DOI: 10.14260/jemds/2014/3873

ORIGINAL ARTICLE

EVALUATION OF TIME TO POSITIVITY IN DETECTION OF MICRO-ORGANISM USING BACTEC™ 9050 IN A TERTIARY CARE HOSPITAL OVER A PERIOD OF ONE YEAR

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HOWTOCITETHISARTICLE:
Anjana Gopi, Arun Kaushik R, Divya Harindranath. "Evaluation of Time to Positivity in Detection of Micro-organism Using BACTEC™ 9050 in a Tertiary Care Hospital over a Period of One Year". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 64, November 24; Page: 13999-13405, DOI:10.14260/jemds/2014/3873

ABSTRACT: Blood stream infections are being reported as one of the most important cause of morbidity and mortality, more commonly in the developing than in the developed countries of the world. Early and accurate identification of the microorganism responsible for the infection permits early initiation of targeted broad spectrum empirical therapy.[1] The automated blood culture systems which have been introduced, have improved in their capacity to detect and isolate the microorganisms much earlier than the conventional culture techniques which were used earlier. The use of culture bottles with specific resins which help in nullifying the antimicrobial effect and improve the growth of the microorganisms are recommended.[1]The following study was done using the BACTEC™ 9050 system to estimate and compare the time to positivity of various microorganisms. 800 samples comprising of blood and body fluids were tested using BACTEC™ 9050 and the time to positivity indicated from the time of processing, to the time of beep were calculated & analyzed. Of 800 samples, 146 (17.9%) were positive for growth by BACTEC™ 9050 culture system. 15 (1.8%) were false positive (no growth on further processing by aerobic culture techniques from a positive beep in BACTEC™ 9050). The microorganisms isolated from the positive cultures included both pathogenic, non-pathogenic bacteria and fungi. The mean detection time, for the isolated microorganisms was around 24 hours. The pathogenic microorganisms isolated had a shorter time to positivity than the nonpathogenic organisms. Since all the cultures were positive by 72 hours, our data supports a four day incubation period for isolating microorganisms using automated blood culture systems. Hence, automated BACTEC™ 9050 is reliable, fast, accurate and cost effective, with high sensitivity, specificity for isolating microorganisms from samples.

KEYWORDS: BACTEC™ 9050, time to positivity, microorganisms, infection, false positive.

INTRODUCTION: Microorganisms play an important role in causing localized or generalized human infection in most of the developing and developed countries of the world.[1] These infectious diseases are associated with increased morbidity, multi organ failure, shock, Disseminated Intravascular Coagulation and death; which has an impact on the length of the hospital stay, health care costs, disability in patients and financial burden on the country.

This necessitates a rapid, accurate, and reliable detection of these microorganisms especially in the case of bloodstream infections (BSI). An early diagnosis is an important step in the workup and better management of febrile patients. This is one of the most challenging problems in clinical microbiology and much work needs to be done to minimise these drawbacks.[2,3]

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A positive blood culture is frequently the trigger for initiation of antimicrobial therapy.[2] Conventional blood culture is time consuming, labour intensive with low sensitivity and the earliest time for positivity being > 36 - 72 hours.[4] This delay in the diagnosis is associated with the culture media used, the method of incubation of the sterile container, the presence of the inhibitors and antibiotics in the sample which prevent the growth of the microorganisms. This results in a delay in initiation of adequate treatment, increased length of hospitalization and long term & poor follow up of patients.[5]

Hence; a system which can overcome these drawbacks and improve the early & accurate diagnosis was devised by many companies. Many studies have been conducted to determine the efficiency of these automated blood culture systems, which is now being used for many body fluids also. These instruments have led to a rapid, accurate and cost-effective detection of the causative organism; but still require repeated manipulations and additional instrumentation for identification.[3]

This BACTEC™ system eliminates cross contamination of cultures while doing repeated subcultures.[1]

Automated blood culture instruments (BACTEC™ 9050) are fluorogenic, non-invasive systems which can be used for rapid, accurate, cost-effective detection of the microorganisms with low cross contamination and a short duration of positivity.[6] The instrument electronically monitors the blood culture bottles 24 hours a day, typically checking each bottle every 8-10 minutes. Time-to-positivity (TTP) is defined as the length of time from the beginning of culture incubation to the detection of bacterial growth by the automated system.[6]

The BACTEC™ 9050 system used in this study has a capacity of 50 bottles; is a small, self-contained, automated system designed for processing around three to five blood cultures per day, with constant agitation of the bottles continuously by rotation and the bottles read one by one by three detectors with positivity indicated by a beep.[1,6]

The instrument design provides optimal, easy-to-use workflow in a compact, modular design. The greater recovery of organisms in the modified media leads to more accurate diagnosis and effective treatment, which in turn leads to shorter hospital stays, lower patient costs and improved laboratory and institutional efficiency.[6] False-positive cultures were defined as those that were indicated by the instrument to be positive but had revealed no microorganisms by routine processing.

