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Evaluation of the Verigene® Blood Culture Nucleic Acid test for rapid identification of gram positive pathogens from positive blood cultures

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Summary

Background. The rapid identification of the etiology and the evaluation of the antimicrobial susceptibility of the bacteria causing bacteremia is of outmost relevance to set up an adequate treatment of sepsis. In this study we evaluated the microarray based method, Verigene Gram-positive blood cultures (BC-GP) nucleic acid test (Nanosphere Inc., Northbrook, IL, USA) for the identification of Gram positive pathogens from positive blood cultures. The panel BC-GP is capable to identify 13 germs and 3 genes associated with antimicrobial resistance.

Materials and Methods. In this study a total of 100 positive, non replicated and monomicrobic blood cultures have been evaluated. For testing on the Verigene platform using the BC-GP assay, 350 L of blood culture media from a positive the blood culture bottle.

Results. A total of 100 positive blood cultures were tested by the Verigene BC-GP assay: out of these a total of 100 Gram-positive cocci were identified. The most frequent bacteria identified included staphylococci, streptococci and enterococci. Among staphylococci, *Staphylococcus aureus* accounted for 25% (15/60), with 38% of *S. epidermidis* 37% (23/60) and 37% (22/60) other CoNS. All the *S. aureus* isolates were correctly identified by BC-GP whereas in 2/45 cases (4%) BC-GP misidentified CoNS. In the case of enterococci 7/10 were *E. faecalis* and 3 *E. faecium*, all of these were correctly identified.

Conclusions. The overall agreement with the results obtained by standard procedure is quite elevated (88%) and as a consequence the BC-GP panel could be used as a rapid diagnostic tool to give a faster response in the case of bacteremia associated with sepsis.

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Introduction

Sepsis, often a consequence of bacteremic infections, is a major cause of mortality worldwide. The rapid identification of the etiology and the evaluation of the antimicrobial susceptibility of the bacteria causing bacteremia is of outmost relevance to set up an adequate treatment of sepsis. The risk of death as a consequence of sepsis increases by 6-10% per hour in the absence of a pathogen effective and targeted therapy and it is well known that during the course of sepsis the efficacy and rapidity of specific treatment are greatly impacting on mortality ratio (6,7,13).

The most commonly isolated bacteria from bloodstream infections belong to Gram positive: in particular many of the isolated strains are coagulase-negative staphylococci (CoNS) that are generally considered as contaminant organisms of little clinical significance (3,10). Blood culture still are the gold standard diagnostic approach for the diagnosis of bacteremia related to sepsis (6).

The common procedure to identify a pathogen from a positive blood culture bottle include preparation of a Gram stained smear and the subsequent isolation of the germs by growing onto solid media, then followed by final identification by biochemical tools. A first empirical indication about the germ found is consequently made available within few minutes following the observation of the slide: this is generally communicated in order to allow an early set up of an empirical treatment (1,2).

A precise identification and a complete evaluation of the antimicrobial susceptibility (AS) of the isolated strain is generally achievable after 18 to 48h of incubation followed by a further 12-24h period that is necessary for the identification and AS testing. This delayed answer is very often of limited impact onto the management of septic patients and as a consequence many efforts have been put in place to identify new techniques that can reduce the time to the final and complete report.

Among these methods, are included fluorescent in situ hybridization (FISH) and Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) (5,8,12). These methods, however, are of limited utility when AS must be evaluated or in the case of polymicrobial infections. The use of genetic microarrays can somehow overcome many of the limitations of either FISH or MALDTTOF, since by using this technology pathogens are identified within a short time and quite a large panel of the genes associated with drug-resistance is generally reported (4,11).

In this study, we evaluated the microarray based method, Verigene Gram-Positive Blood Cultures (BC-GP) nucleic acid test (Nanosphere

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Inc., Northbrook, IL, USA) for the identification of Gram positive pathogens from positive blood cultures.

The system uses oligonucleotide probes associated with gold nanoparticles for the automatic detection of molecular targets by hybridization to genomic sequences species-specific and genetic determinants of antibiotic resistance. Specifically, the panel BC-GP is capable to identify, from a primary volume of 350 L of positive blood culture the following germs: *Staphylococcus* spp., *Streptococcus* spp., *Listeria* spp., *Micrococcus* spp., *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus lugdunensis, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus anginosus group, Enterococcus faecalis, Enterococcus faecium*.

