TB Microbiology
P1108

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P1108 Training, Cape Town, South Africa
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Background

• Challenges of childhood TB
  – Bacteriology confirmed ~ 30%
  – Paucibacillary disease
  – Difficult to obtain sputum: variety of sample types used to diagnose pulmonary TB
Types of samples for TB investigation

- **Respiratory:**
  - Expectorated sputum (older child);
  - induced sputum; gastric aspirate;
  - gastric lavage; nasopharyngeal aspirate; string test; laryngeal swab;
  - ear swab; bronchoalveolar lavage; stool

- **Extrapulmonary:**
  - Lymph node aspirate; lymph node biopsy; CSF; ascitic fluid, pleural/pleural/pericardial/peritoneal fluid; blood;
  - joint aspirate/synovial biopsy; bone; other

Courtesy of Dr E.Walters
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• Challenges of childhood TB
• Challenges of study design (see next slide)
Typical adult TB drug trial:

Screening (can be outside network lab) ➔ Enrollment Day 0 ➔ Study drugs/regimens or OBR + study drug

respiratory specimen for TB lab

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Diagnosis of MDR-TB in outside lab ➔ TB Treatment (OBR) ➔ Screening ➔ Enrollment Day 0 ➔ BDQ

Up to 4 weeks

2 to 12 weeks

Only participants who had lab-confirmed MDR-TB at diagnosis will have bacteriology completed at this visit. Follow-up specimens repeated until 3 negative.
# Appendix I: Schedule of Evaluation for All Care Recipients

<table>
<thead>
<tr>
<th></th>
<th>Screening</th>
<th>Entry/Day 0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CLINICAL EVALUATIONS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Informed consent</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Documentation of HIV status¹</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>History</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Physical exam</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Pill dispensing</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Adherence assessment</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>TB disease status and severity</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Tuberculin Skin Testing²</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>ECG</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>CXR</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Audiology</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td><strong>LABORATORY EVALUATIONS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematology</td>
<td>1.0mL</td>
<td>1.0mL</td>
</tr>
<tr>
<td>Chemistries</td>
<td>2.0mL</td>
<td>2.0mL</td>
</tr>
<tr>
<td>LFT</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>TSH (fT4 if TSH is elevated)</td>
<td></td>
<td>2.0mL</td>
</tr>
<tr>
<td>Serum biomarkers (storage)</td>
<td>0.5-1 mL</td>
<td>0.5-1.0mL</td>
</tr>
<tr>
<td><strong>Cohort 1</strong>: lactate³ to local lab</td>
<td></td>
<td>2.0mL</td>
</tr>
<tr>
<td><strong>Cohort 1</strong>: lactate/pyruvate</td>
<td></td>
<td>2.0mL</td>
</tr>
<tr>
<td>Pregnancy test</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Specimens for TB micro lab</td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>
MDR TB diagnosis (1)

- Were baseline specimens collected optimally?
  - Type? Volume? How? Which TB tests were done on specimen? Not all TB programs perform the same tests

- Specimen more likely to be negative after many weeks
  - Next protocol version: collect specimen at screening: could still be negative but closer to start of treatment

- Considerations when MDR TB diagnosis made in lab other than network approved lab and isolate not available:
  - Isolates cannot be stored for further testing
  - Not all OBR drugs/mutations may have been tested for DST
    - e.g. no INH result with Xpert thus no inhA mutations to guide use of ethionamide and high dose INH mentioned in Annex Constructing regimen
  - Not possible to definitively confirm the MDR diagnosis, even if results on the lab report from outside lab indicates MDR TB.
    - Errors are rare but could occur
• The study is not an efficacy trial. However:

• **Objective 2.2.4** Describe the MDR-TB treatment response up to 120 weeks from the initiation of the study, by HIV status.

• **Objective 2.3.1** Explore longitudinal biomarkers of tuberculosis treatment response in children treated for MDR-TB.

