Original Article

Assessment of Time To Positivity (TTP) and Loading Delay Influencing the Positivity of Blood Culture by an Automated System

Naimika Patel[1], Riddhi Patel[2], Rupal Patel[3]

Background : The presence of living micro-organisms in blood is known as bloodstream infection. It can be leading to sepsis, a critical condition associated with mortality ranging from 14% to 34%. Blood cultures are still considered the gold standard test for the detection of bacteremia and fungemia. The bacterial load in blood culture is assessed by the parameter of time to positivity in an automated system.

Materials and Methods : It was a prospective observational study conducted 3 months after IEC approval. A minimum of one & maximum of three samples were collected from each patient. After receiving them at the microbiology laboratory all bottles were loaded into the BacT/ALERT machine. All the signaled positive bottles were studied for a time to detection and factors influencing it and the second objective was to observe the effect of loading delay on isolation rate and time to detection. The following parameters were prospectively extracted from BacT/ALERT systems™ software the cell number, loading time, signal positive time and unloading time (hour, minute).

Observations : A total of 761 blood culture bottles were received during the study period. Maximum bottles were received from male as compared to female patients. Maximum blood cultures were received from the 0-10 years of age group followed by from 21-30 years and from 51-60 years. The mean TTD for all the isolates was 22.71 hours. 81% of true pathogens were detected within 24 hours and 98% of true pathogens were detected within 72 hours. We observed that inadequate blood volume took longer TTP for GNB & Yeast isolates than adequate volume bottles. The true pathogen positivity rate decreases in case of loading delay. The mean unloading time during routine & emergency hours was 0.6 hours & 1.6 hours.

Conclusion : As positive blood cultures are critical alerts; every step should be taken to decrease the loading & unloading delay of blood culture bottles for the final reduction of turn-around time and timely intimation of positive blood culture results to clinicians.

[*J Indian Med Assoc* 2024; **122(7):** 37-42]

***Key words* : Time To Positivity / Detection, Loading Delay, Unloading Delay, BacT / ALERT.**

The presence of living micro-organisms in the blood is known as a blood stream infection. It can be leading to sepsis, a critical condition associated with mortality ranging from 14% to 34%. Blood cultures are still considered the gold standard test for the detection of bacteremia and fungemia. Positive blood culture results can help clinicians to early diagnosis and therapy against the specific organism/s and provide prognostic value. Today many laboratories use automated blood culture systems for microbial detection methods. Rapid detection and early reports of blood culture are crucial for treating sepsis patients and for reducing hospital stays[1,2].

Time To Positivity (TTP) is defined as the time required from the beginning of culture incubation to the detection of bacterial growth by an automated system. The bacterial load in blood culture is assessed by the parameter of TTP in an automated system. It has been proposed as a diagnostic and prognostic tool and is an independent predictor of fatal outcomes. Time to positivity of blood culture bottles can be influenced by various factors eg, the concentration of a pathogen in the primary sample, collection time, the volume of blood, delay in transfer of sample and culture processing and level of contaminants (coagulase-negative *staphylococci*)[3,4].

At our hospital, the current method for processing blood culture is BacT/ALERT 3D Microbial Detection System (Biomerieux, France). This study aimed (i) to

Editor's Comment :

- Positive blood cultures are critical alerts. Thus, every step is important to follow the manufacturer guideline and laboratory procedure for the final reduction of turn-around time.
- Timely intimation of positive blood culture results to clinicians is helpful for patient managment.

Department of Microbiology, Pramukhswami Medical College - Bhaikaka University, Anand, Gujarat 388325
[1]MD, Assistant Professor and Corresponding Author
[2]MSc, Student
[3]MD, Professor

Received on : 20/07/2023
Accepted on : 23/07/2023

determine TTP for positive blood culture bottles & to study factors influencing TTP and (ii) to observe the effect of loading delay on isolation rate and TTP.

Material and Methods

Source of Data :

The study was conducted at the Microbiology laboratory, a NABL accredited laboratory of Shree Krishna Hospital, Karamsad. The study was conducted after the approval of Institutional Ethics Committee.

