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PII: S1201-9712(17)30166-2
DOI: http://dx.doi.org/doi:10.1016/j.ijid.2017.06.012
Reference: IJID 2968

To appear in: *International Journal of Infectious Diseases*

Received date: 24-4-2017
Revised date: 2-6-2017
Accepted date: 10-6-2017

Please cite this article as: Congestrì Francesco, Pedna Maria Federica, Fantini Michela, Samuelli Michela, Schiavone Pasqua, Torri Arianna, Bertini Stefania, Sambri Vittorio.Comparison of "time to detection" values between BacT/ALERT® VIRTUO™ and BacT/ALERT® 3D instruments for clinical blood cultures samples.*International Journal of Infectious Diseases* http://dx.doi.org/10.1016/j.ijid.2017.06.012

Comparison of "time to detection" values between BacT/ALERT® VIRTUO™ and BacT/ALERT® 3D instruments for clinical blood cultures samples

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Highlights

- A reduction in times to detection of VIRTUO compared to BacT/ALERT 3D is supposed
- Microorganisms isolated from blood culture were grouped into 8 different categories
- Five of the eight groups of microorganisms positive bottles was detected earlier in VIRTUO
- An earlier detection of microrganisms lead to more rapid use of targeted antibiotic therapy

ABSTRACT

Objectives

The early detection of bacteraemia and fungemia is of paramount importance to guide the antimicrobial therapy in septic patients. In this study the "time to detection" (TTD) values for the new blood culture system BacT/ALERT® VIRTUO™ (VIRTUO) was evaluated in 1462 positive clinical bottles and compared with the TTD identified for 1601 positive clinical bottles incubated in the BacT/ALERT® 3D (BTA 3D).

Methods

The most representative microorganisms isolated from bottles incubated in both blood culture systems were divided into 8 categories (in order of frequency): Coagulase-Negative-Staphylococci (CoNS), *Escherichia coli, Enterobacteriaceae* (other than *E. coli*), *Staphylococcus aureus, Enterococcus* spp., viridans group streptococci, *Pseudomonas aeruginosa* and *Candida* spp.

Results

The comparison of TTD values for the 2 blood culture systems strongly indicate that in the first five groups listed above the growth was detected earlier ($p < 0.05$) by using the VIRTUO incubator.

Conclusions

The new VIRTUO blood culture system can reduce the TTD values in more than 75% of isolated microorganisms.

Keywords: Bacteraemia; Sepsis; Blood culture systems; Microorganisms causing sepsis; Time to detection; Early sepsis detection

INTRODUCTION

The incidence of sepsis is increasing dramatically since the last decade, causing a considerable morbidity and a high rate of mortality in patients (Shankar-Hari et al. 2016). A rapid identification of the causal agent is crucial for a correct diagnosis, a consequent pathogen oriented therapy and a better patient outcome (Liao et al. 2009). Blood culture (BC) is still the microbiological gold standard in the diagnosis of sepsis and it is of fundamental importance having a continuous monitoring system that detects any growing microorganism as rapidly as possible.

To date the BacT/ALERT® VIRTUO™ (VIRTUO) system is the only BC incubator that features a fully automated loading and unloading of the bottles.

This improves the stability of the incubation temperature and consequently a reduction of the "Time to Detection" (TTD) is conceivable. TTD is calculated from the beginning of the incubation until a positive signal is detected: this parameter can be influenced by various factors, including the initial bacterial load, the source of the infection, the volume of the inoculum, the speed of growth of each individual microorganism and the presence of antibiotics and host serum factors (Rogers and Oppenheim 1998, Jorgensen et al. 1997). Furthermore, the new VIRTUO BC system uses a different algorithm (BacT/ALERT VIRTUO ™ Microbial Detection System User Manual (2014) bioMeriéux, Inc. Marcy-l'Etoile–France) for the colorimetric detection of microbial growth in respect to the one in use with the BacT/ALERT 3D from the same manufacturer. Some recent reports (Altun et al. 2016, Deol et al. 2016), confirmed a reduction of the TTD values for VIRTUO in comparison with the BacT/ALERT 3D system.

