



## Culture positivism exploitation through automated fluorescent-sensor technology from patients with blood stream infections

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**ABSTRACT:** This study tracks and analyses the culture results of 3615 blood samples received in Popular Diagnostic Centre, Dhanmondi, Dhaka 1205, throughout twelve month from suspected patients with blood stream infections. The samples were prepared by exploitation machine-controlled Fluorescent-Sensor Technology by BACTEC 9120® Culture System (Becton Dickinson and Company, Sparks, USA) choosing a 5 days incubation protocol. A total of 668/3615(18.50%) BACTEC 9120® system positive samples were then sub-cultured in 7% sheep blood agar, MacConkey agar, and chocolate agar plates. A sum of 346/668 (51.8%) were infective organisms and 312/668 (46.70%) positive vial cultures were contaminants. False positivism rate was 1.5% (10/668). The mean detection time for the clinical vital isolates was 17.7 h and for all the isolates was 38.1 h. Microorganisms characterization and antibiotic sensitivity testing was done using typical ways. The majorities (342, 98.84%) of those were Gram-negative microorganisms and solely 4 (1.16%) isolates were Gram-positive pathogens. Clinically vital pathogens recouped on day one, two and three were 91.0%, 7.5% and 1.5% severally. Most of the bacterial isolates were found extremely susceptible to a number of antibiotics along with ceftriaxone (95%), ceftazidime (95%), and cefepime (96%), whereas moderately sensitive to ciprofloxacin (81%), levofloxacin (89%) and chloramphenicol (81%); whereas all bacterial isolates were found to be resistant of nalidic acid (100%). Since all our cultures were positive at intervals the primary 72 h, our data supported 5 days incubation system for the recuperation of medically vital microorganisms in BACTEC 9120® Culture System.

**KEYWORDS:** Blood stream infections, BACTEC 9120® system, culture positivism, antibiotic sensitivity profile.

### INTRODUCTION

Blood stream infection is a danger to each organ inside the body and might have genuine quick results, together with stun, various organ disappointments, dispersed intravascular coagulation and passing (demise frequency at 20% to 50%). Therefore, identification and detection on time of microbial pathogens in blood is one of the vital significant elements of the biological laboratory [1]. As of late, several propelled strategies like as super molecule probes and polymerase chain reaction (PCR)

are created for the assignment of blood contaminations; anyway blood culture despite everything remains the foremost sensible and solid methodology [2].

Typical blood culture techniques include naked eye assessment of blood culture vials once every twenty-four-hour period for the proof of development for 48 hours and then visually impaired sub-culture on the next day on solid culture media. Negative containers of the culture are more re-brooded for 5 to 7 days before coverage. In recent years, sensational development has been occurred in blood culture procedures, culture

media, and in the frameworks. The greater part of the mechanically propelled blood culture systems are completely machine driven constantly observed blood culture frameworks [3]. Every 8-10 minutes vials are screened by these frameworks and final out calculations supported by evaluations of changes related with being development. Presently, 4 frameworks are accessible: Becton Dickinson Microbiology systems, Sparks, Md. (BACTEC®), Trek Diagnostic systems Inc., Organon Teknika, Durham, N.C. (BacT/Alert®), Westlake, Ohio (ESP®), and bioMerieux, Inc. Hazelwood, Mo. (Vital) [1]. All of those systems, there's none of significant distinction in the performances and everyone is extremely strong. Detection of growth is the principal differentiation exists within these systems [3]. For 5-7 days incubation period is programmed by the applicant to incubate samples vials in the systems.

In this research the main point of analysis was to see the range of microscopic bacteria confined from blood samples, their opportunity to identification by BACTEC 9120®, antibiotic sensitivity- resistance profile and to investigate the information to make our mind up that incubation protocol much be a lot of appropriate by machine-controlled BACTEC 9120® culture system. In our study as suggested by the manufacturer, we introduced a five day convention of incubation as there's absence of printed data relating to the ideal duration of incubation for the system from this apart of the nation.

## **MATERIALS AND METHODS**

### **Materials**

All materials and chemicals were maintained in analytical grade in this research project.