• Proportions of children with bacteriological and clinical cure will be presented in analysis.

• There will always be probable TB cases in paediatric TB – however, the more confirmed cases, the more robust the study is.

• Also, helps to better describe drug resistance.
MDR TB diagnosis (3)

• One solution to address these considerations: obtain baseline TB isolate.
  – This is the best scenario and each site should explore carefully how this will be done practically at their site → site specific SOP to obtain isolates from routine TB program laboratory

**Screening Visit Procedures (within 30 days prior to enrollment)**

• If participant is MDR culture positive: Contact the TB laboratory where the MDR diagnosis was made to ask for the isolate to be sent to the site DAIDS approved TB lab for microbiology testing if available.
Site specific SOP to obtain isolates

- Site specific procedure describing
  - How the clinical team will communicate (who, contact details etc.) with the routine program TB laboratory to verify if the participant baseline isolate is available in the lab
  - How the routine TB laboratory will send the isolate (if available) to the network approved TB laboratory used by the site
Collection of respiratory specimens

- Important information described in LPC

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Tube Type</th>
<th>Special Collection Notes</th>
<th>DMC Test Code</th>
<th>Processing</th>
<th>Shipping</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sputum</em> for <em>M. tb.</em> smear, culture, DST</td>
<td>Sterile container</td>
<td>Optimally collect 3-5mL of sputum with a 1.0mL minimum volume or 5-10 mL volume for GA. Store specimen at 2-8°C if not received by the lab within 1 hour of collection to maintain cold chain. Transport specimens to the laboratory in a cool box as soon as possible after collection. Process and culture within 72 hours of collection.</td>
<td>MTBMIC</td>
<td>Send to local or site specific DAIDS approved TB lab as soon as possible. Additional TB respiratory sample collection and processing information including neutralization of gastric aspirates is located in LPC Appendix 2 below and in the P1108 MOP</td>
<td>N/A</td>
</tr>
</tbody>
</table>

- Important information also in MOP
6.2.1 Infection control/Biosafety

- References
- Time between procedures

6.3.4 Collection of specimens for TB testing

- For the purpose of the trial, the type of respiratory specimens collected can be expectorated sputum, induced sputum, and/or gastric aspirate.
- The choice of procedure will be according to local practice with the same technique used for each child for the duration of the study.
- Ideally, the collection of early morning sputum (expectorated or induced) or overnight gastric aspirate should be encouraged as much as possible.
Manuel of Procedures (MOP)  
Relevant sections (2)

• ACTG-IMPAAACT SOP for the Collection Storage and Transport of Expectorated Sputum
  – This is typically more useful in older children (>6 years of age) who are able to expectorate sputum, with or without assistance.

• Network SOPs for collection of specimens other than expectorated sputum for TB testing in children are in development

• Until these SOPs are completed, the following key elements should be observed. References are also provided.
<table>
<thead>
<tr>
<th>Procedure</th>
<th>Key Element/Critical step</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expectorated Sputum collection</td>
<td>Participant is to rinse mouth with boiled/sterile/bottled or distilled water prior to sputum collection</td>
</tr>
<tr>
<td>Expectorated/Induced Sputum collection</td>
<td>Collect at least 3 to 5 mL of sputum. If larger volumes cannot be obtained, a minimum of 1 mL is acceptable.</td>
</tr>
<tr>
<td>Gastric aspirate collection</td>
<td>Collect at least 5 to 10 mL.</td>
</tr>
<tr>
<td>Gastric aspirate collection</td>
<td>Collect the gastric content by aspiration first as lavage introduces dilution. If adequate volumes are not obtained, lavage can be performed.</td>
</tr>
<tr>
<td>Gastric aspirate collection</td>
<td>Neutralise the gastric aspirate as soon as possible after collection. Unless the laboratory is available to pH neutralise the sample within 4h of specimen collection or process it, it should be neutralised at the clinic. See LPC for details</td>
</tr>
<tr>
<td>Transport of all respiratory specimens</td>
<td>Store respiratory specimens in a refrigerator or cool box (2-8°C) if not received by the laboratory within 1 hour of collection to ensure the specimen is maintained on cold chain. Transport specimens to the laboratory in a cool box as soon as possible after collection</td>
</tr>
</tbody>
</table>
Table 2. Specimen collection references

