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# Comprehensive evaluation of the Bact/Alert 3D system for the culture of bodyfluids

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***ABSTRACT***
***Background:****The study was undertaken to evaluate the utility of BacT/Alert 3D automated culture system (BAS) using FA plusaerobic culture bottles for recovery of aerobic bacteria from bodyfluids other than blood.*
***Material and methods:*** *A total of 250 body fluid samples were processed for culture by conventional Culture method (CM) and by BAS system using FA Plus aerobic culture bottles. Isolates were identified by standard bacteriological methods and Vitek 2 Compact system. The mean time to detection was calculated for the BAS. The turnaround time was calculated for both the culture methods.*
***Result:****Out of 250 body fluid specimens BAS give a positive result in 90 (97.82%) clinically significant specimens. Whereas for conventional culture the recorded positivity was for 54 specimens (58.69%). For BAS the calculated mean time to detection for Gram positive cocci was 8 hrs 11 minutes and for Gram negative bacilli was 6 hrs 41 minutes. The mean turnaround time for BAS was 72 hours and for CM was 45 hours 30 minutes.*
***Conclusion:****The BacT/AlerT 3Dsystem using FA plus aerobic bottles is efficient in detection of important pathogen from body fluids.*
***KEY WORDS:*** *BacT/ Alert, body fluids, mean time to detection, turnaround time*

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## I. Introduction:

It is of a great importance to isolate bacteria from sterile body fluids, as microorganisms are present in very low numbers in these samples and are usually missed by conventional culture methods.[1,2]These infections usually are life threatening and the patients are on antibiotics resulting in failure of isolating the organism by conventional methods.[2]Several automated culture system are developed for isolation of bacteria from blood. Some of these are BacT/Alert 3D, Bactec 9000, ESP culture system, Vital blood culture system,Oxoid system.[3] These systems have been evaluated for the culture of blood.[4]There are few studies evaluating BacT/AlerT 3D system for the recovery of clinically significant bacteria from bodyfluid other than blood.[5]The present study has evaluated the BacT/Alert 3D system (Biomerieux) using FA plus aerobic bottles for recovery of aerobic bacteria from bodyfluids. This system was compared with standard conventional culture methodusing solid media.

## II. Materials And Methods:

The present study was carried out in department of microbiology MGM medical college and hospital Aurangabad, Maharashtra. The 250 bodyfluid specimen in the study comprised of pleural fluid (72), Cerebrospinal fluid (41),Pus aspirates (n=59), Bile (n=19), Ascitic fluid (n=34), Dialysis fluid(n=7), Pericardial fluid (n=2), Vitreous fluid (n=1). The pus samples were from deep seated infection collected by aspiration .The samples were processed by CM and BAS within 30 minutes of arrival in the laboratory.

Conventional method: The samples were inoculated on blood, MacConkey andchocolate agar and incubated at $37^0$C for 48 hrs.

BacT/Alert 3D system: A maximum 5ml of the sample was inoculated into BacT/Alert FA plusculturebottles and incubated in the BacT/Alert system for a maximum of 5 days. Bottlesflagged positive were subcultured on blood agar and MacConkeys agar.If no growth was observed on blood agar and MacConkeysagar, chocolate agar was inoculated for fastidious bacteria. The media were incubated for 48 hours at $37^0$C.

Identification of isolates: All isolates were subjected to standard bacteriological method of identification or if necessary were indentified using Vitek 2 compact system.

Criteria for identification of clinically significant specimens (CSS):All body fluids were subjected to microscopic examination using wet mount and gram staining to observe for presence of bacteria& pus cells. Protein level in specimen was procured from pathology department. Specimens showing bacteria, pus cells and raised protein count were considered as clinically significant specimens.

Time to detection (TTD) and mean time to detection (MTD):Time to detection was calculated for the BAS as the time between the loading of the bottles into the systems and the time the bottle is flagged positive by the system. MTD is the average of TTD of all isolates of a species in a give sample type.

Turnaround time (TAT): This was calculated for both the culture methods as the time from inoculation of specimen to when the final report was ready. All samples were processed during office hours i.e 9 AM to 5 PM.Thus the BacT/Alert bottles flagged positive in the BAS couldn't besubculturedimmediately on solid media outside office hours. This was the limitation of our study. To calculate the TAT for BAS we have grouped the specimens into following three groups and the TAT calculated accordingly.

| Group 1 | Group 2 | Group 3 |
|---|---|---|
| Flagged positive 1st day before 5 pm | Flagged positive 1st day after 5 pm to 2nd day before 5 pm | Flagged positive 2nd day after 5 pm |
| Subculture on solid media same day | Subculture on solid media 2nd day | Subculture on solid media 3rd day |
| Overnight incubation | Overnight incubation | Overnight incubation |
| 2nd day ID and AST | 3rd day ID and AST | 4th day ID and AST |
| 3rd day final report | 4th day final report | 5th day final report |
| (TAT of 48hrs) | (TAT of 72 hrs) | (TAT of 96 hrs) |

Statistical analysis:
Fisher exact test was used to know the level of significance.

