

Original Article

Comparative Yield of Solid (L-J) vs Liquid (MGIT 960) Culture for Isolation of *M. tuberculosis* from Pleural Effusion

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Received: 2 January 2023

Accepted: 5 April 2023

doi: <https://doi.org/10.3329/jem.v13i2.84681>

Abstract

Background: Tuberculous pleural effusion (TPE) presents an important diagnostic challenge due to paucibacillary infection characteristics. Precise and early identification of *M. tuberculosis* in pleural fluid is important for successful treatment measures. **Objective:** This study aims to compare the diagnostic sensitivity of solid (L-J) and liquid (MGIT 960) culture in the pleural samples of effusions. **Materials and Methods:** It is a cross-sectional comparative study with 183 sample size conducted in the NIDCH and SSMC & Mitford Hospital in Dhaka, within the period July 2017 to June 2018. Thoracocentesis was performed, gathering pleural fluid samples (n=183) in the cases of clinically suspected tuberculous pleural effusion (TPE) cases. Collected samples were subjected to centrifugation, and the resulting sediments inoculated onto two Lowenstein-Jensen (L-J) slants and into Mycobacteria Growth Indicator Tube (MGIT) 960. L-J slants were incubated at 37°C for a maximum of 8 weeks and checked after every week. PANTA supplementation was enriched in MGIT tubes, and fluorescence values were recorded at 42 days. Ziehl-Neelsen staining and a test to confirm MPB64 ICA were performed on positive cultures. Data analysis was done with MS Excel and SPSS version 25.0 software, and $p < 0.05$ significance was applied. Sensitivity analysis was performed according to MGIT 960, which was called the gold standard. **Results:** Among the 183 suspected cases of TPE, 62.8% were males and 37.2% were females, with a male-to-female ratio of 1.7:1. The mean age of patients was 38.73 ± 19.45 years, and the majority (41%) of the patients belonged to the age range 21-40 years. Mycobacterium tuberculosis grew more positively in liquid media of MGIT 960 (16/183; 8.7%) than in the solid medium of L-J (13/183; 7.1%). In the L-J positive cases, there were 5 grade 1+, 5 grade 2+, and 3 grade 3+. MGIT 960 revealed 13 L-J positive cases, along with 3 extra cases that were not diagnosed by L-J statistics, confirming the diagnostic yield with MGIT 960 being superior. The L-J media sensitivity and specificity, against MGIT 960, were 81.25 percent and 100 percent, respectively. **Conclusion:** *M. tuberculosis* of the pleural effusion exhibited better yield in MGIT 960. It is preferred over L-J due to better detection with TPE diagnosis. These results demonstrate that MGIT 960 has superior diagnostic accuracy to diagnose pleural tuberculosis.

Key words: Tuberculous pleural effusion; Mycobacterium tuberculosis; MGIT 960; L-J medium; Liquid vs solid culture; Pleural TB diagnosis

J Enam Med Col 2022; 13(2): 56--61

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Introduction

M. tuberculosis from pleural effusion, also known as tuberculous pleural effusion (TPE) or tuberculous pleurisy, is a pleural disease caused by the pathogen *Mycobacterium tuberculosis* which infects the pleura and the pulmonary membranes leading to inflammation and oedema in the pleural space.¹ According to WHO statistics, in 2015 the number of TB patients was 10.4 million, and the estimated deaths due to TB were 1.4 million worldwide.² Tuberculous pleural effusion incidents vary from country to country and can occur due to either a new infection before 6–12 weeks or reactivation, with industrialized countries having more reactivation factors.³ A San Francisco study in 2004 identified that pleural TB patients were more likely to be clustered than pulmonary or non-respiratory TB patients⁴ whereas another study in 2010 discovered the reverse.⁵ TB diagnosis has always been a challenge due to the difficulties in sample collection and the minimal sensitivity of diagnostic tests.⁶ Lowenstein-Jensen (L-J) medium is a widely used diagnostic method that can detect first-line drug sensitivity.⁷ Due to some limitations such as it requires longtime (2–8 weeks) to provide result, lack of sensitivity⁸, and the risk of contamination of L-J medium⁷, liquid culture or MGIT 960 is still considered as gold standard for TPE diagnosis according to the World Health Organization⁹. The diagnosis takes a short period to provide a result, but has higher sensitivity.¹⁰ L-J medium is an egg-based solid medium¹¹, whereas the MGIT 960 tube uses Middlebrook 7H9 broth and a fluorescence-based oxygen sensor to detect *M. tuberculosis* from pleural effusion¹². Both procedures are done at 37°C (98.6°F) of temperature.^{10,13} There are limited resources to find out whether that superiority applies to solid (L-J) versus liquid (MGIT 960) culture to pleural fluid, where the bacillary load is generally <100 cfu mL⁻¹. In this research, we aim to compare the yields of the L-J and MGIT 960 cultures that were grown in parallel on collected specimens of pleural effusion.

