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Controlled Evaluation of the New BacT/Alert Virtuo Blood Culture System for Detection and Time to Detection of Bacteria and Yeasts

Osman Altun,[a,b] Mohammed Almuhayawi,[a,c] Petra Lüthje,[a] Rubina Taha,[a] Måns Ullberg,[a] Volkan Özenci[a]

Division of Clinical Microbiology F 72, Karolinska Institutet, Karolinska University Hospital, Huddinge, Stockholm, Sweden[a]; Florida State University, College of Medicine, CoM Pediatric Residency Program, Tallahassee, Florida, USA[b]; King Abdul-Aziz University, Faculty of Medicine, Department of Microbiology, Jeddah, Saudi Arabia[c]

We compared the newly approved BacT/Alert Virtuo blood culture system to the BacT/Alert 3D system using 115 clinical bacterial and fungal isolates in 784 simulated blood culture bottles. The time to detection was reduced by roughly 20% in the Virtuo system ($P < 0.0001$) while the detection rate did not differ.

Bloodstream infections remain a leading cause of death and are associated with high mortality and morbidity (1, 2). Early intervention and initiation of appropriate treatment have been shown to improve patient outcome (3–5). Rapid detection and identification of the causing pathogen are therefore essential in the diagnosis of these invasive diseases.

Blood culture (BC) is the gold standard for detection of bacteria and fungi from blood or other normally sterile body fluids. It has previously been shown that the type of BC bottle used has a substantial impact on the microbiological diagnosis of bloodstream infections (6–10), as these media are complex formulations that provide nutrients and neutralizing antimicrobials in clinical blood samples (11). In addition, the BC system affects workflow and microbiological performance (11, 12). To date, the most widely distributed BC systems are the BacT/Alert 3D (bioMérieux; referred to as 3D hereafter), BD Bactec (Becton Dickinson), and VersaTREK (Thermo Scientific). bioMérieux has recently introduced a new BC system with automatic loading and unloading of BC bottles, the BacT/Alert Virtuo (Virtuo), for bacterial and fungal detection in BC. Enhanced colorimetric technology to detect microbial growth and improved temperature stability suggest improved culture conditions in the Virtuo compared to the 3D system. However, the microbiological performance of Virtuo has not been evaluated.

In this study, the Virtuo system was tested in direct comparison with the 3D system by parallel incubation of a total of 115 clinical bacterial and fungal isolates (Table 1). All isolates were originally collected from clinical BC samples sent for microbiological diagnosis to Karolinska University Laboratory (Stockholm, Sweden) from three tertiary care hospitals in the greater Stockholm area. Isolates were recovered from frozen stocks and were cultured overnight on appropriate agar medium. Approximately 15 CFU in phosphate-buffered saline was inoculated into each BC bottle with 8 ml defibrinated horse blood, and all BC bottles were incubated until positivity was reached or for a maximum of 5 days. We and others have previously demonstrated that the use of horse blood does not significantly influence the performance of BC systems (13–17). Growth was assessed in aerobic (FA and FA Plus) and anaerobic (FN and FN Plus) BacT/Alert BC bottles, except for *Acinetobacter* spp. and *Candida* spp., which were cultured under aerobic conditions only. While the CE-approved version of the Virtuo system is intended to operate with resin-based BC bottles (FA Plus and FN Plus) only, charcoal-based BC bottles (FA and FN) were evaluated in the 3D system and in the investigational-use-only version of Virtuo employed in this study. At the end of the incubation period, the BC medium was subcultured on blood (for bacteria) or Sabouraud dextrose (for yeast) agar plates, respectively, to exclude contamination and to confirm true-positive and true-negative detection results.

Statistical analyses were performed using GraphPad Prism 6. The times to detection (TTD) between BC systems were compared using the Wilcoxon matched-pair signed-rank test or 2-way matched analysis of variance (ANOVA) and the TTD between groups of microorganisms by Mann-Whitney U test. The relation between the TTD values achieved by the two systems was assessed by Spearman correlation. Differences with P values of <0.05 were regarded as statistically significant.

