

Comparative Analysis between Monophasic and Biphasic Methods of Blood Culture

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Abstract

A biphasic blood (BiPB) culture bottle (GIBCO Laboratories, North Andover, USA) with an architectural design that physically separates the agar slant from the broth was compared with a conventional vented monophasic bottle (MPB) (GIBCO Laboratories, North Andover, USA) for routine blood culture. Monophasic bottles contained thioglycollate broth (BioAmerica Inc., USA) while the biphasic contained Brain heart infusion agar-BHIA/Brain heart infusion broth-BHIB (BioAmerica Inc., USA). 120 blood cultures were examined on either of monophasic and biphasic blood culture experimental set up. Out of 240 sets collected, 157(65.4%) were positive for bacterial growth while 83(35.6%) were negative. After subculture a total of 8 isolates (from 154 plates for BiPB and 117 plates for MPB) were recovered during the study. Of these isolates, 4 (from 126 plates for BiPB and 91 plates for MPB) were recovered, 2 isolates (from 28 plates) grew in the BiPB but not in MPB, 2 isolates (from 26 plates) grew only in the MPB but not in BiPB. The BiPB allowed more rapid recovery of *Escherichia coli*, *Staphylococcus* spp., *Klebsiella* spp., *Salmonella* spp. and *Proteus* spp. While *Pseudomonas* spp. and *Enterococcus faecalis* were more readily isolated using MPB. BiPB subcultures was easy enough to permit both early and daily subculture which provided isolated colonies sooner than could be done by using the MPB. Bacteria organisms were recovered at a non-significantly ($P > 0.025$) more frequent rate in the BiPB than in the MPB-A. Either bottle, however, should be used in conjunction with an anaerobic bottle for optimal recovery of anaerobic bacteria.

Key words: Biphasic, Monophasic, Thioglycollate, Brain Heart Infusion Agar/Broth, blood culture.

1. Introduction and Literature Review

Detection of bacteremia traditionally has been one of the most important functions of clinical microbiology laboratories. When blood cultures yield clinically important microorganisms, it is a sign that host defenses have failed to contain an infection at its primary site or that the clinician has failed to adequately eradicate the infectious process.^{3,7} During the past two decades, there have been a number of changes in blood culture practices; these changes have been based on clinical investigations that have established a stronger scientific basis for this diagnostic test.¹²

The accepted practice of routine subculture of conventional MPB bottles is flexible and requires no purchase of expensive instruments but they are labor intensive and time-consuming.^{18,19} Several commercially available products have been introduced to ease the burden of subculture, including biphasic systems. The BiPB with an architectural design that incorporates the agar slant within the bottle in a chamber physically separated from the broth. This design eliminates the need for additional manipulation of the bottle before subculture.^{1,6}

Microbial growth is often identified on the agar rather than in the broth, allowing for prompt preliminary identification and susceptibility testing. Comparisons of this system with other manual systems and with the BACTEC radiometric system have yielded mixed results.^{2,5,16} Certain microorganisms, including *Streptococcus pneumoniae*, other streptococci, *Pseudomonas aeruginosa* and anaerobic bacteria, may not be recovered optimally with this system.¹⁰ Three of these Microorganisms that almost always (>90% of isolates) represent true infection when isolated from the blood include *Staphylococcus aureus*, *Escherichia coli* and other Enterobacteriaceae, *P. aeruginosa*, *S. pneumoniae*, and *Candida albicans*. Isolates from blood that rarely (5% of isolates) represent true infection include *Corynebacterium* species, *Bacillus* species, and *Propionibacterium acnes*. Some authorities have suggested that the number of bottles positive in a culture set is a predictor of the clinical significance of an isolate. However, Mirrett et al., 1996 and Peacock et al 1995 have found that this criterion is unreliable, at least for coagulase-negative staphylococci.^{4,8,14}

A useful interpretative concept is the number of culture sets found to be positive vs. the number obtained. If most or all cultures in a series are positive, regardless of the microorganism recovered, the probability that the organism is clinically important is high.^{11,17} Since the presence of a bloodstream infection is important in terms of both diagnosis and prognosis, correct interpretation of the positive test result is crucial. Misinterpretation of

positive results can be costly both to the institution and to the patient.^{20,21} The purpose of this study was to compare the efficacy of the BiPB with that of a conventional monophasic bottle for the routine culture of blood with respect to types of microorganisms recovered, days to positivity, and time to isolation of colonies.

2. Materials and Methods

Fifteen to twenty milliliters of blood collected aseptically from patients at the Kwara State Polytechnic Medical Centre, Ilorin, were divided equally between the two bottles in each set of BiPB and MPB. The protective caps of the culture bottles were removed and sterilized using an ethanol swab, the top of each bottle was wiped and the protective caps replaced. The culture bottles were gently shaken to mix the blood with the broth thoroughly. Using a lead pencil, the bottles were labeled with the age, number of the patient and sex. The inoculated media were incubated as soon as possible. All isolates from positive blood cultures were identified by routine procedure according to Weinstel, 1996.

