It’s all about the microbiology
- so let’s make sure it’s right

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Critical end points

Both require competent microbiology

Sputum Smear negativity

Culture negativity


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To evaluate the **efficacy**, safety and tolerability at 2 months, 12 months and 24 months in participants with Drug Sensitive and Drug Resistant TB

- Incidence of bacteriologic failure or relapse, or clinical failure at 24 months (104 weeks)
- Proportion of participants with sputum culture conversion to negative status in liquid culture at 4, 6, 12 and 17 weeks
- Time to culture negativity over 8 weeks

**Inclusion Criteria:**

- Results of AFB microscopy & molecular tests on sputum to be obtained during screening period
- If MGIT DST later shows discrepancy with molecular tests, participant may be late exclusion
What are the issues with microbiology?

**Smear**
- Missed organisms
- Miss identified
  - Artefacts
  - Other mycobacteria

**Culture**
- No growth
  - +/-
  - Time to positivity
- Too much growth
  - Contamination

False negative

False positive

False negative

Under estimate of bacterial load

Indeterminate results
Clinical diagnosis: clinical trials: discovery research
Same data - different paradigms

- All laboratories chosen are using acceptable methods for TB diagnosis
- But these methods are not necessarily standardized across laboratories
  - e.g. WHO versus American CDC reporting of smear positivity

<table>
<thead>
<tr>
<th>No. of AFBs (average over 100 fields)</th>
<th>REMoXTB Reporting</th>
<th>WHO Reporting (for conversion only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>No AFB seen (NS)</td>
<td>No AFB seen (NS)</td>
</tr>
<tr>
<td>1-9 per 100 fields</td>
<td>+</td>
<td>scanty/or actual number</td>
</tr>
<tr>
<td>1-9 per 10 fields</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>1-9 per field</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>&gt;9 per field</td>
<td>++++</td>
<td>+++</td>
</tr>
</tbody>
</table>

- Differences could introduce bias
- Limit confidence in cross-comparison of data
Rigour in delivery of microbiology

Shift in zeitgeist

‘Too difficult’ becomes essential
A comprehensive Mycobacteriology Laboratory Manual provided by the sponsor must be followed to ensure the same procedures are used across all laboratories.

- Essential for the **strength** and **integrity** of the trial data

The results generated by the laboratory must be unquestionable for the study to be a success

- Essential to ensure the **consistency** and **validity** of the results obtained

Rigorous assessment, set-up and monitoring of labs, as well as periodic data reviews (remote monitoring) are performed by the sponsor representatives
Elements of a quality framework

- Reliability of data
- Safety
- Training
The sample journey

Each step represents a challenge to reliability of results

1. Same building
   Same site
   Same town
   Same country

2. Key performance parameters

3. Data entry and verification

Result
Key performance parameters:
1. **Sample transfer & receipt**

- Temperature range for sample transfer
- Time from collection to processing

**Consequences:**
1. No growth
2. Contamination
3. Sample lost
Key performance parameters:
2. Sample processing

- Time to Zn
- Time to molecular test
- Time to inoculation in MGIT

- Flag positive
  - Time to Zn
  - Time to blood culture result
  - Time to MGIT speciation

Consequences:
1. Operational
   - Workload accumulation
   - Late exclusion of patient
2. Microbiological
   - Failure to identify contamination
Contamination rates

Acceptable range: 3 – 8%

- Contamination rates reflect the overall performance of a laboratory
- They are multi-factorial:
  - Sample handling
  - Sample type
  - Laboratory environment
  - Staff competence and professionalism
- Too low – depleting mycobacteria in the sputum, false negatives
- Too high – lost data points due to contaminated cultures
Key performance parameters:

3. Results – resolving discrepancies

- Operational
  - Data entry
  - Data verification

- Microbiological
  - Laboratory errors
  - Biological artefacts
  - Unexpected biological observations

- Missing data
  - Redundancy, more samples collected than required for ultimate analysis
Monitoring of laboratory data

• Regular standardised review of all mycobacterial data in database – undertaken remotely

• Output of this review:
  o To direct onsite laboratory monitoring
    o provides overview of laboratory performance
    o identifies areas of concern that may require additional site visits/additional training needs
  o Identify data queries (mistakes with data entry into eCRF)
  o Identify clinical sites that are not recalling patients for additional sputum sampling as required in the protocol
Example overview of laboratory visit schedule from selection to study closeout.

- Pre-study Assessment Visit
- Training Visit
- Initiation Visit
- Lab QC Visit at +5 pt's
- 1st Lab Monitoring at +50 pt's / 3 Mo
- Unscheduled Visit as needed
- Lab Monitoring every +6 Months
- Unscheduled Visit as needed
- Lab Close-Out Visit

'Cradle to grave' site supervision
No

Processes designed for Quality Improvement

Data incursions result in:
• Investigation of cause
• Plan for correction
• Monitored implementation

Success demonstrated by lack of trace in the study database
Rigorous quality management of laboratory procedures minimises uncertainty in the data.
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