Acid Fast Bacilli (AFB) smear microscopy is currently the primary method used for pulmonary TB diagnosis in resource limited settings since it requires minimal laboratory infrastructure. However, it is widely known that the sensitivity of AFB smear microscopy, and particularly with raw sputum samples, depends on the quality of the sputum sample obtained from a patient, bacterial load, the skill of the laboratory personnel performing the staining procedure, and the time taken between collection of the sample and test being performed.

The gold-standard for TB diagnosis is liquid culture, which can detect as few as 10-100 CFU within a given sputum sample. However, this can take up to 6 weeks to complete. If a patient has suspected multi drug resistance (MDR), drug susceptibility testing is then performed to identify the most appropriate antibiotic therapy, but this will take a further month to complete.

Given the inability to elucidate drug resistance using AFB smear microscopy, and the slow growth rates for liquid culture, these classical detection methods can prevent rapid diagnosis, preventing patients from obtaining appropriate and timely therapy at point-of-need.

Molecular testing with CBNAATs (Cartridge Based Nucleic Acid Amplification Tests), such as GeneXpert® is the preferred first diagnostic test (TB testing and diagnosis of TB in India - CBNAAT, tbfacts.org) because it offers an overall sensitivity that is higher than AFB smear microscopy, and also provides genetic information about mutations that confer antibiotic resistance to the primary antibiotic Rifampicin. However, although the use of GeneXpert® is a significant and important breakthrough in the testing of TB, it is limited to larger centralised laboratories and its wider adoption is reduced by the need for air conditioning and continual power requirements, and it's use can require public health funding subsidy in resource limited settings.

WHO guidance on rapid implementation of the GeneXpert® MTB/RIF diagnostic test recommends that temperature control is implemented in the test space used limiting its deployment to larger laboratories since the outreach testing laboratories often do not have the air conditioning required to maintain the air conditioning units. In addition, many laboratories with lower sample throughput will find the cost of the GeneXpert® platform prohibitive.

Whilst smear microscopy has poor sensitivity and issues related to quality control, it remains a mainstay of diagnostic testing for pulmonary tuberculosis. Whilst sensitivity of microscopy has been reported to have greater than 80% sensitivity for identifying pulmonary TB in some settings the sensitivity of the test has been substantially lower and variable in other reports, ranging from 20 – 60%, in India, the sensitivity of ZN-microscopy versus culture has been reported to range from 68% to 86.6%.

There is therefore a need for a molecular test that can replace or supplement AFB smear microscopy in laboratories with a lower sample throughput, and which also provides additional information about the underlying sensitivity to the antibiotic Rifampicin. Genedrive® offers a rapid test for MTB/RIF which is suited to laboratories that have a low sample throughput (up to 8 samples per day). Genedrive® can operate in elevated ambient temperatures (up to 40°C).

The MTB/RIF assay uses PCR to amplify and detect the MTB complex DNA within pulmonary sputum samples. The MTB/RIF kit contains all the materials needed to perform clinical sample preparation and MTB detection in a convenient, temperature-stable reagent format. It includes a test cartridge that contains lyophilised PCR reagents which are designed to target two core sequences within the MTB genome;

- A 130 bp region within a highly conserved multi-copy gene region REP13E12
- A 275 bp region of the single copy rpoβ gene

In addition, each cartridge contains an internal positive control to report the presence of inhibitory substances in the sample that may affect the integrity of the test. Using post-PCR melting analysis, the test is designed for the in vitro detection and genotyping of MTB...
using the broadest possible detection profile to ensure that all clinically relevant Mycobacterium strains and subtypes are detected including the closely related \textit{M. bovis} and \textit{M. africanum} species. The test also detects mutations in the \textit{rpoB} gene associated with resistance to Rifampicin. Rifampicin is a first line MTB medication and thus is the main target to identify in the field prior to treatment.

Here, we describe an assessment of the Genedrive\textsuperscript{\textregistered} MTB/RIF assay using single sputum samples arriving on an “all-comer” basis, processed in ongoing studies in two separate Initiative for Promoting Affordable Quality TB Tests (IPAQT) participating laboratories in India, and comparing its performance against standard AFB smear microscopy and MGIT liquid culture performed by the same labs as part of their routine service. In analysing and presenting the data there are two overviews; (a) data generated to support submission to the Drug Controller General India (DCGI) and (b) meta-analysis of all study site data, including current studies. DCGI approval was granted in April 2015.

This evaluation was performed to determine the clinical diagnostic sensitivity, diagnostic specificity, positive predictive value (PPV) and negative predictive value (NPV) of the Genedrive\textsuperscript{\textregistered} MTB/RIF assay.

Both liquid culture (BACTEC MGIT TB) and microscopy (Ziehl-Neelsen staining) were used as the reference for Genedrive\textsuperscript{\textregistered} MTB/RIF assays.