## III. RESULTS:

In the present study out of 250 body fluids sample 60 (24%) were positive by conventional methodand 104(41.6%)were positive by BAS. All 60 specimens positive by CM were also positive by BAS. Thus BAS identified additional of 44 (17.6%) specimens.(Table-1) There was statistically significant association between the outcome of CM and BAS($P < 0.0001$)Specimen wise the maximum positivity was for pus aspiratesfollowed by pleural fluid and bile.(Table-2)

Out of the 250 specimens the present study identified 92 clinically significant specimens. Out of these 54 (58.69%)were positive by CM and 90(97.82%) were positive by BAS. All 54 CM positive were also positive for BAS. BAS identified additional 36(39.13%) specimens. There were 2 specimens negative by both the culture methods but were clinically significant. These samples had high leucocyte count and this is known to lead to the false positivity.[1,6,7]The association between the outcomes of both the cultural methods was not statistically significant. (Table-3)

If we see the results of TTD for various isolates the least TTD was for *E. coli* and *Enterobacter*species of 2 hours 4 minutes isolated from asciticfluid and the highest TTD was of *E. coli* of 28 hours 40 minutes isolated from pleural fluid. If we see the MTD for various isolates who were more than one in a given sample type the least MTD was for *Pseudomonas* species from pus specimen of 3hours 52 minutes. And the highest MTD was for *S.aureus* from synovial fluid of 16 hours 30 minutes. (Table-4a, 4b, 4c, 4d, 4e, 4f) The calculated MTD for Gram positive cocci was 8 hrs 11 minutes and for Gram negative bacilli was 6 hrs 41 minutes. (Table-5)

Out of the 250 specimens 92 were clinically significant and BAS showed a positive culture result in 104 specimens. Thus12 specimens grew contaminants with a contamination rate of 4.8%. While for the CM 60 specimens were positive of which 54 were clinically significant and thus there were 6 specimens having contamination with a contamination rate of 2.4%.

For CM all specimens showed growth at 18 - 24 hours incubation that is on the 2nd day. On 2nd day the Identification and AST was done and the final report was available on 3rd day with a mean TAT of 45 hours 30 minutes.For BASas mentioned in material and methods forcalculation of TAT 3 groups were made depending on TTD of the blood culture bottles. For group 1 (TAT - 48 hours), group 2 (TAT -72 hours) and group 3 (TAT -96 hours) the number of isolates were 31, 57, and 2 respectively. The mean TAT for BAS was 72 hours (Table-6)

## IV. DISCUSSION:

An important responsibility of a microbiologist is to report accurately the presence and type of the infecting microorganism ii a clinical specimen. This is of greater importance if the culture isof precious samples like sterile body fluids. The result of the present study showed that the BAS using FA plus culture bottles recovered more clinically significant bacteria than CM.Similarly other studies who have used other automatedculture systems and culture bottlesotherthan BacT/Alert FA Plus bottles for culture of body fluids have found increased recovery of organisms in comparison to CM.[1,5,8,9]

The FA plus bottles contain adsorbent polymeric beads (resins) that effectively neutralizeantimicrobials. This property would be useful in increasing the isolation rate if the patient is on antibiotics.Flayhartetal in his studies on blood culture has found resin containing media superior for recovery of bacteria from patients on antibiotics andstandard strains challenged with antibiotics.[10,11] The package insert of BAS mention internal studies have demonstrated that penicillins,polyenes,macrolides,triazoles,ecchinocandins,cefazolin,cefoxitin,aminoglycosides,fluroquinolones,lincosamides,glycopeptides and oxazolidinones were neutralized effectively by resins.Though neutralization is not achieved for ceftazidime and cefepime.Less than complete neutralization is observed for cefotaximeand ceftriaxone.[6]

In this study we have used a maximum volume of 5ml specimen for inoculation of BAS culture bottles. Fluid volume ranging from 0.5 ml to 10 ml has been used in other studies.[5,12,13] However no study has answered about what should be the optimum inoculum.We agree with Lakshmietal that further studies would be required to determine the optimum fluid to broth ratio for a increased isolation rate.[1]

The MTD for BAS for Gram positive cocciand Gram negative bacilli was 8 hour 11min and 6hours 41 minutes respectively.If we see the results of TTD for various isolates the highest TTD was of *E. coli* of 28 hours 40 minutes isolated from pleural fluid. If we see the MTD for various isolates who were more than one in a given sample type the highest MTD was for *S.aureus* from synovial fluid of 16 hours 30 minutes. This suggests that a incubation period of 5 days is more than enough for isolation of organisms. Nita etal in her study concluded that 5 day incubation is sufficient for isolation of bacteria and yeast from blood and body fluids using Bactec system.[14]

