Identification of clinically significant gram-positive pathogens using automated rep-PCR
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BACKGROUND
Gram-positive bacteria can act as opportunistic pathogens and often become invasive to open wounds or burn victims, as well as patients dependent on catheters and other intravascular devices. Bacteria of the genera Staphylococcus, Streptococcus, and Enterococcus are the most common to cause hospital-acquired infections (HAIs). The prevalence of infection and rising occurrence of drug resistance in these bacteria have created the need for a quick and efficient method for identifying gram-positive bacteria not only at the genus level but also at the strain level, particularly in the context of VRE, MRSE, and MRSA. Advances in technology provide both diagnostic and financial benefits through rapid bacterial identification and use of AST. Automated systems such as Vitek 2 and API can provide identification, but only at the genus species level and have difficulty with some gram-positive organisms. Strain typing methods such as PFGE and ribotyping are expensive and time consuming. The DiversiLab System™, a newly developed commercial assay-system that utilizes repetitive-sequence-based PCR (Rep-PCR) and microfluidics, has the potential to provide quick and reliable identification at the species strain level. Rep-PCR has been shown to reduce the number of identifications (38 identifications for 140 bacilli isolates) by rapid fingerprinting without reducing the discrimination power at an intraspecific level. Furthermore, some strains that were misidentified with the API system, can be assigned to the correct genus by rep-PCR. This study reports the performance of the DiversiLab System™ as an alternative molecular genotyping tool for gram-positive bacteria identification for genus, species and strain.

METHOD
A total of 123 previously characterized gram-positive organisms were cultured and DNA was extracted from each culture using the UltraClean® Microbial DNA Kit (Mo Bio Laboratories, Inc., CA). Fifty nanograms of purified genomic DNA was then amplified by rep-PCR using the DiversiLab System. The cycling parameters included an initial denaturation at 94°C for 2 minutes, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 70°C for 90 seconds, and a final extension at 70°C for 3 minutes. The amplicons were separated and detected using lab-on-a-chip technology and the Caliper® 1000 Analyser (Caliper Technologies, Corp., CA). The resulting data were analyzed using the DiversiLab software (v. 2.0) that included Pearson's correlation coefficient for similarity calculation and, unweighted pair group averaging with mathematical averaging (UPGMA) for creating dendrograms.

RESULTS
All 123 samples were processed and analyzed in less than three days by a single technician. Samples analyzed using the DiversiLab System showed >99% concordance to previous characterization by use of traditional methods. The DiversiLab System generated a report that included the dendrogram and gel-like images reflecting each isolate's replicon pattern of genomic DNA. From the complete dendrogram a consensus dendrogram (Fig. 1) was created to demonstrate the distinction between all categories including genus, species, serotype, and strain. Examples of discrimination among species and strains are shown in Figure 2. Data homologous Streptococcus Group B (GBS) as common cause of neonatal infections can easily be distinguished from Group A and C species and strains are shown in Figure 2a. The DiversiLab System showed >99% concordance to previous characterization by use of traditional methods. The DiversiLab System was able to differentiate three groups of Streptococci. As shown in the dendrogram (Fig. 2a), strains of S. pyogenes (Group A), and Group C. Enterococcus are resolved into well-defined groups with further strain differences.

SUMMARY
The latest CDC guidelines recommend that all pregnant women be tested for GBS to prevent transmission to newborns1. GBS is one of the leading infectious causes of neonatal mortality and morbidity2. The DiversiLab System was able to differentiate three groups of Streptococci. As shown in the dendrogram (Fig. 2a), strains of S. pyogenes (Group A), and Group C. Enterococcus are resolved into well-defined groups with further strain differences.

REFERENCES

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