Syringe Method for Processing SASS 3100 Filters

An Alternative Method for Extracting Nucleic Acids

1. Place the filter in a sterile 50ml conical centrifuge tube. The tube should be stored in a cooler (short term) or at -80 °C (long term) until ready to process.

2. When ready to extract nucleic acid from the filter, add 10 ml of NucliSENS® lysis buffer (BioMerieux), to the tube. (50% guanidine thiocyanate, 2% Triton X-100, 1% EDTA)

3. Vortex (agitate) for 20 seconds.

4. Remove the plunger from a sterile 25 mL syringe and place the filter in the syringe. Replace the syringe plunger and draw the lysis buffer into the syringe.

5. Depress the plunger to pass the buffer back into the centrifuge tube. Press the filter down against the bottom of the syringe barrel to extract as much buffer as possible and to collect any adhered particles.

6. Centrifuge the filter extract at 700g for 30 minutes. Store the supernatant and pellet separately.

7. Resuspend the pellet in 550 µl of Power Bead solution (QIAGEN), then transfer to a sterile 2 ml tube containing 600 mg of 0.1 mm zirconia/silica beads (BioSpec Products). Add 60 µl of PowerSoil solution (QIAGEN), then perform bead beating for 1 minute at maximum intensity in a Mini-Beadbeater-8 (BioSpec Products).

8. Centrifuge the 2 ml tube at 13,000g for 2 minutes. Add 250 µl of QIAGEN PowerSoil Kit reagent C2 and 200 µl of reagent C3 to remove PCR inhibitors.

9. Combine the supernatant from the 2 ml tubes to the 10 ml from the filter extraction. Isolate the DNA/RNA using the BioMerieux NucliSENS Magnetic Extraction Reagents kit.