### **Bloodstream sample collection**

In this examination, we directed our study since December 2010 to November 2011 at Popular Diagnostic Centre Ltd, Dhaka 1205. The machine-controlled incessantly monitored blood culture system utilized is BACTEC 9120®. During this study, a complete of 3615 blood samples from individuals of suspected septicemia was received. Blood samples were collected by antiseptic method.

### **Blood culture through BACTEC 9120® method**

A 1-5 milliliter amount of blood sample was inoculated into BACTEC Peds Plus/F for kids and 8-10 milliliter into BACTEC Aerobic/F culture vials for adults. Only aerobic blood cultures were done in this study.

According to the maker's directions, culture bottles were stacked into the BACTEC 9120® system with inoculated blood samples. Five days incubation time was maintained in the complete examination. Every culture vial contained advanced Soybean-Casein Digest broth with CO<sub>2</sub> and resin (nonionic adsorbing resin and cationic exchange resin) to neutralize an enormous kind of antibacterial agents. At the bottom level, each vial has a synthetic sensing element which might observe rising in carbon dioxide (CO<sub>2</sub>) created by development organisms. This sensing element was checked by the machine each 10 minutes for a rise in its fluorescence units that is corresponding to the measure of CO<sub>2</sub> created. A positive perusing demonstrates the hypothetical nearness of feasible microorganisms within the vials. At whatever point there was a signal of microorganism development, the location time was reported by BACTEC 9120® system programming bundle. Days were determined as full 24 h time frames. For instance, disconnects were recognized at 24, 48, and 72 h thought of as distinguished on the very first moment, two and three severally.

### **Bacterial subculture**

All machine signal positive case bottles were sub-cultured on MacConkey agar, blood agar, and chocolate agar media. Also we conducted Gram staining from all of the machine signal positive cases and then the primary results were shared with medical practitioner. Standard biochemical methods were used for identification from sub-culture growth. In this work, negative culture bottles weren't analyzed for sub-culture because it has been demonstrated to be gratuitous [4, 5].

### **Bacterial identification and standardization**

Microscopic organisms bacteria were described and known exploitation customary strategies [6] as antecedently represented [7, 8]. Stocks of isolates were prepared by suspending a loop full of each bacterial growth in 10 milliliter nutrient broth. When incubation at 37°C for 12 h, the turbidity was acclimated to be outwardly practically identical with a 0.5 McFarland's standard.

### **Antibiotic sensitivity testing**

Bacterial antibiotic sensitivity of the pure growth to entirely unexpected antibiotics has made up our minds by the Kirby- Bauer disc diffusion method and supported the rules of the Clinical and Laboratory Standards Institute (CLSI) [9]. The subsequent medical drugs were used that contained agents: Ceftriaxone (30

µg), Ceftazidime (30 µg), Cefepime (30 µg), Ciprofloxacin (5 µg), Levofloxacin (30 µg), Chloramphenicol (25 µg), Nalidic acid (30 µg). Mueller-Hinton agar culture plates were inoculated with bacteria from the stock arrangement effectively acclimated to the 0.5 MacFarland's turbidity standard. The antibiotic discs were henceforth fastidiously superimposed on the agar and incubated at 37° C for 24-48 h.

## RESULTS

### Bacterial growth based on fluorescent-sensor technology

A total of 3615 specimens were gotten for culture over a time of one year. Over the course of our study, 668 (18.5%) culture vials were hailed positive by BACTEC 9120® and pure growth of bacteria were segregated from 346 positive culture vials. There have been 207 males and 139 females. The male feminine magnitude relation was 1.4:1. Pure organisms were recovered from 346 (100%) culture vials and no double organism was found from positive culture bottles. An aggregate of ten positive cases vials (1.5%) were taken as false positive, as they indicated no life form on Gram staining and no development on sub-culture (Table 1).

Infectious agents recouped from positive signal cultural bottle and opportunity to detections is appeared within the Table 2.