Materials and Methods

This comparative analytical study of solid L-J medium and MGIT 960 has been conducted on a cross-sectional basis in the National Tuberculosis Reference Laboratory (NTRL) of the National Institute of Disease of the Chest and Hospital (NIDCH) at Mohakhali, Dhaka and Sir Salimullah

Medical College and Mitford Hospital (SSMC and MH) at Dhaka during July 2017 to June 2018. With ethical considerations, suspected cases in the indoor and outdoor areas of the National Institute of Disease of the Chest and Hospital (NIDCH), Mohakhali, Dhaka during the study period were approached to enroll according to the study-specific inclusion and exclusion criteria.

Clinically and radiologically diagnosed pleural effusion and suspected pleural TB, and pleural TB cases who underwent thoracentesis were included in this study. Patients of all ages and both sexes were enrolled. Informed written consent was taken from all subjects. Patients with thoracic empyema patient haemothorax patients, heart failure, cirrhosis of the liver, or renal failure, and diagnosed pleural effusion were excluded.

Specimen collection and processing: Purposive sample collection techniques were used, and around 50 mL of aspirated fluid was taken in a sterile Falcon tube by a physician for diagnostic testing. The sample was centrifuged at 3000 rpm for 15 minutes, and the deposit was used for culture in L-J and MGIT-960 media.

Solid culture on Lowenstein-Jensen (L-J) medium:

Three drops of the pleural fluid sediment were inoculated on two L-J slants, which were incubated at 37°C first in a slanted position with loosely placed caps and then in the upright position with tightened caps. On day 3, the bottles were inspected to rule out contamination, after day 7 to eliminate the rapid growers, and weekly for 8 weeks on the growth of *M. tuberculosis*. Colonies would generally appear after 3 to 4 weeks and were graded as scanty, 1+, 2+, 3+ depending on the number of colonies. Confirmation of the identification was based on the colony morphology, Ziehl-Neelsen stain that revealed cord formation, and the MPB64-ICA test that revealed the presence of red/pink bands. In case one bottle was contaminated, interpretation was made on the other.¹⁴ A sterile loop (1 µl) was used to scrape a few colonies on the surface of the medium, then suspended in 0.2 mL of extraction buffer to vortex the suspension for homogenization. About 100 L of suspension was transferred into the sample well of the ICA slide test. After 15 minutes, the presence/absence of red to pink bands in both

the control and the test was observed, and the color intensity was recorded.¹⁵

Liquid Culture in BACTEC MGIT 960 System:

Liquid culture uses Mycobacteria Growth Indicator Tube (MGIT), which are filled with 7 ml 7H9 broth containing an embedded fluorescent sensor. Then 0.8 ml enriched growth supplement MGIT PANTA was added to the tube. The processed specimen was transferred into each labeled MGIT tube (0.5 ml) using a sterile pipette, then the tube was recapped to tightly, and then inverted to permit adequate mixing with the growth media. The outside surface of the tube was disinfected with 70% alcohol. The stability of the inoculated tubes was maintained at room temperature for 30 minutes, after which they were inserted into the BACTEC MGIT 960 instrument. Upon loading, the tubes were automatically located and continuously monitored by the BACTEC MGIT 960 system, such that positive cultures were identified by a red alert with an accompanying green marker indicating the tube location (NTBLCP, 2017). The tubes were observed for 42 days at least, and positive and negative ones were eliminated respectively.¹⁶ In positive cultures, a Ziehl-Neelsen stain was used to identify cord formation, which is evidence that *Mycobacterium tuberculosis* exists.

