

Appendix

DNA Extraction and Real-Time PCR

Upon arrival at the next-generation sequencing laboratory, tissue samples were transferred to 2-mL screw-cap tubes, and fluid samples were centrifuged for a period of 10 minutes. This was followed by DNA extraction. DNA extraction was performed using the High Pure PCR Template Preparation Kit (Roche Diagnostics). The extraction process was modified by the inclusion of a beading step for tissue and cell disruption using 5-mm steel beads, 5-mm zirconium oxide beads, and the use of the Qiagen TissueLyser II instrument (Qiagen). The lysate generated from this step was then prepared using the High Pure PCR Template Preparation Kit. The initial step in the performance of the assay was the performance of real-time PCR, using the LightCycler 480 (Roche Diagnostics). This assay provides a quantitative assessment of the bacterial burden of the assay and covers a range of organisms and antibiotic-resistant genes including the following: *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Candida albicans*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serratia marcescens*, methicillin resistance, and vancomycin resistance.