

Blood Agar can be an Effective Alternate Media for Solid Culture of *Mycobacterium tuberculosis* in Resource-poor Settings, a Report from Warangal, India

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Abstract

Introduction: Tuberculosis (TB) diagnosis is mainly based on clinical features and demonstration of *Mycobacterium tuberculosis* (MTb) under microscopy or culture. Solid culture is considered to be gold standard tool in diagnosing MTb. Preparation of Lowenstein and Jensen (LJ) medium for solid culture is time taking and costly.

Purpose: Our cross-sectional study attempts to identify the utility of blood agar (BA) against LJ as medium for solid culture toward reducing cost and time of procedure.

Materials and Methods: Sputum samples were collected at District TB Center, Warangal, Karunapuram Care and Treatment Center for people living with HIV, and processed at Shivani College of Pharmacy in a biosafety Level II laboratory. Sheep BA, LJ, and Middlebrook 7H9 liquid media were used. Samples were homogenized using N-acetyl L-cysteine (NALC) NaOH method; inoculums prepared and processed in the media, incubated at 37°C, and were observed daily for visible growth through naked eyes. DNAs were extracted from all the colonies from BA and sequence confirmed for MTb. Time taken in the growth of MTb and cost were compared for each media.

Results: Sputum samples from 400 TB suspects were tested which included 250 HIV-negative presumptive TB and 150 HIV-TB coinfecting. Of 250 HIV-negative samples, 73 were culture positive in liquid culture, 65 on LJ medium, and 61 on BA medium. Of 150 HIV-TB coinfecting cases, it was 40, 35, and 37, respectively. The highest yield of MTb in BA was before 10 days compared to >50 days with LJ medium.

Conclusion: BA can be preferred over LJ medium with equal efficacy for the growth of MTb irrespective of HIV status. Lower cost and shorter incubation period of BA may outweigh LJ medium in resource-limited settings.

Key words: Blood agar, Lowenstein and Jensen media, Liquid culture, *Mycobacterium tuberculosis*, Solid culture, Tuberculosis-HIV coinfection

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis* (MTb). It typically not only affects the lungs (pulmonary TB) but can also affect

other sites (extrapulmonary TB). TB is one of the major killer public health challenge across the world. According to the WHO, in 2015, there were an estimated 10.4 million new (incident) TB cases worldwide, of which 5.9 million (56%) were among men, 3.5 million (34%) among women, and 1.0 million (10%) among children. People living with HIV (PLHIV) accounted for 1.2 million (11%) of all new TB cases.^[1] Diagnostic tests for TB include sputum smear microscopy, rapid molecular detection, and culture methods. Globally, the use of rapid molecular tests is increasing, and many countries are phasing out the use of smear microscopy for diagnostic purposes; however, microscopy and culture remain necessary for treatment

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monitoring. Despite advances in diagnostics, a considerable proportion of the TB cases reported to the WHO are still clinically diagnosed rather than bacteriologically confirmed. In 2015, 57% of the pulmonary cases reported to the WHO were bacteriologically confirmed.^[1]

MTb was first isolated by Robert Koch from freshly crushed pulmonary tubercles after 10 days of incubation using heat-coagulated sheep and beef serum medium in tubes.^[2] In 1903, MTb isolates were recovered using blood agar (BA),^[3] but this was superseded by an egg-based agar which, by the 1920s, became the standard recommended medium for primary isolation of MTb because of the ease in sterilizing the egg-based medium.^[4] The culture of MTb is done using solid culture method or liquid culture method. Solid culture is considered to be a gold standard tool in diagnosing MTb. Lowenstein and Jensen (LJ) medium is the most commonly used medium for solid culture. However; many media such as BA and nutrient agar (NA) are available as alternate options to LJ medium.

