Implementation of Xpert MTB/Rif Assay in Buhera District, Zimbabwe: Lessons Learned

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Introduction

Zimbabwe is ranked 17th of the 22 high-burden TB countries in the world1. In 2010, the country reported 47,557 cases of tuberculosis with a case notification rate of 633 cases per 100,000 population for all TB cases. 43% of TB cases are notified as smear negative and the HIV co-infection rate is reported as 80%2.

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The burden of HIV in the country is also high at 13.7%³ in the general population with TB being reported as the most frequent cause of death among persons living with HIV/AIDS.

In addition to the high co-infection rates, drug resistant TB is also emerging as an increasing problem. The drug resistance survey performed in 1994 showed a prevalence of <3% multi-drug resistant strains among new cases and 9% among re-treatment cases⁴. In 2007, it was estimated that 1.9% and 8.3% of new and retreatment TB patients were MDRTB respectively⁵.

Current methods to diagnose smear negative TB and MDRTB are slow and cumbersome. The average turn-around time for smear microscopy is 1-2 days but for decentralised sites access to a subsequent CXR needed for diagnosis of smear negative TB may take a further one to two weeks. Access to CXR will also depend on the patient’s ability to afford transport and the investigation itself. Conventional culture techniques used for the diagnosis of drug resistant TB can take 3-8 weeks on solid media, 1-2 weeks in broth media. Drug sensitivity testing following a positive MTB culture takes another 2-4 weeks in solid media and 1 week in broth media⁵.

Recent TB diagnostic research has focused on novel molecular technologies for rapid detection of TB, one such example being Xpert MTB/RIF. Xpert MTB/Rif is a TB-specific, automated, cartridge-based nucleic amplification assay. It is unique in that it has simplified the process of molecular testing, fully integrating sample preparation, amplification and detection required for real-time polymerase chain reaction. Xpert MTB/RIF detects *Mycobacterium tuberculosis* as well as rifampicin-resistance conferring mutations directly from sputum, in an assay providing results in 100 minutes⁵.

Results from a multi-centric validation study coordinated by FIND and from additional single centre studies showed that 92.2% of culture-positive patients were detected by a single direct Xpert MTB/RIF test in controlled clinical validation trials involving 1,730 individuals suspected of

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⁴ DRTB Guidelines Zimbabwe : Draft October 2011

⁵ Roadmap for rolling out Xpert MTB/RIF for rapid diagnosis of TB and MDR-TB, WHO December 2010
TB or MDR-TB. Sensitivity of a single Xpert MTB/RIF test in smear-negative/culture-positive patients was 72.5%, increasing to 90.2% when three samples were tested.

MSF-Belgium in collaboration with MoH has been supporting TB/HIV activities since 2004 in Buhera district, Manicaland, an eastern Zimbabwean province bordering with Mozambique. The district has an estimated population of 238,293 people. There are 30 health facilities in the district, which includes 3 hospitals, 26 government or council clinics/health centers and 1 private mining clinic. Weekly outreach for HIV/TB care to 22 clinics (including BBH) is performed by MSF mobile teams. To date 18,048 patients have been started on ART in Buhera, with 13,603 patients remaining on ART follow-up.

Murambinda Mission Hospital (MMH) and Birchenough Bridge Hospital (BBH), where Xpert MTB Rif machines have been installed, are rural district hospitals. The laboratories in both hospitals are equipped with a fluorescence microscope for auramine staining. This is more sensitive than the light optical microscope for Ziehl-Neelsen staining. Both settings have been equipped with air-conditioning in order to maintain the temperature at levels below 30°C necessary for optimum instrument operation. Although studies indicate that sample preparation does not require handling under a biosafety cabinet, both laboratories utilise this safety measure.

MSF-Belgium in Zimbabwe is one of seven MSF project sites to implement Xpert MTB/Rif during 2011. All sites implemented an initial parallel phase combining conventional smear microscopy alongside Xpert MTB/Rif. In Zimbabwe this parallel phase was implemented in Murambinda Mission Hospital (MMH, May – August 2011) and Birchenough Bridge Hospital (BBH, April – August 2011).

