-SPUTUM-

THE BREAD AND BUTTER OF THE TB LABORATORY

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Sputum Quality

Guidelines and Algorithms for Sputum Collection
Phases of Testing

- Preanalytical
- Analytical
- Postanalytical
Specimen Quality

- **The accuracy of laboratory results are directly related to the quality of the specimen**
- **GOOD sputum**
  - Recently-discharged material from the bronchial tree, with minimal amounts of upper respiratory tract secretions
Specimen Quality

• **Sputum Collection**
  – Requires trained, dedicated individuals

• **Direct observed collection (DOC)**
  – Assures optimal specimens
  – Aids with “uncooperative” patients
    • Patients who want to avoid isolation
    • Cultural issues

• **Sputum induction for patients who cannot produce sputum**
  • Induced sputum resembles saliva
    – Important to label as “induced”
Collection Kits
WSLH Sputum Collection Kit
Storage and Transport

• Specimen storage
  – Refrigerate if not transported to the lab within 1 hour

• Transport
  – Package according to current DOT regulations
  – Ship with refrigerant (e.g. Cool Paks)
  – Preferably overnight delivery
Laboratory Role in Specimen Quality

- Establish a working relationship with the health care personnel who collect patient specimens
- Provide detailed instructions in collection kits
  - Appropriate number of specimens
  - Explain difference between saliva and sputum
  - Need for deep productive cough
  - Rinsing the mouth prior to collection
  - Specimen volume—Optimal 5-10 ml; not less than 3 ml
  - Avoid contamination of the outside of the tube
  - Seal container tightly
Our laboratory asks that specimens be...

1. Shipped with refrigerant
2. Shipped at ambient temperature
3. Shipped by pony express
Specimen Quality Evaluation in the Laboratory

SPIT-ON-A-STICK

REJECT
Specimen Quality Evaluation in the Laboratory

- Assess volume
  - >3 ml

- Time between collection and receipt
  - Should not be > 7 days

- Saliva vs Sputum?
Can we evaluate using the Gram Stain?
Gram Stain Evaluation of Sputum Specimens for Mycobacterial Culture

<table>
<thead>
<tr>
<th>No. of Specimens</th>
<th>Score</th>
<th>M. tb Isolated</th>
<th>NTM Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>115</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>29</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>77</td>
<td>3</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>39</td>
<td>4</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>70</td>
<td>5</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total 400</strong></td>
<td></td>
<td><strong>25</strong></td>
<td><strong>9</strong></td>
</tr>
</tbody>
</table>

Scores

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Not sufficient cells or mucus to make a judgment</td>
</tr>
<tr>
<td>1 or 2</td>
<td>Salivary in origin</td>
</tr>
<tr>
<td>3 to 5</td>
<td>Representative of material from the lower respiratory tract</td>
</tr>
</tbody>
</table>

Contra Costa County Public Health Laboratory
Does your laboratory reject specimens that have a volume of...

1. Less than 3 ml
2. Less than 2 ml
3. Less than 1 ml
4. Less than 0.5 ml
5. If we can see it, we process it
Does your laboratory reject sputa that are...

1. More than 7 days post-collection
2. More than 5 days post-collection
3. Other time criteria
4. Don’t reject sputa based on time post-collection
Does your laboratory reject sputa based on gross appearance i.e. if they look like saliva

1. Yes
2. No
Sputum Collection Guidelines
Initial Diagnostic Specimens—CDC Guidelines

• 3 sputum specimens
  – 8-24 hours apart
  – At least one of which is an early morning specimen
  – Additional specimens may be needed if specimens are smear negative

• Optimally, collect prior to initiation of drug therapy

• Test by both concentrated smear and culture
  – Report smear results within 24 hours
### How Many Sputum Specimens are Enough?

**TABLE 2.** Frequency distribution of the first positive specimen for patients from whom three or more specimens were collected for AFB smear and culture

<table>
<thead>
<tr>
<th>Collection order of specimen</th>
<th>Smear negative</th>
<th>Smear positive&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>39 (61)</td>
<td>41 (73)</td>
<td>41 (73)</td>
</tr>
<tr>
<td>Second</td>
<td>22 (34)</td>
<td>11 (20)</td>
<td>8 (14)</td>
</tr>
<tr>
<td>Third</td>
<td>3 (5)</td>
<td>4 (7)</td>
<td>4 (7)</td>
</tr>
<tr>
<td>Fourth or later</td>
<td>0 (0)</td>
<td>3 (6)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers are for the first culture–positive specimens for all patients, both smear positive and smear negative.

<sup>b</sup> At least one specimen obtained from each member of this group of patients was smear positive.

NAA Testing

• CDC guidelines
  – NAA testing on first sputum of all patients suspected of TB for whom the test result would alter case management or TB control activities
    • NAA should not be ordered routinely when clinical suspicion of TB is low.

• *Can NAA reduce the number of sputum specimens needed?*
### NAA Testing and Culture

**Processing:** 5 days; NAAT 4 days; broth medium monitored 7 days

NAAT (first specimen) - AFB smear and culture (3 specimens) - 797 pt [81 TB]  
*All induced sputa*

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>NPV</th>
<th>Mean TAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB Smear</td>
<td>70</td>
<td>98</td>
<td>79</td>
<td>96.7</td>
<td>1</td>
</tr>
<tr>
<td>NAAT</td>
<td>90</td>
<td>100</td>
<td>100</td>
<td>98.9</td>
<td>2</td>
</tr>
<tr>
<td>Culture x 3</td>
<td>96</td>
<td>100</td>
<td>100</td>
<td>99.6</td>
<td>18</td>
</tr>
</tbody>
</table>

Testing for Release from Isolation

- **Criteria for release from isolation**
  - Adequate course of therapy for minimum of 2 weeks
  - Clinical evidence of improvement
  - 3 consecutive **SMEAR NEGATIVE** sputa or bronchial secretions collected on different days
    - Collect at 8-24 hour intervals, with at least one sputum being an early morning specimen

- **Performance of culture is not necessary on these specimens**
Our laboratory offers a test that is smear only (without culture)...

1. No
2. Yes, but it is not utilized
3. Yes, and it is utilized
4. Yes, but it’s often ordered incorrectly
Monitoring the Course of Treatment

• Obtain specimens at least monthly (for patients with positive cultures before therapy) until the cultures convert to negative
  – If sputum culture becomes contaminated, request a new specimen to avoid gaps in patient monitoring
Monitoring the Course of Treatment

- **MDR-TB patients**
  - Perform cultures monthly for the entire course of treatment
  - Do not release from isolation until 3 consecutive sputa are **CULTURE NEGATIVE**
Monitoring the Course of Treatment

- Patients whose smears or cultures have not become negative or symptoms do not resolve after 3 months therapy should be reevaluated for potential drug-resistance as well as for potential failure to adhere to the treatment regimen
  - Repeat DST

- Consider consultation with the Regional Training and Medical Consultation Center
Monitoring the Course of Treatment

• Second-line DST should be considered ASAP if patient:
  – Has had prior therapy
  – Contact of patients with MDR-TB
  – From a country with endemic MDR-TB
  – Isolate resistant to rifampin or other first-line drugs
  – Has positive cultures after 3 months or more of therapy
Acknowledgements

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