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Original Research Article

Study on continuous monitoring automated culture systems for rapid isolation of microorganisms

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Abstract: Rapid isolation and reliable diagnosis of blood borne infections and characterization of bacterial microorganisms is one of the important tasks for clinical microbiologists. The present study was undertaken to evaluate the Time to Detection of various micro organisms with BACTEC 9120. This study was done on Blood Culture samples obtained from the outpatients as well as admitted patients at IndraprastNadia Sufdar Ali , Lecturer , Department of MLT, College of Applied Medical sciences, Jazan University, Jizan, KSA.ha Apollo Hospital, New Delhi. The BACTEC fluorescent series instruments, BACTEC 9120, are designed for the rapid detection of bacteria and fungi in clinical cultures of blood. Samples are drawn from patient and injected directly into BACTEC culture vials. Positive cultures are immediately flagged by an indicator light on the front of the instrument and displayed on the monitor. When positive vials are identified, the lab technologist pulls them from the instrument for confirmation of results, and for isolation and identification of the organism. Among this 97 blood culture yielded gram positive cocci, 87 yielded gram negative bacilli, 23 were positive for fungi, 36 showed mixed growth and 10 blood culture yielded Gram positive bacilli. All the positive samples from BACTEC 9120 were monitored for positivity and the positive samples were then identified by two methods Vitek-2 and direct methods. In the study it was observed that the maximum percentage of microorganisms isolated within the time period was Gram Positive cocci (37%), followed by Gram Negative Bacilli (33%) and the least isolated was Fungi (9%). From the Time to Detection (TTD) charted out, Fungi were seen taking maximum time to detection (32.4 hrs). This was followed by Gram Positive Cocci (19.3 hrs) and Glucose Non Fermenters (18.4 hrs). The least TTD was shown by Enterobacteriaceae (15.3 hrs). BACTEC 9120 automated Continuous monitoring culture systems are very efficient and important tool in management of critically ill patients and have a great edge over conventional detection in cutting crucial time for detection. Automated Culture, identification and Susceptibility System being versatile system forms inseparable part of management of critically ill patients.
Keywords: continuous monitoring automated culture systems, microorganisms

INTRODUCTION

Microorganisms present in the circulating blood either continuously, intermittently or transiently are a threat to every organ in the body. Microbial invasion of blood stream can have serious immediate consequences including shock, multiple organ failure, disseminated intravascular coagulation (DIC) and death. The detection of micro organisms in a patient's blood has diagnostic and prognostic importance. Blood cultures are essential for the diagnosis and treatment of the etiologic agent of sepsis. Bacterial Sepsis constitutes one of the most serious infectious diseases and therefore the expeditious detection and identification of blood borne bacterial pathogens is an important function of the diagnostic microbiology laboratory [1]. Pathogens of all four major groups of microbes-bacteria, fungi, viruses, and parasites found circulating in the blood during the course of many diseases. Based on the clinical condition of the patient, the physician determines what group is likely to be causing infection. Specific types of blood culture include Aerobic, Anaerobic and Fungal. Most of the blood culture tests

for both aerobic and anaerobic microbes [2]. Blood cultures are done using techniques ranging from manual to totally automated techniques [3]. In the manual method of blood culture, the blood sample obtained is added to 100 ml of a rich growth medium such as Brain Heart Infusion broth and incubated at 37 degree Celsius for 24 hours. The blood culture bottles are watched daily for signs of growth including cloudiness or a color change in the broth, gas bubbles or clumps of bacteria. When there is evidence of growth, the laboratory does subculture and antibiotic sensitivity tests. If there is no immediate visible evidence of growth in the bottles, the laboratory looks for bacteria by doing gram stains and subcultures. These steps are repeated daily for the first several days and periodically after that. The delay in the final culture report remains one of the main drawbacks of Manual blood culture system [4].

The isolation of any significant micro organism from a blood culture is an occurrence that requires careful evaluation by the clinician, and prompt action is usually necessary. If the results of clinical microbiological analyses are to contribute in a meaningful way to the diagnosis and management of patients with bacterimia, they must be made available to the clinician in a relevant time frame [5]. Automated blood culture system is considered as one of the recent technical advances in blood cultures. Automated blood culture is carried out by two fully automated systems known as BACTEC and VITEK4. BACTEC 9000 series of blood culture instruments are designed for rapid detection of micro organisms in clinical specimens. It is also called Continuous monitoring blood culture systems, because the instrument automatically monitors the bottles containing patient blood for evidence of micro organisms, usually every ten minutes. The sample to be tested is inoculated into the vial which is entered into the BACTEC instrument for reading. Each vial contains sensor which responds to the concentration of Carbon dioxide produced by the metabolism of micro organisms or the consumption of oxygen needed for the growth of micro organisms. The sensor is monitored by the instrument every ten minutes for an increase in its fluorescence, which is proportional to the increasing amount of carbon dioxide or the decreasing amount of oxygen present in the vial. A positive reading indicates the presumptive presence of microorganisms in the vial [6].

