Comparison of Rapid Slide Culture with Culture in L-J Medium and MGIT for Isolating *Mycobacterium tuberculosis* from Bronchoalveolar Lavage Fluid

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Abstract

Aims: Rapid Slide Culture (RSC) was compared with the standard diagnostic strategy of simultaneous culture in Löwenstein-Jensen medium (L-J) and Mycobacterial Growth Indicator Tubes (MGIT™) with regard to diagnostic accuracy, turnaround time and cost, for isolating *M. tuberculosis* (MTB) from bronchoalveolar lavage (BAL) specimens.
Materials and Method: Centrifuged deposits of 75 consecutive smear-positive and 75 randomly selected smear-negative BAL specimens were subjected to RSC as well as culture in both L-J and MGIT. Isolates were identified as MTB by standard methods.
Results: One hundred and ten (73.3%) specimens were culture-positive by L-J and / or MGIT; 99 (90%) of these were RSC-positive too. No RSC-positive specimen was culture-negative on both L-J and MGIT. Thus, RSC demonstrated an overall sensitivity, specificity, PPV and NPV of 90%, 100%, 100% and 78% respectively. Significantly, 25 (33%) smear-negative specimens were RSC-positive, indicating the ability of RSC to isolate MTB from specimens with low bacillary loads. The mean time to positivity was seven, 35 and 24 days for RSC, L-J, and MGIT respectively. The cost per test was the equivalent of USD 1.82, 1.34, and 10.42 for RSC, L-J and MGIT respectively.
Conclusion: Rapid Slide Culture proved to be a rapid, effective and low-cost method for culturing MTB from bronchoalveolar lavage fluid, when compared with conventional culture in L-J medium and MGIT.

Keywords: Tuberculosis; Bronchoalveolar lavage; Diagnosis; Culture

Introduction

Mycobacterial culture is the 'gold standard' for the laboratory diagnosis of tuberculosis. Culture on egg-based solid media, e.g., Löwenstein-Jensen medium (L-J), is inexpensive but takes weeks. Culture on liquid media, such as Middlebrook 7H12 in MGIT tubes, is faster but much more expensive. Rapid slide culture (RSC) in lysed-blood medium is an old technique that is attracting attention anew because it is both inexpensive and rapid.(Find diagnostic manual, 2006) We compared the diagnostic accuracy, turnaround time, and cost of RSC against the currently standard approach of culturing specimens simultaneously in L-J slopes and liquid medium. We decided to work on bronchoalveolar lavage (BAL) specimens instead of sputum because the former can yield AFB when patients do not have productive cough, and also when sputum samples are smear-negative, something seen with increasing frequency with the rising incidence of TB-HIV co-infection.(Gupta et al., 1993; Hemavathi Sarmah et al.,2012) Therefore, ours is the first study to test RSC on BAL specimens and also the first one to compare RSC with culture done simultaneously on L-J medium and in MGIT tubes.

Materials and Method

Study setting and specimen selection: The study was conducted at a tertiary-care hospital in northern India on 75 consecutive smear-positive BAL specimens and 75 randomly selected smear-negative BAL specimens as determined by fluorescent microscopy after phenolic auramine staining. This strategy was adopted to enable assessment of the performance of RSC on specimens with both high and low microbial loads.

Laboratory methods: All 150 specimens were homogenised and decontaminated with the NALC-NaOH method, centrifuged at 3,600 g at 4°C for 20 minutes, and finally cultured simultaneously on RSC slides, L-J medium and MGIT.

Rapid slide culture: RSC culture medium was prepared by hemolyzing expired units of banked human blood (tested for HIV, HBV and HCV negativity, and preserved in ACD-A) with an equal volume of sterile distilled water. Anti-bacterial and anti-fungal agents (Polymyxin B 200,000 units / L; Penicillin G 100 mg / L; and Amphotericin B 100 mg / L) were then added to make it selective. Decontaminated and concentrated specimens were smeared on the lower-third of two sterile glass slides. Smears were air-dried and fixed on a heating block at 60°C for 30 minutes.(Jena et al., 1995) Subsequently, one smear was kept as control in a slide mailer, while the other smear was immersed in 35-ml of human blood medium in an ethylene oxide sterilised Coplin jar and incubated at 37°C for seven days.(Kumar et al., 2013; Nair et al., 1998). The H37Rv strain of MTB was used as control with each batch of specimens. On the seventh day, slides were taken out from human blood medium, dipped twice in distilled water to wash off excess blood, fixed, and stained with phenolic auramine. Smears were then examined under a

fluorescent microscope and growth, if any, was graded as 1+, 2+, 3+ and 4+ according to (Purohit et al., 1993)

