain reaction (PCR), have become important molecular diagnostic tools for many infections. Until recently both commercial and 'in-house' NAATs for TB detection have been found to offer high specificity (85 - 98%) and high sensitivity for smear-positive TB (~96%), but poorer sensitivity and specificity for smear-negative TB.[5] The heterogeneity in performance across studies, together with the cost and need for sophisticated laboratory infrastructure and specialised expertise, has limited their widespread use.

The development of an automated, fully integrated DNA extraction and amplification system, the GeneXpert MTB/RIF assay (Cepheid, CA, USA) (illustrated in Fig. 1) has addressed a number of these limitations. GeneXpert can be performed in decentralised locations outside reference laboratories by staff with minimal laboratory training (1 - 2 days). GeneXpert is able to detect the presence or absence of both *Mycobacterium tuberculosis* (Mtb) complex DNA and rifampicin drug resistance (strongly correlated with MDR-TB) in less than 2 hours. A recent demonstration study in 6 648 participants found the sensitivity of a single GeneXpert assay for TB diagnosis to be 97% in smear-positive patients and 76.9% in smear-negative TB cases, with an overall specificity of 99%. The sensitivity and specificity of the assay for genotypic rifampicin resistance was 94.4% and 98.3%, respectively.[6]

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Additionally, GeneXpert decreased the mean time-to-treatment initiation of smear-negative culture-positive TB patients from 56 to 5 days – similar to that of smear-positive patients.[6] The WHO subsequently endorsed the use of GeneXpert for frontline TB diagnosis in HIV-infected and MDR TB suspects in December 2010.[7] This March, on World TB day, the South African Minister of Health, Dr Aaron Motsoaledi, announced that

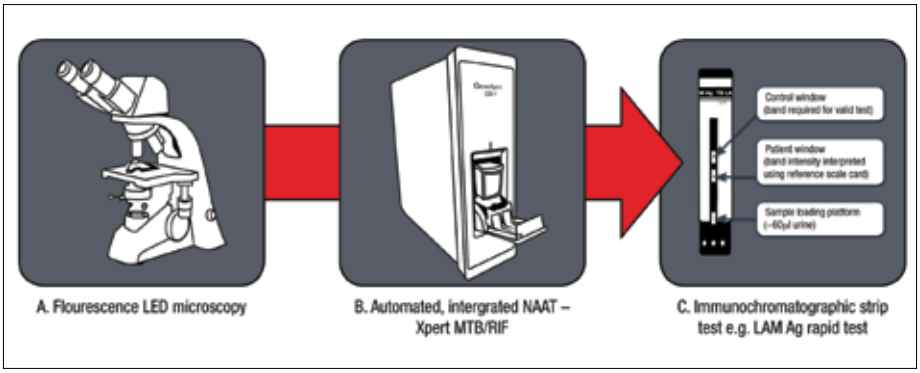

Fig. 1. The progress of frontline TB diagnosis from smear microscopy to molecular methods and onwards toward point-of-care test formats.

the Department of Health intended to replace smear microscopy with a single GeneXpert for frontline TB diagnosis and rifampicin DST for all TB suspects, describing it as a 'bazooka' in the war against TB (*Cape Times*, March 2011).

Frontline TB diagnosis using the GeneXpert assay

The National TB Programme has developed a diagnostic algorithm outlining the routine use of GeneXpert in conjunction with existing TB diagnostic tools currently available to primary care clinics. A preliminary outline of this algorithm is shown in Fig. 2. Key points of the algorithm include the collection of a single initial spot sputum specimen for GeneXpert as the first TB diagnostic test for all persons with suspected TB or MDR TB; the continued need for sputum smear microscopy for TB reporting and treatment monitoring in GX-positive cases; and the treatment of GeneXpert-positive rifampicin-resistant patients with MDR TB regimens while awaiting additional adjunctive mycobacterial culture and genotypic and/or phenotypic DST.