The objectives of implementing Xpert /MTB Rif in this parallel phase were:

- to assess the operational challenges of implementing the Xpert MTB/Rif in a rural district laboratory setting.
- to observe the additional diagnostic value of the tool at rural district level compared to sputum smear microscopy, in particular in the diagnosis of smear negative TB in co-infected patients.
Laboratory implementation

The installation of the Xpert MTB/Rif was performed by staff from PointeCare Company - Harare in presence of the complete staff in both laboratories. The installation was complete in 1 day. The first machine (BBH) was installed in presence of a Senior Laboratory Scientist of Mutare provincial hospital.

Prior to installation the following logistical requirements were fulfilled at both sites:

- Air conditioning: Xpert MTB/Rif is designed for indoor use only. Operating temperature should be between 15-30 degrees, meaning that a functioning air conditioner is required in most African settings.

- The machine should not be placed directly under an air vent or in direct sunlight

- Stable electricity supply: power interruptions can lead to instrument damage and incomplete testing. It is therefore mandatory to have a uninterruptible power supply (UPS) for operation of Xpert MTB/Rif

- Storage of cartridges: The single use cartridges are bulky and require substantial storage space. This needs to be planned for.

- Waste management: Disposal of cartridges is similar to that of other biological waste. i.e sterilisation (autoclave) and appropriate chemical waste incineration.

- Training: laboratory staff can be trained in a one day training provided by the local supplier. Follow up of implementation is however essential to ensure SOPs are followed accurately

Clinical Implementation

Figure 1 demonstrates the clinical algorithm followed during the initial parallel phase. Samples were collected from ALL TB suspect patients, regardless of HIV status. Current WHO recommendations suggest that Xpert MTB/Rif should be used as first test only in co-infected patients or those suspected of DRTB. However there is argument that in high burden HIV/TB settings Xpert MTB/Rif should be made available to all patients; especially in setting with MDR TB patients. This is a crucial
question to be analysed further in terms of direct patient benefit versus presumed additional costs related to Xpert MTB/Rif use.

Validation studies for Xpert MTB/RIF have only demonstrated accuracy in testing sputum specimens, the project focused mainly on sputa, but did not deny extra-pulmonary specimens as having an additional benefit for doctors to diagnose EPTB cases clinically. Children were also included provided they were able to produce sputum. A TB suspect as defined by NTP was any patient who presents with cough of more than 2 weeks, fever more than 3 weeks, night sweat more than 3 weeks, weight loss more than 5% of body weight, chest pain more than 2 weeks or having a close TB contact.
Each TB-suspect was requested to submit 2 sputum specimens: 1 for the conventional smear plus Xpert MTB/Rif and a second for conventional smear alone. PITC was offered (if status unknown) and an empirical antibiotic trial with amoxicillin for 1 week was started for patients seen at health centre level. If Xpert MTB/Rif detected *M.

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**Figure 1: Algorithm for diagnosis of (DR-) TB during the parallel testing period**

**PTB Suspect***

- Offer HIV testing and counseling
- 2 quality sputum specimens for microscopy and one used for GeneXpert
- Give 1° empiric course with amoxicillin

**Negative result**

- Clinical reassessment for other causes
- Give 2° empiric antibiotic course with erythromycin/azithromycin
- CXR

**Positive result with NO Rif-resistance**

- Ensure TB IC measures in place
- Start treatment for drug-sensitive TB

**Positive result with Rif-resistance**

- Repeat Xpert and send for culture and DST
If second Xpert shows rif resistance commence empirical DRTB treatment
If second Xpert sensitive to rifampicin commence first line TB treatment

**PTB still suspected, CXR not suggestive**

One additional quality sputum for repeat of Xpert MTB/RIF assay

**Negative results**

- Clinical reassessment and rule out other causes
- Refer to Doctor for further decision

**Positive result with NO Rif-resistance**

- Ensure TB IC measures in place
- Start treatment for drug-sensitive TB

**Positive result with Rif-resistance**

Follow right side of this algorithm

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**DANGER SIGNS:**
- Resp rate > 30/min and fever > 39° and/or pulse > 120/min and/or unable to walk → REFER FOR HOSPITALISATION

* Suspect = cough > 2 w or, fever >3 w or, night sweats or, weight loss > 5% or, chest pains or TB-contact in the family
tuberculosis and the strain was not rifampicin resistant, then nurses initiated CAT I or CAT II TB treatment and registered the patient based on smear result. If Xpert MTB/Rif detected *M. tuberculosis* and the strain was rifampicin resistant, then the patient was referred to the doctor for further assessment for DR TB.