In addition the presence of the following genes associated with antimicrobial resistance is also made possible: mecA, vanA and vanB, All the procedures get the final end within a maximum time of 2h 30 minutes.

Materials and Methods

In this study, a total of 100 positive non replicated and monomicrobic blood cultures have been evaluated. The method used for the blood culture was the standard procedure based onto the BacTAlert system (Biomerieux, Marcy L'Etoile, France). A first evaluation of the germs performed by Gram staining of a smear was used to discriminate Gram positive from Gram negative bacteria: only the Gram positive were included into the study.

The Verigene platform includes the Verigene Processor *SP* and Verigene Reader. The Verigene Processor *SP* carries out extraction of nucleic acid from specimens using magnetic glass beads.

Patient samples are loaded into an extraction tray, which is then loaded into the processor along with the utility tray, pipette tip holder assembly, and test cartridge. These items are all single-use disposable components that contain all the reagents required for testing. The Verigene Reader controls the processor and is responsible for specimen tracking, test selection, imaging, and analysis of test cartridges and display of the results. For testing on the Verigene platform using the BC-GP assay, 350 L of blood culture media from the positive aerobic bottle is loaded into the extraction tray, which is then placed into the processor *SP* along with all other consumables. The instrument extracts nucleic acid from the sample which is then mixed with the appropriate buffer and transferred to the test cartridge. The target analyze, if present, hybridizes to synthetic gene-specific oligonucleotide capture strands on the test cartridge substrate slide. Another synthetic mediator target-specific nucleotide is introduced to form a hybridization sandwich with the gene of interest.

At this point, a gold nanoparticle-labeled probe is introduced with oligonucleotides complementary to the intermediate oligonucleotide bound to the gene of interest. Finally, the gold nanoparticles are coated with silver to enhance the optical signal. The test cartridge is then removed from the Processor *SP*, and the substrate slide is inserted into the Verigene Reader for analysis. The Verigene Reader projects white light across the substrate slide, detects the relative brightness of each spot due to gold nanoparticles bound to target-specific probes and provides a *Detected* or *Not Detected* result for each of the panel members. The results have been compared with those obtained by standard techniques and the discrepancies were basically investigated by 16S rRNA sequencing after standard PCR amplification (9).

Results

A total of 100 positive blood cultures were tested by the Verigene BC-GP assay: out of these a total of 100 Gram-positive cocci were identified. Of these, 8% (8/100) were found as polymicrobial by routine laboratory investigations. 97% (97/100) of the germs were included in the BC-GP panel, being only 3 out of the identifiable panel.

Table 1 summarizes the results. In detail, the most frequent bacteria identified included staphylococci, streptococci and enterococci

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Table 1. Summary of the results obtained by Gram-positive blood cultures assay.

Microrganism	Total isolates, n.	Isolates correctly identified, n (%)	Isolates not detected, n. (%)	Isolates incorrectly identified, n (%)
Staphylococcus aureus	15	15 (100)		
MSSA*	10	10 (100)		
MRSA**	5	5 (100)		
CoNS***	45	43 (96)		2 (4)
S. epidermidis	23	22 (96)		1 (4)
Other CoNS	22	21(95)		1 (5)
Enterococci	10	10 (100)		
Enterococcus faecalis	7	7 (100)		
Enterococcus faecium	3	3 (100)		
Streptococci	21	18 (86)		3 (14)
Streptococcus pneumoniae	5	5 (100)		
Streptococcus pyogenes	1	1 (100)		
Streptococcus agalactiae	2	2 (100)		
Streptococcus anginosus group	5	5 (100)		
Other streptococci	8	5 (63)		3 (37)
Total monomicrobial isolates	91			
Other[a]	3		3 (100)	
Other[b]	2		2 (100)	
Lack of positive control	4		4 (100)	
Total number of isolates	100			

*Methicillin sensitive *S. aureus*; **methicillin resistant *S. aureus*; ***coagulase negative staphylococc
a, includes isolate not in the panel, one each of P*arvimonas micros, Corynebacterium* spp. and *Kocuria rosea*; b, mixed flora including Gram+ bacteria.

