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Comparison of Lysis Centrifugation with the Conventional Blood Culture Method in Cases of Blood Stream Infection in Tertiary care Hospitals

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Abstract

Background: Bacterimia, septicaemia and sepsis remain a significant health problem in many developing countries due to its considerable morbidity and mortality. Hence, blood cultures have become critically important and frequently performed test in clinical microbiology laboratories for diagnosis and treatment. **Objective:** To compare the conventional blood culture with the lysis centrifugation method for isolation of causative bacteria in cases of bacterimia, septicaemia and sepsis. **Methodology:** Fifty nonduplicate blood samples for culture from cases of septicaemia and sepsis were analyzed using two blood culture methods concurrently for recovery of bacteria from patients diagnosed clinically with septicaemia and sepsis – in conventional blood culture method using trypticase soy and broth-brain heart infusion broth and the lysis centrifugation method using saponin, SPS, PABA and centrifuging at 3000 g for 30 minutes followed by streaking on solid media. **Result:** Overall bacteria recovered from 50 blood cultures were 32%. The conventional blood culture method had a higher yield of organisms, especially gram positive cocci and the lysis centrifugation method was comparable with the former method with respect to gram negative bacilli especially facultative intracellular. The sensitivity only lysis method was 28%, conventional method 24% and both methods in same specimen was 32%. **Conclusion:** Combination of the lysis centrifugation and conventional methods is more sensitive than use of either conventional or lysis method for isolation of bacteria causing blood infection if sample processing in biological safety cabinets for reduces contamination.

Keyword: Blood culture, lysis centrifugation, saponin, SPS, PABA

Introduction

Invasion of the blood-stream by living microorganism causes significant morbidity and mortality, a fact well recognized before the dawn of modern medicine. Hence, blood cultures have become and still remain an essential requisite for diagnosis of sepsis.[1] A positive blood culture not only suggests a definitive diagnosis but also guides specific therapy and prognosis.[2] The final identification of a microorganism by the conventional blood culture method takes about 3 to 4 days, provided the organism grows in the first subculture done 48 hours after the receipt of the sample.[3] The lysis centrifugation method concentrates and separates microorganism from plasma and cells then subculture done, thereby getting result earlier than conventional method. The presence of actual colonies after initial incubation for direct identification, not only makes it rapid but one can also quantify the colony forming units. Based on clinical impression, special media can be chosen for the initial culture setup. It has been found to be an excellent method for intracellular organisms including yeasts and thermal dimorphic fungi. Processing specimens in biological safety cabinets significantly reduces contamination rates, a drawback well known of this method apart from being labor intensive. It is not an optimal method for recovering anaerobes, Haemophilus influenza and listeria.[4] There are few international studies on the lysis centrifugation

method that showed it to be a better method than the conventional one.[5-9] Few Indian reports using lysis centrifugation method for brucella[10], mycobacteria[11] and fungi[12] are available. One documented study done in Mymensingh Medical College Bangladesh for recovery of commonly encountered bacteria in blood cultures by lysis centrifugation method was 22% isolation rate[13]. Hence, this study was undertaken with the intention of comparing the lysis centrifugation method with the conventional blood culture method in terms of yield and recovery rate of bacteria.

Methodology

This cross sectional comparative study was done in the department of Microbiology Prime Medical college hospital, Rangpur and Rafatullah communittty hospital, Bogra. Fifty patients admitted with clinical signs and symptoms of septicaemia and sepsis in these tertiary care hospitals from January 2014 to December 2015 were included in this studied. After written consent was taken, requisite amount of blood (Table 3) was collected aseptically during the fever spike or just before the next dose of an antibiotic, simultaneously for lysis centrifugation and conventional blood culture methods. By the conventional method, blood was inoculated in blood culture bottles (1:10 dilutions) containing trypticase soy broth, brain heart infusion broth, SPS, PABA and 0.1% agar and were incubated at 37°C for 7 days and periodically shaken and three subculture done after 2days to every 24 hours interval upto 6th day. The first subculture was done from each bottle on Blood agar and MacConkey agar plates after 48 hours and then on fourth, fifth and seventh day.[3,4] In the lysis centrifugation method, blood was collected in 10 mL screw capped tubes containing 0.2/0.5 mL solution of saponin (2/5mg) and sodium polyanethol-sulfonate (SPS) (0.8/2.0 mg) (Table-2) for paediatric and adult patients, respectively.[14]