In addition to the clinical diagnostic algorithm, algorithms to indicate clearly the changes required in the patient and specimen flow were developed. These are shown in figures 2 and 3 for the health centres and hospital, respectively.

Communication of results is different in clinics compared to hospitals. In clinics, sputa are collected on the day the MSF mobile team is present in the clinic (on a once per week basis). On the day the team is there, the patient brings 2 morning sputum samples or produces 2 spot samples with an interval of 2 hours. One week later, the result is brought back to the clinic. This turnaround time will hopefully be reduced by the implementation of M-Health technologies in 2012. In the 2 hospital sites – MMH and BBH – 2 sputum samples were collected as spot specimens with an interval of 2 hours. The results were communicated to the clinicians on the same day, or latest 1 day after.
Figure 2: Patient and Specimen Flow at Health Centre level

DIAGNOSIS OF TB FOR PATIENTS ATTENDING PHC CLINICS

Waiting Area Daily
- Cough Triage implemented
- Coughing patients fast tracked to consultation

Consultation room
- TB suspect confirmed using Zim TB guideline screening tool
- Amoxicillin 7/7 given
- PITC offered and result noted on smear request form. Patient instructed to attend on specimen collection day at 8 a.m.
- To be instructed how to produce an early morning sputum at home

On specimen collection day
- Patient instructed again how to cough by nurse aid
- Produce second spot sputum (if on MSF day both sputums to be collected same day)
- Sputum to be checked and repeat once if saliva
- Form MUST have TB suspect number + OI number if HIV +ve
- Patient booked to attend for result in 2 days

Lab processes specimens
- Sputum 1- smear and Xpert. Sputum 2- smear
- At fixed time for each clinic Lab to radio all TB results (NB paper result must follow as usual)

Patient receives results 2 days after specimen collection
- Smear + / Xpert + / Rif Sens= nurse Initiation of TB
- Smear - / Xpert + / Rif Sens= nurse initiation of TB
- If smear - / Xpert - = Nurse to follow algorithm
- If smear + / Xpert - = refer doc
- If smear + / Xpert positive + Rif Resistant = refer to doctor.
Figure 3: Patient and Specimen Flow at Hospital Level

**DIAGNOSIS OF TB FOR PATIENTS ATTENDING MMH AND BBH HOSPITALS**

**Waiting Area**
- Cough Triage implemented
- Coughing patients fast tracked to consultation

**Consultation room**
- TB suspect confirmed using Zim TB guideline screening tool
- Patient instructed will need to stay to do TB tests and HIV test today

**Patient reports to Lab**
- Instructed carefully by lab receptionist on how to produce a sputum sample
- Coughing area identified
- Patient delivers sputum #1 to Lab

**Laboratory**
- Processed sputum #1
- For Smear #1 and Xpert #1

**Patient directed to attend for PITC**
- 2-3 hours after first sputum to produce second sputum and bring to lab.

**Patient receives results of sputum #1 from lab and directed back to OPD consultation room**
- Smear+/Xpert+/Rif Sens= nurse initiation of TB
- Smear-/Xpert+/Rif Sens= nurse initiation of TB
- If smear-/Xpert- = wait for second smear result.
- If remains smear neg nurse to follow algorithm
- If discordant smear+/Xpert- refer doc
- If smear+ or neg/ Xpert positive + Rif Resistant = refer to doctor.
Ensuring adequate training of clinical staff on both the clinical and patient flow algorithms is essential to effective implementation of Xpert MTB/Rif

**Outcomes of parallel phase implementation**

Results for smear and GeneXpert samples were entered into a MSF access based database designed by MSF, called ‘Xact’ (Xpert Accurate Collection Tool). This database requires Microsoft Access 2007 or later.