To date, the performance of the VIRTUO system in term of TTD for clinical samples has never been published (the only set of data available belongs to a poster presented at the 26th ECCMID 2016 meeting (Cheong et al. 2016). A few recent studies (Altun et al. 2016, Deol et al. 2016, Cheong et al. 2016) have compared the TTD between bottles incubated in the VIRTUO and in the BacT/ALERT 3D. The main limitation of these studies is represented by the use of "artificial samples" prepared by spiking different concentrations of distinct bacterial species into blood obtained from healthy donors. This model is of course lacking the potential interfering factors that are well known for clinical samples

(Wilson 2004, Weinstein et al. 1997). The aim of this study is to evaluate the hypothetical differences in the TTD values between the two BC incubation system from bioMérieux, VIRTUO and BacT/ ALERT 3D, using clinical blood cultures.

MATERIAL AND METHODS

The study was performed in two different periods (the first of three months and the second including fours months) from January 1st 2015 to March 31st 2016 and involved a total of 3063 positive BCs derived from the routine workflow at Unit of Microbiology of the Greater Romagna Hub Laboratory, Pievesestina (FC), Italy. In detail: from January 1st to March 31st 2015, 1601 bottles were incubated into the BacT/ALERT 3D and from December 1st 2015 to March 31st 2016, 1462 bottles were incubated in the new system VIRTUO, that was just introduced into the routine workflow of the laboratory. All the diverse categories of bottles (FA Plus (aerobic), FN Plus (anaerobic) and PF plus (pediatric)) sequentially detected positive by the instruments were included in the study and for each bottle the TTD was calculated. In a second step, according to some unexpected results, it was decided to repeat the analysis dividing aerobic from anaerobic bottles. Only bottles showing a polymicrobial growth at the gram staining were excluded.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF MS) was used for microbial identification (VITEK® MS, bioMérieux Clinical Diagnostics). Isolated microorganisms were grouped into 8 different categories as summarized in Table 1. Considering that *Escherichia coli*

was the most frequently isolated microorganism for both the two blood culture systems a single category was created and distinctly evaluated from other members of Enterobacteriaceae. Because of the low frequency of isolation, anaerobic bacteria, alpha-haemolytic streptococci, non fermentative gram negative bacilli (other than *Pseudomonas aeruginosa*), corynebacteria and *Bacillus* spp. were not included in the study. Statistical analyses were performed using the R open source software (available at: https://www.r-project.org and accessed last time on July 12 2016). Before significance testing, normality distribution of analyzed groups was examined by using the Shapiro–Wilk test. It follows from this analysis that all the groups data are not normally distributed. TTD differences between VIRTUO and BacT/ALERT 3D were compared by Mann–Whitney U non parametric test. To confirm our findings the TTD were also compared with the log-rank (Mantel-Cox) test. These further statistical analyses were performed using Stata software version 14.2 (Stata Corp., College Station, TX, USA). Differences with p-value less than 0.05 alpha level were considered statistically significant.

RESULTS

The most frequently isolated microorganisms were, as expected, coagulase-negative staphylococci (CoNS) followed by *E.coli, Enterobacteriaceae* (other than *E. coli*), *Staphylococcus aureus, Enterococcus* spp., viridans group streptococci, *P. aeruginosa* and *Candida* spp.. The above enlisted categories of microorganisms, taken together, represent 95% and 93% of the total

microorganisms isolated with BacT/ALERT 3D and VIRTUO during the study period, respectively. As expected, *S. epidermidis* was the most represented microorganism in the CoNS group. Similarly, *K. pneumoniae* was the most common microorganism among the *Enterobacteriaceae* (other than *E. coli*). *S. pneumoniae* and *E. faecalis* were the main species among viridans streptococci and enterococci, respectively. *C. albicans* followed by *C. glabrata* showed the highest frequency in the yeast group. Although there are significant differences in the numbers of some species of microorganisms isolated in the two systems (i.e. *Klebsiella oxytoca*) we must consider that TTD differences between two systems was calculated comparing the whole entire category and not the single species of microorganisms include in each group. The median TTD differences between the eight groups of microorganisms were calculated and are reported in Table 2.

