

Comparative Evaluation Of Bactec Culture Using Peds Plus F Broth With Standard Microbiological Methods In Detection Of Microbial Aetiologies In Vitreous Aspirates In Infectious Endophthalmitis.

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Abstract

Purpose: Since standard microbiological methods (use of solid media) have low sensitivity to detect aetiological agents in vitreous aspirates in infectious endophthalmitis, a comparative study was undertaken to determine sensitivity of BACTEC Peds Plus F broth for isolation of these aetiological agents. **Methods:** Four hundred and eighty three consecutive vitreous aspirates from clinically diagnosed infectious endophthalmitis were subjected to culture using standard microbiological media and Bactec culture using Bactec Peds Plus F broth. **Results:** Of the 483 consecutive vitreous aspirate specimens processed, Peds Plus F broth yielded microbial growth in 150 (31.1 %) specimens as against standard microbiological culture which revealed growth in 81 (16.8 %) specimens indicating a statistically significant (p value = 0.005 – Non parametric Chi-square test) increased isolation rate of 14.3 % by Bactec method. Bactec method failed to show growth of infectious agent in 8 vitreous aspirates, though it was noted by standard methods. Thus, among the 158 culture positive specimens, bacterial growth was detected in 132 (83.54%) specimens and fungal growth in 26 (16.46%) specimens.

Conclusions: It is recommended that Bactec Peds Plus F broth may routinely be used for culturing the vitreous specimens because of its increased sensitivity and rapidity of growth of the causative agent of infectious endophthalmitis. To the best of our knowledge, this is the first study to be reported in Indian literature.

INTRODUCTION

Infectious endophthalmitis though rare is a severe devastating and blinding complication after an intraocular surgery or penetrating ocular trauma. Successful management of this condition depends on the definitive and early detection of the causative infectious agent to enable ophthalmologist to institute appropriate antimicrobial therapy.¹ Microbiological investigation of vitreous fluid and aqueous humor is the only method permitting reliable identification of the causative organism. In the literature positive cultures, by conventional methods have been reported in 35-85% of the cases.^{2,3,4,5,6} Several studies have demonstrated the utility of blood culture media over standard agar plates and broth culture methods for the isolation of microorganisms from sterile body fluids.^{7,8,9} In literature,

there are many reports denoting the increase in culture yield in a variety of specimens such as joint fluid,¹⁰ pleural fluid,¹¹ pancreatic pseudocyst,¹² ascitic fluid¹³ and other sterile body fluids¹⁴ by using blood culture media. We hypothesize that blood culture media yields an increasing rate of isolation than conventional media and the present study is focused to compare the results of vitreous culture performed using Bactec Peds Plus F broth (used for blood culture routinely) with that of standard microbiological media.

MATERIALS AND METHODS

Four hundred and eighty three consecutive vitreous aspirates collected from patients with clinically suspected endophthalmitis referred to a tertiary eye care hospital from June 2007 to September 2009 were included in the study. All

the specimens were subjected to culture using standard microbiological methods using the following media - Brain heart Infusion broth, Thioglycollate broth, blood agar, chocolate agar, MacConkey agar, Brucella blood agar (incubated anaerobically at 37°C) and Sabouraud's dextrose agar (incubated at 25°C). In parallel, 50 to 100µl of vitreous aspirate was inoculated on to BACTEC Peds Plus F broth and incubated in BACTEC (BD, 9050) rotating incubator. The instrument indicates the growth as soon as it happens. The standard microbiological media and BACTEC Peds Plus F broth were incubated for 48 hours for the growth of bacteria and 12 days for the growth of fungus.

RESULTS :

Of the 483 consecutive vitreous aspirate specimens processed, Peds Plus F broth yielded microbial growth in 150 (31.1 %) specimens as against standard microbiological culture which revealed growth in 81 (16.8 %) specimens indicating a statistically significant (p value =0.005 – Non parametric Chi-square test) increased isolation rate of 14.3 % by Bactec method. In addition 8 specimens showed growth only by standard microbiological methods and not by Bactec method. Thus, among the total 158 (32.7%) culture positive specimens, 132 (83.54%) were bacteria and fungal growth was present in 26 (16.46%) specimens. The results of standard microbiological culture and BACTEC culture performed on vitreous aspirate specimens are shown in Table 1.

ANALYSIS OF CONCORDANT AND DISCORDANT RESULTS

The analysis of results of conventional culture and BACTEC culture performed on vitreous aspirate revealed concordant results in 148 (98.6%) specimens out of 150 positive specimens and discordant results in 2 (1.4%) specimens. The two discordant results were: 1. Growth of *Pseudomonas stutzeri* in conventional culture as against *Microbacterium* species in BACTEC. PCR based DNA sequencing targeting 16SrRNA⁶ was performed on the vitreous aspirate and it revealed the presence of *Microbacterium* species. 2. Growth of *Acinetobacter calcoaceticus* and *Staphylococcus epidermidis* in conventional culture as against *Acinetobacter calcoaceticus* in BACTEC culture.

In the above two instances, the growth revealed by BACTEC correlated well with PCR

based DNA sequencing targeting 16SrRNA ruling out the

discordant results.

