OPTIMAL NUMBER OF SAMPLES REQUIRED TO DIAGNOSE TUBERCULOSIS BY SPUTUM CULTURE AMONG HIV INFECTED SMEAR NEGATIVE SUSPECTS IN KAMPALA, UGANDA

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Introduction

**SPUTUM CULTURE** is recommended for diagnosis of tuberculosis (TB) among HIV infected smear negative TB suspects by W.H.O.

However, it remains unclear as to how many samples are required for culture.
Problem statement

Several studies have documented the usefulness of sputum culture among smear negative HIV infected patients with different prevalence.

- Uganda 56% (Kyeyune R et al., 2010),
- Thailand and Vietnam 63% (Patama Monkongdee., 2009)
- Zimbabwe 39.4% (Dimairo Munyaradzi et al., 2010)

The concern is how many sputum samples should be submitted per TB suspect to maximize efficiency?
Study objectives

To determine the incremental yield and the number of sputum samples needed to find one additional TB case by culture on the second and third sputum samples among HIV infected smear negative TB suspects during a cross sectional study in Kampala, Uganda.

- TB Expert opinion: (maximum number of samples given for the hypothesis)
  - Second sputum culture 12
  - Third sputum culture 17
Study setting and study population

- **Study design:** Cross-sectional

- **Study setting:** Three HIV care clinic and one general TB ward of the National referral hospital in Kampala Uganda.

- **Population:** 203 participants screened.

- **Eligibility:** Males and females, HIV-infected smear negative TB suspects, 18yrs and above.

- **Enrolled:**
  - Consented participants consecutively from routine HIV clinic visits.
Laboratory Methods

- **Sample collection:** Spot – Morning – Spot system.
  - Transported in ice box to the TB culture lab.

- **Laboratory procedures**
  - Repeat smear microscopy ( ZN)
  - Sputum processing ( NAOH/NALC)
  - Culture ( LJ and MGIT 960)
  - Identification using CapiliaTM Neo
Quality Assurance

- **Pre-analytical:**
  - Sample quality and quantity.

- **Analytical:**
  - ATCC H37RV#27294,
  - ATCC#12478 M. kansasii,
  - ATCC #6841 M. fortuitum
  - Artificial sputum, +ve and –ve control in each 12 sample batch.

- **Post analytical: Results cross-checking.**
Data Analysis

- **STATA SE version 10**

- Descriptive for relative frequencies.
  - **A TB case** was defined as: having at least one positive sputum culture for MTBC.
  - **Optimal number:** The number that detects 90% of the TB cases.

- **Incremental yield and number of samples needed** was according to *Ipuge Y et al.*, using an already developed excel database that was also used in previous studies (*Katamba A et al., 2007, Rieder HL, 2005*).
RESULTS

BASLINE CHARACTERISTICS

- Enrolled 203 participants.
- 22 (10.8%) did not consent.
- 11 (5.4%) SM+ on repeat.
- 20 (11.7%) failed to submit the 2nd and 3rd sputum samples.
- 461 (92.0%) sputum samples were obtained.
- 69 (16.3%) were contaminated by MGIT.
- 62 (36.5%) were culture positive.
Table 1: Prevalence of pulmonary tuberculosis (PTB) among sputum smear negative HIV infected TB suspects (n= 170), Kampala (March 2011)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Culture positive/ TB cases ( n=62/36.5%)</th>
<th>Culture Negative (n = 108)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender: Females</strong></td>
<td>34 (54.8%)</td>
<td>68 (63.0%)</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td>28 (45.2%)</td>
<td>40 (37.0%)</td>
</tr>
<tr>
<td><strong>Age: Mean ( SD)</strong></td>
<td>36.5 (9.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Median ( IQR)</strong></td>
<td>35 (32-37)</td>
<td></td>
</tr>
<tr>
<td><strong>Age group, years</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-30</td>
<td>21 (33.9%)</td>
<td>31 (28.7%)</td>
</tr>
<tr>
<td>31-40</td>
<td>24 (38.7%)</td>
<td>45 (41.7%)</td>
</tr>
<tr>
<td>41-50</td>
<td>13 (21.0%)</td>
<td>21 (19.4%)</td>
</tr>
<tr>
<td>51-60</td>
<td>4 (6.5%)</td>
<td>7 (6.5%)</td>
</tr>
<tr>
<td>61-70</td>
<td>0</td>
<td>2 (1.9%)</td>
</tr>
<tr>
<td><strong>Unknown</strong></td>
<td>0</td>
<td>2 (1.9%)</td>
</tr>
</tbody>
</table>
Table 2: Distribution of sputum sample culture results for the six patterns of results according to methods used, Kampala (March 2011)

