EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Unyvero LRT Application and Unyvero System
DECISION SUMMARY

A. DEN Number:

DEN170047

B. Purpose for Submission: *De Novo* request for evaluation of automatic class III designation for the Unyvero Lower Respiratory Tract (LRT) Application and the Unyvero System.

C. Measurand: DNA sequences of the following organisms and antibiotic resistance markers:

Microorganism Targets	Antibiotic Resistance Marker Targets
Acinetobacter spp.	*ctx*-M (bla_{CTX-M}, subgroup 1 only)
Chlamydia pneumoniae	*kpc* (bla_{KPC})
Citrobacter freundii	*mec*A
Escherichia coli	*ndm* (bla_{NDM})
Enterobacter cloacae complex	*oxa*-23 (bla_{OXA-23})
Haemophilus influenzae	*oxa*-24 (bla_{OXA-24})
Klebsiella oxytoca	*oxa*-48 (bla_{OXA-48})
Klebsiella pneumoniae	*oxa*-58 (bla_{OXA-58})
Klebsiella variicola	*tem* (bla_{TEM})
Legionella pneumophila	*vim* (bla_{VIM})
Moraxella catarrhalis	
Morganella morganii	
Mycoplasma pneumoniae	
Proteus spp.	
Pseudomonas aeruginosa	
Serratia marcescens	
Staphylococcus aureus	
Stenotrophomonas maltophilia	
Streptococcus pneumoniae	

D. Type of Test:

Qualitative nucleic acid amplification assay

E. Applicant:

Curetis GmbH

F. Proprietary and Established Names:

Unyvero Lower Respiratory Tract (LRT) Application
Unyvero System

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3985

2. Classification:

   Class II (Special Controls)

3. Product code:

   QBH

4. Panel:

   83-Microbiology

H. Indications for use:

1. Indications for use:

   The Unyvero LRT Application is a qualitative nucleic acid multiplex test intended for the simultaneous detection and identification of nucleic acid sequences from the following microorganisms and antibiotic resistance markers in endotracheal aspirates from adult hospitalized patients with suspected lower respiratory tract infections.

Microorganism	Associated antibiotic resistance marker
Acinetobacter spp. [a]	*ctx*-M [b], *kpc*, *ndm*, *oxa*-23, *oxa*-24, *oxa*-58, *vim*
Chlamydia pneumoniae	-
Citrobacter freundii	*ctx*-M [b], *kpc*, *ndm*, *oxa*-48, *vim*
Enterobacter cloacae complex [c]	*ctx*-M [b], *kpc*, *ndm*, *oxa*-48, *vim*
Escherichia coli	*ctx*-M [b], *kpc*, *ndm*, *oxa*-48, *vim*
Haemophilus influenzae	*tem*
Klebsiella oxytoca	*ctx*-M [b], *kpc*, *ndm*, *oxa*-48, *vim*
Klebsiella pneumoniae [d]	*ctx*-M [b], *kpc*, *ndm*, *oxa*-48, *vim*
Klebsiella variicola	*ctx*-M [b], *kpc*, *ndm*, *oxa*-48, *vim*
Legionella pneumophila	-
Moraxella catarrhalis	-
Morganella morganii	*ctx*-M [b], *kpc*, *ndm*, *oxa*-48, *vim*
Mycoplasma pneumoniae	-
Proteus spp. [e]	*ctx*-M [b], *kpc*, *ndm*, *oxa*-48, *vim*
Pseudomonas aeruginosa	*ctx*-M [b], *kpc*, *ndm*, *vim*
Serratia marcescens	*ctx*-M [b], *kpc*, *ndm*, *oxa*-48, *vim*
Staphylococcus aureus	*mec*A
Stenotrophomonas maltophilia	-
Streptococcus pneumoniae	-

[a] *Acinetobacter* spp. includes: *A. baumannii*, *A. calcoaceticus*, *A. haemolyticus*, *A. junii*, *A.*

lwoffii, *A. nosocomialis*, *A. parvus A. pittii*, (detected by LRT Application) and *A. ursingii* (not detected by LRT Application).
[b] *ctx*-M1 subgroup.
[c] *Enterobacter cloacae* complex includes: *E. asburiae, E. cloacae, E. hormaechei, E. kobei, E. ludwigii*, and *E. xiangfangensis*.
[d] *Klebsiella pneumoniae* includes two variants: *K. pneumoniae* (variant 1), and *K. quasipneumoniae* (variant 2)
[e] *Proteus* spp. includes *P. hauseri*, *P. mirabilis*, *P. penneri* and *P. vulgaris*

The Unyvero LRT Application performed on the Unyvero System is indicated as an aid in the diagnosis of lower respiratory tract infection in adult hospitalized patients with signs and symptoms of lower respiratory infection; results should be used in conjunction with other clinical and laboratory findings. As tracheal aspirates commonly contain colonizing microorganisms, detection of Unyvero LRT microbial targets does not indicate that the microorganism is the cause of the disease. Unyvero positive results do not rule out co-infection with microorganisms not detected by the Unyvero LRT Application. Negative results do not preclude lower respiratory infection, as the causative agent may be a microorganism not detected by this test.

A negative result for any antibiotic resistance marker does not indicate that detected microorganisms are susceptible to applicable antimicrobial agents. Detected resistance markers cannot be definitively linked to specific microorganisms, and may be present in organisms that are not detected by the Unyvero LRT Application such as organisms present as colonizing or normal flora.

Microbiology cultures of aspirates should be performed to obtain isolates for species identification and antimicrobial susceptibility testing, to differentiate quantities of identified microorganisms as well as normal flora present in the specimen and to identify potential microorganisms not targeted by the Unyvero LRT Application.

2. Special conditions for use statement(s):

- For *in vitro* diagnostic use only
- For prescription use only

Limitations:

- Antimicrobial resistance can occur via mechanisms other than the resistance markers detected by the Unyvero LRT Assay. Negative results for the LRT resistance markers do not indicate antimicrobial susceptibility of detected organisms.
- Detected resistance markers cannot be definitively linked to specific microorganism(s), and may be present in organisms that are not detected by the Unyvero LRT assay, including colonizing flora. Standard culture of tracheal aspirates and subsequent testing of the isolated microorganism(s) is necessary to definitively link antimicrobial resistance with a specific microorganism.
- Detection of antibiotic resistance markers in a specimen may not correlate with phenotypic gene expression. Resistance marker results should be used in

conjunction with final culture and antimicrobial susceptibility testing results.

- Detected microorganisms may be indicative of colonizing or normal respiratory flora and may not be the causative agent of pneumonia.

(b) (4)

- The LRT Application is a qualitative test and does not provide a quantitative value for the microorganisms or antibiotic resistance markers detected in the specimen. Compared to standard of care culture methods, the Unyvero LRT Application is not able to assess the presence or amount of normal flora in the specimen or whether the detected microorganism/antibiotic resistance marker is present in predominating amounts as compared to other microorganisms in the specimen. The LRT Application also does not assess if detected microorganisms are present at clinically relevant concentrations, or are present as colonizing flora.
- When three or more microorganisms are detected for a specimen, the user is advised to wait for microbiology culture results to verify the predominant microorganism(s) and to assess for the presence of normal respiratory flora.
- Bacterial nucleic acids may be present in the specimen independent of organism viability. Detection of organism nucleic acid target(s) does not imply that the corresponding organisms are the causative agents for clinical symptoms.
- A negative result for the 'atypical' microorganisms (*C. pneumoniae, L. pneumophila, and M. pneumoniae*) does not exclude the presence of this microorganism in the patient specimen. A positive result should be evaluated in the overall context of the patient's clinical condition and other laboratory results being part of the standard-of-care routine.
- Due to the small number of positive prospective and archived specimens for certain microorganisms and antibiotic resistance markers, performance characteristics for *C. pneumoniae, C. freundii, K. oxytoca, K. variicola, L. pneumophila, M. catarrhalis, M. morganii, M. pneumoniae*, and antibiotic resistance markers *ctx*-M, *kpc*, *ndm*, *oxa*-23, *oxa*-24, *oxa*-48, *oxa*-58, and *vim* were established primarily using contrived clinical specimens.
- Based on *in-silico* analysis and inclusivity wet testing, certain clinically relevant species for some LRT panel microorganism analytes are not detected or detected with reduced sensitivity: *Acinetobacter ursingii* detection is predicted at reduced sensitivity for the *Acinetobacter* spp. assay, *Enterobacter asburiae* detection is predicted at reduced sensitivity for the *Enterobacter cloacae* complex assay.
- Subtypes included in *ctx*-M subgroups *ctx*-M2, *ctx*-M8, *ctx*-M9 (*ctx*-M14), *ctx*-M25 and *ctx*-M45 were not evaluated and are not predicted to be detected by the LRT assay based on *in-silico* analysis.
- The *mec*C variant is not detected by Unyvero LRT and for *mec*C positive S*taphylococcus aureus* strains, the assay will generate negative results for *mec*A.
- Based on *in-silico* analysis and exclusivity wet testing, the following LRT panel microorganism assays are expected to cross-react with closely related clinically relevant species: *Citrobacter freundii* (cross-reactive to *C. braakii*, *Kluyvera georgiana*), *Escherichia coli* (cross-reactive to *E. albertii*, *E. fergusonii* and

Shigella spp. (*S. dysenteriae*, *S. sonnei*, *S. flexneri*, *S. boydii*)), *Haemophilus influenzae* (cross-reactive to *H. haemolyticus*), *Klebsiella oxytoca* (cross-reactive to *K. michiganensis*).