**Table 1:** Analysis results from both BACTEC 9120® culture system & conventional culture methods.

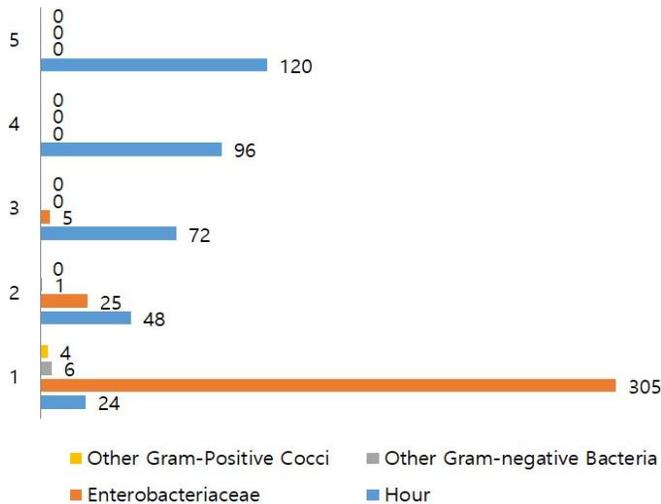
Group	Number of vials	Percent (%)
Single microorganisms (pure)	346	9.6
Contaminated growth	312	8.6
False positive signals	10	0.27
No Growth	2947	81.53
<b>Total</b>	<b>3615</b>	<b>100.0</b>

**Table 2:** Ideal time to identification of bacterial isolates by BACTEC 9120® culture system

Microorganisms	Number (%) of Bacteria recovered on day					Total (%)
	1	2	3	4	5	
Enterobacteriaceae:	1	2	3	4	5	
<i>Salmonella typhi</i>	192	07	05	-	-	204
<i>Salmonella paratyphi A</i>	93	15	-	-	-	108
<i>Escherichia coli</i>	14	02	-	-	-	16
<i>Klebsiella pneumoniae</i>	06	01	-	-	-	07
Other Gram-negative Bacteria:						
<i>Acinetobacter</i> spp.	03	-	-	-	-	03
<i>Pseudomonas</i> spp.	03	01	-	-	-	04
Other Gram-Positive Cocci:						
<i>Streptococcus pneumoniae</i>	01	-	-	-	-	01
<i>Staphylococcus aureus</i>	02	-	-	-	-	02
<i>Streptococcus</i> spp	01	-	-	-	-	01
Group D						
	315	26	5	0	0	346
	(91.0)	(7.5)	(1.5)			(100)

### Sub-culture of clinically vital microorganisms

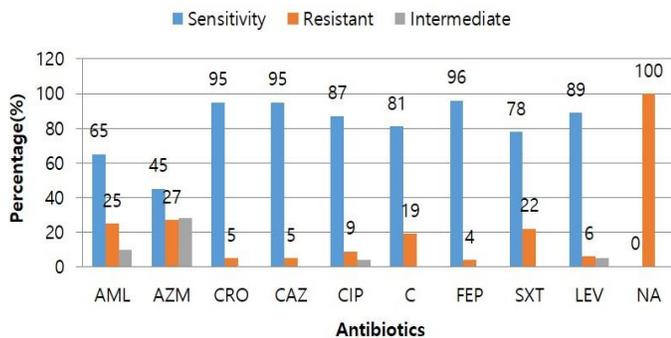
A total of 668 positive vials were sub-cultured, out of that 346 (51.8%) were clinically vital pathogens and 312 (46.7%) were contaminants. False positivism rate was 1.5% (10/668). The segregation ratio was 1.16% (4) for Gram-positive and 98.84 % (342) for Gram-negative bacteria. In this examination, Gram-negative bacteria were foremost pathogens which were under the family of Enterobacteriaceae. In our study a total of 315 (91.0%) Positive cases cultures turned on day one; 26 (7.5%) extra isolates were recovered on day two. Just five isolates recognized on day three were *Salmonella typhi* however when 96 h and one hundred twenty (120) h there was no any single significant isolate during this study (Figure 1). At five (5) days interval incubation period, 312 (46.7%) positive signal vial cultures were contaminants detected and extremely few amounts (1.5%) were showed false positive signals that each culturally and Gram-stain was negative



**Figure 1.** Total range of clinically vital microorganisms and detection time.

### Susceptibility of culturing microorganisms

Antibiotic susceptibility tests by Kirby-Bauer disc diffusion method are shown in figure 2. A considerable range of Gram-negative isolates were sensitive to first-line agents, i.e. Ceftriaxone, Ceftazidime, Ciprofloxacin, Chloramphenicol, Cefepime, Levofloxacin and Nalidixic acid.



