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PCR Identification of Bacteria in Blood Culture Does Not Fit the Daily Workflow of a Routine Microbiology Laboratory

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We have evaluated the GenoType blood culture assay (Hain Lifescience, Nehren, Germany) for the identification of bacteria in 233 positive blood cultures and assessed its suitability in the workflow of a routine microbiology laboratory. In 68/233 (29.2%) samples, the culture result could not be confirmed by the GenoType assay due to a lack of primers in the test, multiple organisms in the sample, or inconsistency with respect to the identification by culture. Although the GenoType blood culture assay gives satisfactory results for bacteria for which primers are available, there are difficulties in applying the test in the routine microbiology laboratory.

Blood culture has been the gold standard for the detection of sepsis pathogens. Continuously monitored blood culture systems have reduced the detection time of positive blood cultures, but there are few rapid and accurate methods for identifying pathogens directly from these samples. Nucleic-acid-based methods (DNA probe method, fluorescent in situ hybridization, PCR) (1, 3, 5, 7–9, 10–14) may offer one solution, and we have studied the accuracy and feasibility of one of them, the GenoType blood culture assay (Hain Lifescience, Nehren, Germany). The two GenoType BC panels identify 41 bacterial species. Additionally, the methicillin resistance-mediating *mecA* gene and the vancomycin resistance-mediating genes *vanA*, *vanB*, *vanC1*, and *vanC2*/*C3* can be detected. The GenoType procedure includes three steps: DNA isolation, multiplex amplification with biotinylated primers, and strip-based reverse hybridization and detection.

The Gram-negative rods detected by the GenoType BC Gram-negative test were *Acinetobacter baumannii*, *Citrobacter freundii*/*C. koseri*, *Enterobacter cloacae*/*E. aerogenes*, *E. intermedium*, *Escherichia coli*/*Shigella* sp., *Haemophilus influenzae*, *H. parainfluenzae*, *Klebsiella oxytoca*, *K. pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella enterica* serovar Enteritidis/*S. enterica* serovar Typhimurium, *Serratia marcescens*, *Stenotrophomonas maltophilia*, and *Enterobacter agglomerans*. The Gram-positive cocci detected by the GenoType BC Gram-positive test were *Streptococcus anginosus*/*S. constellatus*/*S. intermedius*/*S. mutans*/*S. sanguis*, *S. mitis*/*S. oralis*, *S. pyogenes*, *S. agalactiae*, *S. dysgalactiae* subsp. *equisimilis*, *S. bovis*, *S. pneumoniae*, *Staphylococcus aureus*, *S. haemolyticus*, *S. epidermidis*, *S. hominis*, *S. warneri*, *S. simulans*, *Enterococcus faecalis*, *E. faecium*, *E. gallinarum*, and *E. casseliflavus*.

The 233 nonconsecutive samples were collected between December 2008 and November 2010 in the Päijät-Häme Central Hospital, Lahti, Finland, as part of the routine care of patients with suspected sepsis. No additional samples were taken from patients for this study. One blood culture request consists of two blood culture bottles (bioMérieux BacT/Alert FA [aerobic], SN [anaerobic]; one aerobic and one anaerobic bottle per set; from children, a single PF [pediatric] bottle is taken). Normally, two sets (four bottles) are taken with at least 30 min between them. Gram staining from positive blood cultures was carried out to determine whether to use a GenoType BC GP (Gram-positive coccus) or GN (Gram-negative rod) test. The results were compared with those of routine identification methods used in our laboratory, including a DNA probe kit (Accuprobe, Gen-Probe, Inc., San Diego, CA) for preliminary detection of certain Gram-positive bacteria (enterococci, *S. pyogenes*, *S. agalactiae*, pneumococci, and *Listeria monocytogenes*); several biochemical tests, such as self-prepared oxidase (*N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylene diamine dihydrochloride in ethanol; Sigma-Aldrich Co., St. Louis, MO), self-prepared catalase (3% hydrogen peroxide; E. Merck, Darmstadt, Germany), and latex coagulase (Remel Europe Ltd., Crossways, Dartford, Kent, United Kingdom); and commercial test panels. Vitek2 (bioMérieux, Marcy l′Etoile, France) panels were used for the identification of Gram-negative rods complemented with biochemical tests such as oxidase and a self-prepared indole test (dimethylamino-cinnamaldehyde in hydrogen chloride; E. Merck, Darmstadt, Germany) and other commercial test panels (e.g., Api strips [bioMérieux, Marcy l′Etoile, France]). Antibiotic susceptibility testing was performed straight from the positive blood culture bottles by the disk diffusion method according to Finnish national guidelines based on the CLSI standard. Based on our previous findings (4) on specimen handling in the preparation of the GenoType assay, we heated 0.5 ml of positive blood cultures for 5 min at 95°C before DNA extraction. In this previous study of 100 positive blood cultures (152 samples), 15/152 (9.9%) additional samples were found positive in the GenoType assay with the heating pretreatment (122/152 [80.3%] compared to 107/152 [70.7%] nonheated samples). After the heating step, the test was carried out as instructed by the manufacturer (Hain Lifescience, GenoType BC Gram positive/Gram negative, version 3.0 [09/2008]). A universal control (UC) was included on each strip to indicate the presence of bacterial DNA and proper DNA isolation and amplification. Only bands with color intensity equal to or greater than that of the UC were considered positive. A conjugate control was used to check the binding of the conjugate on the strip and the chromogenic reaction.