In the present study the mean TAT for CM is 45 hours 30 minutes is similar to Lakshmietal of 48hours to 72 hrs.[1]For BAS our study gave a mean TAT of 72 hours. Lakshmietal have mentioned a TAT of 24 hours for most specimens for BAS.[1]However the calculation of TAT is not explained. We have a higher TAT for BAS because we couldnot docontinuous processing of samples as processing was done only during office hours. From the MTD for most isolates and mean TAT of 72 hrs it is evident that if continuous processing of BAS flagged positive bottles is done TAT for most specimens would be definitely reduced. However further studies will be required to know what would be the TAT for BAS if continuous processing is done.

In the present study fastidious organisms like *Streptococcus pneumoniae* would be detected by BAS alone is of importance. The present study showed a higher contamination rate of 4.8%.This emphasis the importance of proper aseptic technique duringthe inoculation of BacT/Alert culture bottles.Simor A.E etal using BacT/Alert FAN bottles got a significantly higher contamination rate of 3% over CM of 1 %.[15]Malinietal using Bact/Alert 3 D system got a contamination rate of 2.4%.[7]

## V. CONCLUSSION:

The BacT/Alert 3D system using FA plus aerobic bottles is efficient and superior to conventional culture methods in detection of important pathogen from body fluids. We advocate the use of BAS for the culture of body fluids. If continuous processing of BAS flagged positive bottles is done turnaround time for most specimens would be definitely reduced.

**Conflict of interest:** There is no conflict of interest.

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**Table 1: Comparative culture results of CM versus BAS**

| Conventional | BAS | | Total | Pvalue |
|---|---|---|---|---|
| | Positive | Negative | | |
| **Positive** | 60(24%) | 0 (0%) | 60(24 %) | **P<0.0001S** |
| **Negative** | 44(17.6%) | 146 (58.4%) | 190 (76%) | |
| **Total** | 104(41.6%) | 146(58.4%) | 250(100%) | |

P<0.0001 Significant (S)

**Table 2: Specimen wise culture results for CM and BAS**

| Specimen | BAS Positive /CM Positive | BAS Positive /CM Negative | BAS Negative /CM Negative | BAS Negative /CM Positive |
|---|---|---|---|---|
| Pleural fluid n=72 | 8 | 11 | 53 | 0 |
| Pus aspirates n=59 | 25 | 3 | 31 | 0 |
| Cerebrospinal fluid n=41 | 5 | 9 | 27 | 0 |
| Ascitic fluid n=34 | 8 | 8 | 18 | 0 |
| Bile n=19 | 12 | 7 | 0 | 0 |
| Synovial fluid n=15 | 1 | 2 | 12 | 0 |
| Dialysis fluid n=7 | 1 | 4 | 2 | 0 |
| Pericardial fluid n=2 | 0 | 0 | 2 | 0 |
| Vitreous fluid n=1 | 0 | 0 | 1 | 0 |
| Total n=250 (100%) | 60(24%) | 44(17.6%) | 146(58.4%) | 0 (0%) |

n = number of specimens

**Table 3: Culture results for BAS versus CM for Clinically Significant Specimens**

| CM | BAS | | Total | P value |
|---|---|---|---|---|
| | Positive | Negative | | |
| **Positive** | 54(58.69%) | 0(0%) | 54(58.69%) | **P=0.1679NS** |
| **Negative** | 36(39.13%) | 2(2.17%) | 38(39.13%) | |
| Total | 90(97.82%) | 2(2.17%) | 92(%) | |

NS=Non significant

**Table 4a: MTD of Clinically Significant Bacteria recovered by BAS in Pleural fluid**

| Organism | Total | MTD | S.D | Range |
|---|---|---|---|---|
| *Escherichia coli* | 5 | 9hrs20min | 10hrs 49 min | 3hrs40min to28hrs 40min |
| *Streptococcus pneumoniae* | 3 | 11hrs | 2hrs4min | 8hrs40min to12hrs40min |
| *Streptococcus species* | 3 | 9hrs6min | 20min | 9hrs20min to 10hrs |
| *Klebsiella pneumoniae* | 2 | 4hrs 3min | 4.94min | 4hrs to4hrs7min |
| *Staphylococcus aureus* | 2 | 9hrs | 1hr 15min | 8hrsto10 hrs |

| | | | | |
|---|---|---|---|---|
| *Pseudomonas aeroginosa* | 1 | 5hrs20 min | - | - |
| *Citrobacter species* | 1 | 21 hrs | - | - |
| Total | 17 | | | |

