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Time to Positivity of Microorganisms with BACTEC 9050:- An 18-month Study Among Children of 28 Days to 60 Months in an South Indian Tertiary Hospital

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Abstract: This study tracks and analyses the culture results of 8034 blood samples received in our laboratory during an eighteen month period from children aged between 28-days to sixty months. We documented time required for the culture to become positive, time at which a culture could be considered negative, false positive / negative signal rate of the instrument and the spectrum of isolated organisms. The specimens were processed using BACTEC 9050 (Becton Deckinson Microbiology Systems, Sparks, Md) culture systems selecting a five day incubation protocol. Microorganisms identification / antibiotic susceptibility testing was done on Microscan walkaway - 40 system (Siemens) using synergy panel. The mean detection time for the clinical significant isolates was 21 h and for all the isolates 29 h. A total of 655 (8.15%) positive cultures were pathogenic organisms and 160 (1.99%) positive cultures were contaminants. Of all the signals 143 (1.77 %) were false positives and none were false negative. Among pathogenic microorganisms, Gram- positive, Gram-negative and yeast were 403 (61.52 %), 242 (36.94 %) and 10 (1.52 %), respectively. Since all our cultures were positive within the first 96h our data supports four day incubation protocol for the recovery of clinically significant organisms in BACTEC 9050 system.

Key words: Automated Blood culture system · BACTEC · Optimal Detection Times · Bacteremia · Incubation protocol · Microbial diagnosis

INTRODUCTION

Infectious disease is one of the leading causes of morbidity and mortality in most developing countries. Microbial invasion of the blood stream may lead to serious immediate consequences, including shock, multiple organ failure and disseminated intra vascular coagulation (DIC) and death. The detection of microorganisms in a patient's blood has great therapeutic and prognostic significance [1]. In view of this, timely detection of blood borne pathogens is one of the most important functions of a microbiology laboratory [2]. Blood culture remains central to this quest for identifying bacterial causes of bacteremia and septicemia. Despite recent developments, such as nucleic acid probes, Polymerase chain reaction (PCR) and other molecular techniques for microbial diagnosis, blood culture still remains the most practical and reliable method for the diagnosis of infections in the blood stream [3]. However due to the poor laboratory techniques, laboratory conditions, under developed human skills, wide spread abuse of antibiotics, the rate of growth of bacterial agents is very low [4]. Instrumentation of blood cultures has accomplished rapidness, accuracy and cost- effectiveness. It also eliminates cross contamination of cultures during repeated subcultures.

The BACTEC system (Becton Deckinson Microbiology Systems, Sparks, Md) measures CO_2 produced by the organisms as they metabolize the substrate present in the vial. Increase in the fluorescence of the vial sensor caused by the higher amount of CO_2 is monitored by the Bactec fluorescent series instruments. Analysis of the rate and amount of CO_2 increase enables the instrument to determine if the vial contains viable

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organisms [5]. The BACTEC 9000 series of blood culture systems are fluorogenic, automated, non-invasive. Recently introduced BACTEC™ 9050 with a capacity of 50 bottles, is a small, self-contained, automated system designed for processing three to five blood cultures per day. In addition to the difference in capacity, BACTEC 9050 differ from the larger systems by agitating the bottles continuously (versus intermittently for the other systems) and rotating the bottles to be read by one of three detectors (versus a dedicated detector for each stationary bottle in the large systems). The computer used to monitor the BACTEC 9050 bottles in contained within the system [6].

The aim of this study was to determine detection time, false positive/ negative rate and the distribution of bacteria and yeasts isolated using Peds PLUS TM/F culture vials in BACTEC 9050 system. We have also analyzed the data to decide the suitable and practical incubation protocol.

MATERIALS AND METHODS

This study was conducted in the Clinical Microbiology Laboratory of Kempegowda Institute of Medical Sciences (KIMS) Hospital, Bangalore from February 2009 to Aug 2010. The study group consisted of children between the ages of 28-days to 36-months presenting with fever of 39°C and above and children between 36-months and 60-months with sign of pneumonia, meningitis, arthritis, otitis media, sinusitis etc. 3 ml of blood was collected by aseptic procedures. The blood samples were inoculated into BACTEC Peds Plus/F blood culture bottles and were placed in the BACTEC 9050 blood culture instrument with in 2 h of collection. We did not use anaerobic blood culture bottles because of the inadequate usage of anaerobic blood cultures in the laboratory.