All cultures, positive in liquid media, were applied to the Immunochromatographic Assay (ICA) test slide directly without any manipulation. The test results and the intensity of the color were recorded after 15 min for both the test and the control bands.¹⁵

Data collection and analysis: Patient's data were recorded on an individual case report form (CRF). Following a successful data collection, all the data were analyzed through the use of MS Excel and Statistical Package for Social Sciences (SPSS) version 25.0. The categorical variables were deemed to be analyzed using the Chi-Square test, and a P value <0.05 was assumed statistically significant.

Results

This study enrolled 183 suspected cases of tuberculous pleural effusion (TPE) of which 115

(62.8%) were male and 68 (37.2%) were female with a male: female ratio of 1.7:1.

Fig 1 shows the distribution of subjects according to age. The age range varied from 7 to 90 years. The mean age of plural TB patients visiting the hospitals was 38.73 ± 19.45 years. Among the participants 41% are in the 21–40 years category which holds the highest proportion, followed by 41–60 years of age range with 23.5 %, 20.2% patients in ≤ 20 years, and 15.3% patients were from >60 years of age.

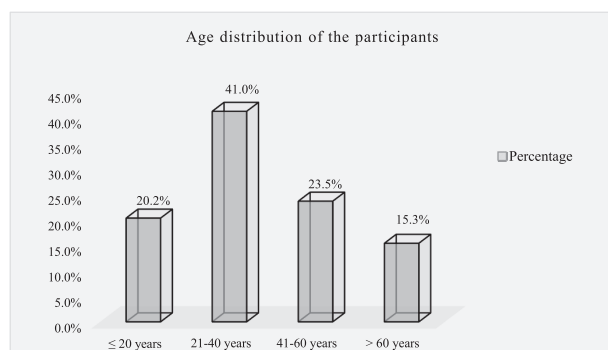


Fig 1. Distribution of patients according to age

Table I shows the detection of *M. tuberculosis* (MTB) in solid (L-J) medium. Out of 183 samples, MTB was detected in 13 (7.1%) samples, and 170 (92.9%) were negative. Among them 5 were 1+, 5 were 2+, and 3 were 3+. Table II shows the presence of *M. tuberculosis* MTB in the liquid media (MGIT 960). Out of the 183 samples, 16 (8.7%) samples were positive for growth, and 167 (91.3%) samples showed negative determinations.

Table I: Isolation of *M. tuberculosis* in L-J medium (n=183)

L-J medium	Number	Percentage
Positive	13	7.1
1+	3	
2+	5	
3+	3	
Negative	170	92.9
Total	183	100

Table II: Isolation of *M. tuberculosis* in liquid (MGIT 960) medium (n=183)

MGIT 960 medium	Number	Percentage
Positive	16	8.7
Negative	167	91.3
Total	183	100

Table III shows comparison of results of solid culture and liquid culture assays. The liquid culture assay shows the highest positive results whereas the solid culture staining showed 7.10% positive results. Table IV illustrates that positive growth in MGIT 960 media were found in 16 samples; among

Table IV: Comparison between L-J media and MGIT 960 media (N=183)

L-J media	MGIT 960 media		Total	L-J media	
	Growth	No Growth		Sensitivity (%)	Specificity (%)
Growth	13	00	13 (7.10%)	81	100
No Growth	03	167	170 (92.90%)		
Total	16 (18.7%)	167 (91.4%)	183 (100%)		

**MGIT 960 media culture was taken as the gold standard

Discussion

Tuberculous pleural effusion (TPE) is a major health problem worldwide, and the main challenge revolves around the detection of *M. tuberculosis* from pleural effusion or tuberculous pleurisy. This study compares two widely used methods between solid (L-J) media and liquid (MGIT 960) culture to detect *Mycobacterium tuberculosis*-induced pleural effusion in human subjects. This study found that male suspected victims are almost one and a half times more likely than female ones, with a percentage of 62.8% and 37.2% respectively. A 2023 Bangladeshi study aligns with this study, recording a 62% prevalence of TPE among male patients, with a predominant age group of 31–40 years (36.26%).¹⁷ The age-based findings partially agree with this study as the maximum subjects belong to the 21–40 years group (41%) and the mean age calculated in this study was 38.73 (± 19.45) years. Porcel¹⁸ claim that the male-female ratio of TPE is 2:1, and this disease them 13 (81.25%) were L-J media positive and three (18.75%) were L-J media

negative. No growth in MGIT 960 media was found in 167 samples, and all were also growth negative in L-J media.