4. Special instrument requirements:

   The Unyvero LRT Application is performed on the Unyvero System which includes the Unyvero Lysator, Unyvero Analyzer and Unyvero Cockpit**.**

I. Device Description:

The Unyvero LRT Application is a qualitative test that includes specimen processing, genomic bacterial DNA isolation and purification, multiplex PCR and array hybridization and detection. The Unyvero LRT Application performed using the Unyvero System detects specific nucleic acid sequences from microorganisms and resistance markers in tracheal aspirates collected from patients with signs and symptoms of lower respiratory infection.

The Unyvero LRT Application consists of the following components:

- Unyvero LRT Cartridge: Contains DNA isolation and purification reagents, a DNA isolation column, eight separate PCR chambers with eight corresponding detection arrays. The Cartridge also contains fluorescently-labeled primers, hybridization and wash buffers and oligonucleotide probes for detection of targeted PCR products using array hybridization technology.

- Unyvero T1 Sample Tube: Contains glass beads and buffers to lyse bacteria and liquefy the sample.

- Unyvero T1 Sample Tube Cap (with Internal Control): Contains proteinase K and a synthetic internal control gene for process monitoring. The T1 Sample Tube Cap seals the Unyvero Sample Tube after which the internal control is combined with each patient specimen. The internal control DNA sequence does not have significant homology to targeted sequences and is amplified independently in each of the eight PCR chambers and the amplified internal control product is hybridized on each array.

- Unyvero M1 Master Mix: Contains reagents for DNA amplification.

- Unyvero T1 Transfer Tool: The Transfer tool can be used to transfer viscous specimens from the primary sample container to the Unyvero Sample Tube.

The Unyvero System consists of the following components:

- Unyvero Lysator: The Lysator lyses the specimen and can process up to four specimens simultaneously in four separate slots.

- Unyvero Analyzer: The Analyzer automates DNA purification, amplification and detection. Each Analyzer can simultaneously process up to two Unyvero Cartridges with each slot available using random access.

- Unyvero Cockpit: The Cockpit provides the main user interface for the Unyvero System, guides the user through the steps to run the Unyvero LRT Application and automatically generates and displays test results. The Cockpit is equipped with a high-resolution touch screen and a barcode reader.

- Unyvero Sample Tube Holder: The Sample Tube holder holds the Sample Tube securely while the specimen is transferred into the Sample Tube.

Other materials required but not provided:

- Pipette capable of dispensing 180µL
- DNase/RNase free, aerosol resistant pipette tips
- 1mL Luer-Lock syringe

J. Standard/Guidance Document Referenced:

- CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline, 2nd edition.
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – 2nd edition.
- CLSI M100, Performance Standards for Antimicrobial Susceptibility Testing, 26th Edition.

K. Test Principle:

The Unyvero LRT Application is a qualitative PCR-based assay that detects DNA sequences of microorganisms and associated resistance markers in tracheal aspirate specimens. The assay includes specimen processing (lysis), DNA extraction and isolation, multiplex PCR with eight parallel multiplex endpoint PCR reactions, and qualitative detection of amplified DNA products using hybridization arrays.

A tracheal aspirate specimen is first pipetted into the Unyvero Sample Tube and closed with the Unyvero Sample Tube Cap. Closing the sample tube automatically adds the lysis reagent and the internal control gene template to the specimen. The sample tube which fits into the Unyvero Lysator only if closed with the Unyvero sample tube cap is then placed on the Lysator. After the specimen is lysed in the Lysator, the Sample Tube and Master Mix are loaded into the Unyvero LRT Cartridge which is then inserted into the position assigned by the Unyvero Analyzer for automated processing and analysis.

In the Unyvero LRT Cartridge, the remainder of the testing steps are automated by the Unyvero Analyzer. The lysed specimen is further processed and then transferred onto a

DNA purification column for nucleic acid (b) (4) . (b) DNA is transferred to a chamber, where mixing with the Master Mix takes place. This mixture is distributed into eight separate PCR reaction chambers each containing multiple primer pairs. After amplification, PCR products are hybridized to the corresponding array probes. Each array has been manufactured (b) (4) (b) (4) (b) (4) Result data are transferred to the Unyvero Cockpit for visualization and result printout. A test run is completed after approximately 4.5 hours, and results for panel microorganisms and associated antibiotic resistance markers are displayed on the Unyvero Cockpit screen.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Reproducibility:

The reproducibility of the Unyvero LRT Application was assessed using a representative panel of samples prepared in artificial respiratory matrix (ARM, adapted from Dinesh S. D.[1]) which was used as a surrogate for tracheal aspirate matrix. In a separate analytical study, the Unyvero LRT Application was shown to demonstrate equivalent performance for samples prepared in ARM matrix and samples prepared in natural tracheal aspirate matrix (See Section L.2.b below).

Samples for the reproducibility study were prepared with whole-organism preparations for the following LRT panel analytes*: E. coli/tem, P. aeruginosa*, *K. pneumoniae*, *M. morganii*, *S. maltophilia* and *S. aureus*/*mec*A. Sample #1 (REPRO 1) was inoculated with each organism at moderate positive concentrations (~5 x LoD) and Sample #2 (REPRO 2) was inoculated with each organism at low positive concentrations (~1.7 x LoD). For the *mec*A target, the REPRO 1 and REPRO2 panel members had concentrations of 12.5 x LoD and 4.2 x LoD respectively. For the *tem* target, the REPRO1 and REPRO2 panel members had concentrations of 6.2 and 2.1 x LoD respectively. Test runs were independently performed by three different operators. Each operator was assigned one of three Unyvero systems, each consisting of 1 Unyvero Cockpit, 2 Unyvero Lysators and at least 4 Unyvero Analyzers. A total of 90 replicate samples were tested for each panel member with each operator testing samples in triplicate on a minimum of five testing days. Positive and negative controls were also tested daily by each operator. The organisms and concentrations used to prepare study samples are presented in Table 1 below. Study results are presented in Table 2.

Table 1: Microorganisms for Reproducibility Study

Analytes	ATCC Strain	LoD (CFU/mL)	Repro1/x-fold LoD	Repro2/x-fold LoD

[1] Dinesh, Artificial Sputum Medium. Protocol Exchange (2010)

E. coli	35218	7.50E+04	3.75E+05	5.0	1.28E+05	1.7
K. pneumoniae V2	700603	5.00E+04	2.50E+05	5.0	8.50E+04	1.7
M. morganii	25830	5.00E+05	2.50E+06	5.0	8.50E+05	1.7
P. aeruginosa	10145	5.00E+04	2.50E+05	5.0	8.50E+04	1.7
S. aureus	33591	5.00E+06	2.50E+07	5.0	8.50E+06	1.7
S. maltophilia	13637	3.00E+04	1.50E+05	5.0	5.10E+04	1.7
mec A	33591	2.00E+06	2.50E+07	12.5	8.50E+06	4.3
tem	35218	6.00E+04	3.75E+05	6.3	1.28E+05	2.1

Table 2: Reproducibility study results, stratified by target, concentration and operator

		REPRO1 (5x LoD)				REPRO 2 (1.7x LoD)			
analyte	**user**	**x-fold LoD**	**Pos.**	**[%]**	**95 % CI**	**x-fold LoD**	**Pos.**	**[%]**	**95 % CI**
E. coli ATCC 35218	user 1	5	30/30	100.0	88.7 – 100.0	1.7	29/29	100.0	88.3 - 100.0
	user 2		30/30	100.0	88.7 – 100.0		29/30	96.7	83.3 - 99.4
	user 3		29/30	96.7	83.3 - 99.4		29/30	96.7	83.3 - 99.4
	all		**89/90**	**98.9**	**94.0 - 99.8**		**87/89**	**97.8**	**92.2 - 99.4**
K. pneumoniae variant 2 ATCC 700603	user 1	5	21/29	72.4	54.3 - 85.3	1.7	14/30	46.7	30.2 - 63.9
	user 2		25/29	86.2	69.4 - 94.5		15/30	50.0	33.2 - 66.8
	user 3		24/30	80.0	62.7 - 90.5		19/29	65.5	47.3 - 80.1
	all		**70/88**	**79.5**	**70.0 - 86.7**		**48/89**	**53.9**	**43.6 - 63.9**
*mec*A (from: *S. aureus* ATCC 33591)	user 1	12.5	30/30	100.0	88.7 – 100.0	4.2	25/29	86.2	69.4 - 94.5
	user 2		27/30	90.0	74.4 - 96.5		29/30	96.7	83.3 - 99.4
	user 3		29/30	96.7	83.3 - 99.4		29/30	96.7	83.3 - 99.4
	all		**86/90**	**95.6**	**89.1 - 98.3**		**83/89**	**93.3**	**86.1 - 96.9**
M. morganii ATCC 25830	user 1	5	28/29	96.6	82.8 - 99.4	1.7 [a]	21/30	70.0	52.1 - 83.3
	user 2		27/29	93.1	78.0 - 98.1		23/30	76.7	59.1 - 88.2
	user 3		28/30	93.3	78.7 - 98.2		24/29	82.8	65.5 - 92.4
	all		**83/88**	**94.3**	**87.4 - 97.5**		**68/89**	**76.4**	**66.6 - 84.0**
P. aeruginosa ATCC 10145	user 1	5	30/30	100.0	88.7 – 100.0	1.7	30/30	100.0	88.7 - 100.0
	user 2		30/30	100.0	88.7 – 100.0		28/30	93.3	78.7 - 98.2
	user 3		30/30	100.0	88.7 – 100.0		30/30	100.0	88.7 - 100.0
	all		**90/90**	**100.0**	**95.9 – 100.0**		**88/90**	**97.8**	**92.3 - 99.4**
S. aureus ATCC 33591	user 1	5	28/30	93.3	78.7 - 98.2	1.7	30/30	100.0	88.7 - 100.0
	user 2		30/30	100.0	88.7 – 100.0		26/30	86.7	70.3 - 94.7
	user 3		30/30	100.0	88.7 – 100.0		29/30	96.7	83.3 - 99.4
	all		**88/90**	**97.8**	**92.3 - 99.4**		**85/90**	**94.4**	**87.6 - 97.6**
S. maltophilia ATCC 13637	user 1	5	30/30	100.0	88.7 – 100.0	1.7	29/30	96.7	83.3 - 99.4
	user 2		28/30	93.3	78.7 - 98.2		28/30	93.3	78.7 - 98.2
	user 3		30/30	100.0	88.7 – 100.0		29/30	96.7	83.3 - 99.4
	all		**88/90**	**97.8**	**92.3 - 99.4**		**86/90**	**95.6**	**89.1 - 98.3**
tem (from: *E. coli* ATCC 35218)	user 1	6.2	29/30	96.7	83.3 - 99.4	2.1	29/29	100.0	88.3 - 100.0
	user 2		29/30	96.7	83.3 - 99.4		22/30	73.3	55.6 - 85.8
	user 3		30/30	100.0	88.7 – 100.0		26/30	86.7	70.3 - 94.7
	all		**88/90**	**97.8**	**92.3 - 99.4**		**77/89**	**86.5**	**77.9 - 92.1**

