Evaluation of a safe sputum processing method for detecting tuberculosis

Ashok Rattan, Kamal Kishore, Sarman Singh, Munir Jaber, Immaculata Xess, Ramesh Kumar

Abstract

***Aims*—To evaluate a safe sputum processing method for detection of tuberculosis in developing countries.**

***Methods*—A sample processing method was developed in which acid fast bacilli were killed with 1% sodium hypochlorite and concentrated by flotation on a layer of xylene before staining by the Ziehl Neelson or auramine O methods.**

***Results*—Best results were obtained by auramine O staining after flotation. Staining by the Ziehl Neelsen method after flotation gave better results than direct Ziehl Neelsen staining without flotation.**

***Conclusions*—The flotation method with Ziehl Neelsen staining offers advantages for smear preparation in the tuberculosis control programmes of developing countries.**

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Sputum examination for the presence of acid fast bacilli in people with symptoms suggestive of pulmonary tuberculosis forms the cornerstone of detection under the National Tuberculosis Control Programme of India.[1] The Ziehl Neelsen method of smear examination requires that nearly 10^4 acid fast bacilli/ml sample should be present for their reliable detection.[2] It has been documented that for detection examination of two smears is as sensitive as culture.[3] It is well known that laboratory workers are at increased risk of acquiring infection during the course of their work.[4] With the advent of AIDS several hospital outbreaks have been reported from the United States and transmission of multiple drug resistant strains of acid fast bacilli to health care workers such as nurses and ward attendants has been documented.[5–7]

It is, therefore, desirable to have a method that both inactivates HIV and *Mycobacterium tuberculosis* and improves the sensitivity of the smear examination. We report our experience in the evaluation of the modified Soltys method.[8]

Methods

The department of laboratory medicine at the All India Institute of Medical Science receives 20 to 30 sputum samples per day for smear examination. Smears are prepared from the sputum samples on an open bench. With the help of a loop they are spread over an area of 2 cm × 1 cm and stained by the Ziehl Neelsen method and at least 300 oil immersion fields examined for the presence of acid fast bacilli. Samples found to be positive are then forwarded to the mycobacteriology laboratory of the department of microbiology for culture.

Over a period of one month, five samples were randomly selected each day after the smears had been prepared in the department of laboratory medicine and before results were ready, and dispatched separately to the microbiology department. The results were expressed semiquantitatively (table 1).

In the mycobacteriology laboratory, the samples were processed by a modification of the Soltys method.[8,9] Briefly, to the tube containing the sample an equal quantity of 1% sodium hypochlorite solution was added. The tubes were left at room temperature for 15 minutes. Xylene (0·5 ml) was then added to each tube and the contents were mixed. The tubes were left undisturbed for another 15 minutes. A creamy layer formed on the surface of the xylene layer. Two smears were prepared from each sample, taking a loopful of sample from the creamy layer; one was stained by the Ziehl Neelsen method and the other with auramine O. All the smears were examined and quantified by different workers, each being unaware of the result obtained by the others. Smears were examined by dry lens (45 ×); acid fast bacilli were counted and divided by the "magnification factor" of four as suggested by Smithwick[10] to obtain equivalent results with the other two staining methods.

To establish the safety of the method, 20 randomly selected samples showing 3 + or 4 + acid fast bacilli after the flotation method and Ziehl Neelsen staining (table 1), were cultured on Lowenstein Jensen medium.

To determine the minimum contact time with 1% sodium hypochlorite solution required to kill or inactivate *M tuberculosis* and maximum contact time before the acid fast

Table 1 Semiquantitative results from smear examination

Acid fast bacilli (No)	*Semiquantitative report*
0 / 300 OIF	Negative for acid fast bacilli
1–2/300 OIF	± Please repeat
1–9/100 OIF	1 +
1–9/10 OIF	2 +
1–9/1 OIF	3 +
> 9/1 OIF	4 +

OIF = oil immersion field

Department of Microbiology
A Rattan
K Kishore
M Jaber
I Xess
R Kumar

Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi, India
S Singh

Correspondence to:
Dr A Rattan, Department of Microbiology, AIIMS, Ansari Nagar, New Delhi 110 029, India.

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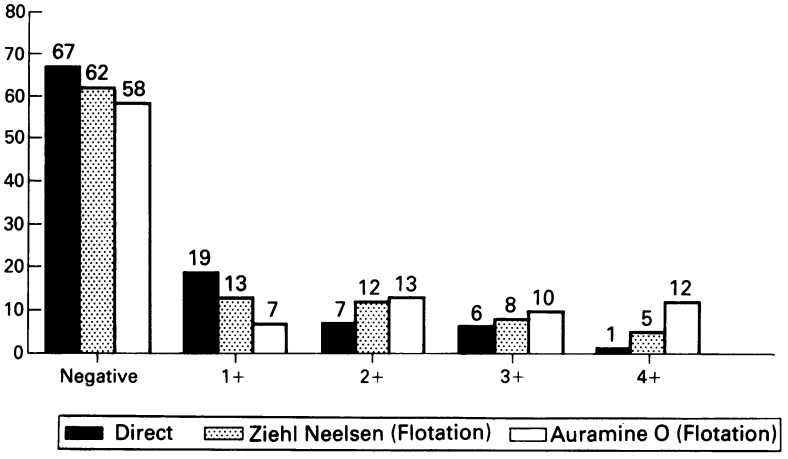

