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Use of Quantitative Broad-based Polymerase Chain Reaction for Detection and Identification of Common Bacterial Pathogens in Cerebrospinal Fluid

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Abstract

Background—Conventional laboratory diagnosis of bacterial meningitis based on microscopy followed by culture is time-consuming and has only moderate sensitivity.

Objectives—The objective was to define the limit of detection (LOD), analytic specificity, and performance characteristics of a broad-based quantitative multiprobe polymerase chain reaction (PCR) assay for rapid bacterial detection and simultaneous pathogen-specific identification in patients with suspected meningitis.

Methods—A PCR algorithm consisting of initial broad-based detection of *Eubacteriales* by a universal probe, followed by pathogen identification using either pathogen-specific probes or Gram-typing probes, was employed to detect pathogens. The 16S rRNA gene, which contains both conserved and variable regions, was chosen as the target. Pathogen-specific probes were designed for *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli*, and *Listeria monocytogenes.* Gram-positive and -negative typing probes were designed based on conserved regions across all eubacteria. The LOD and time to detection were assessed by dilutional mocked-up samples. A total of 108 convenience cerebrospinal fluid (CSF) clinical samples obtained from the Johns Hopkins Hospital (JHH) microbiology laboratory were tested, and results were compared with hospital microbiologic culture reports.

Results—The LOD of the assay ranged from 10^1 to 10^2 colony-forming units (CFU) / mL. Pathogen-specific probes showed no cross-reactivity with other organisms. Time to detection was 3 hours. In clinical specimens, the universal probe correctly detected 16 of 22 culture-positive clinical specimens (sensitivity = 72.7%; 95% confidence interval [CI] = 49.8% to 89.3%), which were all correctly characterized by either pathogen-specific or Gram-typing probes. Adjusted sensitivity after removing probable microbiologic laboratory contaminants was 88.9% (95% CI = 65.3% to 98.6%). The universal probe was negative for 86 of 86 culture-negative specimens.

Conclusions—A broad-based multiprobe PCR assay demonstrated strong analytic performance characteristics. Findings from a pilot clinical study showed promise in translation to human subjects, supporting potential utility of the assay as an adjunct to traditional diagnostics for early identification of bacterial meningitis.

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Keywords

PCR; polymerase chain reaction; meningitis

The most serious infection of the central nervous system (CNS) is acute bacterial meningitis, with an incidence of three to five cases per 100,000 persons per year and a mortality rate of 6% to 26% in the United States.[1] Rates of infection and associated morbidity and mortality are significantly higher in developing countries. For example, in the "Meningitis Belt" of sub-Saharan Africa, bacterial CNS infections cause tens of thousands of cases and thousands of deaths during epidemic years.[2] Permanent serious neurologic sequelae include deafness, seizures, and mental retardation, and behavioral changes can occur in up to one-third of survivors.[3] Among the various methods currently used in clinical laboratories for detection of bacterial meningitis, culture remains the gold standard, but unfortunately takes up to or greater than 24 hours to obtain results. Accordingly, there is a great need for the design and translation of new rapid diagnostic methods that could aid clinical decision-making and would be particularly useful in an adjunctive assay in acute care settings.

Polymerase chain reaction (PCR) assays, which rely on amplification of small amounts of target DNA, have previously been developed and are currently used in clinical settings for definitive identification of viral CNS infections, including enterovirus meningitis and herpes simplex virus meningitis, as well as for slow-growing bacterial CNS infections such as those caused by *Mycobacterium tuberculosis.*[4] More recently, assays for the detection of specific bacterial pathogens that cause meningitis have been developed,[5, 6] but these have limited practical utility in acute care settings because they are pathogen-specific. Broad-based eubacterial PCRs, which exploit the conserved 16S rRNA gene as their target, hold great potential, but published studies to date have reported relatively low sensitivity and prolonged assay performance times.[7–12] Further, many of these broad-based approaches are unable to provide information about the particular infecting pathogen. Accordingly, we have evaluated the limit of detection (LOD) and conducted a pilot study to assess the diagnostic performance characteristics of our unique broad-based quantitative multiprobe PCR assay, which allows rapid bacterial detection as well as simultaneous pathogen identification.

METHODS

Study Design

This was a laboratory study to develop and evaluate a unique broad-based multiprobe PCR assay. The study was approved by The Johns Hopkins University Institutional Review Board.