[a] *M. morganii* was also evaluated at 0.6x LoD in PBS matrix in an initial reproducibility study. Results for this panel member were acceptable based on the microorganism concentration tested (e.g., positivity: 77/88, 87.5% detection for samples prepared at below LoD concentrations).

Testing of reproducibility samples generated acceptable results for all analytes except for *K. pneumoniae* and *M. morganii* for which positivity rates were lower than expected for both the 5x LoD and 1.7x LoD panel members. The reason for these unexpected results was likely due to inaccurate quantitation of organism stocks used for sample preparation. It was noted that testing by different operators/sites generated equivalent results for these analytes (i.e., similarly lower than expected percent positivity at each testing site).

To further evaluate the reproducibility of the Unyvero LRT assay for detection of these two organism targets, ten additional sample replicates for both *K. pneumoniae* and *M. morganii* were prepared using freshly grown and quantitated organism suspensions with final sample concentrations of 5x LoD. For both microorganisms, a positivity rate of 90% was observed with 9/10 samples generating positive results.

Out of 14 positive quality control runs with samples containing moderate positive analyte concentrations (~8x LoD), one quality control sample was false negative for *K. pneumoniae* and one sample was false negative for *M. morganii.*

Three false positive results were observed during the study; two false positive results in reproducibility test sample runs (*S. pneumoniae*, *vim*) and one false positive in a positive control run (*M. catarrhalis*).

Partially invalid results (one PCR chamber only) were observed in 8/180 (4.4%) reproducibility test runs, 2/14 (1.4%) negative quality control runs, and 0/14 positive quality control runs.

Results from the reproducibility study were further evaluated based on Unyvero signal output levels (RBU) for each target and shown in Table 3 below.

Table 3: Reproducibility study, Quantitative analysis of Unyvero signal levels

		REPRO1 (5x LoD)				REPRO 2 (1.7x LoD)			
analyte	**user**	**x-fold LoD**	**Avg Signal**	**% CV**	**Range**	**x-fold LoD**	**Avg Signal**	**% CV**	**Range**
E. coli ATCC 35218	user 1	5	(b) (4)	(b) (4)	(b) (4)	1.7	(b) (4)	(b) (4)	(b) (4)
	user 2								
	user 3								
	all								
K. pneumoniae variant 2 ATCC 700603	user 1	5				1.7 [b]			
	user 2								
	user 3								
	all								
*mec*A (from: *S. aureus* ATCC 33591)	user 1	12.5				4.2			
	user 2								
	user 3								
	all								
M. morganii ATCC 25830	user 1	5				1.7			
	user 2								
	user 3								
	all								
P. aeruginosa ATCC 10145	user 1	5				1.7			
	user 2								
	user 3								
	all								
S. aureus ATCC 33591	user 1	5				1.7			
	user 2								
	user 3								
	all								
S. maltophilia ATCC 13637	user 1	5				1.7			
	user 2								
	user 3								
	all								
tem (from: *E. coli* ATCC 35218)	user 1	6.2				2.1			
	user 2								
	user 3								
	all								

b. Linearity/assay reportable range:

Not applicable.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Internal Control: An internal control consisting of a synthetic DNA sequence without homology to LRT microorganism or resistance marker target sequences is separately processed in each of the eight Unyvero LRT Cartridge PCR chambers. The internal control allows for assessment of DNA purification, amplification, array hybridization, and detection steps. The internal control monitors for the presence of PCR inhibitors

in the specimens and enables the system to detect any failures in the testing process that could potentially result in an incorrect test result. Results generated from Individual PCR chambers are considered valid if successful amplification and detection is completed for either the internal control or any microorganism/resistance marker target in the multiplex PCR reaction.

External Controls

Positive and negative controls were run daily at each of the testing sites during the clinical study. Testing included (b) (4)

External controls are not provided with the Unyvero LRT Application; however, testing of external positive and negative controls are recommended in the assay labeling. Controls may consist of previously characterized positive samples or negative samples spiked with well characterized microorganisms. Previously characterized negative samples may be used as negative controls. External controls should be used in accordance with local, state, and/or federal regulations, accreditation requirements and individual laboratory's quality control policies, as applicable.

Specimen Stability

The Unyvero LRT instructions for use indicates that it is acceptable to store tracheal aspirate specimens at 2-8°C for up to 24 hours prior to testing. To evaluate effects on assay performance for 24 hour refrigerated storage prior to starting an LRT test, an analysis was performed for prospectively tested clinical tracheal aspirate specimens that were tested with the LRT application at various times of storage up to 24 hours. Both qualitative performance (compared to culture) and semi-quantitative Unyvero LRT signal levels for positive analytes were assessed between groups stored for different time periods. Results for specimen which were stored for times close to the 24-hour sample storage time limit did not show a significant difference in clinical performance or a significant difference in assay signal as compared to specimens that were tested immediately after collection.

The sample storage recommendations for the LRT application are consistent with those for traditional culture[2] which include storage and transport of tracheal aspirate specimens at 2-8°C for up to 24 hours. The analysis of the clinical specimen results together with the same recommendation for tracheal aspirate culture support the claim

[2] Baron, E. J. *et al.* A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2013 recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)(a). *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **57,** (2013).

of refrigerated storage of tracheal aspirates for up to 24 hours prior to testing with the Unyvero LRT Application.

d. Detection limit:

A study was conducted to determine the Limit of Detection (LoD) of the Unyvero LRT Application for each targeted analyte. Samples were prepared in Artificial Respiratory Matrix (ARM) which was used as a surrogate for tracheal aspirate matrix. Samples prepared in ARM matrix were shown to generate equivalent results to samples prepared in natural tracheal aspirate matrix (See Section L.2.b below).

For the LoD study, samples were inoculated with multiple organisms (i.e., multi-spiked). To mitigate potential for competitive inhibition, sample compositions were designed to ensure that only one or two analytes were amplified in each multiplex PCR chamber.

LoD estimates were initially determined by testing small numbers of replicates at multiple different organism concentrations. The LoD for each analyte was then confirmed by testing 20 replicates at the estimated LoD concentration. The final LoD is defined as the lowest concentration (reported as CFU/mL, IFU/mL, or CCU/mL) of sample for which ≥95% of sample replicates generate positive results. Tables 4 and 5 below summarize the confirmed LoDs for each LRT panel microorganism and antibiotic resistance marker.