This study was done to determine the time to positivity, false positive / negative rate and the isolation & distribution of bacteria and yeasts isolated.

MATERIALS AND METHODS: The present study was conducted in the Microbiology laboratory at KIMS hospital, Bangalore and the data from January to December 2013 was tabulated and analyzed.

The study was done in the following manner: The samples for the study - 800 samples (blood, body fluid, pus) from clinically suspected febrile patients with infection were collected aseptically to avoid contamination, were inoculated into BACTEC/F or BACTEC Peds Plus/F blood culture bottles depending upon the age of the patient and these bottles were placed in the BACTEC™ 9050 blood culture instrument within 2 hours of collection to obtain optimum results.

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The BACTEC/F blood culture media used with the BACTEC™ System permits screening for bacteria, yeast and fungi present in the blood. These bottles include standard broth media with resins specifically designed for small blood volume inoculation.

The patented BACTEC resin media have shown to be effective in neutralizing a wide variety of antibiotics, allowing growth of microorganisms which do not grow with conventional media and achieving a significantly higher isolation rates in patients on antibiotic therapy than only by broth dilution followed earlier for nullifying the effect of antibiotics.[6]

In the BACTEC™ 9050 instrument, step-by-step directions for bottle processing and test initiation were provided by scanning the barcode test menu. Bottles were tested every 8-10 minutes by the instrument using the sensors. Positive results are flagged for quick processing indicated by a beep. The negative bottles can be batch - scanned out of the system and unloaded at the end of protocol.[6] The BACTEC™ 9050 system, which accommodates 50 test vials, is a continuous monitoring, blood culture testing instrument. The operation of the instrument is based on an easy-to-use icon interface and barcode scanner.

Time of processing and Beep time (flagged by an indicator light, an audible alarm) were noted to calculate the time to positivity. The flagged positive samples were then subjected to routine processing using biochemical tests for isolation and identification of the microorganisms in the sample and the sensitivity pattern was obtained.

The bottles flagged negative by the instrument were processed at the end of 5 days of processing to confirm negativity. All isolates were considered to be clinically significant, except Micrococcus species, Aerobic spore bearers, Diphtheroids' and Coagulase negative staphylococcus (which were considered commensals when not isolated on repeated sample culture) and other contaminants.[4, 5] The data obtained was analysed as below.

RESULTS: In the study period, 800 samples (blood = 785, CSF = 9, Fluid [ascitic fluid, pleural fluid, synovial fluid] = 5, Pus = 1) received in the microbiology laboratory of KIMS, Bangalore were tested for the following.

AGE GROUP (in yrs)	NUMBER OF PATIENTS
0 -1	310
1-18	124
> 18	366

Table 1/Figure 1: Showing distribution of patients' age group

The gender difference was with Males = 475 (59.3%) and females = 325 (40.6%) and a M: F = 1.46:1. Of the 800 samples tested, growth was seen in 143 (17.9%) and 15 (1.8%) were false positives.

In the above study, Gram positive organisms, Gram negative organisms and fungi were isolated and analyzed. The average time of positivity for the organisms were calculated and analyzed. A mean time of positivity of around 20 +/- 4 hours was seen among most of the organisms.

The Average time of positivity for the common pathogenic organisms were as follows - Staphylococcus aureus – 21 hours, Streptococcus pneumoniae – 23 hours, Neisseria meningitides –

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48 hours, Salmonella typhi – 24 hours, Brucella species – 72 hours, Pseudomonas species – 42 hours, Klebsiella species – 23 hours, Candida species – 32 hours. (Table 2 / Figure 2).

The organisms isolated from the culture media were as follows - Staphylococcus aureus - 40, Coagulase negative Staphylococcus - 23, Streptococcus Group A - 1, Streptococcus pneumoniae - 1, Neisseria meningitides - 1, Enterococcus species – 12, Salmonella typhi - 9, Escherichia coli - 11, Pseudomonas species - 7, Klebsiella species – 14, Brucella species – 1, Candida species = 6, others - 17. (Table 3/ Figure 3).

DISCUSSION AND CONCLUSION: In the current study, using BACTEC™ 9050 system, a positive culture was obtained in 143 samples, of the 800 samples. Majority of the microorganisms were isolated within the first 24 hours, with an average time of isolation being 20 +/- 4 hours. Most of the microorganisms isolated were clinically significant in causing human infection. Several other studies with automated instruments have reported time to positivity of < 24 hours.