Median TTD values were shorter for bottles incubated into the VIRTUO system for the following five groups of microorganisms: CoNS, *E. coli*, *Enterobacteriaceae* (other than *E. coli*), *S. aureus* and viridans group streptococci. These five categories represented, during the study period, 84% and 81% of the total isolated microorganisms for the VIRTUO and BactALERT 3D system, respectively. The median TTD reduction in the VIRTUO system was particularly relevant for *Enterobacteriaceae* group (other than *E.coli*) and *E.coli*, respectively 29.8% and 20.8%. It should be specified that the number of positive bottles for *Enterobacteriaceae* (other than *E.coli*) incubated in the VIRTUO

system is approximately twice respect the number of bottles positive for the same group of microorganisms incubated in Bact/ALERT 3D, but despite this, the TTD median difference between the two blood culture systems in this group of microorganisms is still statistically significant. In addition, also for the CoNS a median TTD reduction of 4 hours and 7 minutes was observed. This is of particular relevance because these microorganisms, in most of the blood cultures, are likely contaminants (Hall and Lyman 2006) and their early identification would prevent an unneeded antimicrobial therapy that is associated with increased health care costs and development of multi-drug resistant microorganisms.

In this study *S. aureus* is one of the most commonly isolated pathogen (the third in terms of overall frequency) with a TTD significant reduction of 1 hour 4 minutes for the bottles incubated into the VIRTUO system compared to BacT/ALERT 3D. *S. pneumoniae* is the most represented microorganism of the viridans group streptococci: also in this case, taking together all the isolates of the group, a significant TTD reduction of 2 hours in favour of VIRTUO based incubation was shown. It is needless to underline the relevance of a faster detection in blood culture for these two classes of microorganisms and the crucial role that this shorter detection time could have in term of clinical outcome for the infected patients. Regarding *P. aeruginosa* isolates, the median TTD detected for bottles incubated into the VIRTUO was shorter (2 hours 20 min) than those detected by BacT/ALERT 3D, but this difference was not statistically significant

(P-value: 0.14). A similar feature was detected in the *Candida* spp. group where the median TTD value was shorter for bottles incubated in the VIRTUO system, but the difference was lacking in statistical significance.

This result is in contrast with the findings of a recent study performed with artificially simulated blood cultures (Altun et al. 2016). This discrepancy may be related to the low number of yeasts isolates in our study. Unexpectedly, in the case of *Enterococcus* spp. detection the median TTD was longer for VIRTUO than BacT/ALERT 3D system, as detailed in Table 2. To better understand this result the median TTD of the two systems were compared dividing TTD for aerobic from the anaerobic bottles. This further median TTD differences analysis (data not shown) revealed that aerobic bottles in the case of detection of the following categories, CoNS group, *E. coli*, Enterobacteriaceae (other than E. coli), *S. aureus*, Enterococcus spp., showed a statistically significant differences when incubated in VIRTUO compared to BacT/ALERT 3D. In the case of detection of viridans group streptococci, *P. aeruginosa* and *Candida* spp, the difference was not statistically significant. A further statistical analysis was conducted, considering the data as survival data and applying to TTD the log-rank test that reveal a statistically significant TTD differences for CoNS (p-value= 0.000), E. Coli (p-value= 0.000), Enterobacteriaceae (other than E. coli) (p-value= 0.0002), S. aureus (p-value= 0.049) and viridans group streptococci (p-value=0.000) categories. The cumulative percentages (%) over-time function of positive bottles of eight groups of microorganisms in both incubator systems were

calculated and reported in Table 3. In the CoNS group a marked difference of cumulative % is observed after 10h of incubation where the fraction of positive bottles is over three times greater than those in BactALERT. Also for viridans group streptococci the higher difference was observed at 6 h of incubation, while for E. coli, Enterobacteriaceae (other than E. coli) and S. aureus groups was observed at 4h of incubation. Statistical analysis of results (data shown in Table 3) demonstrated that TTD for the 5 groups (CoNS, E. coli, Enterobacteriaceae (other than E. coli), S. aureus, viridans group streptococci) are shorter in VIRTUO than BactALERT 3D.

DISCUSSION

The differences related to data obtained only from aerobic bottles compared with the analysis performed including the two different categories of (aerobic and anaerobic) bottles together were: the Enterococci group showed a shorter TTD when incubated in VIRTUO compared to BacT/ALERT 3D (P value: 0.049), while viridans group streptococci did not reach a statistically significant values probably because of the low number of samples included in each one (aerobic and anaerobic) type of bottles.