SD =Standard Deviation

**Table 4b: MTD of Clinically Significant Bacteria recovered by BAS inPus**

| Organism | Total | MTD | S.D | Range |
|---|---|---|---|---|
| *Staphylococcus aureus* | 16 | 5hrs 35min | 1hr 31min | 3hrs to 8hrs |
| *Escherichia coli* | 6 | 4hrs40min | 1hr 29 min | 3hrs34min to6hrs47min |
| *Pseudomonas species* | 2 | 3hrs52min | 10.60 | 3hrs45minto 4hrs16min |
| *Klebsiella species* | 2 | 5hrs31min | 1hr 46min | 4hrs16min to 6hrs47 min |
| *Enterococcus species* | 2 | 5hrs45min | 7hrs | 3hrs40min to3hrs50min |
| *Proteus species* | 1 | 3hrs34min | - | - |
| Acinetobacterbaumanni complex | 1 | 13hrs 22min | - | - |
| Total | 30 | | | |

**Table 4c: MTD of Clinically Significant Bacteria recovered by BAS in Synovial fluid**

| Organism | Total | MTD | S.D | Range |
|---|---|---|---|---|
| *Staphylococcus aureus* | 2 | 16hrs 30min | 42.42min | 15hrs34min to16hrs |
| *Pseudomonas species* | 1 | 19hrs | - | - |
| Total | 3 | | | |

**Table 4d: MTD of Clinically Significant Bacteria recovered by BAS inAscitic fluid**

| Organism | Total | MTD | S.D | Range of TTD |
|---|---|---|---|---|
| *Escherichia coli* | 7 | 4hrs54min | 1hr 18 min | 2hrs 4min to 5hrs 35min |
| *Klebsiella species* | 3 | 3hrs35min | 39.25min | 3hrs53min to 5hrs 1 min- |
| *Citrobacter species* | 2 | 12hrs6min | 14.14min | 11hrs50min to 12hrs30min |
| *Salmonella typhi* | 1 | 10hrs | - | - |
| *Acinetobacter species* | 1 | 4hrs30min | - | |
| *Staphylococcus haemolyticus* | 1 | 4hrs30min | - | - |
| *Enterobacter species* | 1 | 2hrs 4min | - | - |
| Total | 16 | | - | - |

**Table 4e: MTD of Clinically Significant Bacteria recovered by BAS in Bile**

| Organism | Total | MTD | S.D | Range |
|---|---|---|---|---|
| *Escherichia coli* | 6 | 5hrs15min | 1hr 31min | 3hrs50min to 7hr 30min |
| *Pseudomonas spp* | 4 | 5hrs56min | 1hr30 min | 3hrs50min to 7hrs 23 min |
| *Klebsiella pneumoniae* | 3 | 5hrs13min | 14.84 min | 5hrs1minto 5hrs30min |
| *Streptococcus species* | 1 | 8hrs 10min | - | - |
| *Enterococcus species* | 1 | 8hrs | - | - |

| | | | | |
|---|---|---|---|---|
| *Acinetobacterbaumanni complex* | 1 | 3hrs50min | - | - |
| *Stenotrphomonas species* | 1 | 7hrs25min | - | - |
| *Achromobacterdenitrificans* | 1 | 13hrs | - | - |
| *Cupriaviduspauculus* | 1 | 3hrs25min | - | - |
| Total | 19 | | - | - |

**Table 4f: MTD of Clinically Significant Bacteria recovered by BAS in CSF**

| Organism | Total | MTD | S.D | Range |
|---|---|---|---|---|
| *Staph aureus* | 4 | 13hrs37min | 10hrs 44min | 2hrs 30min to27hrs |
| *Escherichia coli* | 3 | 8hrs10min | 26.45 | 7hrs40min to8hrs30min |
| *Streptococcus pneumoniae* | 2 | 9hrs 45min | 21.21 | 9hrs30 min to 10hrs |
| *Streptococcus spp* | 1 | 6hrs | - | - |
| Total | 10 | | | |

**Table 5: MTD for Gram positive Cocci and Gram negative Bacillifrom all clinically significant specimens for BAS**

| Organism | MTD | SD | Range |
|---|---|---|---|
| **Gram positive Cocci** | 8hrs 11min | 4hrs 42min | 2hrs 30min to27hrs |
| **Gram negative Bacilli** | 6hrs41min | 4hrs 40min | 2hrs4min to 28hrs 40 min |
| **Average** | 7hrs 26 min | 4hrs 41min | 2hrs 4min to 28 hrs 40min |

**Table6: Turnaround time ofCM and BAS**

| | TAT | Mean TAT |
|---|---|---|
| **CM n= 54** | 42 hrs 15 min to 47 hrs | 45 hrs 30 min |
| **BAS n = 90** | | |
| G1 n=31 | 48hrs | 72 hrs |
| G2 n=57 | 72 hrs | |
| G3 n=2 | 96 hrs | |

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