Methodology :

Study design : Prospective Observational study.

Duration of study : 1st May, 2019 to 31st July, 2019.

Inclusion Criteria : Blood culture requests received during the study duration from all the Indoor & Outdoor patients of all age groups were included in the study.

Exclusion Criteria : Nil.

BacT/ALERT® FA plus and BacT/ALERT® PF plus culture bottles were used for adult and pediatric patients, respectively. After collection at the respective area, all blood culture bottles were transferred to the Microbiology laboratory, a part of the Central Diagnostic Laboratory (CDL), NABL accredited laboratory. All bottles were loaded into the BacT/ALERT® 3D Microbial Detection System as per the standard protocol. Culture bottles were incubated for 5 days/ until signaled positive for growth. Signal-positive bottles were further processed for the identification of micro-organisms and susceptibility testing using the Vitek 2 Compact system.

The Following Parameters for Blood Culture Bottles were Studied :

(1) Loading delay : To observe the effect of rapid loading of bottles into BacT/ALERT, the time interval between various stations was calculated & studied as per the following : (1) collection time to pneumatic receiving time, (2) pneumatic receiving time to Microbiology laboratory receiving time, (3) Microbiology receiving time to loading time into BacT/ALERT machine. The overall time taken from collection to loading into BacT/ALERT was calculated. The effect of loading delay on positivity rate and TTP for true pathogens was assessed.

(2) For TTP calculation, the time interval between loading of bottles and positive signal in BacT/ALERT was calculated & studied. The day of receipt of blood culture in the laboratory was defined as day 0. TTP for different organisms was calculated. Association of TTP with blood volume was compared.

(3) Contaminant : The contaminant was defined as the growth of skin flora from a positive blood culture bottle (*eg, Bacillus spp, Corynebacterium spp, Propionibacterium spp and Micrococcus spp*) Coagulase-negative staphylococci grown from single/ multiple blood culture was clinically correlated to decide whether it was a true pathogen or a skin contaminant. Antimicrobial susceptibility results of Coagulase-negative staphylococci which were identified as contaminants were not reported. All other Gram-positive Cocci (GPC), Gram-negative Bacilli (GNB)/ Coccobacilli (GNCB) & Yeast isolates were considered true pathogens.

(4) Time intervals between (i) loading to signal positive and (ii) signal positive to unloading of bottles during routine (from 9:00 am - 5:00 pm) & emergency (from 5:00 pm - 9:00 am) hours were compared.

Patient Details and Analysis of DATA :

For each blood culture bottle, the following parameters were prospectively extracted from the laboratory information System database: demographic characteristics of the patient (age, sex), collection time, a pneumatic station receiving time, microbiology laboratory receiving time and microbiological result. The following parameters were prospectively extracted from BacT/ALERT system's software: the cell number, loading time, signal positive time and unloading time.All the data were entered and analyzed in Microsoft Excel 2010.

Results

During the study period, a total of 761 blood culture bottles from 604 patients were received for blood culture at the Microbiology laboratory. Maximum bottles were received from 354 (59%) males compared to 250 (41%) females. A total of 182 (30%) blood cultures were received from the 0-10 years of age group followed by 78 (13%)from 21-30 years and 75(12%) from 51-60 years. Out of 761 blood cultures,97 (13%) were identified as True pathogens & 139 (18%) were identified as Contaminants (CONS and *Bacillus spp*). However, 525(69%) blood cultures were negative.

As shown in Table 1, the mean TTD for all the isolates was 22.71 hours. 98% of all isolates were detected by day 2. A total of 73% (173/236) of GPC, GNB and Contaminants were detected within 24 hours and the other 19% (44/236) including Yeast were

Table 1 — *Comparison of TTP in GPC, Yeast, GNB & Contaminants (N=236)*

Organism	Numbers of isolates	TTP-No of blood cultures signaled positive in BacT/ALERT on the day(hours)					
		Mean Time (hours)	0(24)	1(48)	2(72)	3(96)	4(120)
Gram-positive Cocci	24	16.29	21	2	1	0	0
Gram-negative Bacilli	69	17.52	58	8	2	1	0
Contaminants	139	25.13	94	32	10	2	1
Yeast	4	66.98	0	2	1	0	1
	236	22.71	173(73%)	44(19%)	14(6%)	3(1%)	2(1%)

detected within 48 hours. In 81% of true pathogens were detected within 24 hours and 98% of true pathogens were detected within 72 hours. 68% of contaminants were detected in 24 hours.

