Role of Mycobacteriology Laboratory in TB Clinical Trials

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Tuberculosis Trials Consortium
US Centers for Disease Control and Prevention
Pre-antibiotic era TB therapies largely consisted of excluding TB patients in sanatoria.

Development of BCG vaccine in the 1920s
- Much more effective in children
- Variable efficacy in adults

Antibiotic development in 1940s dramatically shifted the outlook for TB patients.
History of TB Drug Therapies

- Single drug therapy or monotherapy often results in resistance to TB drugs
  
  - Treatment of TB in the 1940s with streptomycin or $p$–aminosalicylic acid monotherapy resulted in resistant TB in ~70% of patients
  
  - Streptomycin and $p$–aminosalicylic acid combined therapy reduced TB resistance to ~9%

Introduction of combination therapy including streptomycin, $p$–aminosalicylic acid, and isoniazid (1952)
- Effective reduction in resistance emergence
- 18 month treatment regimen

During the 1950s and 1960s, pyrazinamide, ethambutol, and rifampicin were introduced
- Combined treatment with isoniazid shortened treatment time and helped form the standard TB regimen we have today

Current TB Drug Regimen

- **Initial Phase: 2 months**
  - Rifampicin (RIF)
  - Isoniazid (INH)
  - Pyrazinamide (PZA)
  - Ethambutol (EMB)

- **Continuation Phase: 4 months**
  - Rifampicin (RIF)
  - Isoniazid (INH)

- Two treatment phases target different populations of mycobacteria
Heterogeneous Mycobacteria Populations

- Population A
  - Rapidly multiplying (often in pulmonary cavities)

- Population B
  - Slowly multiplying (due to local adverse conditions)

- Population C
  - Sporadically multiplying (in lung granulomas)

Bactericidal and Sterilizing Effects of TB Drugs

- TB drugs have different effects on mycobacteria
  - Bactericidal
    - Kill active, relatively-fast growing mycobacteria
    - Reduces infectiousness of the patient
  - Sterilization
    - Eliminates dormant but viable bacilli
    - Effective sterilization prevents relapse
Drugs that kill population A are considered bactericidal.

TB drugs bactericidal activity:
- INH ≫ RIF ≫ EMB

Bactericidal and Sterilization Effects of TB Drugs

Drugs that kill populations B and C are considered sterilizing.

- TB drugs sterilization activity population B
  - PZA >> RIF > INH

- TB drugs sterilization activity population C
  - RIF >> INH

Reduction in mycobacteria populations are key predictors of treatment success

- Rate of reduction in bacillary load
- Time to culture conversion
- Proportion culture-negative at 8 weeks
Develop effective clinical strategies to eliminate TB

- Cure TB infections as quickly as possible without relapse
  - Shorten length of TB treatment
  - Prevent community transmission
- Improve patient compliance/adherence
- Reduce emergence of drug-resistant TB
TBTC Study 31

- Substitution of Rifapentine for Rifampicin
- Substitution of Moxifloxacin for Ethambutol
- Shortening of TB Treatment Regimen
Evaluate efficacy of a high dose rifapentine-containing regimen to determine whether the single substitution of RPT for RIF makes it possible to reduce the duration of treatment to 4 months (17 weeks)
  ◦ 2PHZE/2PH

Evaluate efficacy of a 4 month (17 weeks) regimen that substitutes a) high dose RPT for RIF and b) MOX for EMB to determine whether reduction to 4 months (17 weeks) treatment duration is possible
  ◦ 2PHZM/2PHM
S31 Schema

Screen for eligibility

Consent, enroll

Randomize 1:1:1

Regimen 1 (control) 2RHZE/4RH (26 wks)

Regimen 2 (investigational) 2PHZE/2PH (17 wks)

Regimen 3 (investigational) 2PHMZ/2PHM (17 wks)

Evaluation for primary outcome at 12 months after randomization
TB Clinical Trials are complex

- Treatment of multiple drugs lasts several months
- Site staff must watch participant taking most of the pills over several months (DOTS)
- Infection and cure does not protect from reinfection—must determine if relapse is due to reinfection or treatment failure
- Participants must return to the clinic for months or years for follow up
- Success is based on laboratory and clinical data
Importance of Mycobacteriology Labs in TB Trials

- Laboratory data is used for *more than just diagnosis*
- Laboratory data is used to *assess study outcomes*
# Lab Time and Events Schedule

