Journal of Advances in Science and Technology

Vol. VII, Issue No. XIV, August-2014, ISSN 2230-9659

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AN INTERNATIONALLY INDEXED PEER REVIEWED & REFEREED JOURNAL

www.ignited.in

Journal of Advances in Science and Technology
Vol. VII, Issue No. XIV, August-2014, ISSN 2230-9659

Investing Time in Microscopy: An Opportunity to Optimize Smear-Based Case Detection of Tuberculosis

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Abstract – OBJECTIVE: To measure the time spent during routine sputum smear microscopy and assess whether re-examining slides for 10 min translates into higher case detection of smear-positive cases.

DESIGN: A prospective observational study over a12-month period with three components: 1) timing of routine sputum smear examination; 2) blinded re-examination of all slides for 10 min and results compared with initial readings; and 3) blinded re-examination, by the original microscopists, of a portion of false-negative slide smixed with true negatives for 10 min.

RESULTS: A total 612 sputum specimens for screening. The routine examination time The median routine examination time was 5 min. A 10 min examination significantly increased the number of positive smears from 94 to 131. On review by the original readers, more than half of the false-negative slides were reported as positive after 10 min.

CONCLUSION: Sputum smear microscopy has low sensitivity if performed too quickly, and 10 min re-examination significantly increases case detection. Ensuring that smears are examined for the recommended duration may be a simple and low-cost way to improve case detection.

KEY WORDS: tuberculosis case finding; diagnosis; smearpositive; pulmonary; sputum smear microscopy2

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INTRODUCTION

Pulmonary tuberculosis is mainly a disease of the respiratory system, caused by *Mycobacterium tuberculosis* .Tuberculosis is a predominant infectious cause of mortality today. There are various methods for bacteriological diagnosis of tuberculosis. Microscopic examination and culture are still essential elements of the bacteriological diagnosis of tuberculosis in microscopic examination; the diagnosis of tuberculosis is confirmed on the basis of demonstration of tubercle bacilli in the sputum. Smear examination is believed to be simple, cheap, quick and practicable and effective case finding method for developing countries. As tuberculosis bacilli are very slow growing organisms, culture results are available after a period of three or six weeks.

So, Microscopic examination has the advantage of the giving a result at once. The specimen most commonly examined is sputum and mucous secretion coughed up from the lungs . Microscopic examination of Ziehl-Neelson or auramine stained specimen allows detection of most strains in less than an hour. Ziehl-Neelson is the most extensively used procedure for the demonstration of *mycobacterium tuberculosis* in smear. The requisites for the staining procedures are; basic fuchsin, phenol, absolute alcohol; sulphuric acid and methylene blue. Microscopic examination under oil immersion objective reveals mycobacterium are red bacilli.

Two sputum specimens to be examined for each patient i.e. Spot and Morning.. Case detection may thus be expected to increase in locations where the number of new cases would be detected through improved quality of microscopy. Although these

techniques are relatively simple, smear microscopy is labour-intensive and requires significant investment in equipment, supplies, trained personnel, supervision and quality control. examining sputum smears in the routine smear microscopy service and assessed whether examination times shorter than recommended resulted in missing cases.

MATERIALS AND METHODS;-

The study was conducted in the Department of Microbiology, IGIMS PATNA, BIHAR Staining rack to hold smear slide Forceps Slide rack to place stained smear slide to dry in the air 1% carbolfuchsin 25% H2SO4 to decolorise smear solution 0.1% Methylene blue to counterstain decolourised material in the smear Water to rinse the smear Bunsen burner or spirit lamp to flame the stain.

SAMPLE COLLECTION: Early morning sputum samples were collected in clean, sterile, leak proof, wide mouth containers.