Figure 1

Table 1 : Comparative results of standard microbiological culture and BACTEC culture performed on 483 vitreous aspirate specimens

Category	Gram positive bacteria	Gram negative bacteria	Fungi
Growth detected by BACTEC [®] (150)	<i>Staphylococcus epidermidis</i> 25 <i>Staphylococcus aureus</i> 12 <i>Corynebacterium</i> species 4 <i>Viridans Streptococci</i> 5 <i>Enterococcus faecalis</i> 6 <i>Streptococcus pneumoniae</i> 3 <i>Staphylococcus saprophyticus</i> 3 <i>Bacillus cereus</i> 6 <i>Neisseria</i> species 2 <i>Microbacterium</i> species 1	<i>Pseudomonas aeruginosa</i> 31 <i>Pseudomonas stutzeri</i> 6 <i>Alcaligenes faecalis</i> 6 <i>Acinetobacter calcoaceticus</i> 5 <i>Citrobacter</i> species 1 <i>Pseudobacterium</i> species 2 <i>Klebsiella pneumoniae</i> 2 <i>Klebsiella oxytoca</i> 1 <i>Citrobacter koseri</i> 1 <i>Citrobacter freundii</i> 1 <i>Proteus mirabilis</i> 1 <i>Proteus vulgaris</i> 1 <i>Aeromonas hydrophila</i> 1	<i>Aspergillus flavus</i> 3 <i>Candida albicans</i> 5 <i>Candida tropicalis</i> 2 <i>Fusarium</i> species 3 <i>Aspergillus niger</i> 2 <i>Aspergillus fumigatus</i> 2 <i>Aspergillus terreus</i> 1 <i>Candida krusei</i> 1 <i>Bipolaris</i> species 1 <i>Pasillomces</i> species 1 <i>Aureobasidium pullulans</i> 1 <i>Curvularia</i> species 1 <i>Trichosporon</i> species 1
Growth detected by standard microbiological media alone (8)	<i>Staphylococcus epidermidis</i> 2 <i>Viridans Streptococci</i> 1	<i>Pseudomonas aeruginosa</i> 2 <i>Klebsiella pneumoniae</i> 1	<i>Aspergillus flavus</i> 1 <i>Fusarium</i> species 1
Total (158)	70 (44.3%)	62 (39.2%)	26 (16.4%)

NOTE:

- ✦ # Among the 150 (31.1%) specimens in which microbial growth was detected by Bactec, 81 (16.8 %) specimens revealed growth by standard microbiological culture
- ✦ Statistical analysis performed on growth detected by Bactec (150) vs growth detected by standard microbiological culture (81) revealed statistical significance (p value = 0.005 – Non parametric Chisquare test)
- ✦ Standard microbiological media used for isolation of bacteria and fungi are: Brain Heart Infusion broth (BHIB), Thioglycollate broth, Blood agar, MacConkey agar, Brucella Blood agar and Sabouraud's dextrose agar. The criteria used to consider the isolated bacterium or fungus as the causative agent is growth obtained from vitreous aspirate on two or more of the inoculated media i.e growth in any of the two solid media or growth in a single liquid medium and in a single solid medium.

DISCUSSION

In an attempt to improve the recovery of microorganism from intraocular specimens, the blood culture system has been reported to have increasing positive culture results in vitreous fluid.¹⁵ The present study is focused to perform vitreous aspirate culture using BACTEC Peds Plus F broth to determine whether this would improve the microbial isolation rate. The bacterial and fungal isolates recovered by BACTEC and conventional culture showed concordance in 98.6% specimens and discordant results in 1.4% specimens. The application of PCR based DNA sequencing on these two specimens proved that the BACTEC culture results were reliable since DNA sequencing revealed the same identity of the organism similar to that isolated by BACTEC culture.

In the present study, *Pseudomonas aeruginosa* 33 (20.86%) and *Staphylococcus epidermidis* 27 (17.08 %) were the predominant isolates. Fastidious organisms like *Streptococcus pneumoniae*, *Viridans Streptococci* were recovered by BACTEC culture. Our findings i.e. an increase in microbial isolation rate by BACTEC culture yield was 14.3% and the result correlated with the results published with the earlier research groups - Yospaiboon et al 2005²,

Sorlin et al 2000¹⁴ Cetin et al 2007¹⁵ Kratz et al 2006¹⁶ who have also reported a significant increase in microbial culture varying from 10 to 50% with the use of BACTEC Peds Plus F broth. Clinically significant microbes were recovered by BACTEC correlating with the results of Cetin et al¹⁵ who have also reported the isolation of Streptococcus pneumoniae, Viridans Streptococci and Aeromonas hydrophila. The growth of fungi was also well supported in BACTEC Peds Plus F broth facilitating the recovery of yeasts like Candida species, Trichosporon species, hyaline hyphomycetes like Aspergillus and Fusarium species and dematiaceous fungi like Curvularia and Aureobasidium species.

The increase in the culture yield by BACTEC could be attributed due to the presence of resins in Peds Plus F broth. The cationic and strongly acidic resin binds to the positively charged antibiotics (aminoglycosides) and an adsorbant polymeric resin fixes the hydrophobic portion of the antibiotic. The presence of the resins in the BACTEC medium neutralizes the antibiotic effect and facilitates the growth of micro organism. It is also postulated that the purulent fluid exerts an inhibitory effect on the organism. Dilution of this detrimental factor in a large volume of BACTEC medium substantially enhances the chances of recovery.² Microbial culture using BACTEC has multiple advantages over conventional culture like rapidity, cost effectiveness (a single medium alone can be inoculated), recovery of fastidious micro-organisms, simpler transport to the laboratory and possibility of microbial growth even after initiation of antimicrobial therapy. Growth of the infectious agent was noted within a few hours by Bactec method compared 24 – 48 hours by the standard method

In conclusion, the use of BACTEC medium in vitreous aspirate culture is the first to be reported in Indian literature to the best of our knowledge. Direct inoculation of vitreous aspirate into BACTEC medium should be recommended as an acceptable adjunct to conventional solid media and broth.

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