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The need for further investigations in GeneXpert-negative, HIV-infected patients with ongoing symptoms, together with the need for confirmatory DST in patients with rifampicin resistance on GeneXpert highlights two important limitations of GeneXpert that clinicians should be aware of. Firstly, the ability of a single GeneXpert test to 'rule-out' TB in HIV-infected patients is reduced (approximately 1 in 10 HIV-infected GeneXpert-negative individuals are infected with TB) compared with HIV-uninfected patients.[8] Secondly, in low prevalence MDR-TB areas a rifampicin-resistant result on the GeneXpert test may represent a false positive test in up to 1 in 5 cases. In the Cape Town patient cohort of the multicentre GeneXpert demonstration study the positive predictive value of a single GeneXpert for genotypic rifampicin resistance was only 77.1%.[6] Clinicians should also be aware of the paucity of GeneXpert performance data for children, hospitalised TB HIV co-infected patients and extrapulmonary TB. Preliminary studies suggest that GeneXpert performance in children will be similar to that of Mycobacterial-Growth-In-Tube (MGIT) (Becton Dickinson Diagnostics, USA) culture[9] while performance in extrapulmonary samples appears to be superior to smear microscopy in the majority of non-sputum samples evaluated to date, but highly variable depending on the specific sample used (i.e. low in cerebral spinal fluid but high in tissue samples such as lymph node biopsies).[10]

The role of other TB diagnostics

Undoubtedly GeneXpert offers a significant improvement over smear microscopy for rapid TB diagnosis but the test is expensive, true programmatic performance is unknown and GeneXpert will not be able to be performed at the bedside in its current form. Fig. 3 illustrates the comparative performance of a number of both established and novel diagnostic tools for active TB as well as DST and contextualises them within the framework of direct cost, time-to-diagnosis and setting. These tests offer both alternatives for diagnosing active TB in different clinical sub-groups and/or, particularly in the case of mycobacterial culture and DST modalities, adjuncts to frontline diagnostics such as smear microscopy or GeneXpert.

Mycobacterial culture remains the reference standard for both the diagnosis of active TB and phenotypic DST. Automated liquid culture systems have now largely replaced traditional solid culture methods and the MGIT is available in the majority of South African laboratories. MGIT culture offers a 10% sensitivity improvement over traditional solid culture methods and reduced mean time-to-diagnosis, but is expensive and without adequate laboratory infrastructure, and quality control bacterial contamination rates can reach 15%. First- and second-line phenotypic DST is performed by reculturing positive MGIT cultures, taking an additional 14 - 28 days after initial culture positivity to produce DST results. Other liquid culture methods, such as the microscopic observed drugs susceptibility (MODS), offer an inexpensive alternative to MGIT. Comparative performance of both the MGIT and MODS liquid culture methods is shown in Fig. 3. MODS allows for both TB diagnosis and phenotypic DST at the same time in less than 10 days and studies show good performance in resource-limited settings, both for TB diagnosis and MDR detection.[11] MODS is, however, very labour-intensive and consequently seems more suitable for resource-limited countries with poor laboratory infrastructures rather than countries such as South Africa with strong reference laboratories and well-established automated systems.

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The growing threat of MDR- and XDR-TB has necessitated the development of tools for rapid DST. Line probe assays (LPAs), a type of NAAT, were endorsed by the WHO for rapid genotypic rifampicin and isoniazid DST in 2009. Barnard *et al.* demonstrated a sensitivity of >95% for MDR resistance when compared with phenotypic DST.[12] The sensitivity of LPAs for rifampicin resistance is very high, but sensitivity for isoniazid resistance is lower due to the presence of

