original article

Time-to-detection of bacteria and yeast with the BACTEC FX versus BacT/Alert Virtuo blood culture systems

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BACKGROUND: Bloodstream infections are associated with high rates of morbidity and mortality. Rapid detection of bloodstream infections is important in achieving better patient outcomes.

OBJECTIVE: Compare the time-to-detection (TTD) of the new BacT/Alert Virtuo and the BACTEC FX automated blood culture systems.

DESIGN: Prospective simulated comparison of two instruments using seeded samples.

SETTING: Medical microbiology laboratory.

METHODS: Blood culture bottles were seeded in triplicate with each of the standard ATCC strains of aerobes, anaerobes and yeast. TTD was calculated as the length of time from the beginning of culture incubation to the detection of bacterial growth.

MAIN OUTCOME MEASURES: TTD for the various tested organisms on the two microbial detection systems.

RESULTS: The 99 bottles of seeded blood cultures incubated in each of the blood culture systems included 21 anaerobic, 39 aerobic and 39 pediatric bottles. The BacT/Alert Virtuo system exhibited significantly shorter TTD for 72.7 % of the tested organisms compared to BACTEC FX system with a median difference in mean TTD of 2.1 hours (interquartile range: 1.5-3.5 hours). The BACTEC FX system was faster in 15.2% (5/33) of microorganisms, with a median difference in mean TTD of 25.9 hours (IQR: 9.1-29.2 hours).

CONCLUSION: TTD was significantly shorter for most of the microorganisms tested on the new BacT/Alert Virtuo system compared to the BACTEC FX system.

LIMITATIONS: Use of simulated cultures to assess TTD may not precisely represent clinical blood cultures.

CONFLICT OF INTEREST: None.

Bloodstream infections (BSIs) represent a major public health concern as they are associated with high rates of mortality and morbidity.[1] BSIs can cause serious immediate consequences such as shock, multiple organ failure, disseminated intravascular coagulation (DIC), and death. Blood culture is the gold standard method for the detection of BSIs.[2,3] There are several available automated blood culture systems which help in the rapid detection of BSIs. These fully automated blood culture systems electronically monitor blood culture bottles every 8-10 minutes and detect changes associated with microbial growth based on internal algorithms.[2] Rapid detection of BSIs is important in achieving better patient outcomes.[4] Time-to-detection (TTD) is calculated as the length of time from the beginning of culture incubation to the detection of bacterial growth by an automated system.[4] The objective of this study was to compare the TTD (in hours) for the BACTEC FX (Becton Dickinson, Heidelberg, Germany) and the BacT/ALERT Virtuo (VIRTUO, bioMerieux France) systems.

METHODS

This study was conducted in the medical microbiology laboratory of King Khalid University Hospital affiliated to King Saud University Medical City, Riyadh, Saudi Arabia between January 2016 and March 2017. Seeded blood cultures were used for the evaluation of two microbial detection systems: BACTEC FX ((Becton Dickinson, Heidelberg, Germany, the system in use at the time) and the BacT/Alert Virtuo (VIRTUO TM, bioMerieux France, the new system). The blood culture bottles of both systems (BD Bactec Plus aerobic/F, BD Bactec Lytic/10 anaerobic/F and BD Bactec Peds Plus/F, and BacT/Alert FA Plus, BacT/Alert FN Plus and BacT/Alert PF Plus) were seeded in triplicate with each of the standard ATCC or American Type Culture Collection strains of aerobes, anaerobes and yeast.[5]

Aerobic, anaerobic and pediatric blood culture bottles of both systems were simultaneously inoculated. Each bacterial or yeast ATCC strain was suspended in brain heart infusion broth and incubated for 24 hours. After incubation, 0.1 mL of broth culture was then inoculated on appropriate medium, for instance; tryptic soy agar broth (TSAB) was used for aerobic bacteria, chocolate agar was used for fastidious bacteria, Columbia blood agar was used for anaerobic bacteria and Sabouraud dextrose agar was used for yeast, followed by 24 hours incubation. All bacterial and yeast cultures were examined for pure growth; 0.5 McFarland was then created by inoculating 3-5 bacterial or yeast colonies into sterile saline, corresponding to 10^8 CFU/mL and 10^6 CFU/mL, respectively. To simulate blood culture specimens, 5 mL of sterile fresh human blood was added to aerobic and anaerobic blood culture bottles, and 3 mL was added to pediatric bottles. A negative control bottle was inoculated with 5 mL of sterile human blood only.[5]

