



Microbiological validation of smear microscopy after sputum digestion with bleach; a step closer to a one-stop diagnosis of pulmonary tuberculosis

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Summary

Background: Smear microscopy is relatively insensitive for the diagnosis of TB. The digestion of sputum with household bleach prior to smear preparation has been reported to improve its sensitivity. This method has not been validated.

Methods: Seven hundred and fifty six patients with symptoms suggestive of pulmonary TB (PTB) were asked to submit 3 sputum specimens for direct microscopy. One specimen was selected at random for culture and another specimen was digested to prepare a further smear. The WHO case definition (≥ 2 positive smears or one positive smear and positive culture) was used to compare the sensitivity and specificity of the smears.

Findings: Four hundred and fifty five (60%) patients were culture-positive. Of these, 235 (31%) had “definite” PTB and 223 (29%) “very likely” PTB (smear-negative, culture-positive). The WHO case definition identified 51% (235/458) of the patients with “definite” or “very likely” PTB. One digested smear detected 219 (93%) of the 235 patients with “definite” PTB and 10 patients with “very likely” PTB (sensitivity (95%CI) 50% (45–55%); specificity 99% (97–100%)). The positive and negative predictive values for one digested smear were 98% (95–99%) and 56% (52–60%) respectively, which were not different ($p > 0.5$) to the WHO case definition (100% and 57%, respectively).

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Interpretation: One bleach-digested smear is as sensitive and specific as the WHO case definition for the diagnosis of PTB.
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Introduction

Globally, tuberculosis (TB) causes more adult deaths than any other single infectious disease. Approximately 95% of cases and 98% of deaths occur in the developing world. DOTS, the WHO's global strategy for controlling the TB epidemic, relies upon direct sputum smear microscopy for detecting infectious cases. This usually involves patients submitting three sputum specimens over the course of 2 days. The internationally recommended definition of a smear-positive TB case requires at least two positive smears or, where facilities are available, one positive smear together with one culture positive for *Mycobacterium tuberculosis*.¹

Direct smear microscopy is an inherently insensitive technique since large numbers of organisms have to be present in the sputum to be detectable by direct examination.² The sensitivity of the technique is further undermined in areas with high HIV prevalence since HIV coinfection, probably through modifying the TB disease process, increases the proportion of patients with culture-positive, smear negative TB.³ Furthermore, the standard diagnostic process is associated with high drop out rates, since patients need to make repeated visits to the health facility to submit specimens and obtain results.⁴ The requirement to process and examine three specimens from each TB suspect can create heavy workloads for laboratories, which may impact upon the quality of the service.⁵

The global targets for TB control under DOTS include detecting 70% of new smear positive cases by 2005 (case detection). Current estimates suggest the global case detection rate is about 37% and that this target will not be achieved.⁶ It is recognised that health system weaknesses present challenges to improving case detection. These include health service staffing crises in several countries, and a more general lack of access to TB diagnostic services, particularly for poor people.

Various groups have attempted to modify the smear diagnostic process to make it more sensitive, or to reduce the number of smears required for diagnosis. Methods aiming to concentrate the TB bacilli in sputum smears by digestion of the sputum with bleach and subsequent concentration of sputum (by centrifugation or overnight sedimentation) have received much attention.⁷⁻⁹

Previous studies have shown that digestion of sputum with household bleach prior to smear preparation (without a concentration step) can also improve the performance of sputum microscopy. Studies carried out in Ethiopia have reported that a single digested smear can equal the sensitivity of 3 direct smears.¹⁰ These, entirely smear-based, studies reported patients with positive digested smears but three negative direct smears, suggesting that the digestion method was associated with either decreased specificity or increased sensitivity. Mycobacterial culture is a more sensitive method than direct sputum smear microscopy for diagnosing pulmonary TB (PTB). We therefore conducted a study comparing a bleach-digested smear with direct smear microscopy and culture in patients presenting with symptoms suggestive of PTB to district hospitals in Nigeria. If a single digested sputum smear could replace the examination of three direct smears, and provide a one-stop diagnosis, it could make a significant contribution to improving case detection rates while reducing costs and workload.

Material and Methods

This was a cross-sectional, prospective study of patients (aged >15 years) presenting to 8 district hospitals in Abuja, Nigeria, with symptoms suggestive of pulmonary tuberculosis and no previous history of TB treatment. All consenting patients were asked to submit three sputum samples over 2 days (first on-the-spot, early morning and second on-the-spot specimens) as recommended by the International Union Against Tuberculosis and Lung Diseases (IUATLD),¹ and adopted by the national TB and Leprosy Control Programme (NTLCP) of Nigeria.¹¹

All samples were transported to the TB Research Laboratory at Zankli Medical Centre and processed within 24 h. Direct smears were prepared from each specimen and stained using the hot Ziehl-Neelsen (ZN) method.¹ Subsequently, one of the three specimens was randomly selected and used to prepare a digested smear. A second randomly selected specimen was used for mycobacterial culture.

Sputum was digested by adding an equal volume of locally-obtained household bleach (Jik, 3.5% NaOCl, Reckitt Benkiser (Nig) Ltd., Agbara) to the

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