Instruct the patient to rinse his/her mouth with water before producing the specimen. This will help to remove food and any contaminating bacteria in the mouth. Instruct the patient to take two deep breaths, holding the breath for a few seconds after each inhalation and then exhaling slowly. This should produce a specimen from deep in the lungs. Ask the patient to hold the sputum container close to the lips and to spit into it gently after a productive cough. Sputum is frequently thick and mucoid. The colour may be a dull white or a dull light green. Bloody specimens will be red or brown. Thin, clear saliva or nasopharyngeal discharge is not sputum and is of little diagnostic value for tuberculosis.If the sputum is insufficient encourage the patient to cough again until a satisfactory specimen is obtained. Remember that many patients cannot produce sputum from deep in the respiratory track in a few minutes. Give him/her sufficient time to produce an expectoration which s/he feels is produced by a deep cough.If there is no expectoration, consider the container used and dispose of it in the appropriate manner. Check that the container is securely closed and label the *container* (not thelid) clearly. All sputum specimens submitted for AFB screening were processed using the standard hot Ziehl-Neelsen (ZN) technique. Heat-fixed sputum smears were covered by 1% carbol fuschin and heated for 5-8 min, decolourised with 25%sulphuric acid for 3 min, then counterstained with 0.1% methylene blue for 1 min. Reagents used during ZN staining are supplied by theRNTCP. arrive in containers labelled with the names of the reagent only. Every batch of reagents received is subjected to internal quality control: two smears are prepared from sputum specimen available; All sputum slides were then stored and re-read blind at 2-week intervals for 10 min each, using a timer. Smears with AFB were confirmed in all cases during the 10-min re-examination. A selection of smears reported as negative during the routine examination but found to be positive after the 10-min reading were mixed randomly (by tossing a coin) with true negatives and re-examined blind for 10 min. Grading of sputum smears followed the guidelines of the International Union Against Tuberculosis and Lung Disease.

MICROSCOPIC GRADING

RNTCP ZN staininggrading (using 100x oil immersion objective and10x eye piece)	**Reporting /Grading**
>10 AFB/field after examination of 20 fields	Positive, 3+
1-10 AFB/field after examination of 50 fields	Positive, 2+
10-99 AFB/100 field	Positive, 1+
1-9 AFB/100 field	Scanti
No AFB per 100 fields	Negative

RESULTS

A total of 612 sputum smear results were registered in the laboratory sputum register**.** 94 sample (15.2%) were read as AFB-positive receiving a diagnosis of smear-positive pulmonary tuberculosis (PTB) following the standard case definition. Of the 518 slides initially reported as negative, 37 (7.0%) were found to be AFB-positive after 10 min of examination .The median time it took to find AFB in these false-negative slides was 5 min . There were no false positives. Spending 10 min per slide significantly increased both the number of positive slides from 94 to131. When compared with the 10-min reading, the initial process had a sensitivity of only 59% for the diagnosis of smear-positive PTB.

Table 2 Sputum slide results from routine and 10-minute examinations

	Second round: repeat 10-minute examination by controllers		Total
First roundInitial examination by technicians	AFB-positive	AFB-negative	
AFB-positive	94	0	94
AFB-negative	37	481	518
Total	131	481	612

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DISCUSSION

In the present study data correlate performance with microscopy time and clearly demonstrate that routine microscopy under operational condition so may be suboptimal and result in large numbers of cases being missed. In our setting, which we feel is typical of laboratory practice in the region, routine microscopy had a sensitivity of only 59% when compared with the 10-min approach, and missed 41% of the confirmed cases. grading system is based on 100 fields.300 fields should be examined before concluding that a slide is negative. This study shows how simply making sure that the recommended duration of examination is adhered to can result in considerable increases in cases detected. However, in the context of the human resource crisis, overworked laboratories and poor laboratory infrastructure, such a simple intervention may not be all that easy to implement. This study highlights the importance of taking a broad approach in optimising laboratory practice, and future studies could assess the impact the extra time recommended for sputum examinations would have on overall performance.

CONCLUSION

This study shows that a significant number of patients with smear-positive TB are missed if the sputum slides are examined too quickly. In this study, 10-min re-examination of the slides almost doubled the number of cases detected. While a great deal of attention is focused on developing new diagnostic tests and rolling out culture facilities, much can still be gained by optimising smear microscopy. Spending 10 min per slide should be a goal to which all programmes and the human resource limitations preventing the implementation of high quality microscopy need to be addressed. Addressing these practical operational issues would greatly improve the effectiveness of TBcase detection and contribute to the realisation of global TB control.

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