The following ATCC strains were used in this study: **anaerobes:** *Bacteroides fragilis* ATCC 25285, *Bacteroides vulgatus* ATCC 8482, *Clostridium perfringens* ATCC 13124, *Peptoniphilus asaccharolyticus* ATCC 14963; **gram negative:** *Escherichia coli* ATCC 25922, *Haemophilus influenzae* ATCC 10211, *Neisseria meningitidis* ATCC 13090, *Pseudomonas aeruginosa* ATCC 27853, *Stenotrophomonas maltophilia* ATCC 13637; **gram positives:** *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* ATCC 4698, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus agalactiae* ATCC 13813, *Streptococcus pneumoniae* ATCC 6305, *Streptococcus pyogenes* ATCC 19615 and **yeast:** *Candida albicans* ATCC 14053.

The dilution scheme used for aerobic and anaerobic bacteria to achieve a final concentration of 250 CFU/mL was as follows: 0.5 McFarland was adjusted for each bacterial strain to be tested. Three tubes were labeled as 1, 2 and 3. The first 2 tubes had 9.9 mL of sterile saline added and tube 3 had 9.75 mL of sterile saline added. Aseptically, 0.1 mL of bacterial suspension was transferred to tube 1, corresponding to 10^6 CFU/mL. After thorough mixing, 0.1 mL of the suspension was transferred to tube 2 from tube 1, resulting in 10^4 CFU/mL. Finally, 0.25 mL suspension was transferred from tube 2 to tube 3, corresponding to 250 CFU/mL. To verify CFU, 0.1 mL of suspension from the final dilution tube (250 CFU/mL) was inoculated on appropriate medium and a count of 30-300 CFU was considered satisfactory. From tube 3, 0.5 mL of the suspension of the respective ATCC strain was inoculated in triplicate to respective blood culture bottles corresponding to 125 CFU/bottle, and were incubated for a maximum of five days in respective microbial detection system.

The dilution scheme used for yeast to achieve a final concentration of 250 CFU/mL was as follows: 0.5 McFarland was adjusted for the Candida ATCC strain. Two tubes were labeled as 1 and 2. The first tube had 9.9 mL of sterile saline added, and 9.75 mL of sterile saline was added to tube 2. Aseptically, 0.1 mL of yeast suspension was transferred to tube 1, corresponding to 10^4 CFU/mL . After thorough mixing, 0.1 mL of the suspension was transferred to tube 2 from tube 1, resulting in 250 CFU/mL. To verify CFU, 0.1 mL of suspension from the final dilution tube (250 CFU/mL) was inoculat-

ed on appropriate medium and a count of 30-300 CFU was considered satisfactory. From tube 2, 0.5 mL of the yeast ATCC strain was then inoculated in triplicate in respective blood culture bottles resulting in 125 CFU/bottle and were incubated for a maximum of five days in respective microbial detection system.

We used IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA) to analyze the data. To test the differences in the recovery rates from the two systems we used Pearson's chi-squared test or Fisher's exact test. To compare the performance of the two machines we calculated the differences in mean TTDs between BACTEC FX and BacT/Alert machines for each microorganism. Since those differences were not normally distributed, we reported the median values of mean differences in TTDs with their interquartile ranges. Values of $P<.05$ were considered to be statistically significant. Values of $P<.07$ were considered to be marginally significant.

RESULTS

A total of 99 seeded blood culture bottles were incubated in each of the blood culture systems; including 21 anaerobic, 39 aerobic and 39 pediatric blood culture bottles, to evaluate the time difference in detecting a variety of microorganisms. **Table 1** shows the time to detection of the cultured microorganisms using each microbial detection system. The results of three isolates were removed during the analysis, because one of the anaerobic bottles inoculated with *B fragilis* did not show any growth in the BacT/Alert Virtuo system, and two bottles with *H influenzae* and *B vulgatus* showed unusually long detection times. In particular, it took almost 4 times longer (71.8 hours vs. 18.8 hours) for one of three *H influenzae* isolates inoculated in pediatric bottles to grow in the BACTEX FX system and 3.4 times longer (36.9 hours vs. 10.7 hours) for one of three *B vulgatus* isolates incubated in the BacT/Alert Virtuo system using an anaerobic bottle to become positive. The difference in the recovery rates from the two systems was not statistically significant.