**Figure 2.** Anti-biogram sensitivity and resistant pattern of blood culture isolates (AML=Amoxycillin, AZM= Azithromycin, CRO=Ceftriaxone, CAZ=Ceftazidime, CIP=Ciprofloxacin, C=Chloramphenicol, FEP=Cefepime, LEV=Levofloxacin, and NA= Nalidixic acid).

## DISCUSSION

Most of continuous observance machine-controlled blood culture systems are typically three to five days incubation period, suggested once they were initial introduced in Bangladesh. However, 5 days incubation duration is longer for detailing negative results and extra equipment would be needed to suit the accumulated and the accrued range of vials, thus what is more we have a tendency to follow a multi-day brooding convention in

our research facility. In this investigation, we recovered 315 (91.0%) clinically vital microorganism isolates at interval the primary twenty four hours of incubation and 31(9.0 %) by the remainder of four days of incubation. Comparable investigations were performed with alternative machine-controlled culture frameworks for deciding the timeframe needed for these frameworks. There are 4 days blood culture positivism rumored by Nita Pal *et al* [10] was 97.61%, Reisner, *et al.*[3] was 97.35% and Baka, *et al.*[11] 98.5%, while Kara, *et al.*,[12] rumored an occasional culture positivism of 77%. Durmaz, *et al.*, recuperate a large portion of the bacteria at intervals of 5 days [2]. A few agents have reputed at intervals of 3 days in 96-98% positivism cases [5, 13-17].

In this analysis, medically essential Gram-negative and Gram-positive bacteria were 98.84% and 1.16% respectively, comparable isolation rates were accounted by Durmaz, *et al.*, [2] rumored a lot of Gram-negative bacteria. In our investigation, Gram-negative bacteria were the foremost pathogenic bacteria under the family of *Enterobacteriaceae* that corresponds with different examinations. The general defilement rate of blood culture was 8.63 % ( 312/3615), that is somewhat higher contrasted with elective investigations [10, 18, 19]. This might result to the way that in our examination, nursing employees were blame for getting blood for culture rather than explicitly prepared phlebotomists. At whatever point prepared phlebotomists are used in such settings, a decreased defilement may be accomplished as found by Weinbaum *et al.* [20].

Our investigation had 2 attainable impediments. Right off the bat, no consideration was taken the amount of blood sample inoculated in every vial to search out the outcomes with in progress routine day by day follow in this establishment. Besides, we couldn't consider previously used antibiotics. The BACTEC 9120® blood culture media utilized antibiotic neutralize agents. Blood specimens of patient getting antibiotics didn't differ from the pre-antibiotic specimens for the opportunity to identify the microorganisms was appeared by Kara, *et al.*,[12]

Finally, our information bolster a 5-days incubation procedure for recuperation of routine microscopic organisms with the BACTEC 9120® culture system with in general un-wellness prevalence of 9.57% and negative predictive value and positive predictive value of 100% and 51.80% respectively. This is often virtually similar observations by alternative investigators [3-5, 11-14]. All isolates were found extremely susceptible to a number of antibiotics including ceftriaxone (95%), ceftazidine (95%), and cefepime (96%), while moderately sensitive to ciprofloxacin

(87%), levofloxacin (89%) and chloramphenicol (81%); while all of bacterial isolates found to be resistant in Nalidic acid (100%).

## CONCLUSIONS

In conclusion, the most of pathogens were recuperated at intervals of three (3) days. There's no infective being isolated from the remainder of two (2) days. Even if there's not ample printed information relating to the optimum incubation time for this technique, the data in our examination prescribe is that a rebate of the 5-day timeframe that was commonly applied with the BACTEC 9120® framework to three (3) days is feasible, that successively renders the BACTEC 9120® system a simpler device.

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## CONFLICTS OF INTEREST

Authors declared that they have no conflict of interest.