Study Samples

Bacterial Species and Mock-up Samples—Bacterial species were obtained from American Type Culture Collection (ATCC, Manassas, VA) or the Johns Hopkins Hospital (JHH) clinical laboratory (Division of Medical Microbiology, Johns Hopkins School of Medicine, Baltimore, MD). A single isolated colony of *Escherichia coli* was inoculated in 2 mL of trypticase soy broth (Becton Dickinson, Sparks, MD) and incubated at 37°C overnight. For LOD determination of *E. coli*, serial dilutions were spiked into culture-negative and DNA-free cerebrospinal fluid (CSF) samples. For LOD determination of *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Staphylococcus epidermidis, Staphylococcus aureus,* and *Listeria monocytogenes,* the dilutions of organisms were spiked into DNA-free water. Spiked samples were processed

using the DNA extraction step described below. LOD was calculated based on colony-forming units (CFU) / mL.

Clinical Samples—A total of 108 convenience CSF clinical samples were obtained from the JHH microbiology laboratory as follows: from July 2006 to July 2007 we requested that the hospital laboratory retain any "excess" CSF for samples in which microbiologic culture testing had been ordered. These samples were set aside after completion of any clinical testing. Samples were then deidentified and brought to the laboratory for testing with our PCR assay, with results compared to clinical microbiologic culture findings.

Excess convenience samples were processed as follows: 1) samples were assigned random study numbers and taken from the microbiology laboratory to the research laboratory where they were stored at −20°C for later DNA extraction and PCR analysis; 2) a database that included the microbiology accession number and the random study number was created; 3) the microbiology database was queried for culture results; 4) the samples were deidentified; 5) samples were analyzed by PCR by laboratory technicians who were blinded to the microbiologic laboratory results; and 6) PCR results were compared with microbiology culture results.

Study Protocol

Extraction of DNA for PCR—A 500-μL aliquot was made of each CSF sample, to which 50 μL of lysis buffer (MAGNA PURE LC Kit- I, Roche Diagnostics, Indianapolis, IN) was added. After a 30-minute incubation at room temperature, samples were centrifuged at 3,200 × *g* for 10 minutes in a centrifuge (Model 5415D, Eppendorf, Westbury, NY), and the pellet was resuspended in 50 μL of molecular-grade water. The extraction of DNA, which includes a high-yield ultrafiltration step, was performed as previously described by our group.[13]

Design of Primers and Probes—The target site within the 16S rRNA gene (which encompasses the hypervariable V6 region) and design of conserved primers (p891F and p1033R) and probe (Uniprobe) were as previously described.[13, 14] We also designed a pathogen-specific probe to the seven most common organisms causing meningitis (*S. pneumoniae, N. meningitidis, H. influenzae, S. epidermidis, S. aureus, E. coli,* and *L. monocytogenes*). Gram-typing probes to detect Gram-positive and Gram-negative bacteria were also designed. Probes (Table 1) were designed based on 16S rRNA sequence data obtained from GenBank (National Institutes of Health, Washington, DC) and aligned with sequences from various clinically relevant bacterial species using the program ClustalW (http://www.ebi.ac.uk/clustalw.html). Theoretical specificity of all designed primer and probe sequences were further analyzed using the National Center for Biotechnology Information's Basic Local Alignment Search Tool (BLAST) program. Specificity of the pathogen-specific and Gram-typing probes with DNA extracted from 23 Gram-positive organisms and 13 Gram-negative organisms was tested either in CSF or water mock-ups.

PCR Master Mix Preparation—Each PCR procedure was performed in 50 μL total volume, which was composed of 30 μL of PCR master mix and 20 μL of extracted DNA as sample input. PCR master mix contained 25 μL of 2 × TaqMan universal PCR mix (PE Applied Biosystems, Foster City, CA), 1.5 μL of 67 μM forward primer and reverse primer. The 2 × TaqMan Universal PCR mix and the primers underwent an ultrafiltration step using Microcon YM-100 centrifugal filter device (Millipore Corporation, Bedford, MA.) by centrifuging at 3,200 × *g* for 10 minutes to remove potential exogenous background DNA contamination.[14] Following ultrafiltration, an additional 1 μL of 2.5 units of Amplitaq Gold LD (PE Applied Biosystems) and 1 μL of 10 μM probe were added to make up the final master mix before the sample was added. PCR was then performed using the ABI 7900 HT

sequence detection system (PE Applied Biosystems). The cycling conditions used were as follows: preincubation at 50°C for 2 minutes, denaturation at 95°C for 10 minutes and 50 repeats at 95°C for 15 seconds, and annealing / extension temperature at 60°C for 60 seconds. In real-time PCR, the number of cycles required to reach threshold level is dependent on the concentration of the target DNA present. The clinical samples were subjected to 50 repeats to ensure that even very low bacterial loads potentially present in CSF clinical samples are detected.[15]