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TABLE 1 Comparison of conventional methods[a] and GenoType assay[b]

Isolate	No. of isolates:		
	Identified by conventional methods	With consistent GenoType assay results	With divergent GenoType assay results (%)
Gram-positive cocci			
S. aureus	19	14	5 (26), no result
S. epidermidis	17	13	4 (24), no result
S. hominis	10	8	2 (20), no result
S. warneri	1	0	1 (100), no result
S. haemolyticus	2	1	1 (50), no result
S. pneumoniae	14	12	2 (14), no result
S. pyogenes	3	2	1 (33), no result
S. dysgalactiae subsp. *equisimilis*	9	8	1 (11), no result
S. agalactiae	6	6	
S. anginosus group	6	5	1 (17), no result
S. mitis group[c]	4	2	1 (25), *S. anginosus*/*constellatus*/*intermedius*/*mutans*/*sanguis* 1 (25), no result
E. faecium	6	5	1 (17), no result
E. faecalis	6	5	1 (17), no result
Total	103	81	22 (21)
Gram-negative rods			
E. coli	55	52	3 (5), no result
K. pneumoniae	10	9	1 (10), no result
K. oxytoca	7	6	1 (14), no result
P. mirabilis	4	4	
P. aeruginosa	6	5	1 (17), no result
E. cloacae	4	2	2 (50), no result
H. influenzae	2	2	
S. marcescens	3	3	
C. freundii	1	0	1 (100), no result
C. koseri	1	0	1 (100), no result
S. Typhimurium	1	0	1 (100), no result
Total	94	83	11 (12)

[a] Conventional methods: culture, AccuProbe (Gen-Probe), Vitek2 (bioMérieux), biochemical tests.
[b] GenoType BC Gram-positive/Gram-negative tests (Hain Lifescience).
[c] Divergent result: in culture *S. mitis* group and in GenoType assay *S. anginosus*/*constellatus*/*intermedius*/*mutans*/*sanguis*; 16S sequence result *S. sanguinis*.

Based on Gram staining, 7/233 (3.0%) of the positive blood cultures contained multiple pathogens and could not be analyzed by the DNA strip assay. Two hundred twenty-six (97%) of these 233 samples contained a single isolate, and out of these, 127 (54.5%) were Gram-positive cocci and 99 (42.5%) were Gram-negative rods. Twenty-nine (12.4%) of the 233 isolates could not be detected by the DNA strip assay because primers for these species were not included in the assay. These were as follows: 1 isolate of genus *Actinobacillus*, 3 of *Streptococcus*, 4 of *Bacteroides*, 1 of *Fusobacterium*, 10 of *Micrococcus*, and 10 of *Staphylococcus*. Concordant identification results compared to the conventional methods were found in a total of 164 of the remaining 197 specimens (83.2%), i.e., 81/103 (78.6%) of the Gram-positive cocci and 83/94 (88.3%) of the Gram-negative rods (Table 1). We found no methicillin-resistant *S. aureus* or vancomycin-resistant enterococcal isolates during the study period.