Table 1: Composition of Traditional blood culture bottle
Table 1: Composition of Traditional blood culture bottle

Ingredient	Amount
Brain Heart Infusion (BHI) Broth base powder	9.25 gram
Tryptone Soya Broth base powder	7.5 gram
Sodium polyanethol sulfonate(SPS)	0.25 gram
4-Aminobenzoic acid(PABA)	0.025 gram
Agar	0.5gram or (0.1% (w/v)
Distilled water in ml	500 ml
Step-1: Mix well and heat at 80ºC for 30 minutes Step-2: Dispense 100 ml for adult & 50ml for child of media in each bottle. Step-3: Keep rubber cap loosely on the bottle mouth Step-4: Place a piece of Aluminium foil on rubber cap loosely Step-5: Autoclave for 20 minutes Step-6: After removal from autoclave tight the rubber caps and Aluminium foils & disinfectant with hexisole	

Table 2: Composition of Lytic solution

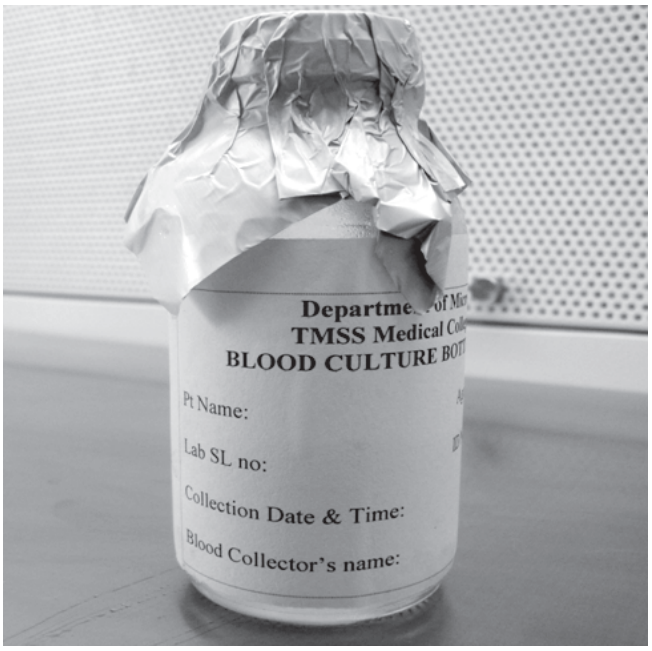
Ingredient	For Infant	For adult
Sodium polyanethol sulfonate(SPS) in gram	0.19	1.53
Saponin in gram	0.80	2.80
Distilled water in ml	100	20
By autoclaving at a temperature of 1210c, pressure of 15ib for 15 minutes. With all aseptic precaution by the help of sterile pipette and sterile tips lytic solution dispensed into Heparinized tubes within a biosafety cabinet. For adult 1.0 ml and for infant 125ul of lytic solution in each heparinized tube.		

Table-03: Amount of blood collected in different Age group for Conventional method & Lytic centrifugation method