Semiquantitation of acid fast bacilli using the three methods.

bacilli lose their staining property a suspension from a clinical isolate of *M tuberculosis* was made, adjusted to a 0·5 Macfarland tube, and 5 ml inoculated into a series of test tubes each containing 5 ml of 1% sodium hypochlorite solution. At intervals of five minutes 0·5 ml of xylene was added and the tube left undisturbed for 15 minutes. Smears were made from the creamy layer and stained by both the Ziehl Neelsen and the auramine O method. Cultures on Lowenstein Jensen media were also made with material from the creamy layer.

Results

Thirty three samples of the 100 examined were positive by the direct staining method, 38 were positive after concentration by flotation and subsequent Ziehl Neelsen staining, and 42 were positive after flotation and auramine O staining. Two of the samples designated positive by the direct method were reported negative after flotation and Ziehl Neelsen staining, and seven samples missed by direct staining were identified positive after flotation and Ziehl Neelsen staining. All the samples reported as positive by these two methods were positive by auramine O staining. Auramine O staining identified nine and four additional positive samples compared with the direct method and flotation followed by Ziehl Neelsen staining respectively; (figure).

Table 2 summarises the comparison of results between direct staining by the Ziehl Neelsen method and staining by both methods after flotation.

All 20 samples cultured after flotation decontamination were negative, testifying to the safety of this method.

Table 2 Results obtained after direct Ziehl Neelsen (ZN) staining and Ziehl Neelsen and auramine O (AO) staining after flotation

Direct ZN	*ZN after flotation*		*AO after flotation*	
	+	-	+	-
+	31	2	33	0
-	7	60	9	58

Direct ZN *v* ZN after flotation p = 0·18 (NS); Direct ZN *v* AO after flotation p < 0·01.

The minimum contact time required to inactivate and kill the mycobacteria was 15 minutes. The bacterial suspension maintained its staining property even after two hours of contact with 1% sodium hypochlorite; thereafter the staining became irregular.

Discussion

Work related infections are a recognised hazard for personnel employed in laboratories where agents of infectious disease are handled. More than 30 years ago it was clearly documented that the incidence of tuberculosis in laboratory workers was nine times higher than in the community.[11] In 1957, Merger[12] found that it was 28 times higher in laboratory workers than the general population and Reid[13] reported that those laboratory workers who handled fresh material were from two to five times more likely to become infected compared with matched controls. Consequently strict laboratory precautions including safety cabinets, prevention of aerosol production, and barrier precautions have been introduced to make the workplace safe for the laboratory worker in the developed world. The use of even a fraction of these precautions in developing countries, however, is the exception rather than the rule. Reliable data are not available, but it is our observation that in over 90% of the laboratories in India smear preparation and examination is carried out on the open bench.

It has been documented that examination of two smears equals culture as far as sensitivity for detection is concerned. Under the Indian National Tuberculosis Control Programme no attempt is made to culture *M tuberculosis* and laboratory workers all over this country process samples for smear examination an open benches. A method that kills *M tuberculosis*, such as the one described, would be of direct benefit to the persons involved in processing these samples. This method also concentrates the bacilli to some extent leading to additional samples being reported as positive. There are also intangible benefits, as most of the debris are left behind. The smear prepared from the creamy layer after flotation has very little background material with the acid fast bacilli, which, if present, are evenly spread out in the smear, making their detection easier.

Sodium hypochlorite is also effective in killing HIV. When HIV infection establishes itself in a tuberculin positive population, the first opportunistic infection that occurs is tuberculosis[14] because the immunosuppression required for its reactivation is minimal.[15] We readily concede that the real risk for acquiring HIV is from needlestick injuries, but barrier precautions do not apply to respiratory secretions such as sputum.[16] As haemoptysis is a frequent presenting symptom of pulmonary tuberculosis, it seems prudent to adopt a method that inactivates the HIV, if and when present in the sample, to safeguard the health care worker in developing countries.

It has been reported, and our results confirm, that staining with auramine O is more sensitive than Ziehl Neelsen staining, but the major drawback in developing countries is the non-availability of a fluorescent microscope. Wherever available, however, it is desirable that auramine O be used for screening of slides. For detection in centres without access to properly equipped laboratories, the flotation method, followed by Ziehl Neelsen staining involves minimal input as far as chemicals and equipment are concerned. It offers, moreover, the possibility of making the samples completely safe to handle for laboratory workers besides slightly concentrating the acid fast bacilli. We suggest, therefore, that this method should be adopted in smear preparation in developing countries for detection in their tuberculosis control programmes.

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