The mean TTP for GPC & Yeast isolates is shown in Table 2. Out of 24 GPC isolates, 88% were detected within 24 hours, 96% were detected within 48 hours and 100% were detected within 72 hours. 72% of S*taphylococcus aureus* isolates and all other GPCs were signaled positive within 24 hours (by day 0).

The mean TTP for Gram-negative bacilli is shown in Table 3. Out of 69 GNB isolates, 84%, 96%, 99% & 100% were detected within 24 hours, 48 hours, 72 hours and 96 hours respectively. None were detected on day 4 (on the 5th day).

As shown in Table 4, the mean TTD for Contaminants was 25.13 hours. 68% (94/1of 39) Contaminants were detected on day 0, and 23% (32/139) were detected on day 1.

The relation between the volumes of blood with TTP is shown in Table 5. Overall TTD from bottles with adequate volume and inadequate volume was 18.45 hours & 19.71 hours respectively.

Table 6 represents the time taken at various stations before loading blood culture bottles into BacT/ALERT machine. Table 7 shows the effect of loading delay on positivity rate and TTP for true pathogens. The mean time from loading to signal positive was 23.32 & 23.39 hours during routine & emergency hours. However, the mean unloading delay during routine & emergency hours was 36.74 & 98.8 minutes respectively. During routine &

Table 2 — *TTP for Gram-positive cocci (N=24)and Yeast (N=4) isolates*

Organism	Numbers of isolates	TTP-No of blood cultures signaled positive in BacT/ALERT on the day(hours)					
		Mean Time (hours)	0(24)	1(48)	2(72)	3(96)	4(120)
GPC :							
Staphylococcus aureus	11	18.58	8	02	01	_	_
Staphylococcus epidermidis	4	18.5	4	_	_	_	_
Staphylococcus hominis	3	13.22	3	_	_	_	_
Staphylococcus haemolyticus	1	16.53	1	_	_	_	_
Streptococcus pyogens	2	11.45	2	_	_	_	_
Streptococcus pneumoniae	1	7.66	1	_	_	_	_
Streptococcus mitis	1	14.11	1	_	_	_	_
Enterococcus faecium	1	11.7	1	_	_	_	_
Total	24	16.29	21	2	01	0	0
Yeast :							
Candida albicans	2	56.49	_	2	_	_	_
Cryptococcus laurentii	2	77.48	_	_	1	_	1
Total	4	66.98	0	2	1	0	1

Table 3 — *TTP for Gram-negative bacilli (N=69)*

Organism	Numbers of isolates	TTP-No of blood cultures signaled positive in BacT/ALERT on the day(hours)					
		Mean Time (hours)	0(24)	1(48)	2(72)	3(96)	4(120)
Escherichia coli	20	15.38	17	3	_	_	_
Klebsiella pneumoniae	18	10.92	18	_	_	_	_
Salmonella typhi	2	14.72	2	_	_	_	_
Salmonella paratyphi A	6	20.98	5	1	_	_	_
Acinetobacter spp	6	11.93	6	_	_	_	_
Pseudomonas spp	4	46.37	2	_	1	1	_
Citrobacter sedalkii	1	8.23	1	_	_	_	_
Enterobacter cloacae	1	7.6	1	_	_	_	_
Shigella sonnei	1	11.81	1	_	_	_	_
Aeromonas hydrophilia	3	17.74	2	1	_	_	_
Brevundimonasdiminuta	1	37.55	_	1	_	_	_
Brucella melitensis	1	44.51	_	1	_	_	_
Burkholderia cepacia	1	22.36	1	_	_	_	_
Ralstonia	1	21.35	1	_	_	_	_
Stenotrophomonas maltophilia	2	16.08	2	_	_	_	_
Sphingomonaspaucimobilis	1	53.73	_	_	1	_	_
Total	69	17.52	58	8	2	1	0