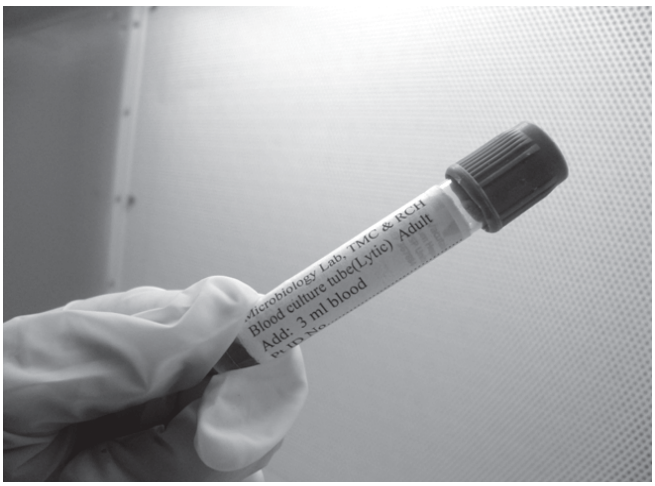
Age group	Conventional	Lytic centrifugation	Total amount of blood
Infant	05 ml	01ml	06 ml
Children	05-07 ml	02ml	08ml
Adult	10ml	03ml	13ml

All the screw capped tubes containing saponin, in which blood was collected were centrifuged within two hours for 30 minutes at 3000 g (g = gravitational force). Supernatant was discarded using sterile Pasteur pipette. Around 0.5 mL of the sediment was spot inoculated on blood agar and

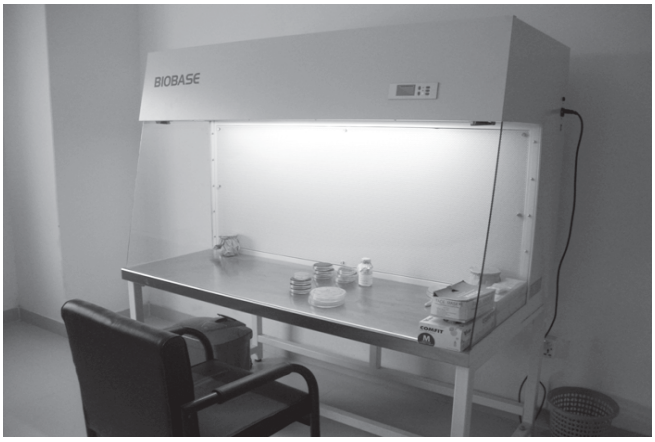
MacConkey agar plates and then with sterile nichrome loop, hexagonal streaking was done. Both the plates were then incubated at 37°C aerobically overnight. The plates were examined for any growth. If there was no growth, they were further incubated for 24 hours after which they were discarded[4].If there was growth by either the conventional or lysis centrifugation method, the organism was further identified by standard laboratory techniques[14].



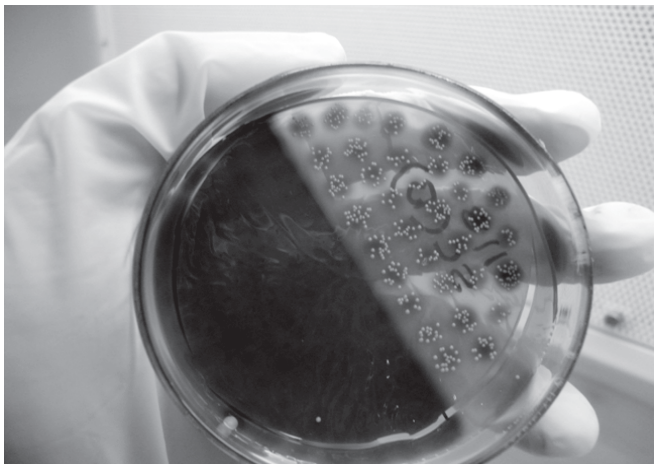
Traditional blood culture bottle



Lytic tube blood culture bottle



Blood culture sample processing in BSC



Colonies in agar media after subculture

Results

Overall bacteria recovered from 50 blood cultures were 32%. The conventional blood culture method had a higher yield of organisms, especially Gram positive cocci. The lysis centrifugation method was comparable with the former method with respect to Gram negative bacilli. The sensitivity and specificity of lysis centrifugation method in comparison to conventional blood culture method was 52.7% and 98.21%. In almost every instance, the time required for detection of the growth was earlier by lysis centrifugation method, which was statistically significant. Contamination by lysis centrifugation was minimal, while that by conventional method was high. Time to growth by the lysis centrifugation method was highly significant (P 0.001) as compared to time to growth by the conventional blood culture method.

