5.2 Specimen DNA/RNA Extraction – Single step extraction using prepGEM

Resuspend Lysozyme – Resuspend lyophilized powder in 100mM Tris pH 8.0 to the volume specified on the label.
Create a 100mM dilution of Tris pH 8.0 from the original 1M Tris pH 8.0 bottle.
100µL of 1M Tris pH 8.0 and 900µL of DNA-free water.
Aliquot 50
µL of Lysozyme and
prepGEM
into 0.6mL tubes.
This will reduce potential for contamination and activity loss. Store lysozyme and
prepGem
at -20
°
C.
Make enough extraction Master Mix for the number of samples to be processed using the following table for reference:

Table 3. Extraction Master Mix Calculations

Extraction Master Mix		
Reagent	Volume per 1 rxn (µL)	Total Reaction Volume (30rxns) (µL)
DNA-free Water	28.1	843
10X Green+ Buffer	3.3	99
prepGEM	0.3	9
Lysozyme	0.3	9
UTI Internal Control	0.333	10

Entero-DR Internal Control	0.333	10
GAPDH Internal Control	0.333	10

Pipette 33
µ
L of extraction master mix and 17
µ
L of patient sample into a 96 well plate, using the following plate map for reference:
Do this for each patient sample and
the
negative control
.

Sections 5.3 through 5.5 omitted from this addendum due to lack of changes to the procedure

5.6 Melt Curve Analysis
Section no longer necessary with GAPDH replacing RNase P as internal control.

Testing personnel will sign this addendum in acknowledgement of changes to the extraction protocol for the Respiratory Panel.

Director Signature Date