<table>
<thead>
<tr>
<th>Myco Lab test and source</th>
<th>Screen</th>
<th>Base</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>17</th>
<th>22</th>
<th>26</th>
<th>Month</th>
<th>Early term. visit</th>
<th>Unscheduled visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum specimen</td>
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<td>Xpert MTB/RIF</td>
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<td>Smear microscopy</td>
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<td>Culture– solid media</td>
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<td>MTB confirmation</td>
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<td>Local storage of isolate</td>
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<td>Ship isolate to CDC</td>
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</table>
How do laboratory data affect trial endpoints?
# Mycobacteriology Laboratory Impacts on Trial Endpoints

<table>
<thead>
<tr>
<th>Primary Endpoint</th>
<th>Critical Mycobacteriology Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB disease-free survival 12 months after study treatment assignment</td>
<td>Liquid and/or solid culture, sequencing of baseline and follow-up isolates</td>
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## Secondary Endpoints

<table>
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<th>Critical Mycobacteriology Test</th>
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<tr>
<td>TB disease-free survival 18 months after study treatment assignment</td>
<td>Liquid and/or solid culture, sequencing of baseline and follow-up isolates</td>
</tr>
<tr>
<td>Time to stable sputum culture conversion</td>
<td>Liquid and solid culture</td>
</tr>
<tr>
<td>Speed of decline of sputum viable bacilli by automated liquid MGIT culture days to detection</td>
<td>Liquid culture</td>
</tr>
<tr>
<td>Proportion of participants who are culture negative at completion of 8 weeks of treatment</td>
<td>Liquid and solid culture at baseline and 8 week follow-up</td>
</tr>
</tbody>
</table>
Endpoint: Disease-free survival at 12/18 months

- Microbiologic evidence of TB disease
  - Positive culture from sputa
- Clinical evidence of TB disease
  - Symptoms, x-ray
## Endpoint: Time to culture conversion

- Culture conversion = 2 consecutive negative sputum specimens, taken ≥28 days apart

- Time to culture conversion = # days of treatment until the first of 2 consecutive negative specimens

- Lab reporting for each specimen:
  - Date inoculated
  - Date Mtb growth observed (yes/no/contaminated)

<table>
<thead>
<tr>
<th></th>
<th>Base</th>
<th>Week 2</th>
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<th>Week 8</th>
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<th>Week 17</th>
<th>Week 22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt. 1</td>
<td>7</td>
<td>10</td>
<td>84 days</td>
<td>16</td>
<td>21</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Pt. 2</td>
<td>12</td>
<td>16</td>
<td>56 days</td>
<td>18</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Pt. 3</td>
<td>5</td>
<td>7</td>
<td>119 days</td>
<td>18</td>
<td>Negative</td>
<td>18</td>
<td>Negative</td>
</tr>
</tbody>
</table>
Endpoint: Rate of change for time to detection

- Time to Detection (TTD) = number of days between inoculation and detection of growth on MGIT
- With treatment, bacillary load is reduced and TTD increases
- Lab reporting for each specimen:
  - Date of positive culture

![Graph showing Time to Positivity with Study Treatment](image)
Endpoint: Proportion who are culture negative at 8 weeks

- For all participants in each arm, the proportion that have a negative culture from sputa collected at the 8-week visit
- Solid and liquid media analyzed separately
- Lab reporting for each specimen:
  - Culture outcome for specimen collected at 8 wk visit
# A tale of identical specimens at BASELINE

<table>
<thead>
<tr>
<th></th>
<th>Lab A</th>
<th>Lab B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transport Time</strong></td>
<td>1 hour</td>
<td>3 days</td>
</tr>
<tr>
<td><strong>Transport Temp</strong></td>
<td>4°C</td>
<td>21°C</td>
</tr>
<tr>
<td><strong>Decontamination</strong></td>
<td>1.5% NaOH</td>
<td>2% NaOH</td>
</tr>
<tr>
<td><strong>Centrifuge</strong></td>
<td>3000 x g at 4°C for 20 min.</td>
<td>3000 x g, ambient temp. for 15 min. with cold PBS</td>
</tr>
<tr>
<td><strong>Resuspension vol.</strong></td>
<td>1.5 mL</td>
<td>2.5 mL</td>
</tr>
<tr>
<td><strong>MGIT inoculum</strong></td>
<td>0.5 mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td><strong>Baseline TTD</strong></td>
<td>7 days</td>
<td>12 days</td>
</tr>
</tbody>
</table>
Controlling what we can control

- Every Myco Lab perfected their system for local diagnostic mycobacteriology
  - Lab A = 7 days → report “positive culture” to physician
  - Lab B = 12 days → report “positive culture” to physician

- For diagnostic purposes, Labs A and B do the same thing ➔ patient has TB, needs treatment

- For a *clinical trial*, comparing results from Labs A and B could affect the trial outcome

- Aim to control factors that introduce variability between labs and study participants
## A tale of identical specimens after 8 weeks of study treatment

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</tr>
<tr>
<td><strong>8 week TTD</strong></td>
<td>21 days</td>
<td>Negative Culture</td>
</tr>
</tbody>
</table>
What happened to skew TTD?

