**Appendix 2: Sample Processing Methods**

**Bacterial test methods:** Swabs and sponges from sampling of surfaces and hot pad towels and foot cozies were transported overnight from the training room to the laboratory for morning delivery, then maintained under refrigeration until tested for total and specific microorganisms. Trypticase Soy Broth (TSB) was aseptically inoculated with measured sections of sponges, towels, and whole foot cozies and swabs. Subsequent agitation and incubation followed to allow detection and quantitation of total and specific microorganisms. To determine APC for each sample ten-fold serial dilutions of inoculated TSB were prepared in phosphate buffer. Aliquots of dilutions were subsequently plated in Microbial Content Agar. Following incubation for 48 hours at 35 °C, all colonies were counted. Total counts in original samples were estimated using appropriate dilution factors.

To determine Coliforms/Escherichia coli (E. coli) 10 to 1 and 10 to 2 phosphate buffer dilutions of each sample were inoculated in 3M Petrifilm E. coli/Coliform Count media. Following incubation, Coliform and E. coli colonies were identified by appropriate growth and reaction and were counted. Total counts were estimated using appropriate dilution factors. To determine Staphylococcus aureus/MRSA, colonies presumed to be S. aureus based on growth and appropriate reaction on Mannitol Salt Agar were confirmed by Gram stain and latex agglutination and coagulase tests. Then isolates confirmed as S. aureus were screened for methicillin resistance (as MRSA) by growth and reaction on Oxacillin Resistance Screening Agar. To determine Enterococcus spp., VRE colonies showing appropriate growth and reaction on M-Enterococcus agar were confirmed as Enterococcus spp. by Gram stain and catalase test. Confirmed Enterococcus spp. were then screened for vancomycin resistance (as VRE) by growth on Brain Heart Infusion Agar supplemented with vancomycin.

**Influenza A test method:** A polyester swab was pre-moistened with Tris-buffered saline with 0.1% Tween 20 and used to swab the surface area of each sampling point in a back and forth horizontal direction. The swab was turned to the unused side and swabbed in a vertical direction back and forth to cover the same area. The swab stick tip was then submerged into a sterile conical tube containing 1.0 mL of the recovery solution. The swabs were sonicated for 5 minutes and vortexed for 1 minute. The swabs were then squeezed to remove liquid into the tube. The liquid solution was centrifuged at 1,000 rpm for 3 minutes, after which the supernatant was collected. The RNA was extracted from 0.45 mL of the supernatant using the QIAamp® Viral RNA Mini Kit (Qiagen, Germany) per manufacturer instruction. The extracted RNA was then analyzed for Influenza A virus RNA copies by reverse transcriptase quantitative PCR (qRT-PCR) in triplicate replicate analyses along with a positive control (influenza A virus) and a negative control (nuclease-free water). The primers used for the influenza A virus qRT-PCR were forward 5’-GACCRATCCTGTCACCTCTGAC and reverse 5’-AGGCATTYTGGACAAAKCGTCTA; and probe 5’-TGC AGT CCT CGC TCA CTG GGC ACG (BHQ). Three PCR reactions were performed for each extracted RNA sample. The average value from the three points was used to assess the viral copies/swab.

**Adenosine triphosphate test method:** A square surface of approximately 5x5 cm was used for most surfaces, however for smaller, irregular surfaces (e.g., door knobs, water bottle lids), an area on the surface as close to 25 cm2 as possible was used for that surface sample consistently for all other similar size sample points. The surfaces were sampled using fresh ATP swabs with the particular ATP meter being utilized. The ATP swabs were rubbed over the surface first left to right, then top to bottom. Values were obtained per manufacturer’s instructions