Table 4 — *TTP for Contaminants (N=139)*

Organism	Numbers of isolates	TTP-No of blood cultures signaled positive in BacT/ALERT on the day(hours)				
		Mean Time (hours)	0(24)	1(48)	2(72)	3(96)	4(120)
Coagulase Negative Staphylococci (CONS)	97	25.4	65	25	5	1	1
Bacillus spp	42	24.1	29	7	5	1	_
	139	25.13	94	32	10	2	1

Table 5 — *Comparison of blood volume with TTP for true Pathogens (N=97)*

	Volume (N)	Mean TTP (hours)
GPC	Adequate (10)	16.26
	Inadequate (14)	16.31
GNB	Adequate (24)	16.21
	Inadequate (45)	18.22
Yeast	Adequate (2)	56.31
	Inadequate (2)	76.98
Total	Adequate (36)	18.45
	Inadequate (61)	19.71

Table 6 — *Time taken at different stations before loading blood culture bottles (N=761)*

	Meantime (minutes)
Collection time- Pneumatic station	28.7
Pneumatic station – Microbiology laboratory	16.23
Microbiology laboratory– Loading time	32.00
Total time	76.9

Table 7 — *Comparison of loading delay (collection to loading duration) with TTP for true Pathogens (N=97)*

Loading delay (hours)	GPC		GNB		YEAST	
	Numbers	Mean TTP (hours)	Numbers	Mean TTP (hours)	Numbers	Mean TTP (hours)
0-1	17	16.99	28	18.43	1	55.81
1-2	7	14.59	28	17.91	3	70.37
2-3	0	-	9	12.41	0	-
3-4	0	-	2	16.5	0	-
4-5	0	-	0	-	0	-
5-6	0	-	1	9.6	0	-
>6	0	-	1	36.93	0	-

emergency hours, the maximum time taken to unload the bottles was 249 & 518 minutes (4.15 & 8.6 hours) respectively.

Discussion

Time to positivity is a parameter provided by the automated blood culture system. Time is calculated from the incubation of bottles until a positive signal is detected[2,5]. Many published studies mention that TTP of blood cultures is a prognostic factor in the cases of bacteremia caused by Gram-positive and Gram-negative micro-organisms such as *S aureus*, *S pneumoniae*, *E coli, Klebsiella pneumoniae* and *Burkholderia pseudomallaei*. They also mention that a short TTP reflects a surrogate marker of bacterial concentration in blood and it suggests severe bacteremia. It can affect clinical outcomes such as an increase in mortality rates, increased length of stays and hospitalization costs[3].

A continuous monitoring system detects all positive signals in a relatively short period, the majority within 24 hours[6,7]. In our study, the mean TTP for all the isolates (true pathogens & contaminants) was 22.71 hours. Lambregts, *et al* found that the median TTP was 15.7 hours which is similar to our findings. The authors also found that neutropenia was a predictor for short TTP and antibiotic pre-treatment was a predictor for prolonged TTP[8].

Previous reports have also shown that clinically significant organism was generally detected within 3 days of incubation[5,7,9,10]. In our study, 81% of true pathogens were detected within 24 hours and 98% of true pathogens were detected within 72 hours. The Mean TTP for GPC, GNB & Yeast was 16.29 hours, 17.52 hours and 66.98 hours respectively. Out of 24 GPC isolates, 88% were detected within 24 hours. A total of 72% of S*taphylococcus aureus* isolates and all other GPCs were signaled positive within 24 hours. Out of 69 GNB isolates, 84% were detected within 24 hours. All (100%) isolates of *Candida albicans* were detected within 48 hours. Kurlat, *et al* reported that all isolates of group B *streptococcus, Escherichia coli, Klebsiella species* and *Staphylococcus aureus* were identified by day 2[10]. In our study, 100% GPC & GNB isolates were detected within 72 hours and 96 hours respectively. Similar to our findings, TTD for fungal isolates varied between 48 hours – 120 hours[10]. Pan F, *et al* found that the average TTP of all positive blood cultures was 30.97 and the TTP of Gram-negative strains was significantly shorter than that of Gram-positive strains and fungi[11].