Table 4: Limit of detection for LRT panel microorganisms

LRT Panel Microorganism	ID of Tested Reference Strain	LoD Concentration [CFU/mL, or other as indicated in footnote]
Acinetobacter spp.	ATCC 19606 (*A. baumannii*)	$1x10^5$
Chlamydia pneumoniae (in IFU/mL) [a]	ATCC VR-2282	$1.5x10^4$
Citrobacter freundii	ATCC 8090	$2x10^5$
Escherichia coli	ATCC 11775	$7.5x10^4$
Enterobacter cloacae complex	ATCC 13047 (*E. cloacae*)	$5x10^6$
Haemophilus influenzae	ATCC 33391	$2x10^6$
Klebsiella oxytoca	ATCC 13182	$2x10^5$
Klebsiella pneumoniae Variant 1	ATCC 13883	$4.5x10^5$
Klebsiella pneumoniae Variant 2	ATCC 700603	$5x10^4$
Klebsiella variicola	ATCC BAA-830	$1x10^5$
Legionella pneumophila	ATCC 33152	$2x10^6$
Moraxella catarrhalis	ATCC 25238	$8x10^5$
Morganella morganii	ATCC 25830	$5x10^5$
Mycoplasma pneumoniae (in CCU/mL) [b]	ATCC 29085	$1x10^5$
Proteus mirabilis	ATCC 29906	$1x10^5$
Proteus vulgaris	ATCC 29905	$6x10^5$
Pseudomonas aeruginosa	ATCC 10145	$5x10^4$
Serratia marcescens	ATCC 13880	$1x10^5$
Staphylococcus aureus	ATCC 12600	$5x10^6$
Stenotrophomonas maltophilia	ATCC 13637	$3x10^4$
Streptococcus pneumoniae	ATCC 49619	$5x10^5$

[a] IFU: inclusion-forming units

[b] CCU: color-changing units

Table 5: Limit of detection for LRT panel antibiotic resistance markers

LRT Panel Antibiotic Resistance Marker	ID of Tested Reference Strain	LoD Concentration [CFU/mL]
ctx-M	NRZ-00751 (*K. pneumoniae*)	$1x10^5$
kpc	NRZ-00281 (*E. coli*)	$5x10^5$
*mec*A	ATCC 33591 (*S. aureus*)	$2x10^6$
ndm	JMI 50067 (*E. coli*)	$5x10^4$
oxa-23	NCTC 13301 (*A. baumannii*)	$2x10^7$
oxa-24	NCTC 13302 (*A. baumannii*)	$5x10^5$
oxa-48	NCTC 13442 (*K. pneumoniae*)	$2x10^6$
oxa-58	NCTC 13305 (*A. baumannii*)	$7.5x10^5$
tem [a]	NCTC 13351 (*E. coli*)	$6x10^4$
vim	DSM-24600 (*P. aeruginosa*)	$5x10^4$

[a] Although the LRT Application reports *tem* only with *H. influenzae*, LoD was determined with an *E. coli* strain positive for *tem*. Note that inclusivity testing was successfully performed with two *tem* positive *H. influenzae* strains.

e. Analytical Reactivity (Inclusivity)

The inclusivity study of the LRT Application was assessed using samples inoculated at low positive concentrations with well-characterized bacterial isolates. Most strains were evaluated in samples prepared at ≤3x LoD. Testing was performed in duplicate

and if 100% detection was not observed during initial testing, additional samples prepared with higher organism concentrations were evaluated.

Analyte LoDs were established using ARM as a surrogate for aspirate matrix. Inclusivity testing was performed with contrived samples prepared in PBS. Analytical testing of PBS and ARM matrices demonstrated that the LoDs for the two matrices are comparable for most analytes; however, for a few analytes, PBS and ARM LoDs differed by a factor between 0.3-3. The differences in LoD between the two matrices for these few analytes was not considered significant and did not raise concerns that the stated reactivity would differ when testing clinical specimens.

Inclusivity/Microorganism Targets:

Inclusivity testing for samples prepared with microorganism concentrations at ≤3x LoD generated 100% detection for the LRT microorganism targets except for the following microorganisms for which one or more strains were not detected or were detected only at higher than LoD concentrations (also bolded in table below):

- One of four strains of *Proteus mirabilis* (detected at 5x LoD and not detected at lower concentrations). Sequencing of the isolate revealed sequence mismatches in a detection probe.
- One of three strains of *Proteus vulgaris* (detected at 5x LoD and not detected at lower concentrations). Sequencing of the isolate revealed sequence mismatches in a detection probe.
- One of six strains of *Moraxella catarrhalis* (detected at 3.8x LoD and not detected at lower concentrations)
- One of seven strains of *Serratia marcescens* (1/2 replicates detected at 5x LoD and 0/2 replicates detected at 10x LoD)
- Two of six strains of *Stenotrophomonas maltophilia* (<100% detection at all concentrations tested):
    - Strain DSM-50173 was positive in 3/4 replicates prepared at 1.7 x LoD and 1/2 replicates prepared at 6.8x LoD; sequencing of this strain revealed multiple mismatches for one internal probe.
    - Strain DSM-21874 was positive in 2/4 replicates prepared at 1.7 x LoD and 0/2 replicates at 6.8x LoD; sequencing of this strain did not reveal any mismatches to primers or probes. Testing was repeated for this strain at 1x LoD (0/2), 1.7x (0/2) and at 3.3x LoD for which 2/2 sample replicates generated the expected positive result.
    -

The following limitation is included in the Unyvero LRT labeling:

- Based on *in-silico* analyses, some strains may either not be detected or be detected with reduced sensitivity due to variations in targeted sequences for the following microorganisms: *C freundii, K. oxytoca*, *L. pneumophilia*, *M. morganii.* Based on wet testing for *Proteus* spp., and *S. marcescens* and based

on *in-silico*/wet testing for *M. catarrhalis* and *S. maltophilia* some strains may be detected with reduced sensitivity due to variations in targeted sequences.

Results from inclusivity testing are shown in Table 6.

Table 6: Microorganism targets, Inclusivity strains

Strain	Strain ID	Test Conc. [CFU/mL]	LoD factor[a]	# Positive/ # Tests
Acinetobacter baumannii	ATCC 19606	$8x10^4$	0.8x	2/2
Acinetobacter baumannii	NCTC 13305	$8x10^4$	0.8x	2/2
Acinetobacter baumannii	NCTC 13301	$8x10^4$	0.8x	2/2
Acinetobacter baumannii	Micromyx 6334	$8x10^4$	0.8x	2/2
Acinetobacter baumannii	Micromyx 6153	$8x10^4$	0.8x	2/2
Acinetobacter baumannii	Micromyx 6149	$1.5x10^5$	1.5x	4/4
Acinetobacter baumannii	Micromyx 4410	$8x10^4$	0.8x	2/2
Acinetobacter baumannii	JMI 49755	$1x10^5$	1x	2/2
Acinetobacter baumannii	NRZ-00449	$8x10^4$	0.8x	2/2
Acinetobacter baumannii	NRZ-00518	$8x10^4$	0.8x	2/2
Acinetobacter baumannii	UCLA A4	$8x10^4$	0.8x	2/2
Acinetobacter baumannii	UCLA A5	$8x10^4$	0.8x	2/2
Acinetobacter calcoaceticus	ATCC 23055	$8x10^4$	0.8x	2/2
Acinetobacter lwoffii	ATCC 15309	$8x10^4$	0.8x	2/2
Acinetobacter haemolyticus	ATCC 17906	$8x10^4$	0.8x	2/2
Chlamydia pneumoniae	ATCC VR-1310	$4.5x10^4$ [b] [IFU/mL]	3x	2/2
Chlamydia pneumoniae	ATCC VR-2282	$4.5x10^4$ [IFU/mL]	3x	2/2
Chlamydia pneumoniae	ATCC 53592	$4.5x10^4$ [IFU/mL]	3x	2/2
Citrobacter freundii	ATCC 8090	$2x10^5$	1x	6/6
Citrobacter freundii	ATCC 43864	$3x10^5$	1.5x	2/2
Citrobacter freundii	NCTC 8581	$2x10^5$	1x	2/2
Citrobacter freundii	NCTC 9750	$5x10^5$	2.5x	2/2
Citrobacter freundii	NRZ-00452	$5x10^5$	2.5x	2/2
Citrobacter freundii	UCLA C1	$2x10^5$	1x	2/2
Citrobacter freundii	UCLA C5	$6x10^5$	3x	2/2
Enterobacter cloacae	ATCC 13047	$1.5x10^7$	3x	2/2
Enterobacter cloacae	ATCC 23355	$3x10^6$	0.6x	2/2
Enterobacter cloacae	ATCC 49141	$6x10^5$	0.1x	2/2
Enterobacter cloacae	ATCC BAA-2468	$6x10^5$	0.1x	2/2
Enterobacter cloacae	JMI 46239	$1.5x10^7$	3x	2/2
Enterobacter cloacae	NRZ-00239	$5x10^6$	1x	2/2
Enterobacter cloacae ssp. *dissolvens*	ATCC 23373	$5x10^6$	1x	2/2
Enterobacter hormaechei	ATCC 49162	$5x10^6$	1x	2/2