Table 4: Number and percentage of isolates by different methods

Method	Growth	No growth	Total
Only Traditional	12(24.0%)	38(76.0%)	50
Only Lysis centrifugation	14(28.0%)	36(72.0%)	50
Both method in same sample	16(32.0%)	34(68.0%)	50

Figure-1: Number and percentage of isolates by different methods

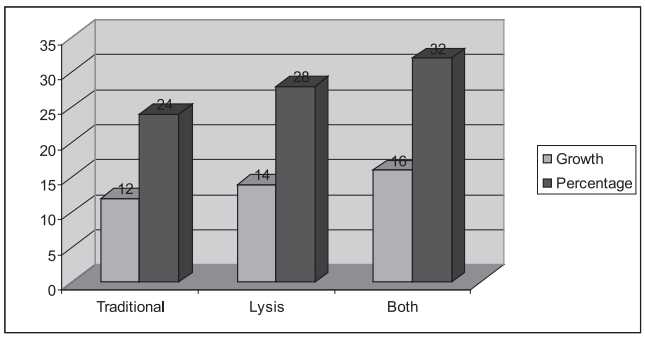


Table 5: Bacterial isolates in Traditional method(n=50)

Name of Isolates	No of Isolates	Percentage
Staphylococcus aureus	04	08
Streptococcus pyogenes	01	02
Streptococcus pneumoniae	01	02
Escherichia coli	02	04
Haemophillus spp.	00	00
Klebsiella spp.	01	02
Salmonella typhi	02	04
Salmonella paratyphi	01	02
Total	12	24

Table 6: Bacterial isolates in Lysis centrifugation method (n=50)

Name of Isolates	No of Isolates	Percentage
Staphylococcus aureus	02	04
Streptococcus pyogenes	01	02
Streptococcus pneumoniae	01	02
Escherichia coli	02	04
Haemophillus spp.	01	02
Klebsiella spp.	01	02
Salmonella typhi	04	08
Salmonella paratyphi	02	04
Total	14	28

Table 7: Bacterial isolates using both Lysis centrifugation & traditional methods(n=50)

Name of Isolate	No of Isolates	Percentage
Staphylococcus aureus	04	08
Streptococcus pyogenes	01	02
Streptococcus pneumoniae	01	02
Escherichia coli	02	04
Haemophillus spp.	01	02
Klebsiella spp.	01	02
Salmonella typhi	04	08
Salmonella paratyphi	02	04
Total	16	32

Discussion

Overall bacteria recovered by using both methods from 50 cases of septicaemia and sepsis were 32.0%. But in traditional method it was 24% and lysis method was 28%. A study from Nepal by lysis method has reported the same in 20%, which is lower than the present study(28%)[15].Culture positivity by conventional blood culture method was 24% in this study but a study from Chandigarh has reported overall blood culture positivity of 9.94%,[16] which is less than this study. Different studies from USA have shown a better recovery of bacteria by the lysis centrifugation method, in comparison to the conventional blood culture method[5,7-9]. In this study, however, the conventional blood culture method yielded greater culture positivity (16%) in gram positive bacteria, than the lysis centrifugation method. This might be explained that all studies in USA used the Isolator system (Wampole Laboratories/ Dupont) containing inert fluorochemical apart from saponin, whereas in this study saponin, SPS and PABA were used. The sensitivity of the lysis centrifugation method in comparison to the conventional blood culture method was 52.5% in this study, specificity was 98.21%. As far as Indian studies are considered, the lysis centrifugation method was compared with the conventional blood culture method for diagnosis of brucellosis and fungal sepsis and both these studies showed a