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum (expectorated)</td>
<td>(1)</td>
</tr>
<tr>
<td>Sputum (induced)</td>
<td>(2) p.122</td>
</tr>
<tr>
<td>Gastric aspirate</td>
<td>(2) p.120, (3)</td>
</tr>
<tr>
<td>Fine needle aspirate (may be done as part of routine care)</td>
<td>(4)</td>
</tr>
</tbody>
</table>


Notes on gastric aspirate/lavage

• Collected early morning, in hospital
• Neutralisation of gastric aspirates with bicarbonates
  – Acidity is acid detrimental to mycobacteria
  – Recommended by many organisations including WHO, American Society of Microbiology
  – Unless processing < 4 h of collection: must consider time to process once in lab!
  – JCM Parashar 2013 questioning need to neutralise GA: more research needed
• Variations:
  – Gastric aspirate vs washing (saline, sterile water): aspirate preferred
  – Inpatient vs outpatient
  – Timing
  – Different neutralisation methods and formulations
    • Solution vs solid/powder form
    • Added by clinical team or by laboratory at reception
    • Measuring pH or not
Neutralisation of gastric aspirates
Refer to LPC Appendix 2

• If gastric aspirate is collected, the specimen must be pH neutralized as soon as possible after aspiration at the site of collection (in clinic), unless the laboratory is available to pH neutralize the sample within 4h of collection or process it.

• To neutralize the specimen, it is placed:
  • in a sterile container with 100 mg of sodium carbonate as per
  • Or a bicarbonate solution as per
TB laboratory technical details

• See LPC Appendix 2
• **Site specific aspects to discuss (site and lab):**
  – Neutralisation of gastric aspirates
  – Obtain isolate from routine lab and send to network lab
  – Drugs to test for DST
• **Appendix 2 Footnote K:**
  – First line drugs and second line drugs that are routinely tested by the laboratory (or drugs in the OBR) are to be tested. The following drugs should be tested at minimum: Isoniazid, Rifampicin, Ethambutol, Streptomycin, Pyrazinamide, Kanamycin (or Amikacin or Capreomycin), and a quinolone (either Levofloxacin, Ofloxacin or Moxifloxacin).
### Appendix 2: Table of TB Mycobacteriology Lab Events for P1108

<table>
<thead>
<tr>
<th>Test</th>
<th>Screening</th>
<th>Entry Day 0</th>
<th>Week 4, 8, 12, 16, 20, 24</th>
<th>Early Study D/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obtain MTB isolate from routine laboratory where diagnosis of MDR-TB was made</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeat phenotypic and genotypic DST of MTB isolate obtained from routine laboratory to confirm MDR-TB&lt;sup&gt;L-N&lt;/sup&gt;</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collection of expectorated sputum, induced sputum, or gastric aspirate&lt;sup&gt;A-B-C-O&lt;/sup&gt;</td>
<td>X&lt;sup&gt;C&lt;/sup&gt;</td>
<td>X&lt;sup&gt;E&lt;/sup&gt;</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Processing of specimens&lt;sup&gt;E&lt;/sup&gt;</td>
<td>X&lt;sup&gt;C&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Smear microscopy&lt;sup&gt;G&lt;/sup&gt;</td>
<td>X&lt;sup&gt;C&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>GeneXpert MTB/RIF&lt;sup&gt;H&lt;/sup&gt; done on sediment after processing</td>
<td>X&lt;sup&gt;C&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>BACTEC Mycobacteria Growth Indicator Tube (MGIT) culture</td>
<td>X&lt;sup&gt;C&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Solid Culture&lt;sup&gt;1&lt;/sup&gt;</td>
<td>X&lt;sup&gt;C&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Identification&lt;sup&gt;1&lt;/sup&gt;</td>
<td>X&lt;sup&gt;C&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>MGIT DST&lt;sup&gt;K-L&lt;/sup&gt;</td>
<td>X&lt;sup&gt;C&lt;/sup&gt;</td>
<td>X&lt;sup&gt;M&lt;/sup&gt;</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>MTBDRplus done on positive culture&lt;sup&gt;N&lt;/sup&gt;</td>
<td>X&lt;sup&gt;C&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Short term storage of MTBC isolate&lt;sup&gt;G&lt;/sup&gt;</td>
<td>X&lt;sup&gt;C&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Long term storage of MTBC isolate&lt;sup&gt;P&lt;/sup&gt;</td>
<td>X&lt;sup&gt;C&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