Time period required for the BACTEC to show positivity varies among different micro organisms. Hence Time to detection or TTD is defined as the time required for the automated system to alert signal which indicates growth in the culture bottle Positive culture vials after processing are put up in the VITEK for results. VITEK automates all the steps needed to perform identification and susceptibility tests, using VITEK cards [7]. Rapid provision of results for diagnosis and treatment of bacteremia remains one of the most important functions of clinical microbiology laboratory. An automated system that monitor culture bottles for microbial growth minimizes the time necessary to detect positive blood cultures. Another way to save time might be to inoculate an automated system for rapid identification and susceptibility testing directly from positive blood culture bottles [8].

MATERIALS AND METHODS

This study was done on Blood Culture samples obtained from all the outpatients as well as admitted patients at Indraprastha Apollo Hospital, New Delhi. All Positive blood culture samples were included in the study for the evaluation of Time to Detection (TTD) irrespective of the organism grown. The specimens were collected using sterile techniques to reduce the chance of contamination. The recommended specimen volume was 8-10ml. It was recommended that the specimen be inoculated into the BACTEC vials at bedside. Most commonly, a 10cc or 20cc syringe with a LUER-LOK bran tip is used to draw the sample. If appropriate, a VACUTAINEDTM Brand Blood Collection Set, VACUTAINERTM SAFETY-LOKTM blood collection set or other tubing “butterfly” set may be used If using a needle and tubing set(direct draw), carefully observe the direction of blood flow when starting sample collection. The vacuum in the vial will usually exceed 10 ml, so the user should monitor the volume collected by means of the 5 ml graduation mark on the vial label [6].

All the blood samples received in BACTEC plus Aerobic Blood Culture Bottles were immediately loaded into the BACTEC 9120 (Continuous Monitoring Automated Blood Culture System). The samples were continuously monitored for positivity. The Blood Culture Vials which had been flagged as positive were immediately followed up. The TTD (Time to Detection) for various organisms was charted out. The Positive Blood culture bottle was taken out of the BACTEC 9120 and kept upright. With the help of needle syringe, a drop of blood was aspirated, and a Gram Stain was made along with Subculture onto Mac-Conkey Agar and Blood Agar for 16-18 hours.

Positive Vial Indicator

A Positive vial indicator is at the top left of the four indicators. This light illuminates YELLOW whenever a new positive culture is detected. System

error indicator The "SYSTEM ERROR" indicator is located to the right of the Positive Vial Indicator. This light illuminates BLUE whenever the system encounters an error condition in the instrument requiring operator attention. System normal indicator The "SYSTEM NORMAL" indicator is located below the positive vial indicator. This light illuminated GREEN whenever the system is operating normally.

Direct Inoculation on biochemical media (From Positive Blood Culture Samples)

1ml blood was aspirated from the positive Blood Culture Vial and transferred on to a sterile test tube. Blood was centrifuged for 3 minutes and supernatant was separated. A Cytochrome Oxidase test was performed with one loop of the supernatant. Then the supernatant was inoculated directly onto biochemical Medias such as Indole, Citrate, Mannitol, Triple Sugar Iron Agar, Methyl Red, Voges Proskauer, Urease, Glucose, Sucrose, Lactose, Lysine decarboxylase, Ornithine decarboxylase, Arginine dihydrolase and Phenylalanine Deaminase. This is incubated for 16-24 hours at 37 degree Celsius.

Positive and Negative Specimens

Although many positive blood cultures will be detected in the first 24 hours after inoculation, ongoing vials must be still kept for several days to assure maximum recovery. With the BACTEC florescent series instrument, vials are typically held for 5-7 days before they are discarded as negative. Vitek-2 is a fully automated Bacteriology system that performs bacterial identification and susceptibility testing analysis using standard inoculums. This system combines both complimentary and highly advanced skills to transform the antibiotic susceptibility test into performances [9].

RESULTS

From a total of 1737 blood culture samples obtained, 263 samples were positive, giving an overall positivity of 15.1%. Among this 97 blood culture yielded gram positive cocci, 87 yielded gram negative bacilli, 23 were positive for fungi, 36 showed mixed growth and 10 blood culture yielded Gram positive bacilli. All the positive samples from BACTEC 9120 were monitored for positivity and the positive samples were then identified by two methods Vitek-2 and direct methods. In the study it was observed that the maximum percentage of microorganisms isolated within the time period was Gram Positive cocci (37%), followed by Gram Negative Bacilli (33%) and the least isolated was Fungi (9%). From the Time to Detection (TTD) charted out, Fungi were seen taking maximum time to detection (32.4h). This was followed by Gram Positive Cocci (19.3h) and Glucose Non-Fermenters (18.4h). The least TTD was shown by Enterobacteriaceae (15.3h) table 1.