Table III: Comparison results of solid culture (L-J) media and liquid culture (MGIT 960 media (n=183)

Results	L-J media Number (%)	MGIT 960 media Number (%)
Positive	13 (7.1)	16 (8.7)
Negative	170 (92.9)	167 (91.3)
Total	183 (100)	183 (100)

affects mostly young individuals, and according to their findings, the average age is 34 years. The possible cause behind male dominance can be the lifestyle-related causes, like smoking and outing, whereas in Bangladesh, women mostly prefer to stay at home. In L-J media, MTB was detected in 13 (7.1%) samples, and 170 (92.9%) were negative. Among them 5 were 1+, 5 were 2+, and 3 were 3+. Lakshmi et al¹⁹ and Siddiqui et al²⁰ revealed MTB growth on L-J medium was positive in 12% and 15% of samples, respectively, which were a little bit higher than the present study. In a study Hillemann et al¹⁰ reported 1.6% positivity in 1730 pleural fluid samples in L-J medium. This is lower than the findings in the present study. These discrepancies might be related to sample size, sampling technique, geographical and bacteriological variations. The MGIT 960 media result found that out of 183 samples, 16 (8.7%) samples were growth positive and 167 (91.3%) samples were negative. A previous 2006 study

proved 3.36% positivity by MGIT 960 media from 1730 pleural fluid samples.¹⁰ In a similar study Saini et al²¹ compared L-J media and MGIT 960 culture, where they found 39.1% positive in L-J medium and 69.5% positive in MGIT 960. Although the positivity rate is more in the current study, but the present study is correlated with the positivity rate being higher in MGIT 960 media than in L-J medium. Comparison of results between solid culture and liquid culture yielded the highest positive result of 8.70% by MGIT 960 isolation, and 7.10% by L-J culture. John & Lusiba²² confirmed 21% pleural TB on L-J culture, whereas Laskar et al²³ showed 74.29% growth positive and 10.48% negative by MGIT 960 media growth. In the present study positive MGIT 960 media was found in 16 samples; among them 13 (81.25%) were L-J media positive and 03 (18.75%) were L-J media negative. Negative MGIT 960 media were found in 167 patients, and all were L-J media negative. A similar observation was reported in a study by Katila et al²⁴ which indicated that the MGIT system recovered 91% of the isolates, and the L-J media revealed 84% of the isolates. Somoskövi et al²⁵ also supported the present observation; the study observed that a single medium, MGIT 960, recovered 96.4% of the 55 M. tuberculosis isolates, and L-J medium recovered 81.8% of the 55 isolates.

Limitations

The study is a breakthrough in comparing solid (L-J) and liquid (MGIT 960) cultures for isolating M. tuberculosis from pleural effusion; but the small sample size and regional focus of this study limit its findings and may reduce generalizability. Additionally, the study does not consider the time lag to detection between media or drug resistance.

The result revealed that MGIT 960 liquid culture were found more sensitive (8.7%) than solid L-J medium (7.1%) to isolate M. tuberculosis in pleural effusion. Nonetheless, MGIT 960 scaled higher despite the minor difference with respect to diagnosis. MGIT also proved being robust because all the L-J positive samples yielded positive results. The outcome confirms the WHO recommendation of liquid culture as a gold standard. The implementation of MGIT 960 in daily diagnostics could increase the number of TB pleurisy identifications.

Abbreviations

TPE – Tuberculous Pleural Effusion

L-J – Lowenstein-Jensen

MGIT – Mycobacteria Growth Indicator Tube

PANTA – Polymyxin B, Amphotericin B, Nalidixic Acid, Trimethoprim, Azlocillin

NIDCH – National Institute of Disease of the Chest and Hospital

SSMC – Sir Salimullah Medical College

ICA – Immunochromatographic Assay

SPSS – Statistical Package for the Social Sciences

Conflicts of interest

The authors report having no conflicts of interest about this work.

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