According to the manufacturer's guide, the inoculated culture bottles were loaded into BACTEC™ 9050 instrument. The culture bottles were arranged in three concentric rings designated A, B and C. and incubated at 35°C and continuously agitated for maximum recovery of organisms. Positive cultures were flagged by an indicator light on the front of the instrument, an audible alarm and were displayed on the LCD screen. The cultures were centrifuged in SSI BD vacutainer® at 3000 RPM/ 20 mins. Smears from the deposit were stained with Gram stain and subcultured onto sheep blood agar plates and incubated in CO_2 incubator. Identification and susceptibility testing was done using automated Siemens Microscan WalkAway® system with synergy panels. Instrument-negative bottles were Gram stained and subcultured at the end of the 5-day to confirm negativity. False-positive cultures were defined as those that were indicated by the instrument to be positive but had revealed no microorganisms by Gram staining and subculture [7]. All isolates were considered to be clinically significant, except *Micrococcus species,* aerobic spore bearers*, Diphtheroids and* Coagulase negative *Staphylococcus* with normal C-reactive protein (CRP) and WBC count. The time for detection was measured in hours, beginning with the loading time of the bottles in the instrument and ending with the positive signal of the instrument.

RESULTS

A total of 8034 blood cultures were received for evaluation over a period of 18-months from Feb 2009 to Aug 2010. The mean age of the study group was 20.20 months. There were 4370 males and 3664 females. The male female ratio was 1.44:1. Out of 8034 samples, 815 (10.14%) samples were identified positive by BACTEC 9050. Clinically significant microbial growth was detected in a total of 655(8.15%) cultures (Error! Reference source not found. and Figure 1.

In this study clinically significant Gram-positive bacteria, Gram-negative bacteria and fungal isolation rates were 403 (61.52%), 242 (36.94%) and 10 (1.52%), respectively. Among Gram-positive bacteria, 196 (29.92%) were determined to be Coagulase-negative *Staphylococci*. Among Gram-negative bacteria, 166 (25.34%) were the members of *Enterobacteriaceae*. Of the 10 fungal isolates five were *Candida species* (0.76%). The mean detection time for the Gram-positive bacteria, the Gram-negative bacteria and the yeasts in our study were 19.33, 19.06 and 24.04 h, respectively. The recovery time for *S.pneumoniae* was 10.05h. Moreover, the mean detection time of 196 Coagulase-negative *Staphylococcus* isolates was found to be 32.51 h (Figure 2). During the 18 month period, 85.71% of all cultures containing pathogens were detected within the first 24 h of incubation (Error! Reference source not found.).

The instrument has given 143 (1.77%) false-positive signals. There were no false negative signals. A total of 160(1.99%) specimens were positive for contaminant organisms.

Table 1: Isolated Microorganisms and their detection times

			Detection times (h)		
Type of Organism	No. of Samples	% of total	Max	Min	Mean
All Gram Positive Bacteria	403	61.52			
Streptococcus pneumoniae	37	5.64	12.25	3.2	10.05
Streptococcus agalactiae	7	1.06	24	7.15	13.15
Streptococcus mitis	41	6.25	22.04	9	12.08
Streptococcus pyogenes	3	0.45	14.2	8.5	10.1
Enterococcus faecalis	39	5.64	36	12.3	21.32
Staphylococcus aureus	78	11.90	43	2.2	13.23
Coagulase-negative *Staphylococcus*	196	29.92	38	7.3	32.51
Actinomycetes comitans	1	0.15	14	14	14
Listeria monocytogenes	1	0.15	47	47	47
All Gram Negative bacteria	242	36.94			
Pseudomonas aeruginosa	33	5.03	46	8.3	22.07
Acinetobacter species	38	5.80	19.3	12	18.47
Vibrio metschnikovii	1	0.15	29.2	29.2	29.2
Vibrio hollisae	1	0.15	14.3	14.3	14.3
Vibrio fluvialis	1	0.15	33	33	33
Bordetella bronchiseptica	1	0.15	10.26	10.26	10.26
Yersinia pseudotuberculosis	1	0.15	10.06	10.06	10.06
Enterobacteriaceae	166	25.34			
Salmonella species	94	14.35	33	9	19.35
Klebsiella species	7	1.06	15	12	13.3
E. coli	19	2.90	30	9.15	22.3
Enterobacter species	19	2.90	33.53	7.2	20.59
Citrobacter species	9	1.37	13.05	8.5	10.77
Morganella morgani	9	1.37	41.3	13.3	22.22
Shigella species	2	0.30	18	16	17
Serratia marcescens	6	0.91	29	18	23
All Fungi	10	1.52			
Candida species	5	0.76	29.53	22	24.04
Cryptococcus neoformans	3	0.45	23	19	21
Trichosporon	1	0.15	35	35	35
Rhodotorula rubra	1	0.15	70	70	70