**Table 1: Demographic description of all TB suspects samples collected during the parallel phase**

<table>
<thead>
<tr>
<th>Samples</th>
<th>MMH (N= 865)</th>
<th>BBH (N=420)</th>
<th>TOTAL (N= 1285)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>359</td>
<td>162</td>
<td>521</td>
</tr>
<tr>
<td>Female</td>
<td>484</td>
<td>255</td>
<td>739</td>
</tr>
<tr>
<td>Unknown</td>
<td>22</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>Adults</td>
<td>729 (84%)</td>
<td>350 (83%)</td>
<td>1079</td>
</tr>
<tr>
<td>Children &lt; 15 y</td>
<td>35 (4%)</td>
<td>45 (11%)</td>
<td>80</td>
</tr>
<tr>
<td>Unknown</td>
<td>101 (12%)</td>
<td>25 (6%)</td>
<td>126</td>
</tr>
</tbody>
</table>

**Table 2.1: Description of the samples (age/ type) submitted both to Smear & GeneXpert testing from MMH**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Adults</th>
<th>Children</th>
<th>Unknown</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum</td>
<td>641</td>
<td>28</td>
<td>95</td>
<td>764</td>
</tr>
<tr>
<td>EP samples</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>644</td>
<td>31</td>
<td>96</td>
<td>771</td>
</tr>
</tbody>
</table>

We noted that not all patients were submitted to both smear and GeneXpert testing; mostly likely due to high workload in the lab and introduction of the new technique. Whilst we noted a total of 865 samples tested in total in MMH during the parallel testing period; only 771 samples
were submitted to BOTH smear and GeneXpert testing and as included in this study.

**Table 2.2: Description of the samples (age/ type) submitted both to Smear & GeneXpert testing from BBH**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Adults</th>
<th>Children</th>
<th>Unknown</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum</td>
<td>317</td>
<td>31</td>
<td>25</td>
<td>373</td>
</tr>
<tr>
<td>EP samples</td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>318</td>
<td>38</td>
<td>25</td>
<td>381</td>
</tr>
</tbody>
</table>

Likewise in BBH only 381 samples of the 420 were submitted to both smear and GeneXpert testing and as such were included in this study.

HIV status was difficult to document since it is currently not routinely recorded on the lab request form and concerns were raised regarding confidentiality should it be added.

**Table 3.1: Comparison between smear and Xpert MTB/Rif results; ADULTS – SPUTUM MMH**

<table>
<thead>
<tr>
<th>MMH (N = 641)</th>
<th>Smear</th>
<th>% additional detection with GeneXpert</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>GeneXpert</td>
<td>Positive</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>7</td>
</tr>
</tbody>
</table>

**Table 3.2: Comparison between smear and Xpert MTB/Rif results; ADULTS – SPUTUM BBH**

<table>
<thead>
<tr>
<th>BBH (N = 317)</th>
<th>Smear</th>
<th>% additional detection with GeneXpert</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>GeneXpert</td>
<td>Positive</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>1</td>
</tr>
</tbody>
</table>
Using Xpert MTB/Rif the laboratory was able to diagnose an additional 48 (MMH) and 23 (BBH) cases of active TB among adults who were smear negative on microscopy; or an increased laboratory detection of TB of 60.7% (MMH) and 56.1% (BBH). Expressed in terms of sputum results this corresponds with an increase in positivity rate from 13.4% (86/641) on smear to 19.8% (127/641) on GeneXpert in MMH and from 13.2% (42/317) on smear to 20.1% (64/317) in BBH.

In the past, these smear negative cases would initially be treated with broad-spectrum antibiotics, followed up after 7-14 days and re-assessed for TB if symptoms persisted. There would be additional delays to TB treatment if these patients did not come back for a re-assessment or actually felt better with the initial treatment. As such it would be equally essential to compare the time to diagnosis for smear negative cases pre and post implementation of Xpert MTB/Rif.