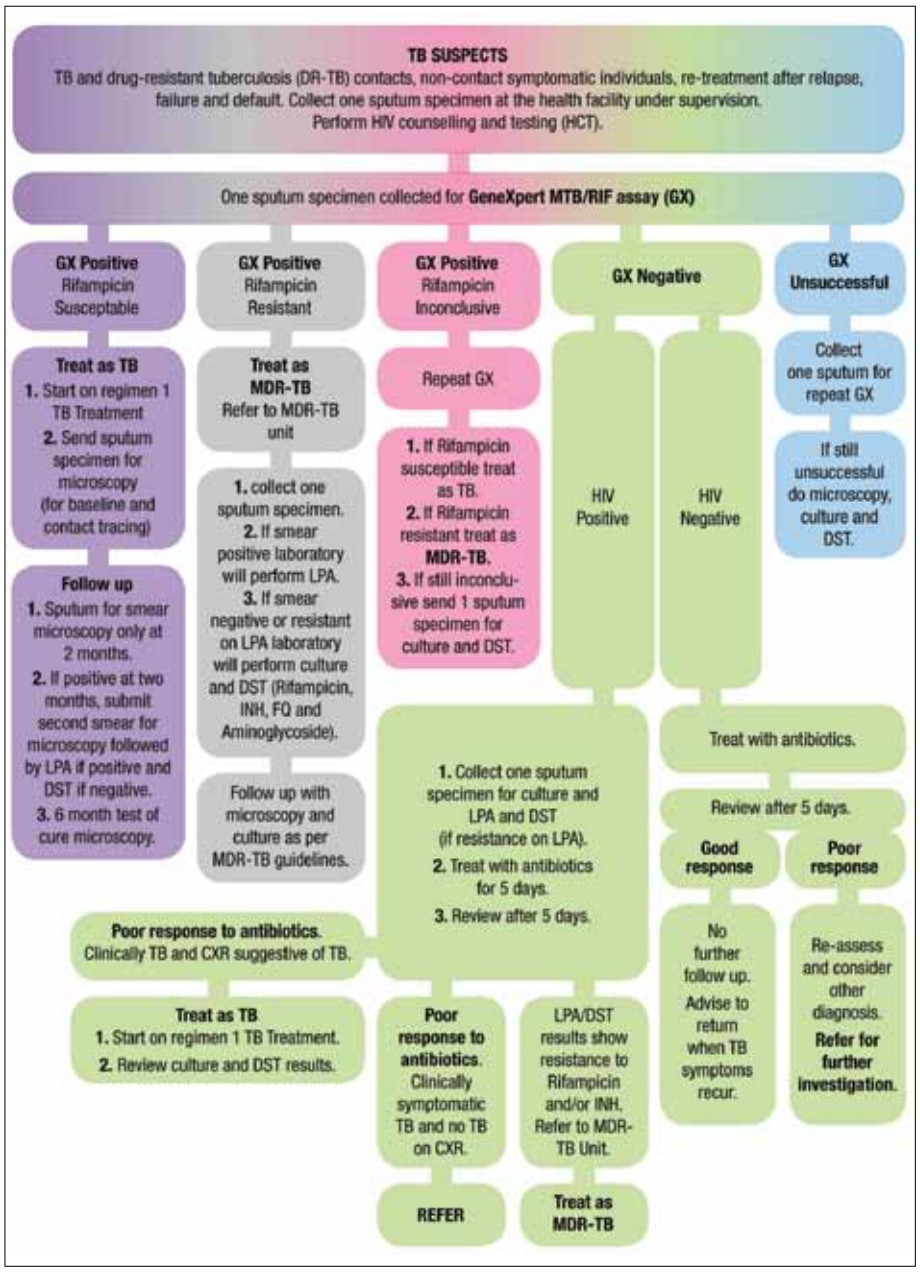

Fig. 2. South African National TB programme preliminary diagnostic algorithm incorporating the GeneXpert MTB/RIF assay for frontline TB diagnosis in TB clinics. LPA = genotypic drug susceptibility testing using GenetypeMTBDRplus (HAIN lifesciences, Nuhren, Germany). DST = phenotypic drug susceptibility testing using automated liquid culture (MGIT).

mutations outside the regions of the *inhA* and *katG* genes that LPAs can detect.[12] LPAs are now routinely available in South Africa for MDR-TB suspects with smear- or culture-positive samples and forms part of the diagnostic algorithm outlined in Fig. 2. A Genotype MTBDRplus sl (Hain Lifesciences; Nuhren, Germany) is currently under evaluation for genotypic second-line DST and rapid XDR-TB diagnosis.

TB immunodiagnostics, such as serological tests, tuberculin skin tests (TSTs) and the interferon-γ release assays (IGRAs) are noticeably absent from Fig. 3. A WHO recommendation, based on the findings of a recent meta-analysis of serological assay,[13] advises against the current use of any of the numerous available blood serological assays for the diagnosis of TB. Multiplex serological assays are under evaluation and may still prove useful for TB diagnosis in the future. Blood-based IGRAs for the diagnosis of active TB in high-burden settings have also been extensively evaluated and currently offer little clinical utility as a frontline TB diagnostic tool in either HIV-infected or -uninfected patients.[14] IGRAs may have some value to 'rule out' TB in smear-negative patients with ongoing symptoms[15] and in TB meningitis patients when applied to CSF in conjunction with existing TB diagnostics.[16] TST remains a useful tool for the diagnosis of active TB in young children and IGRAs offer equivalent, but not superior performance[17] and immunodiagnosis is not a substitute for molecular or microbiological site-of-disease diagnosis. Immunodiagnosis remains important for the diagnosis of latent TB infection and as a means to guide the use of isoniazid preventive therapy, but this is outside the scope of this review.