Positive, Negative, and Exogenous Internal Positive Control Preparation— Ultrapure water was used as nontemplate PCR-negative control (NTC). Culture-negative CSFs were screened as negative controls using our universal probe ("Uniprobe") PCR assay. Samples with a threshold cycle (C_T) value (see "Post-PCR Analysis") equal to or higher than NTC controls were pooled and established for use as a standard negative control. An exogenous internal positive control (PE Applied Biosystems) was used on all clinical samples according to manufacturer's instructions to rule out sample inhibition to PCR.

PCR Assay Algorithm—Clinical CSF samples were tested for presence of eubacteria using Uniprobe PCR. Positive samples by Uniprobe PCR were further analyzed with parallel PCR procedures using our panel of seven pathogen-specific probes. Samples that were negative by our panel of pathogen-specific probes were also tested using Gram-typing probes.

Post-PCR Analysis—Amplification data were analyzed by the SDS software (PE Applied Biosystems), which calculates ΔR_n using the equation $R_n(+) - R_n(-)$. $R_n(+)$ is the emission intensity of the reports divided by the emission intensity of the quencher at any given time, whereas $R_n(-)$ is the value of R_n (+) prior to amplification. Thus, ΔR_n indicates the magnitude of the signal generated. The C_T is the cycle at which a statistically significant increase in ΔRn is first detected. The C_T is inversely proportional to the starting amount of target DNA. Amplification plots were generated by plotting ΔR_n versus C_T.

All standardized pooled negative controls and internal positive control (IPC) controls were performed in triplicate. The mean and standard deviation (SD) for the pooled negative control replicates from each run were calculated. Due to the potential for day-to-day interrun variability, the cutoff C_T value for each run was defined as three SDs below the daily negative control mean.[16] Any sample with a C_T value higher than the cutoff value was considered PCR negative, and samples with lower than the cutoff value were considered PCR positive.

Accuracy of Uniprobe PCR was determined by the observed clinical sensitivity and specificity compared to conventional culture results. Ninety-five percent confidence intervals (95% CI) for clinical sensitivity and specificity were estimated by the exact binominal test method.[17] Adjusted sensitivity and specificity were calculated after removing the samples from analysis that are considered common microbiologic laboratory contaminants.[18–20]

Discordant Analysis—All samples with discordant findings between PCR and microbiology laboratory culture results were plated on 5% sheep blood agar plates (Becton, Dickinson and Company, Franklin Lakes, NJ) to reassess for bacterial growth. Additionally, repeat PCR testing was performed using an alternate protocol for DNA extraction, which consisted of a 1:10 dilution of the sample with molecular-grade water in a total volume of 500 μL. The diluted sample was then processed and tested the same as the normal protocol.[13]

RESULTS

LOD, Analytical Sensitivity, and Specificity of Uniprobe PCR

Limits of detection of the Uniprobe PCR in samples mock-up with serially diluted organisms ranged from 10^1 to 10^2 CFU/ mL, depending on the particular organism being tested (Table 2). Gram-positive and Gram-negative probes were tested against 36 (23 Gram-positive and 13 Gram-negative) clinically common bacterial pathogens, including the seven most common meningitis-causing organisms and were found to have 100% sensitivity and specificity (data not shown).

Assay Performance Time and Performance

Time to detection was 3 hours, which included DNA extraction (70 minutes) and PCR amplification (110 minutes). A total of 108 clinical CSF samples were collected from patients with suspected meningitis and tested using our PCR assay. Among the samples collected, 22 were culture positive and 86 were culture negative. As shown in Table 3, 16 of 22 culture-positive samples tested positive by Uniprobe PCR, and 86 of 86 culture negative samples tested negative, giving a sensitivity and specificity of 72.7% (95% CI = 49.8% to 89.3%) and 100.0% (95% CI = 96.0% to 100%), respectively. Six of the 22 culture-positive samples were negative by the Uniprobe PCR. Of the six false negatives, two samples grew rare colonies of *Pseudomonas aeruginosa* and *S. pneumoniae,* after 2–3 days. The remaining four were considered probable laboratory contaminants (*Micrococcus luteus, Rhodococcus dentocariosa, Corneybacterium* sp.) leaving a total of 18 “true” culture-positive samples and giving an adjusted sensitivity and specificity of 88.9% (95% CI = 65.3% to 98.6%) and 100% (95% CI = 96.0% to 100%).