A limitation of this study is the lack of standardization of blood volume inoculated in the bottles, as it is common in clinical samples were it is quite rare to receive blood culture bottles filled with the exact and identical volume of

blood. Further studies with likely a larger number of clinical blood cultures are needed to better understand the increased TTD values identified for the VIRTUO system in the cases of isolation of Enterococci. The ideal feature of a diagnostic blood culture system is a standardized method that is capable to detect the positivity of the largest possible number of pathogens involved in the etiology of sepsis (Opota et al. 2015). The traditional methods for identifying pathogens in bacteremic patients are considered too slow to give a timely response under the clinical point of view in the large majority of the septic patients, and this is even more relevant when the severity of sepsis is high (Gaieski et al. 2010). Recently introduced methods such as rapid PCR (Polymerase chain reaction) based tests and MALDI-TOF MS (Matrix-assisted laser desorption ionization time-of-flight mass spectrometry) allow the microbiologists to identify the grown pathogens more quickly (Verroken et al. 2016). These technologies, although more faster than the traditional methods, still critically depends on the blood sample positivity after an adequate time of incubation to allow the microorganisms to growth and metabolize.

CONCLUSIONS

The present study showed a reduced TTD for BCs incubated with the VIRTUO system in respect to the predecessor BacT/ALERT 3D incubator for most of the microorganisms isolated in clinical blood cultures. The VIRTUO system enables an earlier management of clinical positive blood cultures thus allowing an appropriate targeted antibiotic therapy in the cases of suspected bloodstream

infection and sepsis, thus allowing for an overall improvement of the patient outcome.

Conflict of interest: none

Acknowledgements

We thank all the personnel of the Unit of Microbiology – Section of Bacteriology-The Greater Romagna Hub Laboratory for their collaboration in managing the positive BCs included in the study. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The policy of the journal on ethical consent has been read and approved by all authors. Standards of animal care (not applicable).

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Table 1 Isolated microorganisms (grouped into 8 categories) for each BC incubation system were evaluated. The number of isolates for each species is reported. The percentage of each species in the overall group composition is also indicated.

		VIRTUO		BTA 3D	
GROUP	MICROORGANISMS INCLUDED IN EACH GROUP	N° of isolates	% of frequency	N° of isolates	% of frequency
CoNS	*Staphylococcus epidermidis*	266	59%	378	64%
	Staphylococcus hominis	86	19%	87	15%
	Staphylococcus capitis	51	11%	52	9%
	Staphylococcus haemolyticus	21	5%	33	6%
	Staphylococcus warneri	8	2%	13	2%
	other CoNS species	19	4 %	27	4%
Escherichia coli	*Escherichia coli*	376	100%	423	100%
Enterobacteriaceae (other than *E. coli*)	*Klebsiella pneumoniae*	116	46%	64	52%
	Klebsiella oxytoca	34	13%	1	1%
	Serratia marcescens	25	10%	7	6%
	Proteus mirabilis	25	10%	14	11%
	Enterobacter cloacae	22	9%	16	13%
	Enterobacter aerogenes	6	2%	1	1%
	Raoultella planticola	10	4%	2	2%
	Providencia stuartii	2	1%	4	3%
	Morganella morganii	2	1%	8	6%
	Proteus vulgaris	1	0%	5	4%
	other species	9	4%	2	2%
Staphylococcus aureus	*Staphylococcus aureus*	139	100%	163	100%
Viridans group streptococci	*Streptococcus pneumoniae*	36	37%	40	45%
	Streptococcus anginosus	23	24%	4	4%
	Streptococcus mitis	20	21%	10	11%
	Streptococcus gallolyticus	5	5%	13	15%
	Streptococcus parasanguinis	4	4%	5	6%
	Streptococcus sanguinis	3	3%	5	6%
	Aerococcus viridans	3	3%	-	-
	Streptococcus salivarius	1	1%	5	6%
	Streptococcus infantarius	-	-	4	4%
	Streptococcus thermophilus	-	-	2	2%
	other species	2	2%	1	1%
Enterococcus spp.	*Enterococcus faecalis*	39	60%	78	76%
	Enterococcus faecium	24	37%	23	22%
	Enterococcus avium	2	3%	0	0%
	Enterococcus casseliflavus	-	-	2	2%
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	46	100%	42	100%
Candida species	*Candida albicans*	24	65%	35	54%
	Candida glabrata	8	21%	19	29%
	Candida parapsilosis	5	14%	11	17%

Table 2 Median differences in the TTD detected by using the VIRTUO and BactAlert 3D systems for the 8 groups of microorganisms. (*: difference with statistical significance, $p<0.05$).