Culture in Löwenstein-Jensen Medium and in MGIT: Simultaneous culture was done on two slopes of L-J medium and in one MGIT tube according to established procedures. Cultures in L-J medium and MGIT were incubated at 37℃ for up to a maximum of eight and six weeks respectively.(Rawat et al., 2013)

Identification: Growth obtained on L-J medium or MGIT was identified as MTB by growth rate, colony morphology and pigmentation, niacin accumulation, nitrate reduction, thermostable catalase test at 68°C, and the ability to grow on medium containing p-nitrobenzoic acid (PNB).(Rawat et al., 2013). The H37Rv strain of *Micobacterium tuberculosis* (ATCC 25618) and *Mycobacterium kansasii* (ATCC 12478) were used for quality control.

Data Management & Statistical Analysis: Data were recorded in MS Excel Sheets.

1. Descriptive Statistics, such as percentages, means and standard deviation, as appropriate, were calculated using MS Excel.
2. Chi-square test was used to evaluate association between categorical variables such as RSC and the standard diagnostic approach of culturing simultaneously on L-J medium and MGIT, and also RSC-positivity and smear-positivity, using MS Excel.
3. Results of RSC were tabulated with results of the standard diagnostic approach on a 2x2 table to calculate the sensitivity, specificity, positive predictive value, and negative predictive value of RSC in comparison to the standard diagnostic approach.
4. Paired 't' test was used to compare the mean time to positivity of RSC with that of culture in L-J medium and MGIT, using SPSS statistical software.

95% confidence intervals of these parameters were calculated assuming a binomial distribution; p value of less than 0.05 was considered statistically significant.

Results

Out of 150 BAL specimens collected from patients with suspected TB, 110 specimens (73.3%) were culture-positive by L-J and / or MGIT, while 40 (26.6%) specimens were culture-negative by both the methods mentioned above (Table 1).

Table 1: Distribution of positive cultures in *L-J medium, †MGIT, and Rapid Slide Culture

Test	Positive (Out of a total of 150 specimens)
L-J	99 (66%)
MGIT	109 (72.6%)
L-J and / or MGIT	110 (73.3%)
Rapid Slide	99 (66% of all samples; 90% of

Culture	L-J / MGIT +ve samples)

*Löwenstein-Jensen medium; †Mycobacterial Growth Indicator Tube

RSC was positive in 99 (90%) out of the 110 BAL specimens which were culture-positive (73.3% of total specimens) in L-J medium and / or MGIT. All 40 specimens which were culture-negative both in L-J medium and in MGIT, were negative by RSC too (Table 2). Therefore, RSC was found to have a sensitivity of 90%, specificity of 100%, positive predictive value of 100%, and a negative predictive value of 78% when compared with the standard approach of simultaneous culture on L-J and MGIT (Table 2).

Table 2: Comparison of Rapid Slide Culture with the *Standard Approach

	†RSC-positive	RSC-negative	p value
Standard approach* positive (110 specimens)	99 (90%)	11 (10%)	<0.0001
Standard approach negative (40 specimens)	0 (0%)	40 (100%)	<0.0001
X^2 with Yates' correction=101.91; p value<.0001			
*Standard approach is simultaneous culture of a specimen in two Löwenstein-Jensen slopes and one Mycobacterial Growth Indicator Tube.			
†Rapid Slide Culture			

Among 75 smear-positive specimens, 74 (98.6%) were RSC culture-positive. Among 75 smear-negative specimens, 25 (33.3%) were RSC culture-positive too, demonstrating the ability of RSC to support growth from specimens with a low bacillary load. (Table 3).

Table 3: Rapid Slide Culture Results in Smear-positive and Smear-negative specimens

	Smear-negative (75)	Smear-positive (75)
*RSC-positive	25 (33.3%)	74 (98.6%)
RSC-negative	50 (66.6%)	1 (1.4%)
*Rapid Slide Culture		

The mean time for L-J medium to show growth was 34.8 days (SD 7.3); for MGIT, the corresponding time was 24.4 days (SD 6.7).The time to positivity in RSC was seven days by design as no specimen was incubated beyond this duration. The mean difference in the time to positivity between L-J and RSC was 26.6 days (SD 6.9), while the mean difference in the time to positivity between MGIT and RSC was 15.9 days (SD 5.1). The p value was <.00005 in both cases.

The cost per test in Indian Rupees (INR; 1 INR ≈ 0.014 USD) was 128, 94, and 730 for RSC, L-J and MGIT respectively. RSC was more expensive than L-J mainly because of its greater need for hands-on time. MGIT was by far the most expensive test of all (Table 4).