Drancourt^[5] in the report termed as “end of dogma” reported the incidental growth of MTb colonies on BA which led to further research on BA for culture of MTb.^[6,7] In addition, the utility of BA against Middlebrook 7H10/11 agar or LJ medium was demonstrated in susceptibility testing of MTb.^[8]

In this study, we evaluated the utility of BA against LJ medium, NA, and Middlebrook 7H10 for susceptibility testing of both in terms of cost-effectiveness and time taken for reporting in a resource-limited setting in Warangal, Telangana State of India.

MATERIALS AND METHODS

In this cross-sectional study, 400 sputum samples were collected during 2013 October–2016 June from District TB Center (DTC) and Karunapuram Care and Treatment Center (KCTC), Warangal, India. Early morning sputum samples from 250 presumptive TB patients from DTC and 150 PLHIV/ADIS visiting KCTC with TB symptoms were transported to Sri Shivani College of Pharmacy (SSCP), Warangal, for further processing. In the biosafety Level II laboratory at SSCP, the sputum samples were processed for microscopy with Zeil–Nelson staining and were treated with culture with various media such as sheep BA, LJ, and Middlebrook 7H9 media. Samples were homogenized using NALC NaOH method; the inoculums were prepared and processed in the media, incubated at 37°C. Visible growth was observed on daily basis, and the visible colonies on BA medium were sequenced by DNA extraction method for confirmation of the presence of

MTb. Time taken in the growth of the colonies in each of the media was noted.

Data analysis was performed using IBM SPSS v20 package. Descriptive analysis method was employed in generating the outcomes of the study.

RESULTS

There were 40% of females in the study population and 60% of males [Table 1]. The mean age of the study population was 43.4 years. However, the mean age of HIV-positive subset was 34.5 years while that of HIV-negative subset was 46.7 years and the subset with unknown HIV status was 55.9 years. The age of the study population ranged from 9 years to 90 years. The mean age of female subset of the study population was 41.6 years while that of male subset was 44.7 years [Table 1 and Figures 1 and 2].

When analyzed for the utility of the LJ medium and BA against the gold standard test, i.e., liquid culture, the sensitivity, specificity, positive predictive value, and negative predictive value of both the tests were almost similar [Table 2].

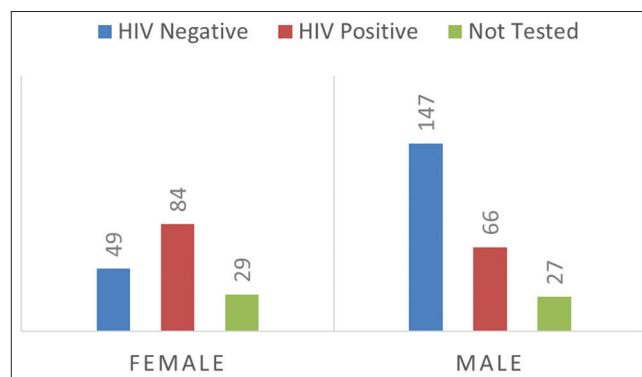


Figure 1: Distribution of study population by gender and HIV status

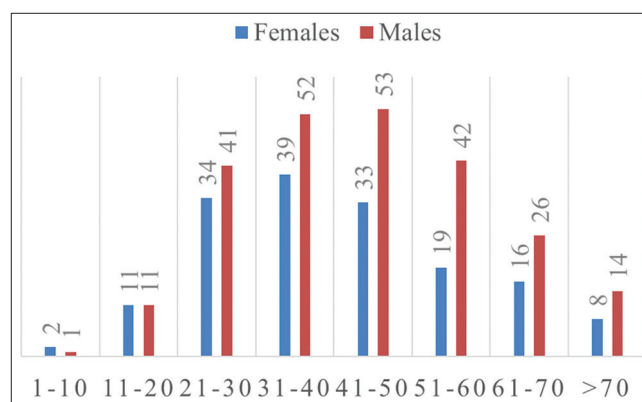


Figure 2: Distribution of study population by gender and age group

Table 1: Demographic profile of the study population by gender, age group, and HIV status