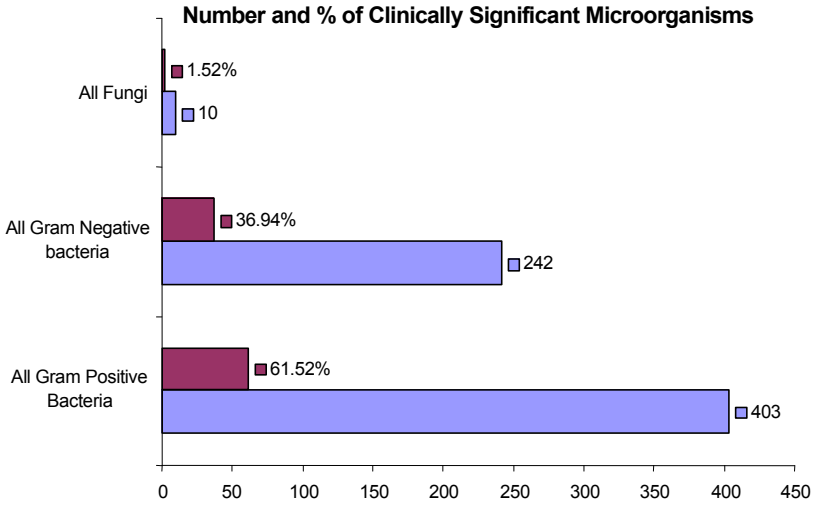

Fig. 1: Number and % of Clinically Significant Microorganisms

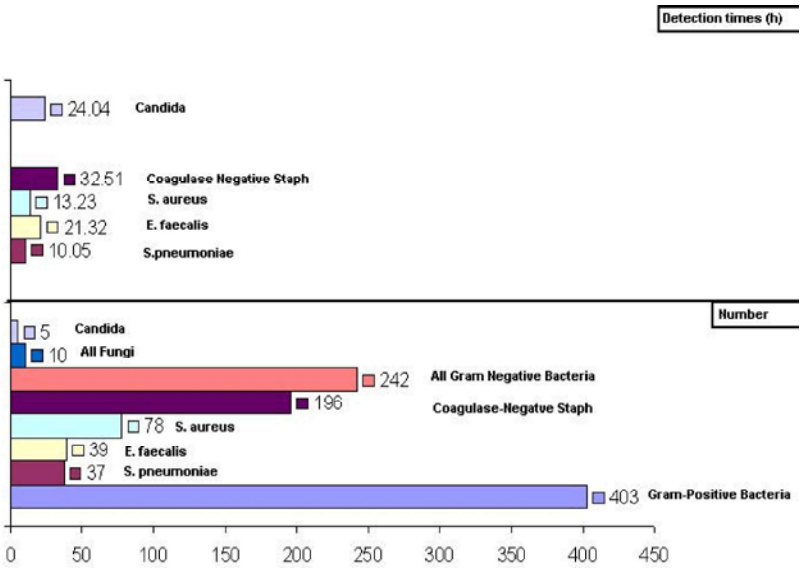

Fig. 2: Number of Clinically significant Microorganisms and Detection times

DISCUSSION AND CONCLUSION

In the current study, using the BACTEC 9050 system more than 75% of clinically significant pathogens were recovered within the first 24 h of incubation and all pathogens were recovered with in 4-days. Cockerill *et al*. [8] found the mean detection time to be 23.0 h for all pathogens. BACTEC 9240 has detected growth in 87.0 to 90.2% of the samples within the first 24 h [Error! Reference source not found. 1]. Durmaz, *et al*. [3], uses BACTEC 9120 to recover majority of pathogens within five days including *Candida* and *Brucella species*. Culture positivity reported after four days of incubation by Baka, *et al*. [10] was 98.5%, Nita *et al*. [11] 97.61% and Reisner *et al*. [12] 97.35%, whereas Kara, *et al*. [13] reported a low culture positivity of 77%. Some investigators have reported 96-98% of positive cultures within three days of incubation [Error! Reference source not found. 1, 14-16].Only four isolates (one strain each of Coagulase-negative *Staphylococci, Listeria, Vibrio* and *Candida species*) were recovered after 30 h. Similar studies have been performed with other automated blood culture systems to determine the incubation period required for these systems. Hardy *et al.* [17] recommended a 5-6 day protocol using the BacT/Alert blood cultures.

In this study, clinically significant Gram-positive isolates were 61.52% and Gram-negative 36.94%, similar isolation rates were reported by most other investigators [16], but *Durmaz et al* [3], reported more Gram-negative isolates.

In most studies, Coagulase-negative *Staphylococcus* was the most frequent isolated Gram-positive bacteria, which was similar to our study (48.63%) [7, 16-20]. Blood cultures yielding Coagulase-negative *Staphylococcus*, in critically unwell febrile patient, is a diagnostic dilemma regarding whether it is a real pathogen or a contaminant. In the current study, Coagulase-negative *Staphylococcus* was considered as a pathogen when it was associated with high CRP and leucocytosis especially in hospitalized patients.

The recovery time for *S. pneumoniae* (10.05 h) was significantly shorter with BACTEC 9050. The detection time of S.*pneumoniae* was reported to be between 20-29 h in various studies [3, 11]. In the IBIS Group-INCLEN multi centric study it was reported that 21% of pneumococcal disease resulted in septicemia. Early diagnosis and appropriate treatment of blood stream infections can make the difference between life and death. It would reduce mortality from septicemia, reduce turn around time and improve patient management [21].

