THE ROLE OF RAPID SLIDE CULTURE IN THE DIAGNOSIS AND MANAGEMENT OF PULMONARY TUBERCULOSIS

Lt Col J JENA*, Lt Col SK NEMA+,
Lt Col BN PANDA#

ABSTRACT

Rapid slide culture method using human blood medium was utilized for the primary culture of *Mycobacterium tuberculosis* and the results obtained were compared with results of smear examination of sputum specimens from fresh cases of pulmonary tuberculosis. Smear and rapid slide culture results of a total of 320 patients were analyzed. Slide culture was positive in 104 cases whereas smears were positive in 90 cases. Early culture confirmation in 7 days coupled with positivity better than smear examination, makes rapid slide culture a better method for diagnosis.

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KEY WORDS : *Mycobacterium tuberculosis*; Rapid slide culture; Bacteriological techniques; Colony count microbial.

Introduction

Tuberculosis has a prevalence of over 40 million cases worldwide, and 10 million new cases are added every year [1]. Early detection of this disease remains an important objective. Numerous unsuccessful attempts have been made to develop serological tests but definitive diagnosis is still based on microscopy and culture [2]. Of these two methods, culture is a more sensitive and specific method with an ability to give positive results on a clinical specimen containing as little as 10-100 bacilli per ml [3]. Conventional culture by Lowenstein-Jensen medium takes 8-12 weeks for primary isolation. This prompted many workers to develop quicker methods for culture of *Mycobacterium tuberculosis* [4,5].

Robert Koch was the first to use a rapid slide culture (RSC) method using coagulated human serum [4]. He successfully obtained growth in seven days time but contamination hindered success. Dickinson and Mitchison used RSC method using out-dated human

blood medium and fluorescent microscopy [5]. Gupta et al substituted fluorescent microscopy with bright-field microscopy [6]. This is a cheap, rapid, simple and safe method and culture results are obtained in 7 days time. This method was used by Purohit et al [7]. Modern radiometric rapid culture method BACTEC - 460 remains a distant dream for a country like ours due to its prohibitive cost.

We have employed RSC using human blood medium (HBM) for primary isolation of *M. tuberculosis* and compared with the results of microscopy.

Material and Methods

Three hundred and twenty patients with clinical and radiological evidence of pulmonary tuberculosis were asked to collect their morning sputum samples in sterile McCartney bottles. Samples were processed by modified Petroff's method [6]. From the deposit of each of the McCartney bottles 2 thick smears, one over the middle 1/3 of a slide (A) and the other over the lower 1/3 of

*Classified Specialist (Pathology & Microbiology); +Classified Specialist (Pathology); #Classified Specialist (Medicine and Chest Diseases); MH (CTC) Pune 411 040.

a longitudinally cut glass slide (B) were made. Smear A was air dried, heat fixed and stained with Ziehl-Neelsen (ZN) stain and examined under a light microscope. For each patient, smear examinations were done on 3 consecutive days whereas only one specimen was subjected to RSC using HBM. Sputum culture by RSC was restricted to one sample only so as to conserve the pure chemicals.

Human blood medium : Outdated, but not more than 4-week-old, citrated blood was diluted with an equal volume of sterile deionized water to cause haemolysis. The medium was then made selective by adding polymyxin-B (2,00,000 units/L), carbenicillin (100 mg/L), trimethoprim (10 mg/L) and amphotericin-B (10 mg/L). The pH of the medium was maintained between 6.5 to 7.5. Seven mL of this medium in a sterile screw capped McCartney bottle constituted one unit of HBM.

Smear B was air-dried and inoculated into HBM and incubated at 37°C for 7 days. On the seventh day smears were taken out with a pair of forceps and dipped individually in separate 10 mL aliquots of sterile distilled water to enable washing off of the excess HBM. Smears were then placed in an oven at 80°C for 30 minutes, stained by ZN method and examined under the oil immersion objective for culture results. A known rapid-grower was cultured by RSC method to compare the microcolonies and the rate of growth at the 2nd, 5th and 7th day of culture by using the same HBM.

Results

Out of a total of 320 patients, 79 patients (24.7%) were found sputum positive by microscopy as well as RSC whereas in 205 patients (64%) both the tests were negative (Table 1).

Smear positivity was found in 90 cases of the total (28.1%) but culture positivity using RSC method was found positive in 104 (32.5%) cases. There were 11 cases (3.4%) positive by smear examination alone whereas 25 cases (7.8%) showed positivity only by RSC (Table 1). The known rapid grower when

cultured in similar fashion showed dense confluent microcolonies after 24 hours of incubation whereas wild strains of *M. tuberculosis* took 7 days for development of microcolonies.

TABLE 1
Correlation of RSC and smear microscopy results

		Smear microscopy		
		Positive	Negative	Total
RSC	Positive	79	25	104
	Negative	11	205	216
	Total	90	230	320

$\chi^2 = 4.69; p < 0.05$

Discussion

Definitive laboratory diagnosis of tuberculosis still revolves around the smear examination and culture confirmation of *M. tuberculosis* even 113 years after the discovery of the pathogen. Conventional culture confirmation by LJ medium has the inherent disadvantage of prolonged incubation period. This can frequently delay the treatment.

In our study of 320 clinically and radiologically diagnosed cases of pulmonary tuberculosis, we found negative results both by direct smear and RSC in 205 cases (64%). Thus 36% were positive by utilizing both the methods. Out of 90 smear positive cases, 79 were also positive by RSC. It is worth noting that in remaining 11 patients though sputum smears were positive for AFB, the bacteria were non-cultivable by RSC. This is probably due to previous chemotherapy or because of the fact that we subjected only one specimen for RSC. Purohit et al [7] found 7 RSC negative cases out of 189 smear positive cases.

In our study, we have included a large number of patients without confining the study to smear positive cases only. We have been amply rewarded by getting RSC positivity in 25 out of 230 smear negative cases, a definite yield of 10.87%. For comparison, we could not locate in literature an identical analytical report. Fortunately in our study, we did not find a single case of contamination

unlike reported by Purohit et al [7]. The beauty of the approach remains in picking up of the microcolonies with the help of light microscope. The possibility of presence of rapid growers was negated by observing the rate of growth employing RSC. The wild strains of *M. tuberculosis* from patients presented as discrete microcolonies on the slide on the 7th day of RSC in contrast to the confluent microcolonies observed on the second day of slide culture of a known rapid grower using the same HBM.

The RSC for mycobacteria is a quick safe, cheap and easy method. The greatest advantage of this method is that it can provide rapid and reproducible results in culture confirmation of suspected cases of tuberculosis which will be a boon to the treating clinician. BACTEC-460 system which is quite effective and quick method of culture is too costly to be used by developing countries like ours. RSC method will also be of great help in the early diagnosis and follow up of treatment failures and drug resistant tuberculosis. It is suggested that the RSC method should be popularized and adopted in the Armed Forces laboratories as a supplement to routine smear examination.

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