Detailed characterization of the 16 Uniprobe-positive and culture-positive samples was performed using our panel of pathogen-specific probes. Based on initial culture findings, eight of the 16 samples contained the most common meningitis-causing organisms and were correctly detected by our panel of pathogen-specific probes (i.e., four *S. aureus*, two *S. epidermidis*, two *L. monocytogenes*; Table 4). The remaining eight samples contained less common meningitis-causing organisms (i.e., *Enterococcus, Pseudomonas*, and *Klebsiella*) not detectable by our panel of pathogen-specific probes; these were instead correctly classified using our Gram-typing probes (Table 5). The 86 culture-negative samples that tested negative by Uniprobe PCR all tested positive by our IPC, indicating no inhibition of the PCR procedure.

Discordant Uniprobe PCR

Six samples showed discordant culture and PCR results (i.e., positive culture, negative by Uniprobe PCR). Repeat culturing of these samples did not show any growth after 3 days of incubation. All six PCR-negative samples remained negative when repeat PCR was performed using the alternate PCR protocol.

DISCUSSION

Although molecular PCR-based assays hold enormous potential for rapid detection of bacterial pathogens in acute care settings, translation from laboratory to the clinical setting has been slow.[14] In the emergency department (ED), the limitations associated with conventional culture-based diagnostic assays are particularly relevant due to prolonged wait times for bacterial growth required for definitive results.[14] These limitations could be potentially offset by PCR-based assays, which are inherently rapid and potentially more sensitive. Our findings demonstrated promising performance for the novel broad-based multiprobe PCR assay, with both rapid detection and species identification.

Broad-based PCR assays for bacterial meningitis have previously been developed and tested in laboratory and clinical settings.[7–9] Although many have shown promising performance characteristics with clinical samples,[21, 22] prior methods either have been restricted to detection only (i.e., identification of the presence of *Eubacteriales*) or have required time-consuming or technically challenging post-PCR detection steps for species identification. Traditional gel-based separation of PCR products from a broad-based PCR assay can take up to 8–12 hours from sample collection to result. Sequencing, while now rapid, is limited by inability to detect multiple pathogens.[23] While multiplex PCR does offer capacity for simultaneous detection of multiple agents,[11] this method requires extensive optimization to eliminate multiple primer set competition[15] and permits only a limited number of targets (maximum three) in one reaction. Our assay circumvents many of these technical limitations by performing simultaneous detection and specific pathogen identification employing a single streamlined platform, which could be integrated into an acute care laboratory. Notably, our sets of primers and probes can detect all of the common agents causing meningitis organisms, including *N. meningitidis, H. influenzae, S. pneumoniae, S. epidermidis, S. aureus, L. monocytogenes,* and *E. coli.*

The LOD of our Uniprobe PCR in mock-up samples ranged from 10^1 to 10^2 CFU/mL (Table 2). With regard to clinical relevance of the LOD, bacterial loads in the order of 10^3 to 10^5 CFU/mL CSF have been associated with severe meningococcal meningitis, with a reported median of 10^3 CFU/mL in a typical case of meningococcal meningitis.[24–26] Accordingly, the LOD of our assay is well below the typical level of bacterial burden seen in clinical cases and is comparable to if not better than that reported by other PCR-based assays for bacterial meningitis.[7, 9, 27]

The overall performance of our Uniprobe PCR assay included an adjusted sensitivity and specificity (88.9% and 100%, respectively) comparable to or better than that reported by others.[6, 8, 11] Of the 22 culture-positive samples tested, 16 were Uniprobe positive, while six were Uniprobe negative. Interestingly, four of the six culture-”positive” samples grew organisms usually recognized as common laboratory microbiologic contaminants, including *Micrococcus, Corynebacterium, and Rhodococcus*.[18–20] The two remaining culture-positive, but Uniprobe-negative samples grew rare colonies of *P. aeruginosa* and *S. pneumoniae,* after 2–3 days. The prolonged growth time required and the report of rare organism growth suggests either low bacterial load or possible contamination. Our multiprobe method yielded successful characterization of all 16 culture-positive and Uniprobe-positive samples. The pathogen-specific probes, which were designed for the seven most common causes of bacterial meningitis,[1] identified the three pathogens included in our multiprobe panel design.