In our study,the mean TTP for Contaminants was 25.13 hours. Amongst them, 68% of Contaminants were detected within 24 hours whereas 32% of Contaminants were detected after 24 hours. A study conducted by Kennedy found that those generally regarded as Contaminants (CoNS & Bacillus spp) were detected much later than other isolates[7]. Although

lower inoculum is associated with longer TTP for Contaminants, there is no consensus on whether the TTP is predictive of contamination versus true infection. This has been emphasized that those isolates that were considered clinically relevant were detected earlier than those regarded as contaminates.

TTP can be influenced by various factors. During the study, we tried to assess the effect of blood volume on TTP. We observed that inadequate blood volume took longer TTP for GNB & Yeast isolates than adequate volume bottles. TTP was not affected by blood volume in GPC isolates. Overall, TTP from bottles with inadequate volume was higher than from bottles with adequate volume indicating the significance of blood volume on the TTP.

The second important parameter that affects TTP is the loading delay of the bottles into the instrument. Published guidelines recommend that the interval between the collection of blood and the entry of the bottles into an automated blood culture system should not be longer than 2 or 4 hours; also, manufacturer instructions indicate that inoculated vials should be transported to the laboratory as quickly as possible[2]. Many studies observed that in half of the investigated laboratories, bottles were not immediately incubated during nightshifts. Studies show that a delay in processing can impact time to positivity, presumptively due to an extended lag phase resulting from storage at suboptimal temperature[8]. In our study, most of the bottles were loaded into the instrument within six hours. With the increase in loading delay, the true pathogen positivity rate decreases in the present study. However, when loading delay was compared with TTD, the mean TTD of GPC & GNB isolates was not affected. Different patient populations might be the reason for such variations. Moreover, contrary to the Italian study where the laboratory in the university hospital is closed on Sundays and holidays, a central diagnostic laboratory at our place operates on weekends and holidays[12]. Janapatla, *et al* conclude that an overnight delay of 15 hours of bottles leads to an increase in the detection time of the pathogen from 25.9 hours to 40 hours, which might influence clinical therapy[13]. In contrast, Panday, *et al* in their study showed that pre-analytical time for the three pre-defined cut-off values did not influence blood culture yield[12].

Time to removal is defined as the time taken from the positive signal in system until the bottle is removed for subsequent sub-culturing for isolation of the organism[2]. In our study, the mean unloading time during routine & emergency hours was 36.74 (0.6 hours) minutes & 98.8 (1.65 hours) minutes respectively. The study conducted by Emeraud, *et al* conclude that the mean unloading time for positive bottles is 5.8 hours which is explained by the fact that the positive bottles are not unloaded during the night duty period this can result in obtaining false negative results[14]. In our study, maximum time taken to unload the bottles was 249 & 518 minutes (4.15 & 8.6 hours) during routine & emergency hours respectively. At our place, diagnostic laboratories were open for 24 hours throughout the year. Blood culture bottles were processed during routine and emergency hours by trained technicians. However, the number of laboratory technicians during routine and emergency hours differ (less number during emergency) which might be responsible for delays in loading & unloading bottles. The reasons for unloading delay during working hours must be identified and necessary actions should be taken. Such a delay in unloading the positive bottles can affect the total time required to identify the organism and inform the result of the clinicians. Positive blood cultures are critical alerts and must be processed immediately to decrease the turn-around time which can save the life of the patients. Our data and results refer to typical operative conditions and describe what was actually occurring in the laboratory. The findings of the study require attention by the laboratory staff and Head of the department for future interventions to improve the quality of blood culture results.

Limitation of study : The study was conducted at a Tertiary Care Teaching Hospital with a smaller number of blood samples (761) & patients (604) within a short period of time and the findings may not be generalizable to all hospitals. Parameters including timing of venepunctures, skin antisepsis, antibiotic treatment prior to sampling, patient comorbidities and manual measurement of blood volume in bottles (as per manufacturer's instructions) might have affected the results.