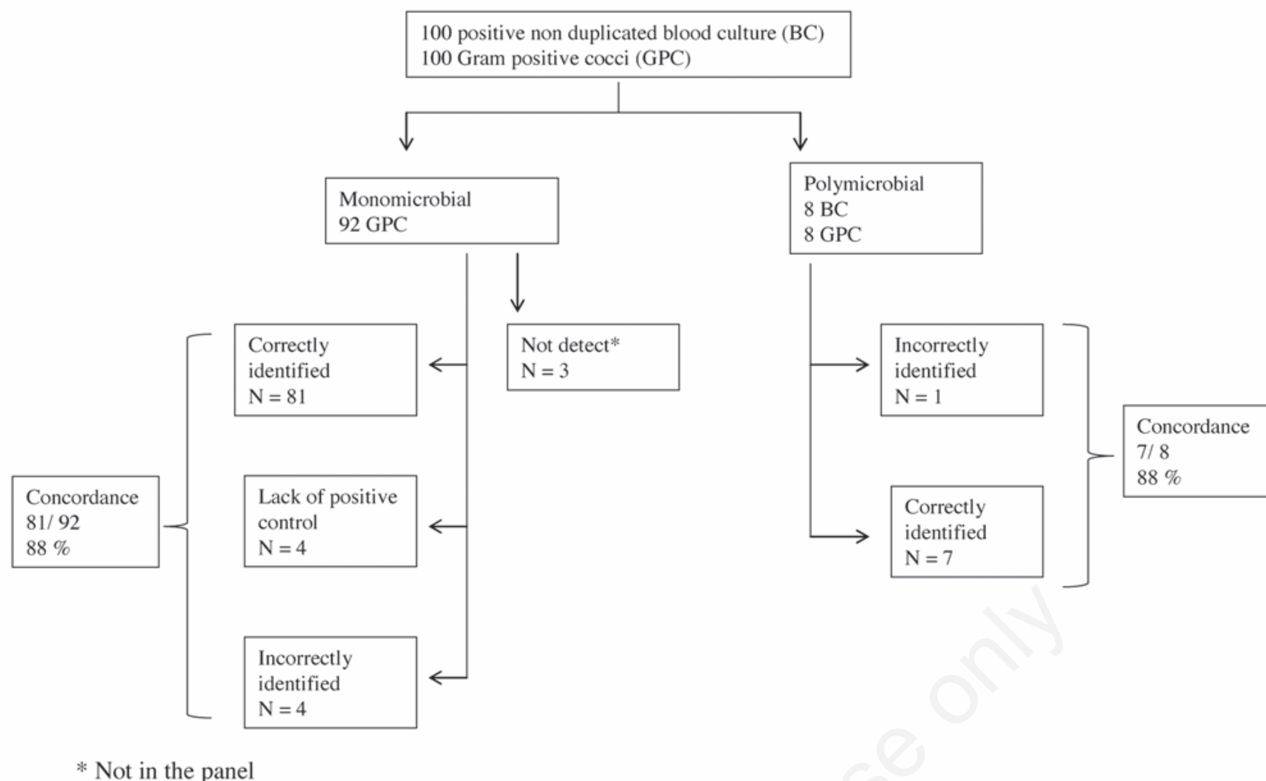

Figure 1. Performance of the Gram-positive blood cultures test for the identification of Gram-positive cocci from positive blood cultures.

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with 60%, 21% and 10% of germs isolated, respectively. Among staphylococci, *S. aureus* accounted for 25% (15/60), with 38% of *S. epidermidis* 37% (23/60) and 37% (22/60) other CoNS. All the *S. aureus* isolates were correctly identified by BC-GP whereas in 2/45 cases (4%) BC-GP misidentified CoNS. In the case of enterococci 7/10 were *E. faecalis* and 3 *E. faecium*, all of these were correctly identified by the Verigene test.

Three isolates (14%) out of the 21 streptococci was misidentified by BC-GP. In total 88% of the bacterial isolates gave a concordant identification between standard colture and BC-GP. The discrepant results were generated by 3 germs that are not included in the BC-GP panel, 3% gave a non-compliant result, and in 4 case no result was generated due to instrumental failure (Figure 1).

Conclusions

The Verigene BC-GP assay was easy and rapid to be performed, The list of the identifiable germs is wide and covers about 85% of the pathogens identified in 2013 from positive blood cultures in the Unit of Microbiology, the Greater Romagna Hub Laboratory (Italy). The overall agreement with the results obtained by standard procedure is quite elevated (88%) and as a consequence the BC-GP panel could be used as a rapid diagnostic tool to give a faster response in the case of bacteriemia associated with sepsis.

In addition, the capability of this method to discriminate between CoNS and other clinically relevant Gram positive species could be important to de-escalate the antimicrobial therapy when the presence of contaminants is highly likely (3,10).

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