Gram-typing probes subsequently correctly characterized all samples containing pathogens that were not included in our panel of pathogen-specific probes. One of the design objectives of the assay is to obtain the most microbiologic information rapidly to allow for early directed antimicrobial selection in the acute care setting. Our Gram type–specific probes demonstrated 100% specificity in both the test panel of organisms and all of the culture-positive clinical samples. Moreover, BLAST search against the microbial database from GenBank under the most stringent criteria confirmed 100% Gram specificity (data not shown). CSF Gram stain is regarded as an important part of the evaluation for patients with suspected bacterial meningitis. The sensitivity of laboratory microscopic CSF Gram staining has been reported to be anywhere between 65% and 89%, and therefore up to one-third of cases of bacterial meningitis may be missed based on that method alone.[28] Further, in instances in which antimicrobial therapy has already been started at the time of lumbar puncture, studies show that the sensitivity of Gram stain is even further reduced.[29, 30] The Gram-typing capacity provided by our PCR assay (which is not dependent on organism

viability and is not affected by antimicrobial presence) thus provides added value for decision-making in acute care settings, important for early targeted antimicrobial therapy.

Our assay algorithm includes a discordant analysis step, which is a 1:10 dilution for DNA sample preparation. This was devised based on previous studies in which false-negative findings may occur due to either highly concentrated DNA or highly viscous samples that require dilution for detection.[31]

For patients with an elevated white blood cell count in the CSF, earlier reliable detection of a bacterial versus viral etiology with identification of the offending pathogen would be clinically useful. An additional clinical application, not tested here, is the potential capability to monitor disease progression and antibiotic responsiveness, made possible by the quantitative nature of real-time PCR.

LIMITATIONS

Our innovative multiprobe-based PCR assay provides a detection time (from specimen collection to result) of 6 hours, which is significantly better than the 1 to 2 days typically required for culture results.[9, 10] Although time to detection is reduced, the multistep nature of the assay algorithm described here (i.e., Uniprobe detection followed by species identification) is still time-intensive, making it not truly point of care. Use of more advanced high-speed thermocyclers[31] could decrease total assay time to under 2 hours.

Although our multiprobe (versus multiplex approach) offers the capacity to identify a larger number of specific pathogens, the method is still restricted to a discrete number of targets, based on the requirement for individual probe design. Accordingly, some of the pathogens detected here by Uniprobe required the use of the less specific Gram-typing method for further characterization. An alternative post-Uniprobe identification method, currently under development by our group, is high-resolution melt analysis.[32] This technique involves a simple, closed-tube, non–probe-based approach to amplicon analysis, based on discrete melt profiles of the amplicon providing the capacity for single nucleotide discrimination and easy integration with PCR. This would not only improve throughput, but result in significant improvements in the breadth of pathogens identifiable, including emerging or those not suspected.

The overall sensitivity of our assay is not high enough to support PCR replacing culture. However, we recognize that molecular diagnostic assays will likely never allow culture to become obsolete, and accordingly our stated goal was to design an assay that could be used as an adjunct to culture, which will remain essential for antibiotic susceptibly testing. From the ED standpoint, the major added value of a rapid molecular diagnostic assay such as this may be to allow earlier identification of true cases in those patients where the initial cell counts yield equivocal findings (i.e., a patient admitted to the hospital awaiting definitive culture results while on presumptive antibiotics). Regarding sensitivity, several potential explanations exist for the false-negative cases, including low bacterial load or possibly pathogen degradation due to sample storage. It is also important to point out that the sample set used for this pilot diagnostic performance study was drawn from a convenience sample. Accordingly, since the study was not designed as a clinical trial, it would be misleading to report predictive values (which are based on the prevalence of disease) or draw direct comparisons with other prospective studies of patients with suspected meningitis. A larger prospective clinical trial currently under way will be required for that and will include quantitative culture, discrepancy analysis, and detailed collection of clinical information.

CONCLUSIONS

We have designed and tested a multiprobe polymerase chain reaction–based algorithm that is rapid, has a low limit of detection, and is capable of etiologic characterization of bacterial meningitis in ED patients. The clinical applicability of our assay as a "molecular triage tool" may prove to be useful not only for those with suspected bacterial meningitis, but ultimately those with systemic bacterial infections. Further large-scale studies are required for clinical validation to establish reliability, feasibility, and ultimately cost-effectiveness.