Progress and exciting developments for point-of-care TB diagnosis

The detection of antigens using immunochromatographic lateral flow tests in non-sputum samples (e.g. urine and volatile organic compounds in exhaled breath systems) hold exciting promise for inexpensive and rapid bedside TB diagnosis. Urinary lipoarabinomannan (LAM), an important Mtb antigen, has high specificity and reasonable sensitivity for TB diagnosis in hospitalised HIV and TB co-infected patients with advanced immunosuppression, but performs poorly in other settings.[18] This test is now available as a point-of-care lateral flow test – LAM Ag rapid test (see Fig. 1) and our preliminary study results in hospitalised HIV-infected patients look promising. A lateral flow point-of-care strip test for unstimulated IFN-γ is under development as this has shown excellent diagnostic utility for use on pleural and pericardial fluid.[19] Additionally, proteomic and metabolomic approaches are being used to identify other TB-specific antigens that may be used for TB diagnosis, while studies of electronic-nose (E-nose) technology to detect volatile organic compounds in exhaled breath and urine are underway.

At the bedside: symptoms and signs as a diagnostic tool and aid to good diagnosis

Despite the availability of new TB diagnostic tools and the improvements in rapid diagnosis using molecular and antigen detection methods, a microbiologically proven TB diagnosis often remains elusive. In particular groups of patients, such as sputum

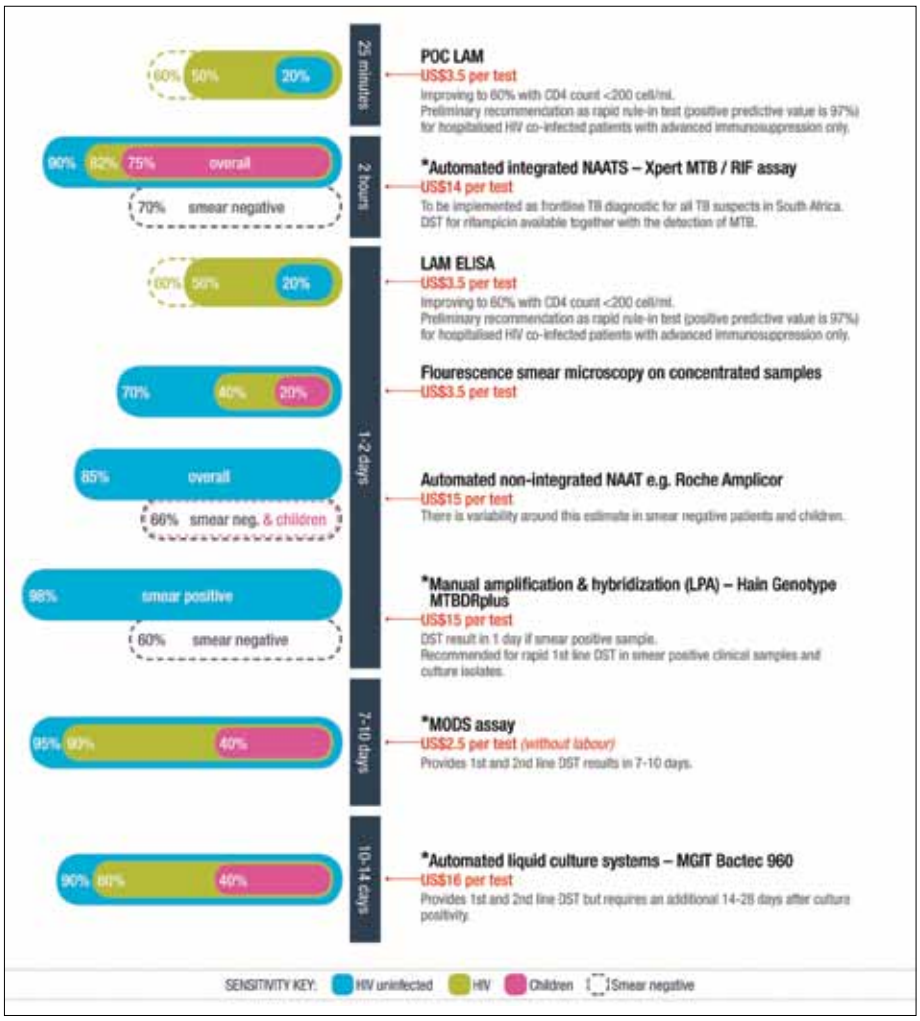

*Fig. 3. Comparison of the sensitivity, time-to-diagnosis and cost of diagnostic tools for active TB and drug susceptibility testing in South Africa indicating areas of reduced performance in HIV TB co-infected patients and children. Only tests commercially available in South Africa and with a specificity >95% for the diagnosis of active TB are included in this diagram. * Indicates the test used for both the diagnosis of active TB and either genotypic or phenotypic drug susceptibility testing (DST). Due to sub-optimal specificity for active TB diagnosis clinical case-definitions, radiology and immunodiagnostics tests are not included in this figure.*