Table 4: Itemised cost of tests in Indian Rupees (INR); (1 INR ≈ 0.014 USD)

	Chemicals and other consumables	Glassware and plasticware	Depreciation of equipment	Electricity	Personnel	Total cost
*RSC	15	40	10	3	60	128
†L-J	10	35	10	9	30	94
‡MGIT	650	35	10	5	30	730
*Rapid Slide Culture; †Löwenstein-Jensen medium; ‡Mycobacterial Growth Indicator Tube							

Discussion

In our study, 110 (73.3%) out of 150 BAL specimens collected from patients with suspected pulmonary TB, were culture-positive by one or more of the culture methods used, i.e., RSC, L-J or MGIT. Similar studies done with sputum specimens, comparing RSC with culture on L-J medium, have shown 42-69% culture-positivity by any of the two culture methods used, i.e., RSC or L-J medium.(Kumar et al., 2013; Kumar et al., 2013) Since no study exists in the published literature on RSC performed on BAL specimens, comparative data are not available.

In our study, 99 (66%) specimens were culture positive in both RSC and L-J medium, while 109 (72.6%) were positive in MGIT. Four (2.6%) of our specimens got contaminated during prolonged incubation in L-J culture. In the study by Rawat et al., L-J and MGIT grew MTB from 46.1% and 51.9% of sputum specimens respectively. (Hemavathi Sarmah et al., 2012) (Kumar et al., 2013) (Sanjeev et al., 2012) found RSC positivity in 64% of specimens, while L-J was positive in 63% of cases.(Kumar et al., 2013) (Hemavathi Sarmah et al., 2012) found 34% of specimens culture-positive by the RSC method and 36.8% positive in L-J medium.(Kumar et al., 2013)

In our study, culture-positivity was significantly higher among the smear-positive specimens than in smear-negative ones, as seen in other studies. Studies on sputum specimens have shown that RSC is capable of detecting AFB in 8.9% – 11% of sputum-negative specimens. (Richter et al., 2011) In our study, the much higher culture positivity rate of 33.3% in specimens, which were smear-negative but L-J and/or MGIT positive, may be due to the fact that BAL samples generally contain AFB in larger numbers than do sputum specimens.

Cultures of four specimens got contaminated in L-J medium; out of these specimens, three were positive by both RSC and MGIT, while the remainder was negative by both RSC and MGIT. None of our RSC cultures were contaminated; this differs from the 5.5% contamination rate reported by (Rodrigues et al., 2011) possibly because we added antibacterial and antifungal drugs to our lysed blood medium.

In our study, the sensitivity of RSC in smear-positive BAL specimens was 98% while its sensitivity in smear-negative specimens was 33.3%. Overall RSC sensitivity of 90% and specificity of 100% tallied closely with those of similar studies done in India on sputum samples that have found a sensitivity of RSC of 88.8 - 89% and a specificity of 97.8 - 100% when compared to culture on L-J medium.(Kumar et al., 2013; Sanjeev et al., 2012)

Culture on L-J medium was found to be inexpensive at Rs. 94 per test and the medium could be produced from ingredients that are either locally available or stable on storage. The main disadvantage of L-J was the long incubation period; in our study, the mean time-to-positivity on L-J medium was 34.8 days (SD 7.3). Similar studies on sputum have shown a time-to-positivity of culture on L-J medium of 31 to 37 days and have calculated the cost of culture on L-J media to be INR 100.(Hemavathi Sarmah et al., 2012; Kumar et al., 2013; Strumpf et al., 1979)The mean duration for culture positivity in MGIT was 24.4 days (SD 6.7). However, it was extremely expensive at INR 730 per test. Rapid slide culture was economical at INR 128 per test; it was also the most rapid of all tests because all specimens underwent final examination at the end of seven days of incubation.

Conclusion

In conclusion, RSC showed good results when compared with culture in L-J medium and MGIT. RSC was only slightly more expensive than culture in L-J medium, and its seven-day turnaround period was a definite advantage over both L-J and MGIT. Therefore, RSC should be further evaluated to fulfil the strongly felt need for a rapid, sensitive and low-cost culture technique in resource-constrained settings.

Ethics Statement

The Ethics Committee of Swami Rama Himalayan University approved our project on January 2, 2014 and sent its consent in Letter No. SRHU/HIMS/ETHICS/2015/5. The project did not entail working with either human subjects or experimental animals; besides, all specimens had, in

any case, been sent for acid-fast bacillus (AFB) microscopy, which is an approved diagnostic test.

Conflicts of Interest

The authors declare no conflict of interest whatsoever.

Acknowledgements

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