Strain	Strain ID	Test Conc. [CFU/mL]	LoD factor[a]	# Positive/ # Tests
Enterobacter asburiae	ATCC 35953	$1.5x10^7$	3x	2/2
Escherichia coli	ATCC 11775	$1x10^5$	1.3x	2/2
Escherichia coli	ATCC 25922	$1x10^5$	1.3x	2/2
Escherichia coli	ATCC 35218	$1x10^5$	1.3x	4/4
Escherichia coli	ATCC BAA-2523	$1x10^5$	1.3x	2/2
Escherichia coli	NCTC 13351	$5x10^4$	0.7x	2/2
Escherichia coli	NCTC 13476	$1x10^5$	1.3x	2/2
Escherichia coli	JMI 50067	$6x10^4$	0.8x	2/2
Escherichia coli	NRZ-00176	$1x10^5$	1.3x	2/2
Escherichia coli	NRZ-00222	$6x10^4$	0.8x	2/2
Escherichia coli	NRZ-00281	$1.8x10^5$	2.4x	2/2
Haemophilus influenzae (serotype a)	ATCC 9006	$2x10^6$	1x	2/2
Haemophilus influenzae (serotype c)	ATCC 9007	$6x10^6$	3x	2/2
Haemophilus influenzae (serotype b)	ATCC 10211	$6x10^6$	3x	2/2
Haemophilus influenzae (serotype b)	ATCC 49247	$6x10^6$	3x	2/2
Haemophilus influenzae (non-typeable/non-capsulated)	ATCC 33391	$6x10^6$	3x	2/2
Haemophilus influenzae (serotype b)	ATCC 49766	$6x10^6$	3x	2/2
Haemophilus influenzae (serotype b)	NCTC 8468	$2x10^6$	1x	2/2
Klebsiella oxytoca	ATCC 13182	$8x10^4$	0.4x	6/6
Klebsiella oxytoca	ATCC 43863	$8x10^4$	0.4x	2/2
Klebsiella oxytoca	ATCC 8724	$1.6x10^5$	0.8x	2/2
Klebsiella oxytoca	ATCC 49131	$8x10^4$	0.4x	2/2
Klebsiella oxytoca	NCIMB 12819	$4x10^5$	2x	2/2
Klebsiella oxytoca	NRZ-22060	$8x10^4$	0.4x	2/2
Klebsiella pneumoniae	ATCC 13883	$4x10^5$	0.9x	4/4
Klebsiella pneumoniae	NCTC 13439	$1x10^6$	2.2x	2/2
Klebsiella pneumoniae	NCTC 13440	$6x10^5$	1.3x	2/2
Klebsiella pneumoniae	NCTC 13442	$4x10^5$	0.9x	2/2
Klebsiella pneumoniae	NCTC 13443	$4x10^5$	0.9x	2/2
Klebsiella pneumoniae	Micromyx 4653	$8x10^5$	1.8x	4/4
Klebsiella pneumoniae	Micromyx 4676	$1x10^6$	2.2x	2/2
Klebsiella pneumoniae	JMI 49831	$6x10^5$	1.3x	2/2
Klebsiella pneumoniae	JMI 49767	$9x10^5$	2x	2/2
Klebsiella pneumoniae	NRZ-00002	$4x10^5$	0.9x	2/2
Klebsiella pneumoniae	NRZ-00103	$1.5x10^6$	3.3x	2/2
Klebsiella pneumoniae	NRZ-00223	$9x10^5$	2x	4/4
Klebsiella pneumoniae	NRZ-00249	$6x10^5$	1.3x	2/2
Klebsiella pneumoniae	NRZ-00472	$9x10^5$	2x	2/2
Klebsiella pneumoniae	NRZ-00751	$9x10^5$	2x	2/2
Klebsiella pneumoniae variant II (K. quasipneumoniae)	ATCC 700603	$6x10^5$	1.3x	2/2

Strain	Strain ID	Test Conc. [CFU/mL]	LoD factor[a]	# Positive/ # Tests
Klebsiella variicola	ATCC BAA-830	$3x10^5$	3x	2/2
Klebsiella variicola	clinical strain 1	$3.9x10^5$	3.9x	2/2
Klebsiella variicola	clinical strain 2	$2.6x10^5$	2.6x	2/2
Klebsiella variicola	clinical strain 3	$1.5x10^5$	1.5x	2/2
Klebsiella variicola	clinical strain 4	$2.4x10^5$	2.4x	3/3
Klebsiella variicola	clinical strain 5	$1.5x10^5$	1.5x	2/2
Legionella pneumophila (serotype 1)	ATCC 33152	$8x10^5$	0.4x	2/2
Legionella pneumophila (serotype 2)	ATCC 33154	$6x10^6$	3x	2/2
Legionella pneumophila (serotype 3)	ATCC 33155	$4x10^5$	0.2x	2/2
Legionella pneumophila (serotype 6)	ATCC 33215	$2x10^6$	1x	2/2
Legionella pneumophila (serotype 8)	ATCC 35096	$4x10^5$	0.2x	2/2
Legionella pneumophila (serotype 10)	ATCC 43283	$4x10^5$	0.2x	2/2
Legionella pneumophila	UCLA L1	$4x10^5$	0.2x	2/2
Legionella pneumophila	UCLA L5	$4x10^5$	0.2x	2/2
Legionella pneumophila	UCLA L6	$4x10^5$	0.2x	2/2
Moraxella catarrhalis	ATCC 25238	$2x10^6$ $3x10^6$	2.5x 3.8x	0/2 2/2
Moraxella catarrhalis	ATCC 43617	$4x10^5$	0.5x	2/2
Moraxella catarrhalis	ATCC 8176	$4x10^5$	0.5x	2/2
Moraxella catarrhalis	ATCC 25240	$4x10^5$	0.5x	2/2
Moraxella catarrhalis	ATCC 23246	$4x10^5$	0.5x	2/2
Moraxella catarrhalis	ATCC 49143	$2x10^6$	2.5x	2/2
Morganella morganii	ATCC 8019	$1x10^5$	0.2x	2/2
Morganella morganii	ATCC 25829	$5x10^5$	1x	2/2
Morganella morganii	ATCC 25830	$1x10^5$	0.2x	2/2
Morganella morganii spp. sibonii	ATCC 49948	$1.5x10^6$	3x	2/2
Mycoplasma pneumoniae	ATCC 29085	$3x10^5$ CCU/mL[c]	3x	2/2
Mycoplasma pneumoniae	ATCC 29343	$3x10^5$ CCU/mL	3x	2/2
Mycoplasma pneumoniae	ATCC 15492	$3x10^6$ CFU/mL	3x	2/2
Mycoplasma pneumoniae	ATCC 15531	$3x10^6$ copies/mL	3x	2/2
Proteus mirabilis	ATCC 12453	$4x10^4$	0.4x	2/2
Proteus mirabilis	ATCC 14153	$3x10^5$	3x	2/2
Proteus mirabilis	ATCC 25933	$4x10^5$ $5x10^5$	4x 5x [d]	0/2 2/2
Proteus mirabilis	ATCC 29906	$3x10^5$	3x	4/4
Proteus vulgaris	ATCC 6380	$2.4x10^6$ $3x10^6$	4x 5x [d]	0/2 2/2
Proteus vulgaris	ATCC 8427	$4x10^4$	0.1x	2/2
Proteus vulgaris	ATCC 29905	$1.8x10^6$	3x	4/4
Proteus hauseri	ATCC 700826	$5x10^4$ [e]	0.1x	2/2
Proteus penneri	ATCC 33519	$5x10^4$ [e]	0.1x	2/2
Pseudomonas aeruginosa	ATCC 10145	$1x10^5$	2x	2/2

Strain	Strain ID	Test Conc. [CFU/mL]	LoD factor[a]	# Positive/ # Tests
Pseudomonas aeruginosa	ATCC 27853	$1x10^5$	2x	2/2
Pseudomonas aeruginosa	DSM-24600	$5x10^4$	1x	4/4
Pseudomonas aeruginosa	NCTC 13437	$2x10^4$	0.4x	4/4
Pseudomonas aeruginosa	Micromyx 2562	$1x10^5$	2x	4/4
Pseudomonas aeruginosa	NRZ-00196	$2x10^4$	0.4x	2/2
Pseudomonas aeruginosa	NRZ-03961	$5x10^4$	1x	2/2
Pseudomonas aeruginosa	UCLA P20	$4x10^4$	0.8x	2/2
Serratia marcescens	ATCC 8100	$3x10^5$	3x	2/2
Serratia marcescens	ATCC 13880	$3x10^5$	3x	2/2
Serratia marcescens	ATCC 14756	$3x10^5$	3x	2/2
Serratia marcescens	ATCC 15365	$3x10^5$	3x	2/2
Serratia marcescens	ATCC 27117 [f]	$2x10^5$ $3x10^5$ $5x10^5$ $1x10^6$	2x 3x 5x 10x	1/2 1/2 1/2 0/2
Serratia marcescens	ATCC 43861	$2x10^5$	2x	2/2
Serratia marcescens ssp. sakuensis	DSM-17174	$3x10^5$	3x	2/2
Staphylococcus aureus	IDEXX VB962455	$8x10^6$	1.6x	2/2
Staphylococcus aureus	IDEXX VB9981353	$8x10^6$	1.6x	2/2
Staphylococcus aureus	IDEXX VB969039	$8x10^6$	1.6x	2/2
Staphylococcus aureus	ATCC BAA-2312	$8x10^6$	1.6x	2/2
Staphylococcus aureus	NCTC 12493	$6x10^6$	1.2x	2/2
Staphylococcus aureus	ATCC 33591	$6x10^6$	1.2x	2/2
Staphylococcus aureus	DSM-17091	$6x10^6$	1.2x	2/2
Staphylococcus aureus	ATCC 12600	$8x10^6$	1.6x	2/2
Staphylococcus aureus	ATCC 29213	$8x10^6$	1.6x	2/2
Staphylococcus aureus	ATCC 43300	$1.5x10^7$	3x	2/2
Staphylococcus aureus	RKI 07-03165	$8x10^6$	1.6x	2/2
Staphylococcus aureus	RKI 01-00694	$8x10^6$	1.6x	2/2
Staphylococcus aureus	RKI 09-00187	$8x10^6$	1.6x	2/2
Staphylococcus aureus	RKI 08-02492	$8x10^6$	1.6x	2/2
Stenotrophomonas maltophilia	ATCC 13636	$5x10^4$	1.7x	2/2
Stenotrophomonas maltophilia	ATCC 13637 [g]	$2x10^4$ $3x10^4$ $5x10^4$ $1x10^5$ $2x10^5$	0.7x 1x 1.7x 3.3x 6.7x	2/2 1/2 5/6 1/2 1/2
Stenotrophomonas maltophilia	ATCC 17666	$2x10^4$	0.7x	2/2
Stenotrophomonas maltophilia	ATCC 49130	$2x10^4$	0.7x	2/2
Stenotrophomonas maltophilia	DSM-50173 [g] [ATCC 17444]	$2x10^4$ $3x10^4$ $5x10^4$ $1x10^5$ $2x10^5$	0.7x 1x 1.7x 3.3x 6.7x	1/4 0/1 3/4 0/2 1/2