smear-negative pulmonary TB, hospitalised HIV-infected patients with advanced and disseminated disease, young children and patients with extrapulmonary forms of TB, clinical case definitions and algorithms can be beneficial for guiding the empiric use of anti-TB treatment. Multiple clinical case definitions and algorithms are available and performance studies have shown variable results, particularly in children. The WHO smear-negative TB guidelines give expanded case definitions for both pulmonary and extrapulmonary TB and are widely available to help guide treatment decisions. Two recent studies evaluated the performance and patient-related impacts of these guidelines in KZN. In an ambulatory setting the sensitivity of the ambulatory WHO algorithm was 80%, but the specificity was only 44% (Wilson and Maartens, 2011). However, implementing the WHO algorithm for seriously ill patients in hospitalised patients to guide early anti-TB treatment initiation resulted in a 10% decrease in 2-month mortality and reduction in length of stay in the group treated according to the guideline as compared with those treated by physician-guided practice only.[20] Thus, despite modest diagnostic performance characteristics these clinical guidelines and case definitions may affect individual patient outcomes. Their role in conjunction with newer rapid diagnostic in hospitalised HIV TB co-infected patients and children warrants further study.

An understanding and use of the following few basic clinical principles can improve both the diagnosis and overall management of TB patients, independently from the use of clinical case definitions to guide treatment decisions:

- The acquisition of an adequate sample is key to successful TB diagnosis regardless of the diagnostic tool used. Adjunctive methods for sputum collection such as sputum induction have been shown to be highly effective in children and hospitalised patients.[21]
- In patients with suspected disseminated TB, tissue sampling can produce a high diagnostic yield, e.g. lymph node aspirate or bone marrow biopsy.
- TB, especially with HIV co-infection, has a wide range of clinical presentations and it is important to maintain a high index of suspicion, and consider the need for empiric anti-TB treatment, while simultaneously balancing the dangers and toxicity of anti-TB drugs.
- The need for close follow-up and monitoring of response to anti-TB treatment for patients given empiric therapy is essential.

Conclusion

The dual epidemics of TB and HIV continue to overburden our hospitals and cause extensive morbidity and mortality. Delayed and misdiagnosis of TB remains a major problem, but promising developments, such as the GeneXpert, and the continued progress towards effective, rapid point-of-care TB diagnostic technology offer hope. Combined with sound clinical skill, these novel technologies will continue to improve our ability to recognise and diagnose the many 'faces' of TB among the co-epidemic of HIV.

References available at www.cmej.org.za

In a nutshell

- Rapid and effective TB and drug susceptibility diagnostics decrease morbidity and mortality and limit the spread of disease, especially in HIV co-infected patients.
- Smear microscopy and chest X-ray (CXR) are sub-optimal for TB diagnosis in high HIV prevalence settings.
- The GeneXpert MTB/RIF assay is a point-of-care molecular test which produces results within 2 hours. It is a more sensitive diagnostic test than smear in sputa, and was shown to diagnose 3 out of 4 smear-negative pulmonary TB cases. It also provides rapid test for rifampicin resistance. Preliminary evidence suggests that GeneXpert substantially outperforms smear microscopy for the diagnosis of extrapulmonary TB.
- The GeneXpert assay is to replace smear microscopy as the frontline pulmonary TB diagnostic tool in South Africa following endorsement by the World Health Organization.
- Detection of the lipoarabinomannan (LAM) antigen in urine on strip test offers a potential inexpensive point-of-care 'rule-in' test for TB in hospitalised HIV co-infected patients with advanced immunosuppression.
- Effective sample acquisition using techniques such as sputum induction and needle aspiration of lymph nodes remain important clinical adjuncts for effective TB diagnosis.
- Automated liquid culture remains the reference tool for both TB detection and phenotypic first- and second-line drug susceptibility testing.
- Line probe assays (LPA) offer rapid genotypic drug susceptibility testing (DST) for multidrug-resistant TB (MDR-TB) using smear-positive clinical samples or culture isolates.
- Immunodiagnostics (serology and interferon-γ release assays (IGRAs)) are not useful in the diagnosis of active TB in adults and are no more effective than tuberculin skin testing (TST) in children from high-burden settings.