Of note there were 7 cases in MMH and 1 case in BBH found to be smear-positive yet Xpert MTB/Rif-negative. 4 of the 8 samples were recorded as smear positive scanty. Based on controlled clinical validation trials cited by the WHO, specificity for the Xpert MTB/Rif assay is reported as 99%. Taking this into consideration, it would imply that these 8 specimens are most likely not MTB, and may be explained by visualization of non-tuberculous Mycobacteria (NTM) (which would also appear as acid-fast bacilli in auramine staining), technical inaccuracy in reading the slides, contamination or improper sample processing with Xpert MTB/Rif. Unfortunate no culture results are available for those specimens. With respect to adequate specimen quantity, it is recommended by Cepheid that at least 1 mL of sputum is available for testing; a paucibacillary specimen would normally still turn out GeneXpert-positive if indeed it was positive for the MTB in the first place. Collection of a minimum of 2 ml of sputum has been implemented in both laboratories to standardize the procedure and ensure enough sample both for sputum smear microscopy and GeneXpert testing.
Table 4: Comparison between smear and Xpert MTB/Rif results on CHILDREN: SPUTUM (MMH and BBH combined data)

<table>
<thead>
<tr>
<th>SPUTUM MMH + BBH (N = 59)</th>
<th>Smear</th>
<th>% additional detection with GeneXpert</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>GeneXpert</strong></td>
<td>Positive</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
</tr>
</tbody>
</table>

Using Xpert MTB/Rif 3 additional were diagnosed PTB; which were not picked up on microscopy.

Table 5: Comparison between smear and Xpert MTB/Rif results for EP samples (all facilities & ages combined).

<table>
<thead>
<tr>
<th>EP samples MMH + BBH, all ages combined (N=15)</th>
<th>Smear</th>
<th>% additional detection with GeneXpert</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>GeneXpert</strong></td>
<td>Positive</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
</tr>
</tbody>
</table>

There is a growing body of evidence to support the use of Xpert to test EP specimens. Using Xpert MTB/Rif 1 additional case of EP TB was picked up at BBH. Results of extra-pulmonary sample were carefully checked by doctors with clinical findings before making final decision.

Among the extra-pulmonary samples we noted: 1 CSF, 4 pleural fluid, 2 lymph node aspirates, 2 ascitic samples and 6 gastric lavage.

See annex 1,2,3 for more details on data per health facility, age group (including the unknown) and sample type.

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6 http://jcm.asm.org/content/50/2/513.abstract?sid=7ffdb22c-1f97-4013-9e8e-afa9433f600
Table 6: Inconclusive results

Xpert MTB/rif testing results in 1 of 5 possibilities: MTB detected, MTB not detected, Error, Invalid, or No Result. The latter 3 possibilities all represent ‘inconclusive’ results.

Inconclusive results were analysed from May to November. The average inconclusive rate in MMH project was 17% (range 9% to 28%). This rate is considerably higher than the standard of <3% reported in FIND demonstration studies. The two sites in Zimbabwe also showed higher rates of invalid and error rates than the 5 other MSF projects sites which have implemented Xpert/MTB/RIF in 2011.

<table>
<thead>
<tr>
<th>Month</th>
<th>Total tests done</th>
<th>INCONCLUSIVE (TOTAL)</th>
<th>Error (% from total inconclusive)</th>
<th>% error due to 5011</th>
<th>Invalid (% from total inconclusive)</th>
<th>No Result (% from total inconclusive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>125</td>
<td>35 (28%)</td>
<td>27 (78%)</td>
<td>59%</td>
<td>4 (11%)</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>June</td>
<td>262</td>
<td>50 (19%)</td>
<td>32 (64%)</td>
<td>81%</td>
<td>17 (34%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>July</td>
<td>284</td>
<td>48 (17%)</td>
<td>30 (63%)</td>
<td>70%</td>
<td>17 (35%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>August</td>
<td>316</td>
<td>34 (11%)</td>
<td>25 (74%)</td>
<td>76%</td>
<td>1 (2%)</td>
<td>8 (24%)</td>
</tr>
<tr>
<td>September</td>
<td>335</td>
<td>63 (19%)</td>
<td>55 (87%)</td>
<td>86%</td>
<td>7 (11%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>October</td>
<td>355</td>
<td>77 (22%)</td>
<td>68 (88%)</td>
<td>74%</td>
<td>8 (10%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>November</td>
<td>251</td>
<td>22 (9%)</td>
<td>18 (82%)</td>
<td>50%</td>
<td>2 (9%)</td>
<td>2 (9%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1928</td>
<td>329 (17%)</td>
<td>255 (77%)</td>
<td>74%</td>
<td>56 (17%)</td>
<td>18 (6%)</td>
</tr>
</tbody>
</table>