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Table 1

Primer and Probe Sequences

Target Organism	Probe Sequence
Forward primer	5′-TGGAGCATGTGGTTTAATTCGA-3′
Reverse primer	5′-TGCGGGACTTAACCCAACA-3′
Uniprobe	5′-VIC-CACGAGCTGACGACARCCATGCA-3′-MGB
Gram-negative organisms	5′-VIC-ACAGGTGCTGCATGGCTGTCGTCAGCT-3′-MGB
N. meningitidis	5′-VIC-TCCGTCTCCGGAGGATTCCGTAC-3′-MGB
H. influenzae	5′FAM AAGGCACAAGCTCATCTCTGAGCTCTTCTTAGG 3′-MGB
E. coli	5′-FAM-ACATTCTCATCTCTGAAAACTTCCGTGGATGTC-3′-MGB
Gram-positive organisms	5′-FAM-AGGTGGTGCATGGTTGTCGTCAGC-3′-MGB
S. pneumoniae	5′-FAM-CCTTTGACAACTCTAGAGATAGAGCCTTCCC-3′-MGB
L. monocytogenes	5′TET-AAGGGAAAGCTCTGTCTCCAGAGTGGTCAA-3′-MGB
S. epidermidis	5′-TET-AAAACTCTATCTCTAGAGGGGCTAGAGGATGTCAAG-3′-MGB

Table 2

LOD of the Most Common Meningitis-causing Bacteria Using Our Broad-based PCR

Organisms	CFU/mL
S. pneumoniae	70
S. aureus	50
L. monocytogenes	110
N. meningitides	20
H. influenza	10
E. coli[*]	30
S. epidermidis	10

CFU = colony-forming units; CSF = cerebrospinal fluid; LOD = limit of detection; PCR = polymerase chain reaction.

[*] *E. coli* was tested by spiking organism in both sterile CSF and water. The LOD was comparable for both. Due to limited supply of pooled negative CSF, other organisms were spiked in molecular grade water to test for the LOD.

Table 3

CSF Samples: Uniprobe PCR Versus Culture Results

	Culture		
Uniprobe	**+**	**−**	**Total**
+	16 (16) *	0 (0)	16 (16)
−	6 (2)	86 (90)	92 (92)
Total	22 (18)	86 (90)	108 (108)

CSF = cerebrospinal fluid; PCR = polymerase chain reaction.

* Values in parentheses are the adjusted 2 × 2 table after assigning the common microbiologic contaminants (*M. luteus, R. dentocariosa, Corneybacterium* sp.) to the culture-negative cell; these numbers were used to calculate adjusted sensitivity and specificity.

Table 4

Pathogen-specific PCR Results for Uniprobe-positive / Culture-positive Samples

Sample No.	Culture Results	Uni	Pathogen-specific PCR results
760	*S. aureus*	+	STAU
679	*S. aureus*	+	STAU
561	*S. aureus*	+	STAU
425	*S. epidermidis*	+	STEP
278	*S. epidermidis*	+	STEP
1199	*S. aureus*	+	STAU
1049	*L. monocytogenes*	+	LIMO
1063	*L. monocytogenes*	+*	LIMO

LIMO *L. monocytogenes* + = positive for Uniprobe PCR; *S. aureus* (STAU) = *Staphylococcus aureus; S. epidermidis* (STEP) = *Staphylococcus epidermidis;* Uni = Uniprobe PCR. PCR = polymerase chain reaction.

* Uniprobe-positive tested with 1:10 dilution.

Table 5

Gram-typing PCR Results for Uniprobe-positive / Culture-positive Samples

Sample No.	Culture Results	Uni	Gram-typing
681	*E. faecium*	+	GN
1145	*P. aeruginosa*	+	GN
435	*E. cloacae*	+	GN
1594	*E. cloacae*	+	GN
431	*E. cloacae*	+	GN
1279	*K.. pneumoniae*	+	GN
1238	*K. pneumoniae*	+*	GN
1132	*Pseudomonas* and *Enterococcus* spp.	+	GN

+ = positive for Uniprobe PCR; GN = Gram-negative; GP = Gram-positive; UA = unavailable; Uni = Uniprobe PCR. PCR = polymerase chain reaction.

* Uniprobe positive tested with 1:10 dilution.