All 11 of the Gram-negative rods with divergent results (Table 1) gave no result in the GenoType assay, with either nonvisible or nonmatching bands or a band color intensity too weak compared to that of the UC. Of the 22 divergent Gram-positive coccus results (Table 1), 21 samples gave no GenoType result. The one sample identified as *S. mitis* group by culture and *S. anginosus*/*S. constellatus*/*S. intermedius*/*S. mutans*/*S. sanguis* in the GenoType assay was later sequenced as *S. sanguinis* belonging to the *S. mitis* group, thus confirming the culture result. *S. sanguinis* (formerly *S. sanguis*) is also included, as *S. sanguis*, in a complex of the five species identified by the GenoType assay (see the lists of species detected above). The test is, however, unable to differentiate among the five species.

Eigner et al. (2) evaluated the GenoType BC assay with Bactec blood culture bottles for 279 positive samples with a spectrum of bacteria similar to ours. Of the 279 organisms isolated, 87.1% (n = 243) were covered by specific probes, compared to our 87.6%. They found that 97.4% of the Gram-positive cocci (148/152) and 97.8% (89/91) of the Gram-negative rods were correctly identified by the assay, both values that are higher than ours. Of the two discrepant results for Gram-negative rods (2/91, 2.2%), one *E. coli* strain gave no result in the GenoType assay and one *E. aerogenes*, confirmed by 16S rRNA gene sequencing, was reported as *K. pneumoniae* by the GenoType assay. Of the four discrepant results for Gram-positive cocci, PCR failed to detect one *S. aureus* culture, one *S. bovis* culture, and two *S. epidermidis* cultures. All four of the species negative in direct testing of blood cultures gave a positive signal when retested from pure agar culture.

Prère et al. (12) evaluated the test with bioMérieux BacT/Alert FA, SN, and PF bottles, as we did, but did not list the species isolated. All of their 60 positive blood cultures, for which primers were available, were in agreement with the results of the conventional microbiological procedures, but if they had isolates not covered by probes, they do not provide this information.

Their conclusion was that the test is fast and reliable in detecting the most important sepsis pathogens, as well as the *mecA* and *van* genes. They also found that charcoal did not cause any inhibition of the PCR. Compared to these two studies, there was one major difference in our study: we heated the samples prior to extracting DNA. However, as mentioned above, we have shown previously (4) that this change improves rather than impairs the efficiency of the test.

During the study period, we had 1,438 positive blood cultures in our laboratory and 233 (16.2%) of them were included in this study, representing well the bacterial species found in blood cultures in our laboratory during the last 5 years (data no shown).

Blood culture samples were analyzed in the BacT/Alert culture system around the clock, but further Gram staining, culturing, and antibiotic susceptibility testing was done by the technicians of the microbiology laboratory only during business days and during weekends. Only those samples giving a positive signal in the BacT/Alert culture system on business days in the morning could be analyzed by the GenoType assay in this study.

Although the assay includes a very fast, simple, and effective DNA extraction step, the other steps in the protocol occupy the technician and the equipment so tightly (requiring a minimum of 2 h of hands-on time) that the samples must be analyzed in batches. Also, in order to maintain the skill to perform the assay, we could not train all of our technicians (15 persons) to carry out the test and a skilled technician was not available at all times. On the other hand, the results were available relatively quickly (79.8% of the samples were processed within a total time of approximately 4.5 h) and their interpretation was easy.

Lin and Yan (6) have estimated the turnaround times for identification of sepsis pathogens by conventional methods, and Tissari et al. (14) concluded that by using PCR, the species of the pathogen is found 18 h earlier. The time saved by using the GenoType assay is very similar.

In summary, the GenoType blood culture assay gives reliable results for bacteria for which primers are available, but there are technical and time-related difficulties when applying the test in a routine microbiology laboratory. We have used the Gen-Probe AccuProbe test in our routine for many years (7), and despite the fairly narrow selection of probes, its benefits are clear: the test is rapid (30 min), is easy to use, and can be run at any time when the personnel of the microbiology laboratory are available. Unfortunately, commercially available PCR methods, such as the GenoType blood culture assay, do not yet offer the same benefits.

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