The error rate table gives a total of 987 samples analysed in MMH (May to August) whilst the total in the demographic table is 865. This difference is due to the fact that this error rate comes from the GeneXpert software which gives individual sample results; whilst in Xact we only recorded patient results (ex 1 GenX error and 1 GenX neg for same patient will have only 1 entry in Xact, but 2 in GeneXpert software).

The most common cause of an inconclusive result in MMH was an error result (77%). Technically, an error indicates that the probe check
control (PCC) failed and the assay was aborted possibly due to the reaction tube being filled improperly, a reagent probe integrity being detected, the maximum pressure limits were exceeded or there was a GeneXpert module failure. Practically, an error is associated with the cartridge or the machine itself; a particular error will present as a code which the troubleshoot section of the GeneXpert manual will associate with the problem causing the error. An error should be addressed with the company for cartridge or module replacement.

The most common type of error experienced in MMH was E5011 (74% of the inconclusive results). This specific error is linked to cartridge or module problems. Gxx files from the instrument were sent to Cepheid for technical assistance on these errors. Cepheid can propose cartridge or module replacement free of cost according to their analysis of the gxx files. For MMH Cepheid agreed to reimburse 137 cartridges from analysing the error results as well as 2 modules replacement.

Troubleshooting included a thorough revision of sample processing in the laboratory with the following points being reinforced:

- Specimen volume not less than 2 mL
- Reference volume containers (1 mL, 2 mL and 3 mL) used for sputum volume estimation
- Ratio specimen: buffer (1:2) is ensured through the use of volumetric plastic pipettes rather than by adding unmeasured buffer.
- A minimum of 2 mL MUST be transferred to the cartridge
- Bubbles should be avoided

In spite of the points reinforced inconclusive rate remained high throughout the year with a considerable decrease in November; during this month a new lot number was introduced and this might have contributed to the drop.

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7 Results of Xpert MTB/RIF in MSF Projects Report No 1. GeneXpert SubGroup
In BBH a total of 40 inconclusive errors were recorded, being invalids the most common reason. Unfortunately due to a computer virus we were unable to trace back the total number of samples examined at BBH. But from extrapolation we estimate the inconclusive rate at BBH to be around 8.6% (40/460).

Inconclusive results are caused either by an invalid result, an error or no result. Whenever an error or invalid result was found the test was repeated; as such in our setting did not cause any delay in diagnosis or treatment of patients. The high number of tests needing to be repeated translates into an increase in cost and personnel workload.

In BBH, invalid results were the more common causes for inconclusive results. Invalid results indicate that the sample processing control (SPC) failed; either because the sample was not properly processed or PCR was inhibited. This implies possible problems in sample processing and/or sputum collection. Refresher trainings of laboratory personnel will minimize this kind of result in the future.

Errors are an operational risk that comes with the installation of a new technology, implying an additional cost to the project. During the parallel testing phase, the clinicians could still rely on the smear result for further management. In the future, a smear will still be performed if GeneXpert gives repeatedly error results.

