Evaluation of the Verigene Gram-Positive Blood Culture Test (BC-GP)

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Abstract

Background: Rapid detection, identification and antimicrobial testing of blood borne pathogens improve antimicrobial stewardship and patient care. The Verigene BC-GP is a multiplexed, automated nucleic acid test for the direct identification from positive blood culture broth of genus, species and genetic resistance determinants for a panel of common Gram-positive bacteria. We prospectively evaluated the Verigene BC-GP Test (Nanosphere) using positive patient blood cultures.

Methods: Positive BACTEC plus aerobic/F blood culture bottles with a direct Gram stain showing Gram positive organisms were enrolled. Positive cultures were limited to one per patient and were processed using the Verigene System within 12 h of BACTEC detection. Phenotypic identification using conventional biochemical testing established accepted identification. The Verigene System is an automated random access instrument that requires 5 min of hands-on and 2.5 h of run time. The BC-GP Test, which runs on the Verigene System, can identify *Staphylococcus* spp., *S.aureus, S. epidermidis, S. lugdunensis, Streptococcus* spp., *S. pneumoniae, S. pyogenes, S. agalactiae, S. anginosus group, E. faecalis, E. faecium, Micrococcus* spp., and *Listeria* spp,, in addition to methicillin resistance marker *mecA* in *S. aur*eus and *S. epidermidis*, and vancomycin resistance markers *vanA* and *vanB* in *E. faecalis* and *E. faecium*. Conventional identification and BC-GP testing were performed by separate technologists in a blinded fashion.

Results: 136 Gram-positive bacteria were detected. 129/136 (95%) isolates included targets detected by BC-GP test. 126/129 isolates were correctly identified by BC-GP, including 31 *S. aureus*, 10 beta streps, 6 *S. pneumoniae*, 11 viridans streptococci, 8 enterococci and 2 *Micrococcus* spp. 9/126 were correctly identified when repeated following an error message during the first run. 2 viridans streptococci and 1 *E. faecium* were not detected by BC-GP, but should have been.

Discussion: The Verigene BC-GP Test accurately and rapidly detects most Gram-positive pathogens and resistance mechanisms directly from positive blood culture bottles in less than 3 hours. The automated Verigene System allows 24/7 processing by laboratory technicians. Results are available 1-2 d sooner than those provided by conventional testing.

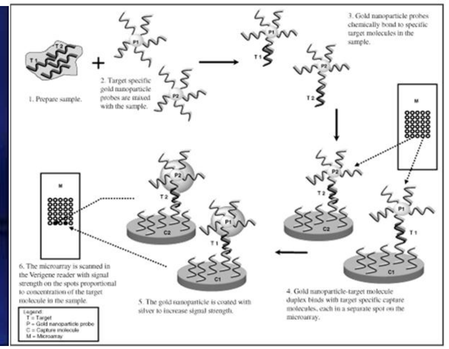
Introduction

Rapid identification of blood isolates is important to patient care as well as antimicrobial stewardship. Conventional identification of blood isolates can take up to 48 h, while PCR based identification of colonies can take 25 h (Thomson *et al*). The Verigene BC-GP Test (Nanosphere) is a random access, multiplexed, automated nucleic acid test for the direct detection and identification of genus, species and genetic resistance determinants of common Gram-positive bacteria from blood culture broth. Using the Verigene BC-GP Test, positive identification of a wide panel of common blood pathogens can be obtained in 3-4 h depending on laboratory work flow.

References

Thomson et al. Abstr.Gen.Meet.ASM, May, 2003.

Verigene Processor SP (width, height, depth): 7.6”x18.7”x22.7”
Verigene Reader (width, height, depth): 11.7”x12.4”x20.5”



“NANOSPHERE INC - FORM 10-K - March 11, 2010.” N.p., n.d. Web. 19 Sep 2011. <http://www.faqs.org/sec-filings/100311/NANOSPHERE-INC_10-K/>.