- Longer transport time and higher temperatures allow for contaminants to grow.
- Contaminants will:
  - Grow faster and compete for nutrients in culture.
  - Lead to false positive MGIT results.

Lab A
1 day at 4°C

Lab B
3 days at 21°C
Transit time at RT affects culture results

Longer transport time = ↓ rate of positivity and contamination

Reference: Ken Jost, Texas State Department of Health
NaOH during processing kills contaminants and mycobacteria

- Too low and contaminants flourish
- Too high and too many mycobacteria are killed

Lab A 1.5% NaOH

Lab B 2.0% NaOH
After weeks of treatment, sputum volume or number of mycobacteria may be very low.

Key time points for measuring drug efficacy:

- **1.0-1.5% NaOH**
- **≥2.0% NaOH**

**NaOH (pH) effect with low bacillary load**

- **Positive** MGIT
- **Negative** MGIT
- **False Positive** MGIT
NaOH (pH) affects culture results

- Final pH of the specimen concentrate greatly affects the recovery and TTD
  - High pH will lower the positivity rate and increase the TTD of positive culture
  - High pH may also cause false fluorescence (false positive)

- MGIT was validated and cleared by FDA for use with 1.0 to 1.5% NaOH
Resuspension volume can dilute the mycobacteria
- If number of mycobacteria are high, not an issue
- If number is low, no mycobacteria may be inoculated on media

Lab A
1.5 mL

Lab B
2.5 mL
How endpoints are affected by variation in Myco Lab methods

### Culture Conversion

<table>
<thead>
<tr>
<th>Lab A</th>
<th>Base</th>
<th>Week 2</th>
<th>Week 4</th>
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<td>18</td>
<td>Negative</td>
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</tbody>
</table>

**Mean=87 days**

<table>
<thead>
<tr>
<th>Lab B</th>
<th>Base</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 8</th>
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</tr>
<tr>
<td>Pt. 2</td>
<td>14</td>
<td>19</td>
<td>96 days</td>
<td></td>
<td>Negative</td>
<td>21</td>
<td>Negative</td>
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<tr>
<td>Pt. 3</td>
<td>8</td>
<td>28 days</td>
<td>15</td>
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<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
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</tbody>
</table>

**Mean=60 days**
How endpoints are affected by variation in Myco Lab methods

Proportion with Negative Culture Results at 8 Weeks

Lab A

30% Negative

Lab B

70% Negative
How do identical specimen analogy affect endpoints?

- Time to Culture Conversion
  - Lab A = 12 weeks
  - Lab B = 8 weeks

- Proportion negative at 8 weeks
  - Lab A = 0/1
  - Lab B = 1/1

- Bias towards drug efficacy at Lab B

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 6</th>
<th>Week 8</th>
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</tr>
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<tbody>
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<td>Lab A</td>
<td>7</td>
<td>10</td>
<td>16</td>
<td>20</td>
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</tr>
<tr>
<td>Lab B</td>
<td>12</td>
<td>16</td>
<td>18</td>
<td>25</td>
<td>Negative</td>
<td>Negative</td>
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</tbody>
</table>
Centrifugation is used to sediment mycobacteria
- Speed too low and mycobacteria are still floating and will be discarded
- Too high and mycobacteria can be damaged or killed

Centrifugation temperature keeps mycobacteria happy
- Too high and mycobacteria will be damaged or killed

<table>
<thead>
<tr>
<th>Lab A</th>
<th>3000xg @ 4°C</th>
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<tbody>
<tr>
<td>Lab B</td>
<td>3000xg @ 21°C</td>
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</tbody>
</table>
# A tale of identical specimens after 8 weeks of study treatment

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<tr>
<td>Transport Time</td>
<td>1 hour</td>
<td>3 days-1 hour</td>
</tr>
<tr>
<td>Transport Temp</td>
<td>4°C</td>
<td>21°C 4°C</td>
</tr>
<tr>
<td>Decontamination</td>
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<td>2%-1.5% NaOH</td>
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</tr>
<tr>
<td>MGIT inoculum</td>
<td>0.5 mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Baseline TTD</td>
<td>7 days</td>
<td>~7 days</td>
</tr>
<tr>
<td>8 week TTD</td>
<td>21 days</td>
<td>~21 days</td>
</tr>
</tbody>
</table>
Labs have different resources and constraints, and (realistically) every step will not be the same.