Among Gram-negative bacteria isolates, *Enterobacteriaceae* were found to be the most frequent isolates (25.34%). Gray *et al*. [Error! Reference source not found.] drew attention to a striking increase in the isolations of *Enterobacteriaceae* (56.8%) in their study, conducted using the BACTEC 9240 blood culture system. This might be due to their antibiotic resistance.

Fungi were isolated from 10 (1.52%) samples. Out of five *Candida species*, four were isolated within 24 h of incubation. Smith *et al.* [22] reported that the detection time of the yeasts is 41.0 h. *Candida isolation* rate in

the current study was found to be lower than the other researchers probably because the study sample was not restricted to ICU patients.

In general a contamination rate of > 3% of total blood cultures in a study is not up to the standard for accurate evaluation. The overall contamination rate in this study was 1.99 %, which is low compared to other studies (3, 11, 22). This may be due to the appropriate aseptic conditions maintained at collection site, during transportation, during culture etc. Trained nurses and phlebotomists were employed in such activities to reduce contamination. There was a low false-positive rate (1.77%) reported during the study. Nolte *et al*.[23] reported highest false-positive rate of 2.2% followed by Nita *et al*. [11] of 1.5% and then Smith *et al*. [14] 0.5%.

At the end of 5 day incubation period the negative samples were sub-cultured on sheep blood agar, re-incubated for 24 h and none of these samples showed any growth. Longer incubation periods have been recommended for isolation of fastidious organisms like *Brucella*. Durmaz, *et al*. [3], isolated 65% *Brucella strains* within 72 h of incubation which is well within the incubation protocol followed by this study.

In conclusion, BACTEC 9050 has been proven as a reliable and fast method to identify the blood stream pathogens in blood cultures. However there is limited published data about the optimal detection time for this system. Based on this results the incubation period of 4-days is recommended as all the culture bottles flagged positive within 96 h. Routine subculture at the end of incubation is not advocated as none of them showed growth in the current study. Added advantage of reducing the incubation period to a four day protocol would be that larger number of samples could be accommodated in a single instrument.

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