Improvement of microscopic screening of pulmonary tuberculosis in low-income countries

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> 1/3 cases in the South-East Asia Region

DOTS strategy (1995)
Directly Observed Treatment Short course

5 key components:
- Government commitment to sustained TB control activities
- Case detection by sputum smear microscopy among symptomatic patients self-reporting to health services
- Standardized treatment regimen of 6-8 months
- A regular, uninterrupted supply of all essential anti-TB drugs
- A standardized recording and reporting system

Sputum smear microscopy
- Ziehl-Neelsen staining
- The most cost-effective method of pulmonary TB screening
- Rapid, specific, inexpensive
- Can detect AFB > 10^4/ml sputum = highly infectious TB cases
- But low sensitivity < 50%

Factors limiting the case detection of pulmonary TB in Lao PDR

- Sensitivity of sputum smear microscopy: 47% in 2007
- Constraint of international criteria for positivity: ≥ 2 sputum samples/3 AFB+
- Distance to laboratory screening
- Quality problems: materials, techniques, technicians. Culture of mycobacteria not performed
- Prevalence of HIV co-infection (3.3% of incident TB cases): decrease the sensitivity of smear microscopy < 20%
How to improve detection of pulmonary TB by 2015 in low income countries?

- Increase the number of samples:
  - 3 consecutive sputum: sensitivity 2-5%
  - 2 sputum on the same day: workload performances

- Increase the contrast:
  - Fluorescence examination: sensitivity 10%
  - Limits: costs and stability of reagents

- Increase the concentration of AFB in sputum:
  - Liquefaction (sodium hypochlorite) + centrifugation

The bleach method

1. Add a solution of NaOCl 5% to the sputum (vol / vol) in the vial
2. Mix thoroughly and incubate for 15 min at room temperature
3. Transfer 2 to 15 ml in a conical tube with distilled water (vol / vol)
4. Centrifuge at 2000 rpm for 15 min
5. Put a drop of the pellet on a slide dry, fix and stain with Ziehl Neelsen

First study in Lao PDR, 2008
(612 patients – 1675 sputum samples)

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The bleach method improves the detection of pulmonary tuberculosis in Laos
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Design of the study

- 3 months: central lab
  - Mahosot hospital
  - 560 patients
  - 1552 samples

- 1 month: peripheral lab
  - Attapeu hospital
  - 52 patients
  - 123 samples

Double blind reading

2 independent microscopists
20 mlfrottis (200 champs)

Matching of results

Agreement

Discrepancy

Validation

3rd reading

Contradictory

Semi-quantitative scale of the IUATLD

<table>
<thead>
<tr>
<th>Number of AFB</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>No AFB* on at least 100 HPF**</td>
<td>0</td>
</tr>
<tr>
<td>1-9 AFB / 100 HPF</td>
<td>±</td>
</tr>
<tr>
<td>10-89 AFB / 100 HPF</td>
<td>+</td>
</tr>
<tr>
<td>&gt;10 AFB / HPF on at least 50 HPF</td>
<td>++</td>
</tr>
<tr>
<td>&gt;10 AFB / HPF on at least 20 HPF</td>
<td>+++</td>
</tr>
</tbody>
</table>

* AFB: acid-fast bacilli
** HPF: high-power microscopic field

First study in Lao PDR, 2008
(612 patients – 1675 sputum samples)
Qualitative results

<table>
<thead>
<tr>
<th>Smear microscopy</th>
<th>Direct method</th>
<th>Bleach method</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>1469 (87.7)</td>
<td>1400 (83.6)</td>
</tr>
<tr>
<td>positive</td>
<td>206 (12.3)</td>
<td>275 (16.4)</td>
</tr>
<tr>
<td>Total</td>
<td>1675 (100)</td>
<td>1675 (100)</td>
</tr>
</tbody>
</table>

p = 0.0007

Quantitative results (according to the semi-quantitative scale of UICTMR)

- 1-9/100
- 10-99/100
- 1-10/0
- >10/1

9 serial samples from a HIV+ patient

Second study in Lao PDR, 2009

Application de la méthode de fluidification-centrifugation à l’eau de Javel au dépistage de la tuberculose pulmonaire chez les patients infectés par le VIH en RDP LAO

Provisional results (60 patients – 121 samples)

<table>
<thead>
<tr>
<th>Culture</th>
<th>Smear microscopy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct method</td>
<td>Bleach method</td>
</tr>
<tr>
<td></td>
<td>neg</td>
<td>pos</td>
</tr>
<tr>
<td>negative</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>positive</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>107</td>
<td>14</td>
</tr>
</tbody>
</table>
Cost-effectiveness analysis

<table>
<thead>
<tr>
<th>Effectiveness</th>
<th>direct method</th>
<th>bleach method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - suspect patients</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>2 - patients AFB+</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Costs (US$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 - unit (one patient)</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>4 - total (60 patients)</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>5 – cost-effectiveness (4/2)</td>
<td>8.6</td>
<td>8.2</td>
</tr>
<tr>
<td>6 – marginal cost-effectiveness (4/4 of 2)</td>
<td>7.5</td>
<td></td>
</tr>
</tbody>
</table>

Bleach method: advantages

- inexpensive, available everywhere
- easy to implement in peripheral and poorly equipped laboratory
- does not require special technical skills
- better sensitivity and negative predictive values, than direct method
- improves the yield of case detection, especially for paucibacillary samples
- reduces the number of samples to examine
- reduces the risk of laboratory contamination

Bleach method: disadvantages

- Lack of standardization:
  - what concentration of the solution of NaOCl? 2 to 5%
  - how long the incubation period? 15 min ± ?
  - how long and what speed centrifugation? 15 min, 2000 rpm ± ?
- Additional costs and delays:
  - centrifuge and disposable (conical centrifuge tubes)
  - preparation 30 min before staining
- samples must be doubled if culture:
  (sputum treated with the bleach can not be then cultured)
- Technician Training & Quality Assurance

Conclusion

The bleach method can improve the case finding of pulmonary TB in low income countries, especially
- when culture is not yet available
- when HIV co-infection is prevalent

Applied in all laboratories involved in the NTP, it might effectively strengthen the DOTS strategy provided that training and quality control are ensured

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