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7 Results of Xpert MTB/RIF in MSF Projects Report No 1. GeneXpert SubGroup
<table>
<thead>
<tr>
<th>Lab No</th>
<th>Pts Name</th>
<th>HIV status</th>
<th>GenX1</th>
<th>Gen X2</th>
<th>Culture +DST (R/H/S/E)</th>
<th>MDR Treatment started</th>
<th>Date sample sent for DST</th>
<th>Date DST result received</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1636</td>
<td>Patient A</td>
<td>Pos</td>
<td>Rif Res</td>
<td>Rif Res</td>
<td>RRSS</td>
<td>Y</td>
<td>26/6/11</td>
<td>12/09/11</td>
<td></td>
</tr>
<tr>
<td>1641</td>
<td>Patient B</td>
<td>Pos</td>
<td>Rif Res</td>
<td>Rif Res</td>
<td>RRRR</td>
<td>N</td>
<td>07/03/11</td>
<td>13/6/11</td>
<td>Not motivated</td>
</tr>
<tr>
<td>1285</td>
<td>Patient D</td>
<td>Pos</td>
<td>Rif Res</td>
<td>Rif Res</td>
<td>RRSR</td>
<td>Y</td>
<td>Died M6 on Tx</td>
<td>17/06/11</td>
<td>July 2009</td>
</tr>
<tr>
<td>1365</td>
<td>Patient E</td>
<td>Pos</td>
<td>Rif Res</td>
<td>Rif Res</td>
<td>MGIT pos, contaminated for DST</td>
<td>N</td>
<td>29/06/11</td>
<td>23/09/11</td>
<td>Died, pat from rural home in Gutu</td>
</tr>
<tr>
<td>BBH</td>
<td>Patient F</td>
<td>Neg</td>
<td>Rif Res</td>
<td>Rif Res</td>
<td>RRRR</td>
<td>Y</td>
<td>28/06/11</td>
<td>12/09/11</td>
<td></td>
</tr>
<tr>
<td>1384</td>
<td>Patient G</td>
<td>Neg</td>
<td>Rif Res</td>
<td>Rif Res</td>
<td>RRRR</td>
<td>Y</td>
<td>29/06/11</td>
<td>19/12/2011</td>
<td></td>
</tr>
</tbody>
</table>
There were 7 cases (6 from MMH and 1 from BBH) diagnosed as rifampicin-resistant. Among these, 5 (71%) have been confirmed by culture/DST as MDRTB. Of the two unconfirmed cases, one was a resident of Harare and was not motivated to go back to Murambinda, thus did not submit a sample for culture; while the other one turned out MTB positive on culture but isolate was contaminated for final DST. So far there were no cases of discordance between culture/DST and GeneXpert Rif-resistance identified.

Also worth mentioning is the HIV status of the above patients. Two of the seven (29%) were HIV-negative, while the rest (5 of 7 or 71%) were co-infected.

**Workload**

MMH laboratory was able to process a total of 865 specimens in June, July and August (66 working days). This translates to 13 specimens per day (a 4 module Xpert MTB/Rif machine can process 16-20 samples per day). The parallel testing actually meant doubling of work for laboratory personnel since they had to process sputum for both smear and Xpert MTB/Rif at the same time. However, in the future, if Xpert MTB/Rif is adopted as the first diagnostic test used in detecting TB the laboratory staff confirm it will considerably reduce their workload as it is automated and easy to operate. If Xpert is used as first test, baseline smear microscopy is still performed for all Xpert MTB+ results; since monitoring at 2 and 5 months will continue to be by microscopy. As such further analysis on the impact on workload should be undertaken.

In Murambinda Mission Hospital and Birchenough Bridge Hospital laboratories the GeneXpert machines are run by microscopists. The microscopists received an in-house training on the machine by the lab technicians who were in turn trained by the Cepheid agent in Zimbabwe when the GeneXpert system was installed. The use of the GeneXpert machine is simple and straightforward especially to computer literate personnel since it uses a computerised interface. One day is adequate for someone to be proficient in using it.

**Maintenance/ Infection Control:**

The GeneXpert machine is disinfected at least once every month whereby the instrument surfaces, cartridge bay and cartridge plunger
rods are disinfected according to the User Manual using 70% alcohol and 1% hypochlorite as disinfectants. The preliminary processing of the sample is done in a bio-safety cabinet (BSC) as these are available in the two labs though the manufacturer states that the BSC is not essential for infection control. Also, personnel preparing samples adorn the N95 masks and other standard lab protective clothing. Furthermore, the TB sample processing room is separate from the Xpert machine room and isolated from other activities for infection control reasons.