Data inclusion criteria for analysis included samples having (a) Valid culture, Genedrive\textsuperscript{\textregistered} and AFB smear microscopy results, (b) Sputum not being from defaulting, previously-treated or unknown-history patients and (c) Only live Mycobacteria-free sputum (true negative) and live MTBC-containing sputum (true positive) were included in the analysis i.e. no sputum with non TB complex bacteria were included. These studies directly compared the sensitivity of Genedrive\textsuperscript{\textregistered} and its MTB/RIF assay against the primary method of AFB smear microscopy and the gold standard of liquid culture. Data represents the detection of MTB from raw unprocessed sputum collected from all-comers that meet the above noted inclusion criteria. Specific training was provided to teach the operator to select and process the sputum since this influences the quality of the test. As with AFB smear microscopy it is important to select the yellow, mucoid or purulent portion of sputum which will contain the highest density of bacilli\textsuperscript{10}. Thin saliva contains little or no bacilli.

### Results & Discussion

In a total of 300 specimens analysed for the purposes of regulatory submission to DCGI, Genedrive\textsuperscript{\textregistered} MTB/RIF demonstrated a sensitivity and specificity of 93\% and 96\% for smear positive/culture positive and smear negative/culture negative specimens respectively (n = 300) (Figure 1).

![Figure 1: DCGI Cohort (Culture confirmed microscopy)](image1)

When expanded to include current IPAQT testing site data in India, from a total of 614 samples, Genedrive\textsuperscript{\textregistered} MTB/RIF showed a sensitivity of 95\% in smear positive/culture positive and a specificity of 93\% in smear negative/culture negative specimens (n = 614) (Figure 2a).

Of 26 false positives, where Genedrive\textsuperscript{\textregistered} MTB/RIF returned positive results from smear negative culture negative samples, for those that have corresponding GeneXpert\textsuperscript{\textregistered} data approximately half were also detected positive by GeneXpert\textsuperscript{\textregistered}, likely indicating these false positives result from dead bacilli. Of 12 false negatives noted, where Genedrive\textsuperscript{\textregistered} MTB/RIF returns undetected results for culture positive smear positive samples, for those with available GeneXpert\textsuperscript{\textregistered} data all were detected positive by GeneXpert\textsuperscript{\textregistered}. This may be reflective of sputum quality.

![Figure 2a: DCGI & 2 IPAQT laboratories Cohort (Culture confirmed microscopy)](image2)

The results for raw sputum AFB smear positive samples obtained in these studies are consistent with published studies for GeneXpert\textsuperscript{\textregistered}, which has been shown to have a sensitivity of ≥95\% for a single raw sputum sample shown to be AFB smear positive sputum\textsuperscript{12}. Clinicians using GeneXpert\textsuperscript{\textregistered} are recommended to use two tests where samples are negative in order to achieve higher sensitivity\textsuperscript{16}. We would expect to see a similar improvement in sensitivity if negative Genedrive\textsuperscript{\textregistered} tests were similarly repeated with a second test.

![Figure 2b: Multicentre analysis (Culture confirmed microscopy)](image3)
Smear grades in this cohort of 614 samples is presented in Figure 3, showing the spread of International Union Against Tuberculosis and Lung Disease (IUATLD) grades. In addition to this cohort of culture confirmed microscopy cases, across all studies just 4.79% of TB cases were culture positive and smear negative. In this population Genedrive® detected 10 of 31 cases giving a sensitivity in this group of 32.26%.

Published frequencies of lowest measurable AFB scanty grade sputum represents between 5-10% of all smear positive samples17, 18. Genedrive® sensitivity was lower in this population (76.92%) than in the 1+ to 3+ IUATLD smear grade groups.

Overall, in the ongoing current laboratory cohorts described above, Genedrive® showed a control failure rate of 6% across the two sites and across 5 manufactured batches of cartridges, demonstrating that the assay sample processing cassette was able to process sputum and minimise transferral of inhibitory substances from the sputum into the PCR. This compares favourably to invalid rates for GeneXpert® which are have been shown by others to be in the region of 7%.9

Conclusions

Genedrive® MTB/RIF assay offers a molecular solution for MTB detection that in the cohorts described here, showed a high level of concordance with AFB smear microscopy across two microscopy reference laboratories in India. Additionally, the test captured a percentage of AFB smear negative, culture positive samples and showed good agreement with GeneXpert® albeit with a slightly lower overall sensitivity. Specificity was matched to GeneXpert®.

Genedrive® is a more affordable, rapid, molecular diagnostic system which can allow for the detection of MTB/RIF for use in small to medium sized laboratories which wish to use molecular testing as an adjunct or a replacement to microscopy. In addition to the MTB result the Genedrive® MTB/RIF assay can also offer printing and storage of results, RIF status testing and is not subjective like smear microscopy.

We believe that Genedrive® offers a molecular tool that can be performed at AFB smear microscopy centres, including those low sample throughput settings where the use of GeneXpert® is not practical to perform because of cost, requirement for air conditioning, or ancillary laboratory equipment such as vortexes or centrifuges. We believe that this should widen the number of sites able to perform molecular screening of MTB from pulmonary sputum samples.

References

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