Both bacteria and fungus were isolated from the cultures in this study. Automated blood cultures have significantly reduced the time for isolation of organisms. Many automated instruments are available and many studies have been conducted to evaluate the instruments. Many studies have reported a false positive rate of around 1-2%, which is similar to our study.

One of the studies done previously by Anjana Gopi et.al, had positivity rate of 10.14%, of which a total of 655 (8.15%) positive cultures were pathogenic organisms and 160 (1.99%) positive cultures were contaminants. The average time for positivity around 24 hours, this is similar to that reported in our study. The mean detection time for the clinically significant isolates was 21 h and for all the isolates 29 h according to this study. Among pathogenic microorganisms; Gram- positive, Gram negative and yeast were 403 (61.52 %), 242 (36.94 %) and 10 (1.52 %), respectively which was similar to our study.[1]

Another study done by Ates Kara et.al, reported a positivity rate of 11.18%, time to positivity of around 54.48 hours.[7] A study done by Zadroga et.al obtained a 13% positivity rate of the 9395 samples tested, with a 4.5 hour faster detection time.[8]

A study done by Durmaz et al. isolated 65% Brucella strains within 72 h of incubation which is well within the incubation protocol obtained in this study.[9]

A study done by Sullivan KV et.al compared the sensitivities and time to detection (TTD) of BacT/Alert Pediatric FAN (PF) and Bactec Peds Plus blood culture bottles. They reported a better recovery rate with a time to positivity of 4 hours shorter in test samples than controls tested in both BACTEC and BacT/Alert systems. The control bottles were inoculated with 3 ml of banked blood and organism suspensions only, the test bottles were inoculated with 2 ml of banked whole blood, 1-ml aliquots of antibiotic suspension and organisms diluted to simulate a bacteremia level of 10 to 100 CFU/ml.[10]

A study was done by Flayhart D et.al, for the identification of the bacterial pathogens in samples containing antibiotics at therapeutic levels. The antibiotics were chosen for testing represented those most frequently used at the Johns Hopkins Hospital. Antibiotics were measured and prepared on each day of use. The final concentrations of the antibiotics were as follows: ampicillin, 3 μg/ml, 22 μg/ml, and 47 μg/ml; cefepime, 10 μg/ml, 87 μg/ml, and 164 μg/ml; cefoxitin, 10 μg/ml, 60 μg/ml, and 110 μg/ml; ceftriaxone, 94 μg/ml, 125 μg/ml, and 250 μg/ml; gentamicin, 1

μg/ml, 4 μg/ml, and 8 μg/ml; gentamicin/penicillin combination, 0.5/0.08 μg/ml, 1.75/10 μg/ml, and 3/20 μg/ml; oxacillin, 13 μg/ml, 57 μg/ml, and 230 μg/ml; piperacillin-tazobactam, 5/0.7 μg/ml, 100/10 μg/ml, and 240/24 μg/ml; vancomycin, 10 μg/ml, 25 μg/ml, and 50 μg/ml. BACTEC™ system isolated 95.1% of the cultures from test bottles, in comparison BacT/Alert systems isolated 25.1% of samples and was able to recover Gram positive and Gram negative organisms in the presence of antibiotics at therapeutic levels.[11]

A study was done by Murray et.al. for comparing between BACTEC™ 9050 and BACTEC™ 9240 systems for overall recovery of organisms and time to detection. A total of 4,383 compliant aerobic (Plus Aerobic/F) blood culture sets showed no significant difference in the recovery of individual groups of organisms with the two systems, but with the exception of Streptococcus pneumonia which was isolated more frequently with BACTEC™ 9050. False-positive signals occurred more often with BACTEC 9240 (58 cultures) than with BACTEC™ 9050 (43 cultures), but a false-negative culture were uncommon with both systems. The time to detection of positive cultures of clinically significant organisms was essentially the same with both instruments.[12]

A study done by Galo Peralta et. al. on S. pneumoniae had a time to positivity of 22 hours.[13] The recovery time for S. pneumoniae was 10.05 hours in the study done by Anjana et.al[1] but in our study the time to positivity was 23 hours similar to other studies. The reason for a longer time to positivity could be due to the fastidious nature of the organism, the care that is needed while transporting and processing the sample and the usefulness of the constant 360^0 rotation in BACTEC™ 9050 instrument.

BACTEC™ 9050 system has proved to be a reliable and efficient instrument for rapid isolation of microorganisms from culture. The rapid time to positivity significantly reduces time of isolation for faster institution of treatment.