The mean TTD was statistically significant ($P<.05$) or marginally significantly ($P<.07$) shorter for 72.7% (24/33) of microorganisms incubated in the BacT/Alert Virtuo system compared to the BACTEC FX system, with a median difference in mean TTD of 2.1 hours (interquartile range [IQR]=1.5-3.5 hours), ranging from 0.9 hours to 13.8 hours. The BACTEC FX system was faster in 15.2% (5/33) of microorganisms, with a median difference in mean TTD of 25.9 hours (IQR=9.1-29.2 hours; min.=0.4 hours, max.=30.9 hours). There was no statistically significant difference in mean TTD for the remaining 4 microorganisms between the two systems. For those 24 microorganisms, where the BacT/Alert Virtuo system performed faster compared to the BACTEC FX system, there was no statistically significant difference ($P=.163$) in mean TTD between anaerobic, aerobic and pediatric bottles.

DISCUSSION

In this study, the TTD was significantly shorter in 72.7% using the BacT/Alert Virtuo system compared to the BACTEC FX system. Differences among organisms could be explained by the low numbers of certain tested organisms.[6] Consistent with our findings, one study observed a lower TTD for the BacT/Alert Virtuo system compared to the BACTEC FX system.[7] On the other hand, another study that tested 405 bottles reported 96.8% agreement between the two systems and no statistical differences were observed in recovery rate and TTD between the BACTEC FX and the BacT/Alert Virtuo systems.[8]

With regard to anaerobic bacteria, the mean TTD of *B fragilis* was strikingly shorter in the BACTEC FX (mean=18.3 hours) compared to the BacT/Alert Virtuo system (mean=49.2 hours) and slightly shorter for *Clostridium perfringens* and *Peptoniphilus asaccharolyticus* (**Table 1**). However, the TTD was shorter for *B vulgatus* on the BacT/Alert Virtuo system compared to the BACTEC FX. Similarly, Cheong et al found the TTD for *B fragilis* group to be 2.03 days using the BacT/Alert Virtuo system in their comparison to the BacT/Alert 3D system (1.38 days).[6] Contrary to our findings, one study found a major difference in the TTD of *B fragilis* using the BacT/Alert Virtuo system (24.78 hours) when they compared it to the Bact/Alert 3D system (71.45 hours).[9] The BacT/Alert 3D system is a previous generation system from the Bact/alert Virtuo system. Comparatively the BacT/Alert Virtuo system is more automated, sensitive and specific with a shorter TTD for microbial growth.[10,11]

Regarding *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae*, reduction in TTD was prominent using BacT/Alert Virtuo system compared to the BACTEC FX. However, a striking difference in TTD was observed for *H influenzae* and *N meningitidis*, with the BACTEC FX having a shorter TTD compared to the BacT/Alert Virtuo system. This could be explained by the effect of SPS (sodium polyanethol sulfonate). SPS is an anticoagulant that inhibits the growth of *N meningitidis*. SPS concentration in the BacT/Alert Virtuo FA Plus bottle is higher (0.3%) compared to SPS concentration (0.05%) in the Bactec Plus aerobic/F bottles used with the BACTEC FX. In contrast to our findings, this dif-

Table 1. Time to detection (in hours) of blood culture positivity for BACTEC FX and BacT/Alert systems