Table 1: Average TTD of different groups of Micro organisms

Microorganisms	Average TTD
Enterobacteriaceae	15.3h
Non-Fermenters	18.4h
GPC	19.3h
Fungi	32.4h

Table 2: Average TTD of Gram Positive Cocci

Microorganism	Average TTD
Streptococcus Pneumoniae	6.09h
Staphylococcus Aureus	11.77h
Enterococcus	12.01h
Non-Enterococcus Streptococci	16.62h
Alpha Haemolytic Streptococcus	18.05h
Coagulase Negative Staphylococcus	25.02h
Micrococcus	46.15h
Average TTD	19.39h

Among the Gram Positive Cocci, Micrococcus has the maximum TTD whereas Streptococcus Pneumoniae showed the lowest TTD.

Table 3: TTD of Enterobacteriaceae

Enterobacteriaceae	Average TTD (hours)
Enterobacter cloacae	8.83
Providencia spp	11.26
Klebsiella pneumoniae	11.27
Salmonella paratyphi A	17.22
Salmonella typhi	20.27
Escherichia coli	23.52
Average TTD	15.39

In the Enterobacteriaceae family, Escherichia coli showed the maximum TTD while Enterobacter cloacae showed the least TTD.

Table 4: TTD of Nil Fermenters

Nil Fermenters	Average TTD (hours)
Burkholderia cepacia	11.05
Acinetobacter baumanii	14.05
Stenotrophomanas maltophilia	16.5
Pseudomonas aeruginosa	16.66
Alcaligenes spp	33.76
Average TTD	18.40

Table 5: Average TTD of fungi

Nil Fermenters	Average TTD(hours)
Candida albicans	24.67h
Candida spp	40.2h
Average TTD	32.43h

DISCUSSION

The present study was designed to determine the accuracy and rapidity of diagnosis with BACTEC 9120 and VITEK-2 systems. Rapidity of identification and performing sensitivity test can lead to rapid therapy in appropriate directions which are be of immense benefit clinically. Blood stream infection is one of the most serious problems in all infectious diseases [1]. The blood is normally sterile, but bacteria occur transiently in the bloodstream after vigorous chewing or dental surgery or the instrumentation of Genito urinary tract or bowel. Bacteremia may be a phase in the natural course of some infections such as typhoid fever and brucellosis and meningococcal infection; it also occurs as a spill-over effect in a serious infection when the patient's defenses become inadequate [10]. However the source of organisms may not be determined in up to one third of bacteremia [2] Blood culture is currently the only routine method for detecting bacterial bloodstream infections. Despite recent developments, like nucleic acid probes, PCR, and other molecular techniques for microbiological diagnosis, blood cultures still remain the most practical and reliable method in the diagnosis of blood stream infections. Blood culture is one of the most important tools of clinical microbiology laboratory. Rapid isolation and identification of micro organisms in the blood samples and directing of the treatment accordingly are critically important in order to reduce the mortality rate [11]. In our study of TTD (time to detection), it was observed that significant pathogens like Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus, took lesser time to detection than the less pathogenic organisms like Coagulase Negative Staphylococci, Micrococci. Our study also observed that the maximum of significant organisms were detected within 24 hours. Glucose Non-Fermenters and Fungi on an average yielded a slightly higher TTD than the rest of the microorganisms. Identification of Bacteria was done by both Conventional and Automated Techniques. Both the techniques identified all the Enterobacteriaceae members to the species level. However a few of the isolates could not be identified conventionally to the Species level. This difficulty was not faced with the Automated Identification (Vitek-2 Compact). It was also seen that many of the Non-Fermenters could not be differentiated conventionally Ex. Burkholderia spp, Ralstonia mannitolytica, Brevundimonas diminuta,

Alcaligens denitrificans, Stenotropomonas maltophila could not be differentiated on the basis of Conventional biochemical tests alone. However the Automated System could identify it fully. In a study by KS Chatzigeorgiou *et al.;* comparing phoenix 100 versus Vitek-2 for identification of gram positive and gram negative bacteria found Vitek-2 was hiving higher identification of gram negative fermenters versus Non-fermenters at genus levels [12]. It was similar to our study in which the Vitek was able to identify all the non fermenters which were not identified by the conventional methods. In one study by Shalani Duggal et al; for comparison of automated system with conventional system for identification and microbial susceptibility found that automated system are useful tools for early identification and susceptibility pattern of aerobic bacteria in routine microbiology labs [13]. Similarly we found that the automated methods have advantages both in reducing detection time and accuracy.

CONCLUSION

BACTEC 9120 automated Continuous monitoring culture systems are very efficient and important tool in management of critically ill patients and have a great edge over conventional detection in cutting crucial time for detection. Automated Culture, identification and Susceptibility System being versatile system forms inseparable part of management of critically ill patients.

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