<table>
<thead>
<tr>
<th>Pattern of Result</th>
<th>LJ Alone n (%)</th>
<th>MGIT Alone n (%)</th>
<th>LJ &amp; MGIT Combined n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Px</td>
<td>36 (21.2)</td>
<td>44 (25.9)</td>
<td>52 (30.6)</td>
</tr>
<tr>
<td>NPx</td>
<td>5 (2.9)</td>
<td>13 (7.7)</td>
<td>7 (4.2)</td>
</tr>
<tr>
<td>NNP</td>
<td>2 (1.2)</td>
<td>3 (1.7)</td>
<td>3 (1.7)</td>
</tr>
<tr>
<td>NNN</td>
<td>82 (48.2)</td>
<td>67 (39.4)</td>
<td>67 (39.4)</td>
</tr>
<tr>
<td>NN9</td>
<td>29 (17.1)</td>
<td>26 (15.3)</td>
<td>24 (14.1)</td>
</tr>
<tr>
<td>N99</td>
<td>16 (9.4)</td>
<td>17 (10.0)</td>
<td>17 (10)</td>
</tr>
<tr>
<td><strong>Total patterns /patients:</strong></td>
<td><strong>170</strong></td>
<td><strong>170</strong></td>
<td><strong>170</strong></td>
</tr>
</tbody>
</table>

10/28/2011
Table 3: Yield of first and Incremental yield of the second and third serial sputum culture among HIV infected suspects, Kampala (2011)

<table>
<thead>
<tr>
<th>Missed TB cases %¥</th>
<th>LJ method</th>
<th>MGIT method</th>
<th>LJ &amp; MGIT method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positives missed by not doing a 2nd sputum culture</td>
<td>0.7</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Positives missed by not doing a 3rd sputum culture</td>
<td>1.1</td>
<td>1.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Total positives missed</td>
<td>1.8</td>
<td>3.8</td>
<td>2.9</td>
</tr>
<tr>
<td>Expected yield from 3 sputum cultures (Exp)π</td>
<td>44.8</td>
<td>63.8</td>
<td>64.9</td>
</tr>
<tr>
<td><strong>Expected Yield ( Fraction)†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed pattern (n) + missed TB cases /Exp</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Expected from the first sputum culture‡₁</td>
<td>0.805</td>
<td>0.690</td>
<td>0.801</td>
</tr>
<tr>
<td>Expected from second sputum culture ‡₂</td>
<td>0.127</td>
<td>0.236</td>
<td>0.126</td>
</tr>
<tr>
<td>Expected from third sputum culture ‡₃</td>
<td>0.068</td>
<td>0.075</td>
<td>0.073</td>
</tr>
</tbody>
</table>

¥ = Number of estimated TB cases missed among those who never submitted the 2nd and 3rd sputum sample for culture, assuming a same proportion of TB cases as in those who submitted the 2nd and 3rd sputum for culture, respectively. † = The proportion of TB cases expected if each of the suspects had a complete set of three sputum cultures, \( \Pi = \text{Total positives observed + total positives missed} \), \( \hat{\xi}_1 = P x / \text{Exp} \), \( \hat{\xi}_2 = N P x + \text{missed by second} / \text{Exp} \) and \( \hat{\xi}_3 = N N P + \text{missed by third} / \text{Exp} \).
Number of sputum cultures required to detect one additional TB case on the second and third sputum culture using LJ or MGIT method (95%CI)

- **LJ Method:** 29.9 (16.6, 156.5) and 55.6 (26.4, 500.4)

- **MGIT Method:** 11.3 (7.6, 21.9) and 35.7 (19.0, 313.8)

- **LJ plus MGIT methods:** 20.8 (12.5, 62.7) and 36.1 (19.1, 330.9)

**TB Expert opinion: (maximum)**
- Second sputum culture 12
- Third sputum culture 17

CONCLUSIONS

- Among HIV infected smear negative TB suspects in Kampala Uganda, more than 93% of TB cases are diagnosed with two serial sputum sample cultures, two sputum sample cultures are optimal for TB diagnosis.

- On average, 15 and 35 sputum culture specimens need to be examined to identify one TB case on second and third sputum sample using LJ and or MGIT methods respectively.

- We recommend resource limited settings to consider two sputum samples for culture, however, a cost-effectiveness analysis of these findings in a similar setting in needed.
Acknowledgements

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