A total of 784 BC bottles were included in this study. The vast majority ($n = 760$; 97%) signaled positive within the 5-day incubation period (Table 1). There was no difference in the number of negative bottles per BC system ($n = 12$ each). Mostly, the paired BC bottles from one isolate were affected (alpha-hemolytic streptococci, $n = 7$ bottle pairs; coagulase-negative staphylococci [CoNS], $n = 3$ bottle pairs; *Acinetobacter*, $n = 1$ bottle pair), except for one alpha-hemolytic streptococcal isolate that grew in the Virtuo system and one *Acinetobacter* isolate that grew only in the 3D system. Subcultures of the BC medium confirmed the absence of bacterial growth in these bottles. Thus, false-negative BC results were not observed.

The TTD values were compared among the 379 bottle pairs that signaled positive in the two systems (Table 2; Fig. 1). Overall, the TTD was significantly shorter for bottles incubated in Virtuo than those incubated in 3D (median 12 h and 15 h, respectively; $P < 0.0001$) and was reduced by 18.5% (median)

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Address correspondence to Osman Altun, osman.altun@ki.se.

TABLE 1 Clinical isolates tested in the BacT/Alert 3D and the BacT/Alert Virtuo blood culture systems

Species/group	No. of isolates[a]	No. with positive blood cultures within 5 days							
		FA		FA Plus		FN		FN Plus	
		3D	Virtuo	3D	Virtuo	3D	Virtuo	3D	Virtuo
Gram-negative species	39/32	38	38	39	38	32	32	32	32
Escherichia coli	10	10	10	10	10	10	10	10	10
Klebsiella oxytoca	3	3	3	3	3	3	3	3	3
Klebsiella pneumoniae	7	7	7	7	7	7	7	7	7
Serratia marcescens	12	12	12	12	12	12	12	12	12
Acinetobacter spp.	7	6	6	7	6				
Gram-positive species	49	48	48	47	48	44	44	46	46
Staphylococcus aureus	10	10	10	10	10	10	10	10	10
CoNS	5	5	5	5	5	2	2	5	5
Enterococcus faecalis	5	5	5	5	5	5	5	5	5
Enterococcus faecium	5	5	5	5	5	5	5	5	5
Group A streptococci	3	3	3	3	3	3	3	3	3
Group G streptococci	2	2	2	2	2	2	2	2	2
Streptococcus pneumoniae	13	13	13	13	13	13	13	13	13
Alpha-hemolytic streptococci	6	5	5	4	5	4	4	3	3
Yeast	27/0	27	27	27	27				
Candida albicans	12	12	12	12	12				
Candida glabrata	15	15	15	15	15				
Total (aerobic/anaerobic)	115/81	113	113	113	113	76	76	78	78

[a] Number of isolated tested under aerobic/anaerobic conditions.

overall without striking differences between bacterial species or bottle types. Among bacterial cultures ($n = 325$ bottle pairs), 90% of the bottles reached positivity within 21 h in the 3D system, while this was already achieved after 16 h in the Virtuo system. The TTD was reduced by 2.8 h (median) in the Virtuo system ($P < 0.0001$). Among cultures with *Candida* ($n = 54$ bottle pairs), 90% reached positivity at the fourth day (81 h) in the 3D system and within 3 days (71 h) in the Virtuo system. The median TTD was reduced by 4.8 h ($P < 0.0001$). The two *Candida* species included in this investigation showed marked differences in TTD, with 24 h for *Candida albicans* and 66 h for *Candida glabrata* ($P < 0.0001$). *C. glabrata* is an increasingly common pathogen that is isolated from bloodstream infections (18, 19). Treatment of these infections is challenging due to reduced

TABLE 2 Time to detection in four different types of blood culture bottles incubated in two blood culture systems

