Addendum – UTI/Wound/DR Panel with GAPDH Internal Control Procedure

To simplify the protocol, a new internal control (GAPDH gene) was designed to replace RNase P. This will result in the Melt Curve Analysis (Section 5.6 of PT:0033.01) no longer being needed.

4.0 Materials
4.1 Reagent/Consumables
NOTE: RNase P primers, probe, and control will no longer be needed for PT:0033.01.

KKiKit	Item	Part or Cat Num.	Vol.	Shipping Conditions	Storage Conditions (°C)
N/A	GAPDH Forward Primer	N/A	1 tube	Ambient	-25 to -15
N/A	GAPDH Reverse Primer	N/A	1 tube	Ambient	-25 to -15
N/A	GAPDH Probe	N/A	1 tube	Ambient	-25 to -15
N/A	GAPDH Control	N/A	1 tube	Ambient	-25 to -15

Table 1. Reagents

5.0 Procedure
5.1 Reagent Aliquoting

Table 2. Aliquot Instructions

Reagent	Abbreviation	# Tubes Needed	Reagent Vol. (µL)	Diluent Vol. (µL)	Vol. per tube (µL)	Expiration Date

Sul1 Forward Primer	DR Panel 2 Primer/Probe Mix	DR2 PP	20	49.2	1139	100	1 month from preparation
Sul1 Reverse Primer				49.2			
Sul1 Probe				24.6			
Sul2 Forward Primer				49.2			
Sul2 Reverse Primer				49.2			
Sul2 Probe				24.6			
Sul3 Forward Primer				49.2			
Sul3 Reverse Primer				49.2			
Sul3 Probe				24.6			
mecA Forward Primer				49.2			
mecA Reverse Primer				49.2			
mecA Probe				24.6			

mecB Forward Primer				49.2			
mecB Reverse Primer				49.2			
mecB Probe				24.6			
mecC Forward Primer				49.2			
mecC Reverse Primer				49.2			
mecC Probe				24.6			
GAPDH Forward Primer				49.2			
GAPDH Reverse Primer				49.2			
GAPDH Probe				24.6			

qnrB Forward Primer	DR Panel 3 Primer/Probe Mix	DR3 PP	20	49.2	647	100	1 month from preparation
qnrB Reverse Primer				49.2			
qnrB Probe				24.6			
qnrS Forward Primer				49.2			
qnrS Reverse Primer				49.2			
qnrS Probe				24.6			
nfsA Forward Primer				49.2			
nfsA Reverse Primer				49.2			

nfsA Probe				24.6			
nfsB Forward Primer				49.2			
nfsB Reverse Primer				49.2			
nfsB Probe				24.6			
dfrA1 Forward Primer				49.2			
dfrA1 Reverse Primer				49.2			
dfrA1 Probe				24.6			
dfrA5 Forward Primer				49.2			
dfrA5 Reverse Primer				49.2			
dfrA5 Probe				24.6			
dfrA8 Forward Primer				49.2			
dfrA8 Reverse Primer				49.2			
dfrA8 Probe				24.6			
dfrA12 Forward Primer				49.2			
dfrA12 Reverse Primer				49.2			
dfrA12 Probe				24.6			
dfrA14 Forward Primer				49.2			
dfrA14 Reverse Primer				49.2			

dfrA14 Probe				24.6			
dfrA17 Forward Primer				49.2			
dfrA17 Reverse Primer				49.2			
dfrA17 Probe				24.6			
GAPDH Forward Primer				49.2			
GAPDH Reverse Primer				49.2			
GAPDH Probe				24.6			

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
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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