Materials & Methods

133 unique patient blood cultures collected between July and December 2011 were enrolled, with each patient being limited to one sample. 350 µL from the BACTEC plus aerobic/F blood culture bottles was tested on the Verigene System within 12 hours of BACTEC detection, after a Gram stain containing Gram positive organism was read. Standard phenotypic identification was concurrently conducted by a separate technologist in a blinded manor.

After breaking down the bacteria in the blood sample into 300-500 bp DNA fragments by a sonication process, the Verigene Processor SP introduces oligonucleotides, attached to gold nanoparticles, that are complimentary to one of the 13 bacterial target DNA sequences. Following a washing step, the DNA+ gold nanoparticle complex is introduced to a second set of complimentary capture probes, this time attached to a micro array (glass slide). Each capture probe has a specific spot on the micro array, forming a grid like pattern. The micro array is then fed into the Verigene Reader where the gold nanoparticles scatter light parallel to the slide surface, allowing for optical detection of the nucleic acid targets.

Table 1. Verigene BCGP Identifiable Targets

Gram-Positive Blood Culture (BC-GP) Test			
Genus	*Staphylococcus* spp.	Species	•*Staphylococcus aureus* •*Staphylococcus epidermidis* •*Staphylococcus lugdunensis*
	Streptococcus spp.		•*Streptococcus pneumoniae* •*Streptococcus anginosus* Group •*Streptococcus agalactiae* (GBS) •*Streptococcus pyogenes* (GAS)
	Micrococcus spp.		
	Listeria spp.		•*Enterococcus faecalis* •*Enterococcus faecium*
Resistance	*mecA*		
	vanA		
	vanB		

Results

The Clinical Microbiology lab detected 136 Gram positive isolates from the 133 patients enrolled in the study. Of those 136 isolates, 129 were intended target organisms of the Verigene System, capable of being detected (95%). When compared to conventional phenotypic identification methods, the Verigene System correctly identified 126/129 (98%) of its intended target organisms, and a total of 126/136 (93%) of all bacterial isolates it was presented. The Verigene System correctly identified 13 *mecA* positive *Staph aureus* (MRSA) isolates, and 18 *mecA* negative *Staph aureus* (MSSA) isolates. The Clinical Microbiology lab did not test Coagulase negative Staph species for the presence of *mecA* so its accuracy in that set of organisms cannot be compared. Similarly, the Clinical Microbiology lab did not identify to species the coagulase negative staphylococci organisms, so the accuracy of *Staph epidermidis* and *Staph lugdunensis* detection was based on correct genus identification. Additionally, no patient samples contained Enterococcal isolates possessing the *vanA* or *vanB* genes.

The Verigene System experienced technical errors (“No Call-Negative Control Error”/“No Call-Internal Control Error”) on 9 trials (7%), with all subsequent trials (re-runs) following a technical error producing successful identifications. 2 Viridans group streptococci and 1 *E. faecium* were not identified when they should have been.

The Verigene System requires roughly 5min of hands-on preparation, 2.5h of run time, and approximately 5 minutes of wrap-up time per sample. In comparison, conventional identification methods require 24-48 hours, thus the Verigene System decreased the time from blood positivity to results.

Table 2. Successful Identification Rates of Bacterial Isolates

Isolated Organism	Verigene Result/Micro Lab Result
Staphylococcus	87/87 (100%)
Streptococcus	29/31 (93%)
Enterococcus	8/9 (89%)
Micrococcus	2/2 (100%)
Corynebacterium*	0/4 (0%)
Aerococcus*	0/1 (0%)
Bacillus sp*	0/1 (0%)
Lactobacillus*	0/1 (0%)
Total # of isolates	126/136 (93%)
Total # of identifiable isolates	126/129 (98%)

*Not an intended target of the Verigene BCGP Test

Conclusions

- Relative ease and hands free nature of the device allows for 24/7 testing without extensive training.
- BC-GP panel accounts for large percentage of blood isolates seen in a hospital setting (95%).
- BC-GP panel achieves correct identification of intended bacterial targets (98%) and total organisms seen (93%) when compared to traditional diagnostic techniques.