## REFERENCES

- [1] Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology. 11th ed. St. Luise Mosby; 2002.
- [2] Durmaz G, Tercan US, Aydinli A, Kiremitci A, Kiraz N, Akgun Y. Optimum detection times for bacteria and yeast species with the BACTEC 9120 aerobic blood culture system: Evaluation for a 5-year period in a Turkish University Hospital. *J Clin Microbiol* 2003; 41:819-21.
- [3] Reisner BS, Woods GL. Times to detection of bacteria and yeast in 9240 Blood culture bottles. *J Clin Microbiol* 1999; 37: 2024-6.
- [4] Doern GV, Brueggemann AB, Dunne WM, Jenkins SG, Halstead DC, McLaughlin JC. Four-day incubation period for blood culture bottles processed with the Difco ESP blood culture system. *J Clin Microbiol* 1997; 35: 1290-2.
- [5] Hardy DJ, Hulbert BB, Migneault PC. Time to detection of positive BacT/ Alert cultures and lack of need for routine subculture of 5- to 7- day negative cultures. *J Clin Microbiol* 1992; 30: 2743-5.
- [6] Barrow GI, Feltham RKA. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd edition, Cambridge University Press 1993; Pp331.
- [7] Ehwarieme DA, Egbule OS, Okonjo NP. Antibiotic resistance profile of soil-borne enteric bacteria isolated from parts of Delta State, Nigeria. *Journal of Applied Sciences* 2010; 13(1): 8949-8958.
- [8] Enabulele OI, Ehwarieme DA, Aluyi HSA. Resistance pattern of *Salmonella* isolates from food, animal and human sources, to common antimicrobial agents. *Global Journal of Pure and Applied Sciences* 2008; 14(2): 179-182.
- [9] Wayne, PA. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 17th International Supplement. CLSI M100-S17 2001.
- [10] Pal N, Sharma R, Rishi S, Vyas L. Optimum Time to Detection of Bacteria and Yeast Species with BACTEC 9120 Culture System from Blood and Sterile Body Fluids. *J. of Laboratory Physicians* 2009; 1(2): 69.
- [11] Baka S, Logginidis I, Efstratiou V, Panagiotopoulou E, Kaparos G, Gerolymatos K. Time to positivity of BACTEC blood culture bottles, abstr. No. 1733\_441. *In Abstracts of the 17th European Congress of Clinical Microbiology and Infectious Diseases ICC, Munich, Germany, 2007.*
- [12] Kara A, Kanra G, Cengiz AB, Apis M, Gür D. Pediatric blood culture: Time to positivity. *Turk J Pediatr* 2004; 46: 251-5.
- [13] Bourbeau PP, Pohlman JK. Three days of incubation may be sufficient for routine blood cultures with BacT/Alert FAN blood culture bottles. *J Clin Microbiol* 2001; 39: 2079-82.
- [14] Bourbeau PP, Foltzer M. Routine incubation of BacT/Alert FA and FN blood culture bottles for more than 3 days may not be necessary. *J Clin Microbiol* 2005; 43: 2506-9.
- [15] McGowan KL, Foster JA, Coffin SE. Outpatient pediatric blood cultures: Time to positivity. *Pediatrics* 2000; 106: 251-5.
- [16] Endimiani A, Tamborini A, Luzzaro F, Lombardi G, Toniolo A. Epidemiology of bloodstream infections and time to detection of positive blood culture: An evaluation of the automated BacT/Alert and BACTEC 9240 systems. *New Microbiol* 2002; 25:9-16.
- [17] Johnson AS, Touchie C, Haldane DJ, Forward KR. Four-day incubation for detection of bacteremia using BACTEC 9240. *Diagn Microbiol Infect Dis* 2000; 38: 195-9.
- [18] Smith JA, Bryce EA, Ngui-Yen JH, Roberts FJ. Comparison of BACTEC 9240 and BacT/Alert blood culture system in an adult hospital. *J Clin Microbiol* 1995; 33: 1905-8.
- [19] Nolte FS, Williams JM, Jerris RC, Morello JA, Leitch CD, Matushek S. Multicenter clinical evaluation of a

continuous monitoring blood culture system using fluorescent-sensor technology (BACTEC 9240). *J Clin Microbiol* 1993; 31: 552-7.

- [20] Weinbaum FI, Lavie S, Danek M, Sixsmith D, Heinrich GF, Mills SS. Doing it right the first time: Quality improvement and the contaminated blood culture. *J Clin Microbiol* 1997; 35: 563-5.



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