Gender	HIV negative	HIV positive	HIV status unknown	Total (%)
Female	49	84	29	162 (40.3)
Male	147	66	27	240 (59.7)
Total	196	150	56	402 (100)

Age group	Females	Males	Total (%)
1–10	2	1	3 (0.7)
11–20	11	11	22 (5.5)
21–30	34	41	75 (18.7)
31–40	39	52	91 (22.6)
41–50	33	53	86 (21.4)
51–60	19	42	61 (15.2)
61–70	16	26	42 (10.4)
>70	8	14	22 (5.5)
Total	162	240	402

Age group	HIV negative	HIV positive	HIV status unknown	Total
1–10	0	3		3
11–20	9	13		22
21–30	31	43	1	75
31–40	32	51	8	91
41–50	40	33	13	86
51–60	45	5	11	61
61–70	26	2	14	42
>70	13	0	9	22
Total	196	150	56	402

Mean age	HIV negative (years)	HIV positive (years)	HIV status unknown (years)	Grand total (years)
Females	46.1	33.8	56.5	41.6
Males	47.0	35.3	55.2	44.7
Overall	46.7	34.5	55.9	43.4

The growth of MTb in either medium (BA or LJ medium) did not differ by the HIV status of the patient [Table 3 and Figures 1 and 3].

When analyzed for the duration for observing the growth of MTb in the media, it was seen that 100% of possible growth of MTb has been achieved within 10 days of incubation [Figure 4]. While it took 21 days to achieve 100% of possible positive culture in liquid culture medium, the last possible positive growth in LJ medium went up to >50 days. This clearly demonstrates the benefit of BA over LJ medium for the achievement of the early growth of MTb.

DISCUSSION

In our study, it is observed that the highest MTb growth yield is achieved by liquid culture in comparison to both LJ media as well as BA medium in identifying 15% more positive MTb growth as expected from a gold standard test. However, when compared between BA and LJ media, the BA medium seems to achieve equal amount of growth of MTb in 1/5 duration taken by LJ medium which is very critical for early diagnosis of MTb in settings where liquid culture cannot be undertaken. Especially among

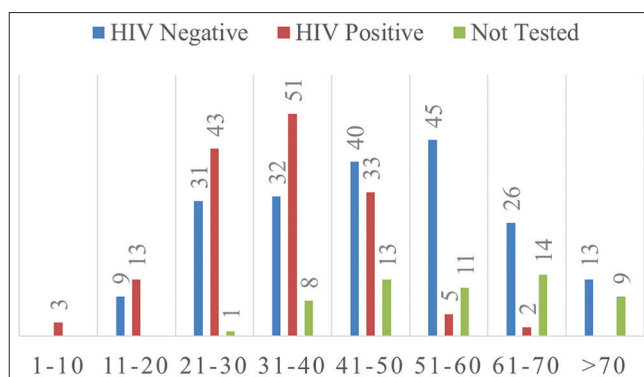


Figure 3: Distribution of study population by HIV status and age group

HIV-TB coinfecting cases, reduction in time for achieving positive growth (maximum of 10 days in BA compared to >50 days in LJ medium) can have major implications in early diagnosis, early initiation of treatment, and thus saving the lives of PLHIV. Similar studies across the world have shown that the mean time to detect MTb from smear-positive pulmonary sample on LJ slants has been found to be in the range of 19–24 days by most workers.^[9-13]

The utility of BA media for the recovery of MTb was reported early in the last century but has been removed from contemporary microbiology manuals.^[11,14] However,

