Standardisation of the bleach method

A report from Mathare

Maryline Bonnet\textsuperscript{1}, Andrew Ramsay\textsuperscript{2,3}, Willie Githui\textsuperscript{4}, Laramie Gagnidze\textsuperscript{1}, Francis Varaine\textsuperscript{5}, Philippe J Guerin\textsuperscript{1}

\textsuperscript{1}Epicentre, \textsuperscript{2}Liverpool School of Tropical Medicine, \textsuperscript{3}TDR/WHO, \\
\textsuperscript{4}Centre for Respiratory Diseases Research, Kenya Medical Research Institute, \\
\textsuperscript{5}Médecins Sans Frontières
Optimised smear microscopy

- Public health impact and patients’ benefit
- Sputum specimen processing
  - Use of commercial bleach
  - + Overnight sedimentation
Bleach method: no consensus

- Variability of results due to deficiencies in study designs and evaluation
  - Different target populations
  - Use of different criteria to define a TB suspect
  - Lack of blinding routine
  - Lack of attention to bleach quality, storage and stability

Bleach method: no consensus

- No answer to 3 important operational questions
  - Can bleach method be of benefit in peripheral clinics?
  - Can bleach method improve diagnosis in HIV co-infected patients?
  - Benefit of bleach method when using very sensitive acid fast bacilli cut-off?

Objectives

- To evaluate the bleach method in a peripheral clinic and high HIV prevalence country
  - To compare the smear positive case detection between direct and bleach method microscopy
  - To measure the reproducibility of the smear reading
  - To assess the operational aspects of the bleach method
Methods

- Population based prospective study
  - >15 years pulmonary TB suspects: cough > 2 weeks
  - HIV/TB urban health clinic: Mathare (Nairobi)
  - 75% co-infection in smear-negative suspects

- Consecutive patients’ sampling

- Collection of 3 sputa in 2 days
  - 1\textsuperscript{st} on spot, 2\textsuperscript{nd} morning and 3\textsuperscript{rd} on spot
  - Coaching for specimen collection

- Hot Ziehl Neelsen method
Commercial Bleach 3.5%

Specimen liquifaction with bleach → Overnight sedimentation on the bench → Ziehl Neelsen microscopy on the sediment
**Method**

- Weekly monitoring of the chlorine concentration using pool tester and prior dilution at $d = 2.10^4$

- Case definitions
  1. AFB on 2 smears out of 3, 1 of which $>10$ AFB/100 HPF
  2. $>1$ AFB/100 HPF on 2 smears out of 3
  3. $>10$ AFB/100 HPF in 1 smear out of 3 smears
  4. $>1$ AFB/100 HPF in 1 smear out of 3 smears

- McNemar test and T-test for comparison of matched data

- Kappa coefficient for inter and intra-reader reproducibility
Results: Study profile

Total screened
N= 788

Less than 15 years old = 1
Impossibility to produce sputum = 7
Cough < 2 weeks = 20
ICF not signed = 63
Other = 1

Included
N= 696

Analysed
N= 644 patients

52 no sputum
General characteristics

- 644 patients
  - Mean age: 32.5 years
  - Sex ratio, M/F: 0.8
  - Past TB history: 121 (18.8%)
  - Antibiotic intake in the last 2 weeks: 37 (5.7%)
  - Production of 3 sputa: 614 (95.3%)

- 1879 specimens
  - 1401 (74.6%) purulent
  - 414 (22%) mucoid
  - 56 (3%) blood stained
  - 8 (0.4%) salivary
Smear-positive specimens detection

- N=1879 specimens

- 10 AFB/100 HPF threshold
  - Bleach method: 19.3% (363 positive smears (PS))
  - Direct: 16.0% (301 PS)
  - \( p < 0.001 \)

- 1 AFB/100 HPF threshold
  - Bleach method: 19.9% (374 PS)
  - Direct: 24.5% (460 PS)
  - \( p < 0.001 \)
Smear-positive patient detection

<table>
<thead>
<tr>
<th>Case definitions</th>
<th>Direct</th>
<th>Bleach method</th>
<th>Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>≥ 2PS (1PS&gt;10AFB)</td>
<td>621</td>
<td>116 (18.7)</td>
<td>136 (21.9)</td>
</tr>
<tr>
<td>&gt;2PS (≥1AFB)</td>
<td>621</td>
<td>126 (20.3)</td>
<td>155 (25.0)</td>
</tr>
<tr>
<td>≥1PS (≥10AFB)</td>
<td>644</td>
<td>120 (18.6)</td>
<td>138 (21.4)</td>
</tr>
<tr>
<td>≥1PS (≥1AFB)</td>
<td>644</td>
<td>140 (21.7)</td>
<td>172 (26.7)</td>
</tr>
</tbody>
</table>
Test reproducibility

- **Inter-reader**
  - Kappa 0.81 (95% CI 0.71-0.85)

- **Intra-reader**
  - Kappa 0.93 (95% CI 0.89-0.95)

K >0.8: very good reliability
Practical aspects

- Bleaching and sedimentation
  - Bleaching: mean duration 18.6 min
  - Sedimentation: mean duration 16.8 hours
- Slide preparation and reading (mean duration)
  - Smearing & drying: 52.9 min vs 21.4 min
  - Staining: 45.0 min vs 47.1 min
  - Reading of a positive smear: 3.1 min vs 3.0 min
  - Stability of chlorine concentration
- Increase of fragility of smears
- + 0.27€/slide (reagents+consumables): 38% increase
Conclusions

- Effective, simple and affordable method
- Answer to the 3 operational questions
  - HIV prevalence context
  - Peripheral clinic
  - Using sensitive AFB threshold
Perspectives

- Further research needed
  - Cost-effectiveness analysis
  - Replicability of the method in different contexts
  - Shorter sedimentation time
  - Bleach method and simplified fluorescence microscopy