higher positivity by the former method.[10,12] In another Indian study from Vellore, 16.2% Mycobacterium tuberculosis were detected by the lysis centrifugation method.[11] Brucella species, M. tuberculosis and fungi are all intracellular microorganisms, but in this study only the common bacterial pathogens of sepsis, which are extracellular were looked for. Thus, this study revealed that the lysis centrifugation method is not suitable for extracellular microorganisms. Kelly et al. have reported 3% contamination by the lysis centrifugation method.[7] By the conventional method, contamination as reported by Henry et al. was slightly higher (3.2%) than the lysis centrifugation method (3%).[9] However, few studies have reported greater contamination rates with the lysis centrifugation method (4.4-9.6%), than conventional method (0.6-2.7%).[5,7-9] In this study, contamination was remarkably less two in fifty samples (4% only) by the lysis centrifugation and 5% in conventional method.

Contamination in the conventional method could have been reduced, if all aseptic precaution is done in Biological safety cabinet. According to the standards published by the American Society for Microbiology, the rate of blood culture contamination should not exceed 3%.[17] Therefore, in this study; the contamination rate by the conventional method is acceptable. A recent study from the United Kingdom has also reported 7.4% contamination from blood cultures.[18] Therefore, this highlights the need for regular training and education of health care professionals, who collect blood samples and laboratory technicians who process the samples. Kelly et al. have reported that both the methods were equally effective for detection of S. aureus, the conventional blood culture method was more effective for detection of Streptococcus species.[7]In this study, sensitivity of the lysis centrifugation method for Gram-negative bacilli was higher than that of Gram-positive cocci (64.71%), as compared to the conventional blood culture method. Henry et al. also reported significant recovery of S. aureus by lysis centrifugation method, but Streptococcus species was significantly recovered by conventional blood culture,[9] which is similar to this study. Therefore, the lysis centrifugation method can be used for recovery of Gram-negative bacilli and the conventional blood culture method is more effective for the detection of S. aureus, streptococci and enterococci.

Amongst the Gram-negative bacilli, Klebsiella pneumoniae predominated with a recovery rate of 41.18% (7/17) by the conventional blood culture method and 50% (6/12) by lysis centrifugation method. De et al. have also isolated K. pneumoniae as the commonest Gram-negative bacilli (34.3%) from blood cultures of hospitalized children suspected of sepsis.[19] Bacteria grown by only conventional blood culture method took minimum 48 hours to grow, whereas bacteria by only lysis centrifugation method grew within 24 hours. By t-test, two-methods assuming equal variance, time to growth for Enterobacteriaceae was significant ($P < 0.001$) by the lysis centrifugation method as compared to the conventional blood culture method. Kiehn et al.[8] have also reported the superiority of the lysis centrifugation method over the conventional blood culture method by detecting the bacteria on an average of 1 day earlier and yeasts on an average of 2 days earlier, which is exactly similar to this study. Henry et al. have detected Gram-positive cocci and Pseudomonas aeruginosa 1 day earlier by the lysis centrifugation method.[9] The commercially available Isolator system (Wampole/Dupont) is definitely better than the method used in this study in recovering blood culture isolates but it is costly. There is still no single blood culture system capable of offering optimal recovery of aerobic and facultatively anaerobic bacteria, anaerobic bacteria, and yeasts. Thus, the use of at least two blood culture systems is advocated.[9]

The lysis centrifugation method minimizes contamination and has a faster recovery of bacteria and fungi, whereas the conventional blood culture method has a greater yield of microorganism. Therefore, in resource restricted settings, where automated blood culture systems like BACTEC, BacT/ALERT, etc. are not feasible, a combination of lysis centrifugation method and conventional blood culture method with trypticase soy broth or biphasic media is advocable in order to achieve faster recovery and a better yield of microorganisms. Latter are simple methods, do not require any specialized equipments and uses chemicals and media which are readily available. The rapid identification of aetiological agents would permit the clinician to institute appropriate therapy, thereby reducing hospital stay, morbidity and mortality in patients with sepsis.

Conclusion

For the diagnosis of sepsis, combination of the lysis centrifugation method and the found to be an excellent method for intracellular organisms including yeasts and thermal dimorphic fungi. Processing specimens in biological safety cabinets significantly reduces contamination rates, a drawback well known of this method.