Medium and Microorganisms	BACTEC FX			BacT/Alert			χ^2	*P* value
	No. of Isolates	Mean TTD (hours)	95% CI	No. of Isolates	Mean TTD (hours)	95% CI		
Anaerobic bottles								
Bacteroides fragilis	3	18.3	17.6-18.7	2	49.2	49.2-49.2	3.446	*.063*
Bacteroides vulgatus	3	18.7	18.5-18.9	2	10.7	10.3-11.0	4.263	**.039**
Clostridium perfringens	3	9.5	8.7-10.3	3	9.7	9.5-9.9	0.000	.988
Peptoniphilus asaccharolyticus	3	9.2	9.1-9.3	3	9.6	9.5-9.7	4.738	**.030**
Escherichia coli	3	9.4	9.1-9.6	3	7.8	7.6-8.0	4.738	**.030**
Staphylococcus aureus	3	12.8	12.7-12.9	3	9.4	9.2-9.6	5.052	**.025**
Streptococcus pneumoniae	3	16.2	15.8-16.6	3	12.6	12.3-12.9	5.052	**.025**
Aerobic bottles								
Escherichia coli	3	10.3	10.1-10.4	3	8.7	8.6-8.8	5.052	**.025**
Haemophilus influenzae	3	15.6	14.6-16.5	3	41.5	39.0-44.0	5.052	**.025**
Neisseria meningitidis	3	15.9	15.5-16.4	3	33.8	17.6-50.0	5.052	**.025**
Pseudomonas aeruginosa	3	14.2	14.2-14.2	3	12.2	12.1-12.4	5.052	**.025**
Stenotrophomonas maltophilia	3	35.2	34.0-36.3	3	33.9	33.4-34.5	1.667	.197
Enterococcus faecalis	3	10.7	10.3-11.1	3	9.3	8.9-9.6	5.052	**.025**
Micrococcus luteus	3	17.6	17.5-17.8	3	15.7	15.4-16.0	5.052	**.025**
Staphylococcus aureus	3	14.5	14.2-14.8	3	11.3	11.2-11.3	5.052	**.025**
Staphylococcus epidermidis	3	17.6	17.2-18.0	3	15.5	15.0-15.8	5.052	**.025**
Streptococcus agalactiae	3	8.8	8.4-9.2	3	7.9	7.7-8.0	5.052	**.025**
Streptococcus pneumoniae	3	14.3	13.8-14.8	3	13.1	12.7-13.5	5.052	**.025**
Streptococcus pyogenes	3	10.9	10.8-11.0	3	9.4	8.8-10.0	5.052	**.025**
Candida albicans	3	35.9	33.1-38.6	3	22.1	20.7-23.6	5.052	**.025**
Pediatric bottles								
Escherichia coli	3	11.0	11.0 -11.1	3	8.9	8.8-9.0	5.052	**.025**
Haemophilus influenzae	2	18.8	18.7-18.8	3	13.7	13.4-13.9	3.446	*.063*
Neisseria meningitidis	3	18.1	17.4-18.7	3	14.2	13.7-14.7	5.052	**.025**
Pseudomonas aeruginosa	3	14.2	14.0-14.3	3	12.1	12.0-12.3	5.052	**.025**
Stenotrophomonas maltophilia	3	44.6	42.3-46.9	3	72.1	72.0-72.1	4.738	**.030**
Enterococcus faecalis	3	10.1	10.1-10.1	3	9.7	9.3-10.2	0.181	.671
Micrococcus luteus	3	16.9	16.3-17.4	3	13.8	13.2-14.5	5.052	**.025**
Staphylococcus aureus	3	13.5	13.0-14.0	3	11.2	10.6-11.8	5.052	**.025**
Staphylococcus epidermidis	3	17.0	16.5-17.4	3	14.1	13.6-14.6	5.052	**.025**
Streptococcus agalactiae	3	8.9	8.7-9.1	3	7.7	7.7-7.8	4.738	**.030**
Streptococcus pneumoniae	3	12.6	7.8-17.3	3	13.1	12.8-13.4	1.182	.277
Streptococcus pyogenes	3	10.9	10.6-11.2	3	9.7	9.2-10.1	5.052	**.025**
Candida albicans	3	33.9	27.6-40.3	3	25.6	23.4-34.5	5.052	**.025**

TTD, time to detection. Shaded areas represent faster growth; bold and in green grid box values denote *P*<.05; italic values denote *P*<.07.

ference for both organisms was not observed in other studies comparing the BacT/Alert Virtuo system and the BacT/Alert 3D system.[9,11] The volume of blood used in our study could explain this discrepancy. A higher volume of blood (8-10 mL) could lead to neutralization of the inhibitory effect of SPS on sensitive organisms, thus optimizing the recovery of these organisms. Fastidious organisms like *H influenzae* require growth factors such as NAD and factor X especially if sterile body fluid is inoculated into blood culture media; however, this is likely not a factor as both are provided by the blood culture media used in both systems.[12] Another explanation of the long TTD for both these organisms could be due to the cell density of the inoculum.[12] Of note is that adequate blood volume is the most important parameter for the detection of BSI because bacterial or fungal density is very low in most cases of BSIs.[1] We used 5 mL fresh blood for aerobic and anaerobic blood culture bottles in our study, which may explain the longer TTD. *N meningitidis* and *H influenzae* bacteraemia/septicaemia are serious infections with devastating consequences particularly in pediatric patients. Using advanced and accurate microbial detection systems for rapid and definitive isolation is important for the rapid detection of BSI due to these fastidious bacteria.