Species/group	No. of isolates	Time to positivity (median, h)								*P* value[a]
		FA		FA Plus		FN		FN Plus		
		3D	Virtuo	3D	Virtuo	3D	Virtuo	3D	Virtuo	
Gram-negative species	38	14.5	11.4	14.0	11.4	13.2	10.7	14.5	11.7	<0.0001
Escherichia coli	10	14.7	11.4	11.5	9.2	13.1	10.1	11.4	8.8	<0.0001
Klebsiella spp.	10	12.6	9.8	14.0	11.9	12.1	9.7	15.0	12.1	<0.0001
Serratia marcescens	12	15.3	11.9	15.5	12.0	14.5	11.5	15.5	12.5	<0.0001
Acinetobacter spp.	6	16.5	14.0	14.0	12					<0.0001
Gram-positive species	43–48	16.3	13.3	13.0	11	16.8	13.7	14.0	11.0	<0.0001
Staphylococcus aureus	10	16.6	13.8	13.1	11.3	18.7	14.3	13	10.9	<0.0001
CoNS	2–5	21.8	16.0	19.6	15.5	33.5	30.0	19.6	17.7	<0.0001
Enterococcus spp.	10	16.0	12.9	13.0	10.5	15.0	11.8	13.5	10.1	<0.0001
Beta-hemolytic streptococci	5	21.0	14.0	12.0	10.0	17.0	13.8	14.0	12.1	<0.0001
Streptococcus pneumoniae	13	15.0	11.0	13.0	10.3	16.0	13.0	14.0	11.0	<0.0001
Alpha-hemolytic streptococci	3–5	21.0	15.0	17.9	14.6	18.9	14.1	19.4	13.6	<0.0001
Yeast	27	50	39	56	48					<0.0001
Candida albicans	12	23.9	20.8	26.5	24.4					<0.05
Candida glabrata	15	79.2	68.0	65.0	54.0					<0.0001
Total	108–113	16.6	13.3	14.0	12.0	15.0	12.0	14.0	11.1	<0.0001

[a] Comparison of BacT/Alert 3D and BacT/Alert Virtuo using 2-way matched ANOVA.

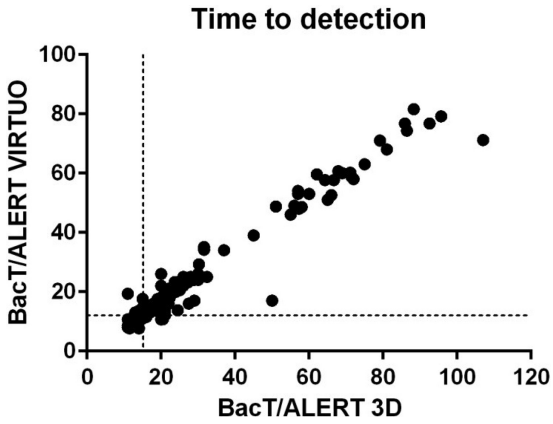

FIG 1 Relation between time to detection (TTD) in the BacT/Alert Virtuo and the BacT/Alert 3D blood culture systems. Time to detection was evaluated in 379 pairs of blood culture bottles incubated in parallel in the two BacT/Alert blood culture systems. TTD is expressed in hours, and median TTD are indicated by broken lines.

sensitivity of *C. glabrata* to antifungal agents (20). It has previously been observed that *C. glabrata* was more strongly affected by BC system and medium than *C. albicans* (6, 21), which in general grew faster and with less variable results (21–24). Similarly, TTD for *C. glabrata* was reduced by 14.7% (median, 9 h), while TTD for *C. albicans* was only 9.1% (2 h) shorter in Virtuo.

A limitation of this study is the usage of simulated BC. While our results indicate a systematic influence of the BC system across all microorganisms tested (Fig. 1) ($r = 0.91$; $P < 0.001$), it cannot be ruled out that the performance might differ in the presence of antibiotics and other inhibitory factors in clinical samples. Also, the effect on anaerobe bacteria and polymicrobial growth were not assessed. In addition, practical integration of the two different BacT/Alert systems into clinical routine and processing time prior to incubation were not considered.

The present results strongly indicate that the Virtuo BC system allows faster detection of pathogens from bloodstream infections, which is of high relevance in clinical microbiological diagnostics. Further controlled clinical studies are warranted to evaluate the performance of the Virtuo system on clinical specimens.

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