LIMITATIONS AND FUTURE PLANS: The above study will be continued and inclusion of more samples for a better analysis. The anaerobic culture was not performed due to difficulty in sample procuring and processing.[1] Among the fungi, only Candida species was isolated. This study will be done using more samples and tested for bacterial, Mycobacterium tuberculosis and fungi, using BD BACTEC™ Plus Aerobic/F, BD BACTEC Paeds Plus/F, BD BACTEC Myco / F lytic culture vials respectively.

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ORGANISM	AVERAGE TIME TO POSITIVITY (hrs)
Staphylococcus aureus	21
Streptococcus pneumoniae	23
Coagulase negative Staphylococcus	30
Streptococcus Group A	59
Streptococcus Group B	20
Enterococcus species	26
Klebsiella species	23
Pseudomonas species	42
Salmonella typhi	23
Citrobacter species	21
Salmonella paratyphi	41
Acinetobacter species	23
Enterobacter species	20
Escherichia coli	17
Neisseria meningitidis	48
Brucella species	72
Candida species	32

Table 2/Figure 2: Average Time to positivity of the organisms

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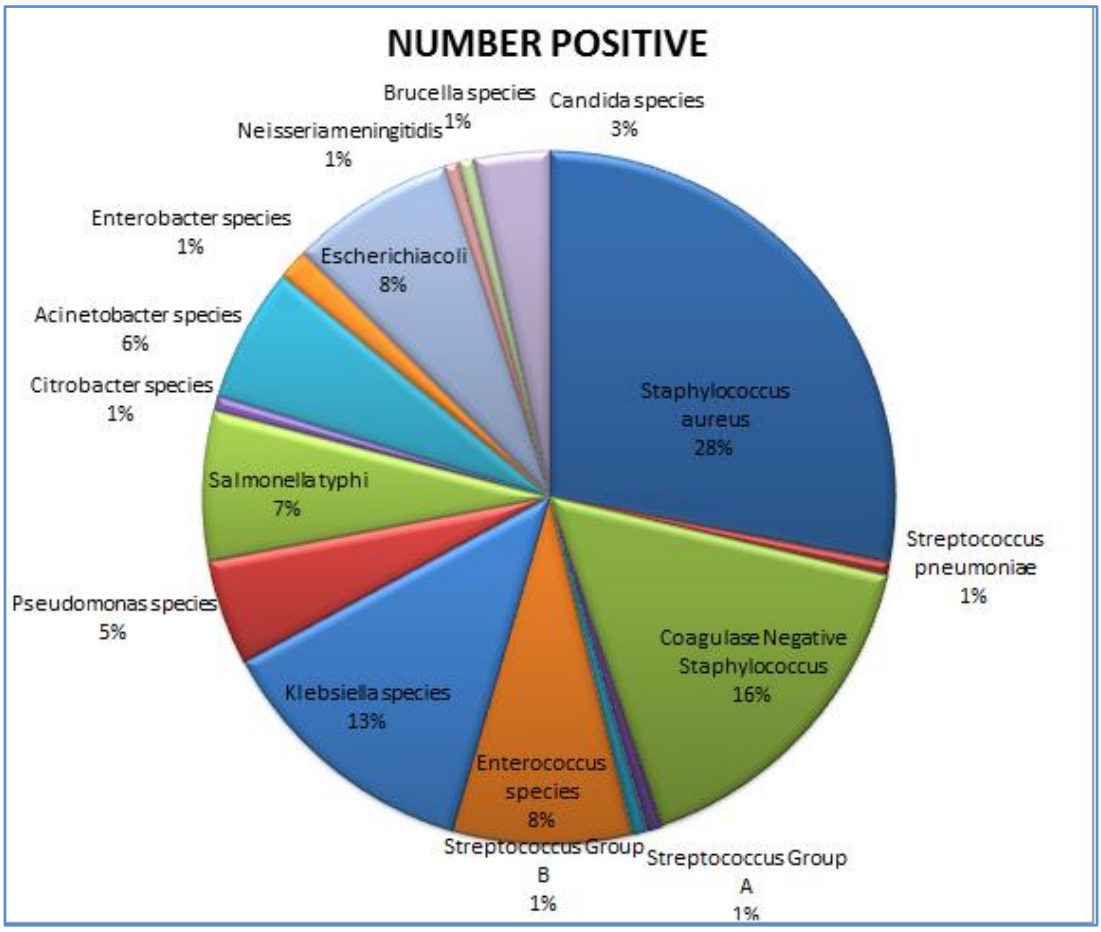

Table 3/ Figure 3: Graph showing the number of organisms positive in the samples

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Date of Submission: 12/11/2014.
Date of Peer Review: 13/11/2014.
Date of Acceptance: 17/11/2014.
Date of Publishing: 21/11/2014.