Conflict of Interest: None declared.

References

1. Reimer LG, Wilson LM, Weinstein MP. Update on detection of bacteraemia and fungaemia. Clin Microbiol Rev. 1997;10:444–65.
2. Hall KK, Lyman JA. Updated review of blood culture contamination. Clin Microbiol Rev. 2006;19:788–802.
3. Borriello SP, Murray RP, Funke G. 10th ed. Vol. 1 and 2. Hodder Arnold: London; 2005. Topley and Wilson's Microbiology and Microbial Infections: Bacteriology; pp. 509–54. (811).
4. Forbes BA, Sahm DF, Weissfeld AS. 12th ed. Missouri: Mosby Elsevier; 2007. Bailey and Scott's Diagnostic Microbiology; pp. 871–83.
5. Henry NK, McLimans CA, Wright AJ, Thompson RL, Wilson WR, Washington JA., 2nd Microbiological and clinical evaluation of the isolator lysis-centrifugation blood culture tube. J Clin Microbiol. 1983;17:864–9.
6. Dorn GL, Land GA, Wilson GE. Improved blood culture technique basedon centrifugation. clinical evaluation. J Clin Microbiol. 1979;9:391–6.
7. Kelly MT, Buck GE, Fojtasek MF. Evaluation of a lysis-centrifugation and biphasic bottle blood culture system during routine use. J Clin Microbiol. 1983;18:554–7.
8. Kiehn TE, Wong B, Edwards FF, Armstrong D. Comparative recovery of bacteria and yeasts from lysis-centrifugation and a conventional blood culture system. J Clin Microbiol. 1983;18:300–4.
9. Henry NK, Grewell CM, Van Grevenhof PE, Ilstrup DM, Washington JA., 2nd Comparison of lysis-centrifugation with a biphasic blood culture medium for the recovery of aerobic and facultatively anaerobic bacteria. J Clin Microbiol. 1984;20:413–6.
10. Mantur BG, Mangalgi SS.Evaluation of conventional castaneda and lysis centrifugation blood culture techniques for diagnosis of human brucellosis. J Clin Microbiol.2004;42:4327-28.
11. David ST, Mukundan U, Brahmadathan KN, Jacob TJ. Detecting mycobacteraemia for diagnosing tuberculosis. Indian J Med Res. 2004;119:259–66.

12. Sinha K, Tendolkar U, Mathur M. Comparison of Conventional Broth Blood Culture Technique and Manual Lysis Centrifugation Technique for Detection of Fungemia. Indian J Med Microbiol. 2009; 27:79–80.

13. Washington WC, Jr, Allen S, Janda W, Koneman E, Procop G, Shreckenberger P, et al. 6th ed. Philadelphia: Lippincott Williams and Wilkins; 2006. Koneman's Color Atlas and Textbook of Diagnostic Microbiology; pp. 100–5. (1219,1443-81).

14. Sultana S. Comparison of different test methods including polymerase chain reaction for early and realiable diagnosis of typhoid fever. M.Phil thesis 2012.

15. Cheesbrough M. 6th ed. Vol. 2. Cambridge: Cambridge University Press; 2006. District Laboratory Practice in Tropical Countries; p. 127.

16. Shrestha P, Das BK, Bhatta NK, Jha DK, Das B, Setia A, et al. Clinical and Bacteriological Profiles of Blood Culture Positive Sepsis in Newborns. J Nepal Paediatr Soc. 2007;27:64–67.

17. Ernst DJ. Controlling blood-culture contamination rates. (quiz 20-1).MLO Med Lab Obs. 2004;36:14–8.

18. Raja N, Parratt D, Meyers M. Blood culture contamination in a district general hospital in the UK: a 1 - year study. Healthc Infect. 2009;14:95–100.

19. De A, Saraswathi K, Gogate A, Fernandes A. Bacteraemia In Hospitalised Children – A One Year Prospective Study. Indian J Med Microbiol. 1995;13:72–75.