A. More than one respiratory specimen may be collected. See protocol and Manual of Procedures (MOP) for details.
Completing TB Lab CRFs

• Challenging
  – Requires understanding of the different tests done in the TB laboratory
  – Results can be complex, especially for MDR-TB
  – Drug susceptibility results can be done by different methods.
    • Example: RIF resistance tested by GeneXpert or by MGIT DST (culture based or phenotypic)
  – Variety of diagnosis algorithms
Variety of TB diagnosis algorithms

Algorithm 1. Using microscopy, solid or liquid culture, species identification and drug-susceptibility testing to diagnose TB

Algorithm 2. Using microscopy and line-probe assays in conjunction with drug-susceptibility testing (with solid or liquid media) to diagnose TB

Algorithm 3. Using the Xpert MTB/RIF assay as an initial diagnostic test for TB followed by drug-susceptibility testing for second-line anti-TB agents when necessary

Algorithm 4. Using LPA and the Xpert MTB/RIF assay as follow-up diagnostic tests to microscopy for TB with drug-susceptibility testing for second-line anti-TB agents when necessary

Specimen

Direct tests

Processing

Culture

Further tests if positive culture
Main steps for testing in the TB laboratory:

Specimen

Processing

Direct tests

- Smear
- NAAT (Xpert, LPA, etc.)
- DST

Culture

Further tests if positive culture:

- DST (phenotypic, genotypic)
- LPA
- WGS
- Genotyping

Storage of isolate
Drug susceptibility testing (DST)

• Genotypic/molecular: done on sample or on culture
  – GeneXpert
  – LPA: Hain MTBDRplus and MTBDRsl
  – Whole genome sequencing

• Phenotypic (culture-based)
  – On sample (direct) or on culture (indirect)
  – On liquid culture or on solid culture
  – Methods: proportion method (most used), absolute concentration method, and resistant ratio method

• Discordants
  – Different genotypic tests
  – Genotypic and phenotypic tests
  – Genotypic and/or phenotypic tests and clinical response
Clinical implications of molecular drug resistance testing for *Mycobacterium tuberculosis*: a TBNET/RESIST-TB consensus statement


13. If the results of molecular and culture-based drug susceptibility testing differ, what is the gold standard?

The level of discordance between molecular and culture-based DST depends on the drug and the genomic region evaluated. Despite the fact that results of phenotypic methods do not always correspond to response to clinical treatment, culture-based methods are still regarded by most experts involved in this document as the gold standard for DST.