2.1 Data Analysis

The BiPB and MPB-A were compared with respect to the day of positivity, the frequency of positive cultures, the time to isolation of colonies and the types of microorganisms recovered.

3. Results

There was early isolation and confirmation of microorganisms on the biphasic setups. Unlike the monophasic setups, growth of isolates were easily seen after 48 hours of incubation on the agar slants of about 75% of the biphasic setups as shown in table 2. It took 3 days to 6 days for the positivity and isolation of pure colonies on the monophasic setups. After subculturing on blood and MacConkey agar media, 154 plates were positive for biphasic setups with 6 isolates recovered while 117 plates were for monophasics with 6 isolates also recovered as shown in table 3. *Staph. aureus* is the most frequent isolates followed by *Staph. epidermidis* and *Escherichia coli* on biphasic and monophasic setups. *Pseudomonas* spp. and *Enterococcus faecalis* were isolated mainly on the monophasics while *Klebsiella* spp. and *Proteus* spp. mainly on the biphasics as in table 3. Also *Salmonella* spp. was isolated on both setups.

4. Discussion

The higher recovery of isolates by the biphasic setups was a clear indication of its efficiency and selectivity. This was because, coupled to the rapid detection of isolates in the liquid phase was the isolation on the upper portion of the slant two days later. This means we had a two stages check; an early detection and followed by primary isolation and confirmation.

Al-Sulami et al (2002) stated that, the high performance of biphasic is due to the changes in the composition of the liquid and solid media, and the manner in which they are set up. This was as a result of the simple monophasic-biphasic culture setup (MBCS) which provided efficient isolation and identification of microorganisms. Due to longer period of recovery in the monophasic setups some microorganisms were not easily recovered as in the biphasic.

Al-Sulami et al (2002) also noted that, the diagnostic value and applicability of any method depends on its simplicity, rapidity, reproducibility and cost. Assessment of biphasic media appeared to be the simplest, most rapid culture procedure with the lowest cost. The conclusion was arrived at by comparing the performance of biphasic with the monophasic.

5. Conclusion

The advantage of the biphasic lies in its versatility. It permits a solid phase to be in direct contact with a liquid one both of which are below a solid phase. Biphasic is inexpensive because of the small quantities of liquid and solid media consumed, the omission of transport media and the short incubation time within one test tube. It also obviates the need for frequent opening of the culture to subculture which prevents contamination and human infection.

Monophasic setup, on the other hand, forms the basic units of any biphasic setup. Their combination leads to biphasic setup. Unlike biphasic setup, monophasic setup is easy to prepare without much technical knowledge. Cultural characteristics of colonies are easily determined on a solid sub-cultured monophasic medium. Certain confirmatory tests are performed on monophasic setup like sensitivity test, haemolysis on blood agar, lactose fermenting on MacConkey agar and so on. Also anaerobic culture of microorganisms is easily performed on the monophasic setups. Monophasic media; liquids, solids and semi-solids are the main means of microbial culture while biphasic media are just arrangement of monophasics for better and quicker recovery of microbial isolates.

Based on these findings biphasic media setup is suitable for a wide range of medical and non-medical

applications, particularly since current procedures and serological tests are relatively insensitive and culture is also time consuming.

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Table 1: Distribution of patients by age and sex

Age range	Number of patient	Male	Female
0-9	6	2	4
10-19	9	6	3
20-29	11	7	7
30-39	20	12	8
40-49	29	11	18
50-59	24	14	10
60-69	18	10	8
70 above	3	1	2
Total	120	63	57

Table 2: Age range with number of positive growth culture

Age range	Biphasic Media		Monophasic Media	
	No	%	No	%
0-9	5	5.56	2	2.99
10-19	8	8.89	5	7.46
20-29	9	10.00	9	13.43
30-39	15	16.67	11	16.41
40-49	19	21.11	15	22.39
50-59	20	22.22	11	16.41
60-69	11	12.22	13	19.40
70 above	3	3.33	1	1.49
Total	90	100	67	100

Table 3: Types of Microorganisms isolated with frequency of occurrence

Microorganism Isolated	Biphasic Media		Monophasic Media	
	No	%	No	%
Staph. aureus	61	39.61	48	35.90
Staph. epidermidis	30	19.48	25	21.37
Escherichia coli	22	14.29	10	9.40
Pseudomonas spp.	-	-	19	16.23
Klebsiella spp.	19	12.34	-	-
Salmonella spp.	13	8.44	8	6.84
Enterococcus faecalis	-	-	7	5.99
Proteus spp.	9	5.84	-	-
Total	154	100	117	100

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