

Standardisation of the bleach method

A report from Mathare

Maryline Bonnet¹, Andrew Ramsay^{2,3}, Willie Githui⁴, Laramie Gagnidze¹, Francis Varaine⁵, Philippe J Guerin¹

¹Epicentre, ²Liverpool School of Tropical Medicine, ³TDR/WHO,

⁴Centre for Respiratory Diseases Research, Kenya Medical Research Institute,

⁵Médecins 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?

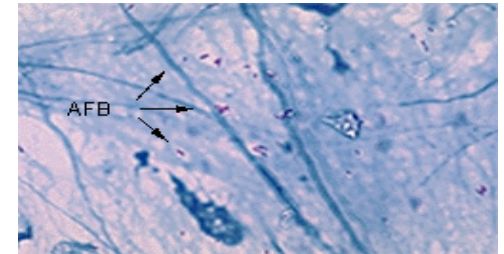
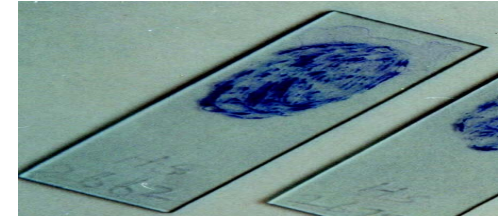
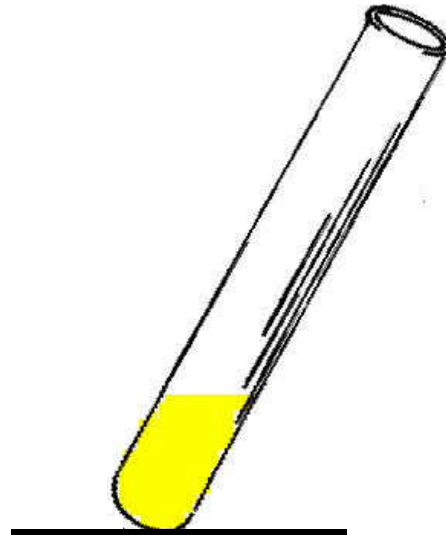
Objectives

- 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
 - 1st on spot, 2nd morning and 3rd on spot
 - Coaching for specimen collection
- ❑ Hot Ziehl Neelsen method

Commercial Bleach 3.5%



Specimen
liquifaction with
bleach



Overnight
sedimentation on
the bench

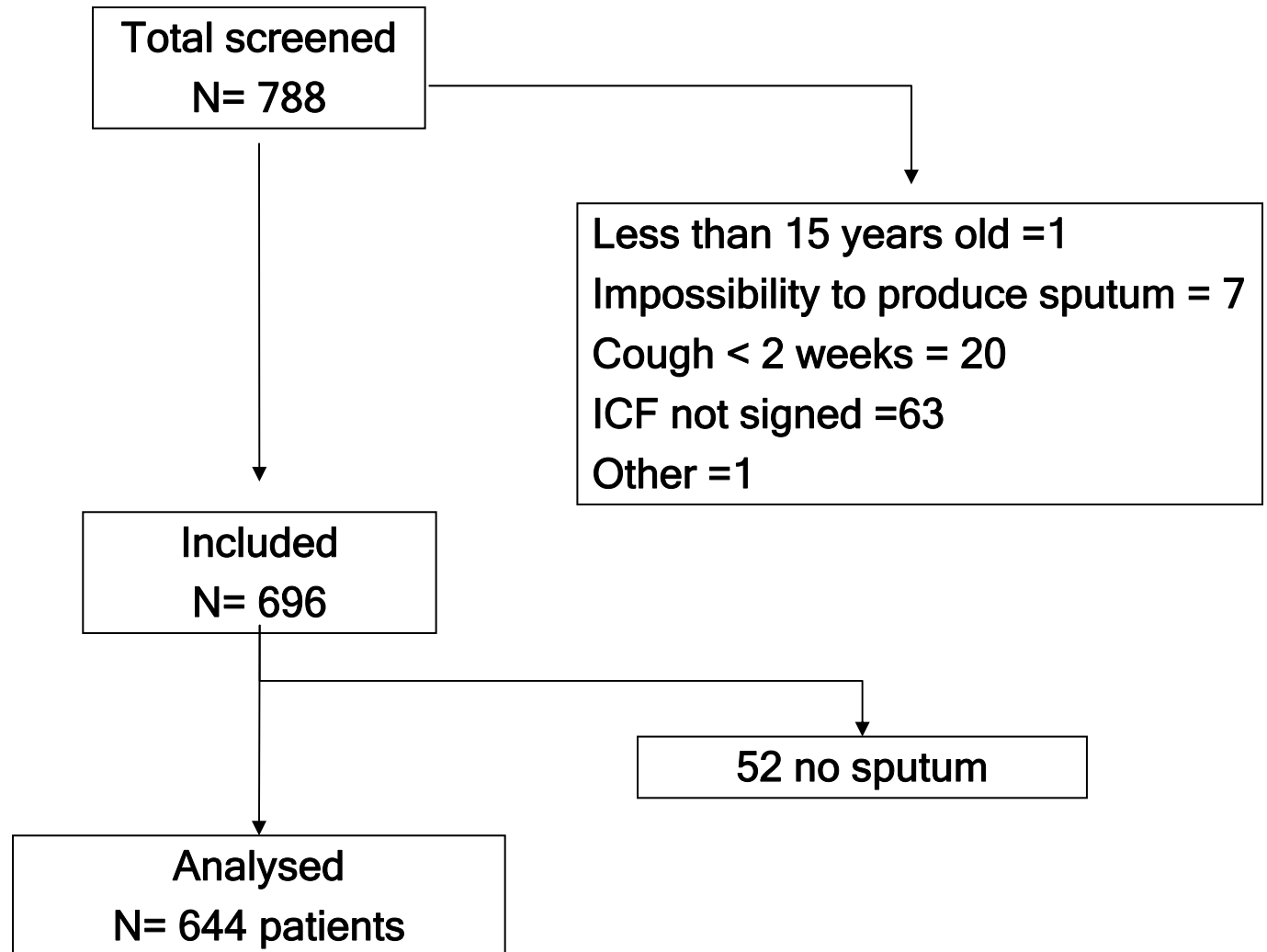


Ziehl Neelsen
microscopy on the
sediment

Method

- ❑ Weekly monitoring of the chlorine concentration using pool tester and prior dilution at $d=2 \cdot 10^4$
- ❑ 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



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

Case definitions	Direct		Bleach method		Gain n (%)
	N	n (%)	n (%)	<i>P</i>	
≥ 2PS (1PS ≥ 10AFB)	621	116 (18.7)	136 (21.9)	<0.001	20/116 (17.2)
≥ 2PS (≥ 1AFB)	621	126 (20.3)	155 (25.0)	<0.001	29/126 (23.0)
≥ 1PS (≥ 10AFB)	644	120 (18.6)	138 (21.4)	<0.001	18/120 (15.0)
≥ 1PS (≥ 1AFB)	644	140 (21.7)	172 (26.7)	<0.001	32/140 (22.9)

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