Mycobacterium tuberculosis
Case Study

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Microbiologist
WSLH
Coming up next...

1. Background on the patient
2. Patient’s *M. tuberculosis* infection chronology
3. Treatment and follow up testing
4. Ana Guaracao Ph.D  summer study
5. Significance of evaluating *M. tuberculosis* cell viability
6. Review of new assays on development to assess *M. tuberculosis* cell viability
Background on the patient

Demographics:
- Age: 58 yrs
- Father: Native American-Alaskan
- Mother: Caucasian

Life style:
- Vietnam veteran
- Heavy smoker
- Recovering Alcoholic
- Previous use of Cocaine
- Hobbies: Native American rituals
  Motorcycle riding
- Lives with mother and brother

Medical history:
- Post Traumatic stress disorder
- Hepatitis C
- Sinusitis
- Ankylosing spondylitis
From Motorcycle Accident to TB infection

April 16, 2004: Motorcycle accident
- Concussion
- Broken nose
- Memory loss
- Pain in left anterior chest
- Hemoptysis
- Subjective fevers
- Sputum blood tinged
- SPOT in LUNG

5 months

September 09, 2004: Pulmonologist appointment
- Lung lesion increased

September 22, 2004: BAL collected
- Smear Positive (2+)

September 23, 2004: Admitted to the hospital as presumptive TB pt
Treatment and Follow up Testing

1st line drugs
-IIRE and PZA

September 23 - 1st week of April

6 sputum collected
5 smear negative
1 smear positive
1 culture positive

2010
Diagnosed *M. avium complex*
# Follow up Testing

<table>
<thead>
<tr>
<th>Specimens</th>
<th>DOC</th>
<th>SMR</th>
<th>TB/MAC PCR</th>
<th>MGIT</th>
<th>TB/MAC PCR (from MGIT)</th>
<th>CULTURE</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>4/4/2015</td>
<td>+ Few AFB</td>
<td>TB CT=34.30</td>
<td></td>
<td>TB CT=0</td>
<td>TB NEGt</td>
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<td></td>
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<tr>
<td>2</td>
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<td>4/17/15</td>
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<td>-</td>
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<tbody>
<tr>
<td>10</td>
<td>6/24/2016</td>
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<td>8/5/16</td>
<td>TB CT=0 MAC CT=0</td>
<td>MAC POST</td>
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</table>
Using PCR CT values to predict viability

Ana Guaracao Ph.D
Senior microbiologist at WSLH

<table>
<thead>
<tr>
<th></th>
<th>1st TB PCR</th>
<th>Days between PCRs</th>
<th>2nd TB PCR</th>
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</thead>
<tbody>
<tr>
<td>Average for 31 Positive TB cultures</td>
<td>Ct=25.18</td>
<td>19</td>
<td>Ct= 18.6</td>
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<tr>
<td>Average for 5 Negative TB cultures</td>
<td>Ct=37.2</td>
<td>25</td>
<td>Ct= 28.38</td>
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<tr>
<td>Average for 36 total cultures</td>
<td>Ct=26.85</td>
<td>20</td>
<td>Ct= 19.95</td>
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</table>

- Decrease in Ct values between 1st and 2nd PCR are not indicative of viable M. tuberculosis.
- Include more specimens for more statistically significant data.
- Continue this study including Follow-Up specimens to compare CT values from before and after commencing treatment.
Significance of Evaluating *M. tuberculosis* Viability

1. Assessing treatment response

2. Minimize toxicity

3. Prevent development of drug resistance
Ongoing Research on Cell Viability Assay Development

1. *M. tuberculosis* Viability by PMA Real-Time PCR

2. *M. tuberculosis* Viability by Microscopy (Fluorescein Diacetate (FDA))
3. Assessing *M. tuberculosis* viability by Flow Cytometry
4. Nile-Red labeled Auramine-O staining smear
**M. tuberculosis Viability by PMA Real-Time PCR**

**Advantages**
- Comparable with NALC-NaOH
- Used in primary specimens

**Disadvantage**
- Cells with compromised membrane integrity
**M. tuberculosis Viability by Microscopy**

**Fluorescein Diacetate (FDA)**

- Does not require sophisticated laboratory infrastructure
- Used as early indicator of poor treatment response
- Permits early identification of drug resistance

**Advantages**

**Disadvantage**

- Only useful to assess sputum from smear positive pulmonary TB pt
- Requires trained lab personal
- Does not identifies the cause of treatment failure
Assessing *M. tuberculosis* viability by Flow Cytometry

**Calcein-violet with Acetoxy-Methyl Ester**
- permeates cell membranes
- intake or efflux depends on growth rate

**Sytox Green**
- penetrates through damaged cell membranes
- binds to DNA

M. tuberculosis In vitro studies show:

- MTB becomes persistent
- Metabolic downregulation
- Stops replication
- Decrease ATP production
- Shifts energy from carbohydrates to fatty acids

Tuberculosis 2015 95, 770-779 DOI: (10.1016/j.tube.2015.08.001)