As for maintenance, PointeCare is the local Cepheid agent that attends to the immediate problems of the GeneXpert machine. The machine is supposed to be recalibrated once every year or after 2000 tests per module, whichever comes first. This process can be done online or technician can come and perform it on the ground. The local IT department performs routine antivirus updating as the system is very vulnerable to attacks. For any technical problem with the machine, the company PointCare Harare can be contacted by e-mail on ray@pointecare.org.

**Cost**

MSF purchased the items at the following rates:

- 4 module Xpert MTB/RIF machine with desktop computer: €14,661.77 (excluding transport)
- UPS, ON-LINE DOUBLE conversion, 2000VA, 230 VAC 50 Hz: €458.46
- Cost per cartridge: €15.59 (Feb 2011)
- Installation of the machine by Pointecare: USD$ 276 (installation is free; cost is for transport – accommodation only)

Cepheid has announced price reductions based on annual global volumes to USD$14 if >1’700,000 cartridges are sold and further to USD$10.72 if >3’700,000 are sold. According to the latest data from the WHO monitoring of GeneXpert roll-out 329,350 cartridges have been sold globally until Q3 2011. So price reduction will take few years to reach the agreed volumes. For that reason WHO and GLI are currently looking for mechanisms such as advance purchase commitments and company competitors of GeneXpert to further lower the price of GeneXpert cartridges.
Conclusions

- There was a demonstrated increase in laboratory based TB detection of TB using Xpert MTB/Rif compared to smear microscopy (Auramine staining) at 47.7% and 52.4% in MMH and BBH, respectively.
- Diagnosing more cases of smear negative TB at laboratory level enables more cases of TB to be initiated by nurses and reduces the need for patients to travel to hospital for CXR.
- In 3 children Xpert MTB/Rif was positive versus smear negative and assisted clinicians to diagnose TB with more certainty. A higher proportion of children however should be able to benefit from this diagnostic tool and sputum induction could be proposed to increase access for children.
- We have not been able to collect information on HIV status of the patients. This information is not required on the standard TB smear request form. Sub-analysis of these results according to HIV status would have additional interest in order to guide on the decision as to whether to use Xpert MTB/Rif as first test in all TB suspects or only those who are HIV co-infected.
- Good sputum collection remains the cornerstone in the diagnosis of TB and efforts to improve this are to be sought including the use of sputum induction.
- Spot specimens proved effective and at hospital level enabled same day diagnosis of TB. The programme should continue to run a 2 spot sputa collection strategy; if only 1 is used for diagnosis 2nd sample to be used for inconclusive results, for confirmation of Rif resistant case and to run smear for classification of patients in cases where MTB is detected by Xpert MTB/RIF.
- Preparation of clinical staff for introduction of Xpert MTB/RIF is important. This includes training on TB diagnostic algorithms, specimen and patient flow pathway and identifying ways for fast transmission of results; to assure maximum benefit of this new technology in term of increased laboratory based TB case detection and reduction in time to diagnosis. In our setting a training of 1 day proved to be sufficient.
- We noted a high rate of inconclusive results; 17% in MMH and 8.6% in BBH which is higher than the standard of < 3% demonstrated in FIND studies. This is a concern if Xpert MTB/Rif is to be rolled out on a larger scale and should be further investigated with the company.
There were 7 cases of Rifampicin-resistant TB detected. Among these, 5 (71%) have been confirmed by culture/DST. Also, 5 (71%) were HIV positive. No discordant results were observed.

A policy decision from MOH is needed to allow rifampicin-resistant cases (proven on Xpert MTB/Rif on 2 different sputa from the same patient) to start MDRTB treatment awaiting culture and DST results. This will greatly reduce the waiting time to start treatment and consequently the risk to further transmit the disease.

There is a need to ensure accurate data collection when introducing Xpert MTB/Rif.

Xpert MTB/Rif as first diagnostic test for all patients has now been adopted in Buhera.

Consideration of abandoning category 2 treatment with streptomycin should be considered in settings with access to Xpert MTB/Rif.

Further analysis to assess the reduction in time to diagnosis using geneXpert (cascade) and the cost-effectiveness are recommended.