Strain	Strain ID	Test Conc. [CFU/mL]	LoD factor[a]	# Positive/ # Tests
Stenotrophomonas maltophilia	DSM-21874 [g] [NCIMB 9528]	$2x10^4$ $3x10^4$ $5x10^4$ $1x10^5$ $2x10^5$	0.7x 1x 1.7x 3.3x 6.7x	1/4 0/2 2/4 0/2 0/2
Streptococcus pneumoniae	ATCC 33400	$1.5x10^6$ $2.5x10^6$	3x 5x	1/2 2/2
Streptococcus pneumoniae (serotype 19F)	ATCC 49619	$1.5x10^6$	3x	2/2
Streptococcus pneumoniae (serotype 3)	ATCC 6303	$1.5x10^6$ $2.5x10^6$	3x 5x	1/2 2/2
Streptococcus pneumoniae (serotype 5)	ATCC 6305	$2x10^5$	0.4x	2/2
Streptococcus pneumoniae	ATCC 49150	$1.5x10^6$	3x	2/2
Streptococcus pneumoniae (serotype 1)	ATCC 6301	$2x10^5$ $1x10^6$	0.4x 2x	1/2 2/2
Streptococcus pneumoniae	ATCC 10015	$2x10^5$	0.4x	2/2
Streptococcus pneumoniae (serotype 2)	ATCC 27336	$1.5x10^6$	3x	2/2
Streptococcus pneumoniae (serotype 9V)	DSM-11865	$2x10^5$	0.4x	2/2
Streptococcus pneumoniae (serotype 23F)	DSM-11866	$1.5x10^6$	3x	2/2
Streptococcus pneumoniae (serotype 6B)	DSM-11867	$1.5x10^6$	3x	2/2

[a] Analyte LoDs were established using "artificial respiratory medium" (ARM) as sample matrix surrogate for aspirates. Inclusivity testing was performed by contrived samples using PBS as sample matrix. Test concentrations are given as multiples of claimed ARM LoDs. PBS and ARM LoDs are comparable for most analytes; for few analytes PBS and ARM LoDs differ by a factor between 0.3-3.

[b] Concentration of *C. pneumoniae* strains was determined as IFU (inclusion-forming units) / mL.

[c] For *Mycoplasma pneumoniae* inclusivity testing different source materials were used: strain cultures determined in CCU (color changing units) / mL or CFU / mL, or a genomic DNA extract (in copies / mL); test concentrations are referenced against an LoD of $1x10^5$ CCU/mL that correlates approximately to $1x10^6$ CFU / mL or $1x10^6$ copies / mL.

[d] Sequencing of one *P. mirabilis* and one *P. vulgaris* strain with reduced performance revealed single mismatches for internal probes.

[e] Test concentrations for *P. hauseri* and *P. penneri* are shown as LoD multiples as determined for *P. vulgaris*.

[f] One strain did not perform consistently at concentrations close to the LoD; sequencing attempts have failed.

[g] Strain ATCC 13637 (type strain) was used to establish LoD. During inclusivity testing, single failures at concentrations above LoD were observed, although no primer or probe mismatches were present. Inclusivity testing was repeated for this strain at 1x ARM LoD and 2/2 positive replicates were obtained.
Strain DSM-50173 showed reduced sensitivity and sequencing revealed multiple mismatches for one internal probe.
Strain DSM-21874 showed reduced sensitivity, however, sequencing did not reveal any mismatches to primers or probes. Testing was repeated for this strain at 1x LoD (0/2), 1.7x (0/2) and 3.3x LoD (2/2).

To supplement inclusivity testing for each LRT panel microorganism target, *in-silico* analysis was performed to assess Unyvero LRT primer and probe sequences for the predicted detection of microorganism strains with applicable sequences available in the GenBank database (search performed January 2018).

Results from the *in-silico* analyses identified strain entries that are predicted to be

detected when the organism is present at LoD concentrations (match of relevant primer and hybridization probe sequences), predicted to be detected with reduced sensitivity (typically, single relevant primer or probe sequence mismatches; detection likely at higher than LoD concentrations only) or predicted to be not detected at clinically relevant concentrations (multiple relevant primer mismatches in primer and probe sequences).

Table 7 lists microorganisms that were evaluated in inclusivity testing that are also predicted to be detected at LoD concentrations based on *in-silico* analysis. Table 8 lists microorganisms evaluated in inclusivity testing for which one or more strain entries are predicted to be detected with reduced sensitivity.

Additional microorganisms were evaluated by *in-silico* analysis only. For these microorganisms, Table 9 lists those that are predicted to be detected when present at LoD concentrations and Table 10 lists microorganisms that are predicted to be detected with reduced sensitivity for one or more strain entries.

The following language was included in the device labeling along with results from the *in-silico* analyses performed:

- *In-silico* analysis results were provided as supplementary data. The results are not intended to be a surrogate for wet testing and do not assure that specific strains will be detected.

- The performance of the Unyvero LRT Application has not been established for those microorganism species that were evaluated by *in-silico* analysis only.

Table 7: Microorganisms with reference strains detected at or near LoD concentrations in inclusivity wet testing that are predicted to be detected at LoD based on *in-silico* analysis for all strain entries

Microorganism	Microorganism
Acinetobacter baumannii	*Mycoplasma pneumoniae*
Acinetobacter calcoaceticus	*Proteus mirabilis*
Acinetobacter lwoffii	*Proteus vulgaris*
Acinetobacter haemolyticus	*Proteus hauseri* [c]
Chlamydia pneumoniae	*Proteus penneri* [c]
Enterobacter cloacae [a]	*Pseudomonas aeruginosa*
Escherichia coli	*Serratia marcescens*
Klebsiella pneumoniae [b]	*Staphylococcus aureus*
Klebsiella variicola	*Streptococcus pneumoniae* [d]

[a] Including *E. cloacae* ssp. *dissolvens*
[b] Including *K. pneumoniae* variant 2 (*K. quasipneumoniae*)
[c] No Genbank entries available, BLAST search was performed using the whole genome shotgun (wgs) database
[d] *In-silico* analysis includes serotype 7F

Table 8: Microorganisms with reference strains detected at or near LoD concentrations in the Inclusivity study and are predicted to be detected with reduced sensitivity for one or more strain entries

Microorganism	# entries predicted to be detected at LoD	# entries predicted to be detected with reduced sensitivity
Citrobacter freundii	11	3
Enterobacter asburiae	-	9
Enterobacter hormaechei	11[a]	1
Klebsiella oxytoca	8	3
Legionella pneumophila	31	36[b]
Moraxella catarrhalis	11	4
Morganella morganii	1	5
Stenotrophomonas maltophilia	17	2

[a] *In-silico* analysis includes subspecies. *oharae* and *steigerwaltii*

[b] Inclusivity wet testing included three strains predicted with reduced sensitivity by *in-silico* analysis (ATCC 33215, ATCC 33152, ATCC 33154); all strains were detected at concentrations at LoD.

Table 9: Microorganisms predicted to be detected at LoD based on *in-silico* analysis only

Microorganism	
Acinetobacter nosocomialis	*Acinetobacter oleivorans*
Acinetobacter pittii	*Acinetobacter schindleri*
Acinetobacter junii	*Enterobacter kobei*
Acinetobacter parvus	*Enterobacter ludwigii*
Acinetobacter lactucae	*Enterobacter xiangfangensis*

Table 10: Microorganisms predicted to be detected with reduced sensitivity for one or more strain entries based on *in-silico* analysis only

Microorganism	# entries predicted to be detected at LoD	# entries predicted to be detected with reduced sensitivity
Acinetobacter ursingii	-	9 [a,b]
Acinetobacter soli	-	1
Acinetobacter guillouiae	-	1

[a] No Genbank entries available, BLAST search was performed using the whole genome shotgun (wgs) database

[b] Tests with reference strain DSM-16037 were negative at 10^7 CFU/mL

Based on the results from the Inclusivity study and *in-silico* analyses for microorganism targets, the following limitation is included in the Unyvero LRT labeling:

- Based on *in-silico* analyses, some strains may either not be detected or be detected with reduced sensitivity due to variations in targeted sequences for

the following microorganisms: *C freundii, K. oxytoca*, *L. pneumophilia*, *M. catarrhalis, M. morganii.* Based on wet testing for *Proteus* spp. and *S. marcescens* and based on *in-silico*/wet testing for *M. catarrhalis* and S. *maltophilia* some strains may be detected with reduced sensitivity due to variations in targeted sequences.