GCLP helps to ensure that labs participating in a trial have minimized the variability among sites.

Key Elements in SOPs at all sites:
- All labs across the network use the same Key Elements in their SOPs
- Minimizes variability
Key Elements of Mycobacteriology Laboratory Procedures

- Key Elements were designed to:
  - Reduce variability across mycobacteriology labs
  - Provide high quality data to assess study outcomes

- Adherence to Key Elements provides confidence in laboratory data and resulting study outcome assessment
# Key Elements

## Sputum Collection and Transport

- The participant is advised to rinse his/her mouth with water (boiled/bottled/sterile) prior to sputum collection.

- At least 3 – 5 mL of sputum is collected; however, if larger volumes cannot be obtained, a minimum of 1 mL is acceptable.

- Sputum induction is performed when the spontaneously expectorated sputum volume collected is less than 1 mL.

- The sputum specimen is stored in a refrigerator or cool box if not transferred to laboratory within 1 hour of collection.

- Transport the sputum specimen to the laboratory in a cool box with ice packs as soon as possible and within 24 hours after collection.

- If refrigeration is not accessible, specimens collected by participants at home must be kept as cool as possible and delivered to the laboratory within 2–3 hours of collection.
### Key Elements

#### Sputum Receipt and Processing

- The specimen must be refrigerated at 2–8°C unless it is processed within 1 hour of receipt by the laboratory.

- Sputum specimens must be decontaminated with a final sodium hydroxide (NaOH) concentration of 1–1.5%.

- Sputum specimens must be processed in a centrifuge capable of generating a relative centrifugal force (RCF) of 3000–3500g (centrifuge must be calibrated annually per manufacturer's instructions).

- Use of a refrigerated centrifuge is preferred.

- During specimen processing, the sputum is decontaminated in NaOH for 15–20 minutes before adding buffer. Do not exceed 20 minutes.

- The digested and decontaminated sputum must be washed and re-suspended in 1.5 mL Phosphate Buffered Saline (PBS) pH 6.8.

- Positive and negative controls must be included in each processing batch.
### Key Elements

<table>
<thead>
<tr>
<th>LJ Solid Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Inoculate each LJ slope/plate with (200 \mu\L) of the decontaminated sputum sediment.</td>
</tr>
<tr>
<td>- Solid media must be incubated for at least 8 weeks before being reported as negative.</td>
</tr>
<tr>
<td>- Appropriate controls (e.g., containing low amounts of MTB) must be tested before each batch of media is used, regardless if purchased commercially or prepared in-house.</td>
</tr>
<tr>
<td>- All positive solid media must be examined by ZN staining and subcultured on blood agar to confirm purity.</td>
</tr>
<tr>
<td>- Semi-quantitative colony counts must be reported according to the WHO/IUATLD reporting scheme for solid cultures.</td>
</tr>
<tr>
<td>- Growth of MTB complex must be confirmed using an appropriate identification test method (e.g., PNB or MPT64 TB Antigen Test).</td>
</tr>
</tbody>
</table>
### Key Elements

**MGIT Culture**

- All MGIT cultures (positive and negative) are worked up according to the FIND MGIT Manual.
- MGIT cultures must be inoculated with 0.5 mL of the decontaminated sputum sediment.
- All positive MGIT cultures must be examined by ZN staining and subcultured on blood agar (BAP) to confirm purity.
- The machine generated time-to-positivity (TTP) must be recorded for all positive MGIT cultures.
- Growth of MTB complex must be confirmed using an appropriate identification test method (e.g., PNB or MPT 64 TB Antigen Test).
Role of Your Myco Lab in TB Trials

- Fast and accurate screening of participants
- Quality Mycobacteriology Testing using Key Elements
- Accurately reporting Mycobacteriology test results
- Shipping isolates
- Retaining accurate and auditable source documents
Laboratories Control the Success of TB Clinical Trials

- Questions?