Conclusion

As positive blood cultures are critical alerts; every step should be taken to decrease the loading & unloading delay of blood culture bottles for the final reduction of turn-around time and timely intimation of positive blood culture results to clinicians. For analytic issues of loading and unloading delays at the laboratory, all the concerned staff should be informed about the importance of the blood cultures and the effect of such delays on patient outcomes.

References

1 Byun JH, Kim SW, Koh EH, Kim S, Kim SC — Comprehensive Analysis of Blood Cultures Using BacT/Alert 3D FA Plus and FN Plus Media. *Laboratory Medicine Online* 2016; **6(3):** 176-82.

2 Dreyer A W — Blood culture systems: From patient to Result. In: Azevedo L, editor. Sepsis. An ongoing and significant challenge. Rijeka: InTech; 2012. Ch-15.

3 Tang PC, Lee CC, Li CW, Li MC, Ko WC, Lee NY — Time-to-positivity of blood culture: an independent prognostic factor of monomicrobial Pseudomonas aeruginosa bacteremia. *Journal of Microbiology, Immunology and Infection* 2017; **50(4):** 486-93.

4 Ning Y, Hu R, Yao G, Bo S — Time to positivity of blood culture and its prognostic value in bloodstream infection. *European Journal of Clinical Microbiology & Infectious Diseases* 2016; **35(4):** 619-24.

5 Kumar Y, Qunibi M, Neal TJ, Yoxall CW — Time to positivity of neonatal blood cultures. *Archives of Disease in Childhood-Fetal and Neonatal Edition* 2001; **85(3):** F182-6.

6 Lamy B, Dargere S, Arendrup MC, Parienti J-J and Tattevin P — How to Optimize the Use of Blood Cultures for the Diagnosis of Bloodstream Infection? *A Stat-of-the Art Front Microbiol* 2016; **7:** 1-13

7 Kennedy GT, Barr JG, Goldsmith C — Detection of bacteremia by the continuously monitoring BacT/Alert System. *J Clin Pathol* 1995; **48:** 912-4.

8 Lambregts MMC, Bernards AT, van der Beek MT, Visser LG, de Boer MG — Time to positivity of blood cultures supports early re-evaluation of empiric broad-spectrum antimicrobial therapy. *PLoS ONE* 2019; **14(1):** e0208819. https://doi.org/10.1371/journal.pone.0208819

9 Hardy DJ, Hulbert BB, Migneault PC — Time to Detection of Positive BacT/Alert Blood Cultures and Lack of Need for Routine Subcultures of 5- to 7- Day Negative Cultures. *J Clin Microbiol* 1992; **30:** 2743-5.

10 Kurlat I, Stoll BJ, McGowan JE Jr — Time to positivity for detection of bacteremia in neonates. *J Clin Microbiol* 1989; **27(5):** 1068-71. doi: 10.1128/jcm.27.5.1068-1071.1989. PMID: 2745679; PMCID: PMC267484.

11 Pan F, Zhao W, Zhang H — Value of time to positivity of blood culture in children with bloodstream infections. *Canadian Journal of Infectious Diseases and Medical Microbiology* 2019; 2019. doi: 10.1155/2019/5975837.

12 Nannan Panday RS, Wang S, van de Ven PM, Hekker TAM, Alam N, Nanayakkara PWB — Evaluation of blood culture epidemiology and efficiency in a large Europenteching hospital. PLoS ONE; **14(3):** 1-7.

13 Janapatla RP, Yan JJ, Chien ML, Chen HM, Wu HM, Wu JJ— Effect of overnight storage of blood culture bottles on bacterial detection time in the BACTEC 9240 blood culture system. *J Microbiol Immunol Infect* 2010; **43(2):** 126-32. doi: 10.1016/S1684-1182(10)60020-5. PMID: 20457429.

14 Emeraud C, Yilmaz S, Fortineau N, Cuzon G, Dortet L — Quality indicators for blood culture: 1 year of monitoring with BacT/Alert Virtuo at a French hospital. *Journal of Medical Microbiology* 2021; **70(3)**. 001300. doi: 10.1099/jmm.0.001300