Inclusivity/Resistance Marker Targets:

Inclusivity testing for resistance marker targets included testing of samples prepared with well-characterized strains determined to carry resistance markers targeted by the Unyvero LRT Application. Samples were prepared at near LoD concentrations (≤3x LoD) for each resistance marker and tested in duplicate for each strain. Results are shown in Table 11 below. When phenotypic antibiotic susceptibility testing results (AST) were available, the corresponding information is also shown in the table (e.g., Carbapenem^{R} = resistant to Carbapenems, Carbapenem^{S} = susceptible to Carbapenems).

Inclusivity testing resulted in detection of targeted resistance markers for most strains evaluated. For the following microorganism/resistance markers, one or more strains were not detected or were detected only at higher than LoD concentrations (also bolded in Table 11 below):

- One strain carrying *ctx*-M (*E. cloacae*) - detected at 6x LoD but not detected at lower concentrations (one of eight *ctx*-M carrying strains tested)
- Four of eight strains carrying *vim*:
  -Two strains carrying *vim*-1 (*E. cloacae*, *K. pneumoniae*) – not detected at any concentration tested.
  -One strain carrying *vim*-1 (*C. freundii*) – detected in one of two replicates at 4x LoD and detected in both replicates at 10x LoD.
  -One strain carrying *vim*-10 (*P. aeruginosa*) – detected in one of two replicates at 2x LoD and 2/2 replicates detected at 8x LoD.

Table 11: Resistance marker targets, Inclusivity strains

Marker	Subgroup	Host Strain	Strain ID	Test Conc. [CFU/mL]	LoD [a]	# Positive/ # Tests
ctx-M	*ctx*-M3	*Klebsiella pneumoniae*	NRZ-00751	$6x10^4$	0.6x	4/4
ctx-M	*ctx*-M15	*Klebsiella pneumoniae*	NRZ-00249	$6x10^4$	0.6x	2/2
ctx-M	NA	*Klebsiella pneumoniae*	NCTC 13443	$1x10^5$	1x	2/2
ctx-M	**NA**	***Enterobacter cloacae***	**JMI 46239**	**$1x10^5$** **$6x10^5$**	**1x** **6x**	**0/2** **2/2**
ctx-M	NA	*Escherichia coli*	JMI 50067	$6x10^4$	0.6x	2/2
ctx-M	NA	*Klebsiella pneumoniae*	NRZ-00002	$6x10^4$ $4x10^5$	0.6x 4x	1/2 2/2
ctx-M	NA	*Klebsiella pneumoniae*	JMI 49767	$6x10^4$	0.6x	2/2
ctx-M	NA	*Klebsiella pneumoniae*	NRZ-00472	$1.2x10^5$	1.2x	2/2
kpc	*kpc*-2	*Escherichia coli*	NRZ-00281	$8x10^5$	1.6x	2/2

Marker	Subgroup	Host Strain	Strain ID	Test Conc. [CFU/mL]	LoD [a]	# Positive/ # Tests
kpc	*kpc*-2	*Klebsiella pneumoniae*	NRZ-00103	$6x10^4$	0.1x	2/2
kpc	*kpc*-3	*Escherichia coli*	NRZ-00222	$8x10^5$	1.6x	2/2
kpc	*kpc*-3	*Klebsiella pneumoniae*	NRZ-00223	$1x10^5$	0.2x	2/2
kpc	*kpc*-3	*Klebsiella pneumoniae* (Carbapenem^R)	Micromyx 4653	$4x10^5$	0.8x	2/2
kpc	*kpc*-3	*Klebsiella pneumoniae* (Carbapenem^R)	Micromyx 4676	$8x10^5$	1.6x	2/2
ndm	*ndm*-1	*Acinetobacter baumannii*	JMI 49755	$1x10^5$	2x	2/2
ndm	*ndm*-1	*Enterobacter cloacae* (Imipenem^R, Ertapenem^R)	ATCC BAA-2468	$1x10^5$	2x	2/2
ndm	*ndm*-1	*Enterobacter cloacae*	JMI 46239	$1x10^5$	2x	2/2
ndm	*ndm*-1	*Escherichia coli*	JMI 50067	$6x10^4$	1.2x	2/2
ndm	*ndm*-1	*Klebsiella pneumoniae*	JMI 49767	$6x10^4$	1.2x	2/2
ndm	*ndm*-1	*Klebsiella pneumoniae*	NCTC 13443	$1.5x10^5$	3x	2/2
ndm	*ndm*-1	*Klebsiella pneumoniae*	JMI 49831	$1x10^5$	2x	2/2
oxa-23	*oxa*-23	*Acinetobacter baumannii*	NCTC 13301	$2x10^7$	1x	3/3
oxa-23	NA	*Acinetobacter baumannii* (Carbapenem^R)	Micromyx 4410	$2x10^7$	1x	2/2
oxa-23	NA	*Acinetobacter baumannii* (Carbapenem^R)	Micromyx 6148	$2x10^7$	1x	2/2
oxa-23	NA	*Acinetobacter baumannii* (Carbapenem^R)	Micromyx 6149	$2x10^7$	1x	2/2
oxa-23	NA	*Acinetobacter baumannii* (Carbapenem^R)	Micromyx 6153	$2x10^7$	1x	2/2
oxa-23	NA	*Acinetobacter baumannii* (Carbapenem^R)	Micromyx 6334	$2x10^7$	1x	2/2
oxa-23	NA	*Acinetobacter baumannii* (Imipenem^R, Meropenem^R)	UCLA A5	$2x10^7$	1x	2/2
oxa-24	*oxa*-25	*Acinetobacter baumannii*	NCTC 13302	$5x10^4$	0.1x	4/4
oxa-24	*oxa*-72	*Acinetobacter baumannii*	NRZ-00449	$5x10^4$	0.1x	4/4
oxa-24	NA	*Acinetobacter baumannii* (Imipenem^R, Meropenem^R)	UCLA A4	$5x10^4$	0.1x	3/4
oxa-24	NA	*Acinetobacter baumannii* (Imipenem^R, Meropenem^R)	clinical strain 1	$5x10^4$	0.1x	2/2
oxa-24	NA	*Acinetobacter baumannii* (Imipenem^R, Meropenem^R)	clinical strain 2	$5x10^4$	0.1x	2/2
oxa-48	*oxa*-48	*Escherichia coli* (Ertapenem^R)	ATCC BAA-2523	$2x10^6$	1x	2/2
oxa-48	*oxa*-48	*Escherichia coli*	NRZ-00176	$2x10^6$	1x	2/2
oxa-48	*oxa*-48	*Klebsiella pneumoniae*	NRZ-00002	$2x10^6$	1x	2/2
oxa-48	*oxa*-48	*Klebsiella pneumoniae*	NCTC 13442	$2x10^6$	1x	2/2
oxa-48	*oxa*-162	*Escherichia coli*	NRZ-00361	$3x10^6$	1.5x	2/2
oxa-48	*oxa*-162	*Klebsiella pneumoniae*	NRZ-00472	$5x10^6$	2.5x	2/2
oxa-48	*oxa*-232	*Klebsiella oxytoca*	NRZ-22060	$2x10^6$	1x	2/2
oxa-58	*oxa*-58	*Acinetobacter baumannii*	NRZ-00518	$4x10^5$	0.5x	2/2
oxa-58	*oxa*-58	*Acinetobacter baumannii*	NCTC 13305	$4x10^5$	0.5x	2/2

Marker	Subgroup	Host Strain	Strain ID	Test Conc. [CFU/mL]	LoD [a]	# Positive/ # Tests
tem	*tem*-1	*Escherichia coli*	ATCC 35218	$1x10^5$	1.6x	2/2
tem	*tem*-3	*Escherichia coli* (ESBL)	NCTC 13351	$5x10^4$	0.8x	2/2
tem	NA	*Citrobacter freundii*	ATCC 43864	$1.8x10^5$	3x	2/2
tem	NA	*Enterobacter cloacae*	JMI 46239	$1x10^5$	1.7x	2/2
tem	NA	*Klebsiella pneumoniae*	JMI 49767	$6x10^4$	1x	2/2
tem	NA	*Escherichia coli*	NRZ-00281	$1.8x10^5$ $2.5x10^5$	3x 4x	1/2 2/2
tem	NA	*Haemophilus influenzae* (Ampicillin^R, Cefinase^R)	clinical strain 1	$1x10^5$	1.6x	5/5
tem	NA	*Haemophilus influenzae* (Cefinase^R)	clinical strain 2	$1x10^5$	1.6x	5/5
vim	***vim*-1**	***Citrobacter freundii***	**NRZ-00452** [b]	**$2x10^5$** **$5x10^5$**	**4x** **10x**	**1/2** **2/2**
vim	*vim*-1	*Pseudomonas aeruginosa* (Ceftazidime^R, Imipenem^R)	DSM-24600	$5x10^4$	1x	4/4
vim	***vim*-1**	***Enterobacter cloacae***	**NRZ-00239** [b]	**$5x10^4$** **$1x10^5$**	**1x** **2x**	**0/2** **0/4**
vim	*vim*-1	*Klebsiella pneumoniae*	NCTC 13439	$5x10^4$	1x	2/2
vim	***vim*-1**	***Klebsiella pneumoniae***	**NCTC 13440** [b]	**$4x10^5$**	**8x**	**0/2**
vim	***vim*-10**	***Pseudomonas aeruginosa***	**NCTC 13437** [b]	**$1x10^5$** **$4x10^5$**	**2x** **8x**	**1/2** **2/2**
vim	NA	*Pseudomonas aeruginosa*	UCLA P20	$3x10^4$	0.6x	2/2
vim	NA	*Pseudomonas putida* (Carbapenem^R)	Micromyx 1612	$4x10^4$	0.8x	2/2
vim	NA	*Pseudomonas aeruginosa* (Carbapenem^R)	Micromyx 2562	$1x10^5$	2x	4/4
*mec*A	SCCmecI	*Staphylococcus aureus*	RKI 07-03165	$4x10^6$	2x	2/2
*mec*A	SCCmecII	*Staphylococcus aureus*	RKI 01-00694	$4x10^6$	2x	2/2
*mec*A	SCCmecIII	*Staphylococcus aureus* (Methicillin^R)	ATCC 33591	$3x10^6$	1.5x	2/2
*mec*A	SCCmecIV	*Staphylococcus aureus*	RKI 09-00187	$4x10^6$	2x	2/2
*mec*A	SCCmecV	*Staphylococcus aureus*	RKI 08-02492	$4x10^6$	2x	1/2
*mec*A	NA	*Staphylococcus aureus* (Methicillin^R, Cefoxitin^R)	NCTC 12493	$6x10^5$	0.3x	2/2
*mec*A	NA	*Staphylococcus aureus* (Methicillin^R)	DSM-17091	$3x10^6$	1.5x	2/2