Agreed: 13; disagreed: 0; abstained: 0.
• No perfect DST method
• For example, limitations of line probe assays

Test accuracy LPA for direct testing compared with phenotypic DST (done on sputum):
Sensitivity: 0.89 (95% CI: 0.86–0.92); specificity: 0.98 (95% CI: 0.97–0.99)

Test accuracy LPA for indirect testing compared with phenotypic DST (done on culture):
Sensitivity: 0.91 (95% CI: 0.89–0.93); specificity: 1.00 (95% CI: 0.99–1.00)

A 90% prevalence of isoniazid resistance is likely to occur in a population of MDRTB patients when a patient is diagnosed by the Xpert MTB/RIF assay

When the test (LPA) shows a negative result for isoniazid, phenotypic culture-based DST can be performed, especially in persons with a high pre-test probability of isoniazid resistance, such as patients with rifampicin-resistant TB.
MDR TB Diagnosis in P1108

• Different possibilities for diagnosis of MDRTB
• Phenotypic DST
  – MGIT and/or solid media DST confirmed MDR with isolate available/viable in network approved lab
  – MGIT and/or solid media DST confirmed MDR made in outside lab but isolate not available (from report)
• Molecular DST (done directly on specimen and/or on culture)
  – Xpert RIF R
  – Hain DRplus RIF R (± INH R)
  – Other molecular assay RIF R
• Probable TB → important to document well adult source case
4.1.5 Either confirmed or probable MDR-TB:
Confirmed intra-thoracic (pulmonary) MDR-TB, with or without one of the following forms of extrathoracic TB:
- Peripheral TB lymphadenitis
- Pleural effusion or fibrotic pleural lesions
- Stage 1 TB meningitis
- Miliary and abdominal TB,
- Other non-disseminated forms of TB disease

Rifampin mono-resistant TB (RMR-TB, routinely treated as MDR-TB), or where additional INH resistance has not been confirmed (i.e., isolated Xpert MTB/RIF rifampicin resistance) pre-XDR (MDR plus resistance to either a fluoroquinolone or a second-line injectable agent) and XDR-TB disease will be included, according to case definitions of pediatric TB described as per international consensus definitions (12) and as per local pediatric TB guidelines (35). RMR-TB, MDR-TB, pre-XDR-TB and XDR-TB are therefore collectively referred to here as “MDR-TB”, for the purposes of the protocol.

8.10 Criteria for Premature Discontinuation of Study Drug

- Participant diagnosed as having drug-susceptible TB despite initial diagnosis of DR-TB.
- Pregnancy
Completing TB Lab CRFs

• 1) Results from specimens collected before the study and sent to routine lab ➔ LBW0162
• 2) Results from specimens collected by the research team and sent to network lab ➔ LBW0163
• 3) Results from specimens collected before the study and sent to the routine lab: baseline TB isolate sent to network lab ➔ LBW0163
  – Same form used as situation 2 since testing done in the network lab
  – Select “8-Isolate from non-network laboratory for retesting” instead of specimen type
TB Lab reports

• Important to understand what tests are done
  – Contact laboratory before study
• Verify results for all specimens collected
  – Other labs? Name variations?
• Verify if final report or not
• Contact laboratory if unclear, discordants, etc.
**LBW0162: P1108 Historical TB Microbiology**

- If TB microbiology testing including drug susceptibility testing (DST) was performed on 2 or more specimens, results from all relevant specimens that document the MDR diagnosis and DST of all drugs tested must be recorded.
  - For example, if the first sputum specimen has an Xpert done and the 2nd specimen gets a Hain MTBDRplus line-probe assay and a culture, results from both specimens must be recorded on 2 separate forms.

- Complete for each child participant enrolled in study.
- Complete for any known adult MDR-TB source case(s) if the child participant is bacteriologically negative (i.e. the MDR-TB diagnosis was made clinically) at screening.
- Use one eCRF per specimen taken.
- Part A is for specimen information. Part B is for results from tests done directly on the specimen (results are usually available within 1 to 3 days). Part C is for culture results or for tests done on the culture only if the culture was positive (results usually take longer).
After training

- Site SOPs for specimen collection
- Indicate neutralisation method
- Site SOP to obtain isolate
- TB lab CRF exercise?
Acknowledgements

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