The TTD for *Stenotrophomona maltophilia* was shorter using the BACTEC FX compared to the BacT/Alert Virtuo system for pediatric bottles ($P \leq .05$), while a longer TTD was observed for the BACTEX FX aerobic bottles. Similarly, one study reported a longer TTD for *S maltophilia* with the BacT/Alert Virtuo system compared to the BacT/Alert 3D system (29.4 hours using 3D compared to 36.6 hours using Virtuo).[11] For *Candida albicans*, we found that the TTD was shorter on the BacT/Alert Virtuo system (22.1 hours vs 35.9 hours). Other studies demonstrated similar mean TTDs for *Candida albicans* using the BacT/Alert Virtuo system.[3,11]

The performance of the BacT/Alert Virtuo system was recently evaluated in a large-scale clinical study where they compared its performance to the BacT/Alert 3D system for the detection of bacteremia and fungemia using four bottle types.10 Overall TTD by the BacT/Alert Virtuo system was 2 hours faster than the BacT/Alert 3D system (mean 15.9 hours versus 17.7 hours). For most tested organisms, our mean TTD on the BacT/Alert Virtuo system was similar to or shorter than what they observed.

In addition to our findings of a shorter TTD of microbial growth for the BacT/Alert system over the BACTEC FX system for most tested organisms, the new BacT/Alert Virtuo system has other advantages. The BacT/Alert Virtuo provides automated processes such as having a robotic apparatus for automated loading and unloading of bottles and provides a total reduction of processing steps (from 11 to 4).[7,11] Furthermore, the BACTEC FX system reportedly provides reliable estimates of blood volume in blood culture bottles with one limitation, the volume of blood is monitored in batches and not for each individual bottle,[1] while the BacT/Alert Virtuo system provides automated volume estimates for each bottle.[1]

Although our study findings are mainly in agreement with other studies comparing the performance and TTD of the BacT/Alert Virtuo system and the BACTEC FX or other BACTEC systems, it has some limitations. First, the use of simulated bacteremia/fungemia in the blood culture bottles may have led to the differences in the recovery rates of the organisms that resulted in inaccurate measurements of TTD compared to bacteremia/fungemia in clinical specimens. Second, the inoculum used in our study was high (30-300 CFU/bottle) compared to microorganism concentration in real bacteremia/fungemia, which often varied between 0.1-10 CFU/mL.[1] Third, the choice of tested organisms in our study was small and did not include other commonly isolated gram-positive and gram-negative organisms and other species of *Candida* and molds that are seen in clinical practice (such as *Acinetobacter*, methicillin-resistant *Staphylococcus aureus*, and other gram-negative bacteria including extended spectrum β-lactamase producing *Enterobacteriaceae*), plus the performance of the two systems was not assessed in the presence of antibiotics. Fourth, the volume of blood added to aerobic and anaerobic blood culture bottles probably was not enough, which potentially influenced the TTD. TTD is considered an important parameter that is consistently associated with disease severity and risk of death independently of the detection system.[4] Fifth, concentration of SPS in the BacT/Alert Virtuo aerobic bottles may have resulted in a long TTD for *H influenzae* and *N meningitidis* compared to the BACTEC FX system. Further comparative studies are recommended using clinical specimens to validate the performance of the new BacT/Alert Virtuo MDS for common microorganisms isolated in clinical settings.

In conclusion, our study demonstrated that TTD was significantly shorter for most of the microorganisms tested on the new BacT/Alert Virtuo system compared to the BACTEC FX system. Due to its many advantages, we would recommend use of the BacT/Alert Virtuo system as a microbial detection system in medical microbiology laboratories. However, further studies are needed to evaluate its performance in clinical settings, especially for fastidious organisms.

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