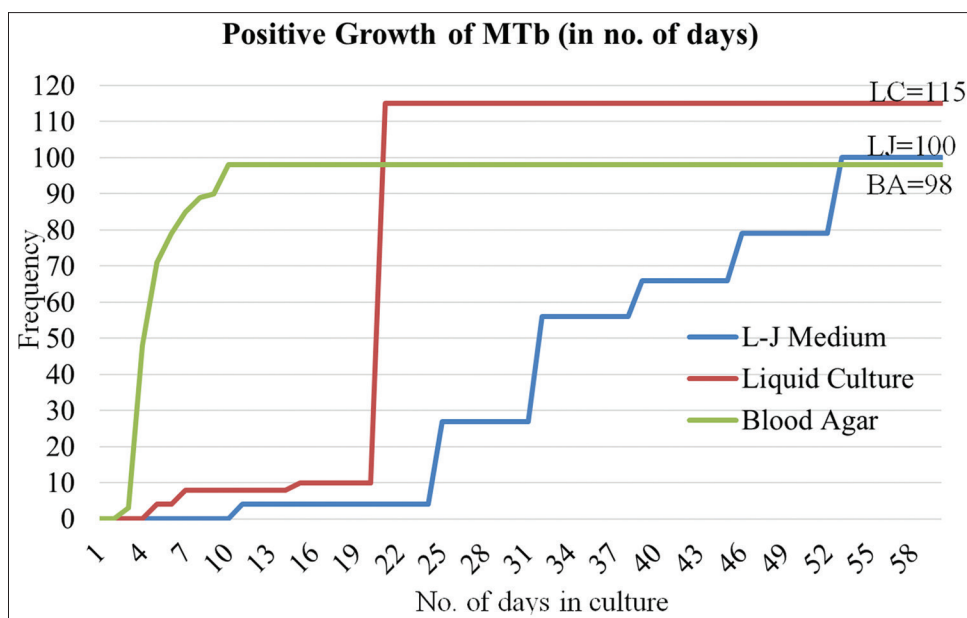


Figure 4: Growth of *Mycobacterium tuberculosis* in culture by LJ and BA media against liquid culture

Table 2: Utility of LJ medium and blood agar medium against the liquid culture

	Liquid culture			Total
		Growth positive	No growth	
L-J medium	Growth positive	97	3	100
	No growth	18	284	302
	Total	115	287	402
	Sensitivity	84.3%		
Specificity	99.0%			
Positive predictive value	97.0%			
Negative predictive value	94.0%			
Blood agar	Growth positive	96	2	98
	No growth	19	285	304
	Total	115	287	402
	Sensitivity	83.5%		
Specificity	99.3%			
Positive predictive value	98.0%			
Negative predictive value	93.8%			

LJ: Lowenstein and Jensen

there have been recent reports, including one from Mathur *et al.* in 2009 which isolated MTb clinical isolates from pulmonary smear-positive patients and suggested that the sensitivity of BA media was equivalent to LJ media.^[6]

In our study, MTb growth in BA medium was well comparable to the growth achieved in liquid culture using Middlebrook 7H9 medium with even early achievement of positive culture which helps the laboratories in choosing a

Table 3: Differential growth of MTb in blood agar and LJ medium

HIV Status	Growth positive	No growth	Total
LJ medium			
Negative	65	131	196
Positive	35	115	150
Unknown	0	56	56
Grand total	100	302	402
Blood agar			
Negative	61	135	196
Positive	35	115	150
Unknown	2	54	56
Grand total	98	304	402

MTb: *Mycobacterium tuberculosis*, LJ: Lowenstein and Jensen

right medium when liquid culture facility is not available. The sensitivity and specificity of both BA and LJ media against the gold standard liquid culture are nearly equal which establishes BA as an alternative to LJ medium for solid culture of MTb. We observed that the growth of MTb in either medium (BA or LJ medium) did not differ by the HIV status of the patient.

CONCLUSION

We conclude that BA can be a good alternative medium to LJ medium for achieving the growth of MTb. especially among HIV-TB coinfecting cases, reduction in time for achieving positive growth can have major implications in early diagnosis, early initiation of treatment, thus saving the lives of PLHIV, and hence can be preferred over LJ medium in the absence of facility for liquid culture.

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