Microorganisms categories	BTA 3D			VIRTUO			**Median TTD difference (hours and minutes** between VIRTUO and BTA 3D)	**Variation (%)**	**P–value**
	N° positive bottles	**Frequency of isolation over the total number of bottles evaluated**	**Median TTD**	**N° positive bottles**	**Frequency of isolation over the total number of bottles evaluated**	**Median TTD**			
CoNS	590	36.9%	22h42m	451	30.8%	18h35m	4hours 7 min*****	-18,1%	< **0.0001**
Escherichia coli	423	26.4%	10h36m	376	25.7%	8h35	2hours 1 min*****	-20,8%	< **0.0001**
Enterobacteriaceae (other than E. coli)	124	7.7%	11h02m	252	17.4%	8h	3hours 2 min*****	-29,8%	< **0.0001**
Staphylococcus aureus	163	10.2%	13h54m	139	9.5%	12h50m	1hour 4 min*****	-12,2%	**0.035**
Viridans group streptococci	89	5.6%	13h	97	6.6%	11h	2 hours*****	-16%	**0.0303**
Enterococcus species	103	6.6%	10h42m	65	4.4%	11h54m	-1 hour 12 min	11,2%	0.96
Pseudomonas aeruginosa	42	2.5%	16h30m	46	3.1%	14h10	2hours 20 min	-13.9%	0.14
Candida species	65	4.1%	22h48m	37	2.5%	20h47m	2hours 1 min	-9,2%	0.431

Table 3 Cumulative percentages comparison of the two incubation systems.

Cumulative percentages of the positive blood colture bottles among the eight groups of microrganisms. The TTD (expressed in hours) is also indicated. Microrganism categories with higher cumulative percentages of positive bottles in Virtuo respect BTA 3D are shown in bold.

CoNS		**Enterobacteriaceae (other than *E. coli*)**		**E.coli**		Enterococcus species		P. aeruginosa		**S. aureus**		**Viridans group streptococci**	
VIRTUO	BTA 3D	VIRTUO	BTA 3D	VIRTUO	BTA 3D	VIRTUO	BTA 3D	VIRTUO	BTA 3D	VIRTUO	BTA 3D	VIRTUO	BTA 3D
0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
0.00%	0.00%	5.16%	4.84%	0.53%	1.66%	1.54%	3.81%	0.00%	0.00%	0.72%	0.00%	0.00%	0.00%
2.22%	0.34%	19.44%	11.29%	9.31%	3.79%	4.62%	6.67%	2.17%	7.14%	10.79%	3.07%	4.12%	3.37%
5.56%	0.85%	30.95%	22.58%	24.73%	10.90%	9.23%	17.14%	13.04%	7.14%	15.11%	12.88%	11.34%	5.62%
9.56%	1.53%	50.4%	32.26%	47.07%	25.12%	20.00%	24.76%	19.57%	9.52%	30.22%	21.47%	18.56%	10.11%
12.22%	3.90%	64.68%	46.77%	65.43%	45.97%	23.08%	45.71%	30.43%	14.29%	38.13%	29.45%	39.18%	19.10%
56.89%	37.35%	92.46%	80.65%	92.82%	86.73%	95.38%	94.29%	76.09%	71.43%	78.42%	70.55%	76.29%	85.39%
84.89%	73.68%	96.43%	93.55%	97.34%	93.36%	.	.	84.78%	90.48%	92.09%	85.89%	86.60%	94.38%
94.89%	90.15%	97.62%	96.77%	98.40%	96.68%	.	.	89.13%	95.24%	97.84%	90.80%	88.66%	97.75%
96.22%	93.72%	98.02%	97.58%	98.94%	97.87%	.	.	97.83%	95.24%	98.56%	96.93%	93.81%	97.75%
97.11%	96.60%	99.21%	98.39%	99.47%	98.82%	.	.	97.83%	97.62%	99.28%	98.77%	95.88%	98.88%
.	99.83%	.	.	.	99.76%	.	.	.	.	.	.	.	.

TTD (h)	Candida species	
	VIRTUO	BTA 3D
0	0.00%	0.00%
2	0.00%	1.54%
4	5.41%	7.69%
6	8.11%	9.23%
8	10.81%	13.85%
10	21.62%	16.92%
20	48.65%	41.54%
30	72.97%	70.77%
40	78.38%	76.92%
50	78.38%	81.54%
60	86.49%	83.08%
120	.	.