[a] Analyte LoDs were established using "artificial respiratory medium" (ARM) as sample matrix surrogate for aspirates. Inclusivity was performed by contrived samples using PBS as sample matrix. Test concentrations are given as multiples of claimed ARM LoDs. PBS and ARM LoDs are comparable for most analytes; for few analytes PBS and ARM LoDs differ by a factor between 0.3-3.

[b] For three *vim*-1 strains and one *vim*-10 strain positive results were only obtained at 8-10x LoD or were negative at applied concentrations. For strain NCTC 13437 (*vim*-10) sequencing did not show any primer or probe mismatches. For strains NRZ-00239, NRZ-00452, NCTC 13440 (all *vim*-1) sequencing revealed mismatches for all internal probes.

To supplement inclusivity testing for specific resistance marker variants and subgroups, *in-silico* analysis was performed with Unyvero LRT primer and probe sequences compared to sequences available in the GenBank database. Variants predicted to be detected at LoD (match of relevant primer and probe sequences), variants predicted to be detected with reduced sensitivity (typically, single relevant

mismatches of primer or probe sequences; detection likely at higher than LoD concentrations only), and variants predicted to be not detected at clinically relevant concentrations (multiple relevant mismatches in primer and probe sequences) are listed in Table 12.

The following statements are included in the assay labeling:

- *In-silico* analysis results were provided as supplementary data. Results are not intended to be a surrogate for wet testing and do not assure that specific resistance marker variants will be detected by the assay.
- The performance of the Unyvero LRT Application has not been established for those resistance marker variants that were evaluated by *in-silico* analysis only.

Table 12: *In-silico* predicted detection, resistance marker variants

Resistance Marker: Subgroup	Variants predicted at LoD	Variants predicted at reduced sensitivity	Variants not predicted
***ctx*-M:** *ctx*-M1 subgroup	1, 3, 10, 11, 15, 22, 23, 28-30, 32-34, 36, 37, 42, 52-55, 57, 58, 61, 66, 69, 71, 72, 79, 80, 83, 88, 101, 103, 108, 109, 114, 116, 117, 132, 136, 138, 139, 142, 144, 150, 155-158, 162-164, 166, 167, 170, 172, 173, 175-177, 179-184, 186, 188, 189, 190	12, 60, 62, 64, 68, 82, 96, 107, 133, 169	-
***ctx*-M:** *ctx*-M2 *ctx*-M8 *ctx*-M9 *ctx*-M25 *ctx*-M45 subgroups	-	-	2, 4-9, 13, 14, 16-21, 24-27, 31, 35, 38-41, 43-51, 56, 59, 63, 65, 67, 73-78, 81, 84-87, 89-95, 97-100, 102, 104-106, 110-113, 115, 121-126, 129-131, 134, 137, 141, 147, 148, 152, 159-161, 165, 168, 171, 174, 185, 191
kpc	1-32	-	-
ndm	1, 3-19, 21	2	-
***oxa*:** *oxa*-23	23, 27, 49, 73, 134, 146, 165-171, 225, 239, 366, 398, 422, 423, 435, 440, 469, 481, 482, 483, 565	103, 133	-
***oxa*:** *oxa*-24	24-26, 33, 40, 72, 139, 160, 207, 437	-	-
***oxa*:** *oxa*-48	48, 48b, 162, 163, 181, 199, 232, 244, 245, 247, 252, 370, 405, 416, 438, 439, 484, 505, 514, 515, 517, 519, 538, 546, 566, 567	204, 547	54, 436, 535
***oxa*:** *oxa*-58	58, 96, 97, 164, 397, 467	512	420

Resistance Marker: Subgroup	Variants predicted at LoD	Variants predicted at reduced sensitivity	Variants not predicted
tem	1-4, 6, 8-12, 15-17, 19, 20-22, 24, 26, 28-30, 32-36, 40, 43, 45, 47-49, 52-55, 57, 60, 63, 67, 68, 70-72, 76-88, 90-96, 101, 102, 104-116, 120-139, 141-150, 152-160, 162, 164, 166-169, 171, 176-177, 181-199, 201, 204-217, 219, 220, 224-228	97, 98, 99, 151, 163	178
vim	1-4, 6, 8-12, 14, 15-20, 23, 24, 26-29, 30, 31, 33-37, 39- 46, 48, 50-52, 54, 55	5, 25, 38, 49	7, 13, 47

Although the Unyvero LRT Application can detect multiple *tem* variants as shown in Table 13 above, positive or negative *tem* results are reported by the assay only if *H. influenzae* is concurrently detected in the specimen. It is noted that the *tem*-1 variant is carried by *H. influenzae* while other gram negative rods species can carry *tem*-1 or other *tem* variants. If the source of positive *tem* result is not *H. influenzae* and *H. influenzae* is not detected, the *tem* results will not be reported by the LRT software (i.e., it will be masked). However, if the source of a positive *tem* result is another microorganism and *H. influenzae* is also detected in the specimen, the *tem* result will be reported.

The following limitation is included in the Unyvero LRT Application labeling:

- Because the *tem* gene is ubiquitous in members of the Enterobacteriaceae, positive LRT results for *tem* may be due to the presence of Enterobacteriaceae in the specimen.

In summary, based on the results from wet-testing and *in-silico* analyses for targeted resistance markers, the following limitation is included in the Unyvero LRT labeling:

- Wet testing was not performed for all known resistance marker types and/or subtypes. Based on *in-silico* analyses and inclusivity wet testing, some antibiotic resistance marker variants may either not be detected or detected with reduced sensitivity due to variations in targeted sequences for *ctx*-M1 subgroup, *ndm*, *oxa*-23, *oxa*-48, *oxa*-58, *vim*.

The potential for false negative results for targeted resistance markers is further mitigated by inclusion of the following limitation in the Unyvero LRT Application labeling and report:

- Antimicrobial resistance may occur via multiple mechanisms other than the resistance markers detected by the Unyvero LRT assay. Negative results for LRT resistance markers do not indicate antimicrobial susceptibility of

detected organisms.

f. Cross-Reactivity/Exclusivity:

A study was conducted to evaluate the potential for cross-reactivity (exclusivity) of Unyvero LRT Application targets with closely related microorganisms as well as commensal microorganisms that are commonly present in the respiratory tract. Study samples were prepared with microorganisms at high concentrations (~10^7 CFU/mL) and testing was performed in duplicate.

In addition to testing of exclusivity samples, *in-silico* (BLAST) analysis was used to evaluate the potential for cross-reactivity for any microorganism strain entries with applicable sequences available in the Gen-Bank database.

The following cross-reactivity with Unyvero LRT targets was either observed in the evaluation of test samples or predicted based on *in-silico* analysis.

- Cross-reactivity with the *Citrobacter freundii* target is predicted with *Citrobacter braakii* and *Kluyvera georgiana*.
- Cross-reactivity with the *Enterobacter cloacae* complex target is predicted with *Enterobacter soli*, *Enterobacter mori*, and *Enterobacter nickellidurans*.
- Cross-reactivity with the *E. coli* target is predicted with *Shigella dysenteriae*, *Shigella boydii*, *Shigella flexneri*, *Shigella sonnei*, *Escherichia albertii* and *Escherichia fergusonii*.
- Cross-reactivity with the *Haemophilus influenzae* target was demonstrated with *Haemophilus haemolyticus* and *Haemophilus parainfluenzae*. Note that wet- testing of one *Haemophilus parainfluenzae* strain generated expected negative results.
- Cross-reactivity with the *Klebsiella oxytoca* target is predicted with *Klebsiella michagenensis*.
- Cross-reactivity with the *Staphylococcus aureus* target is predicted with *Staphylococcus argenteus* and *Staphylococcus simiae*.
- Cross-reactivity with the *Stenotrophomonas maltophilia* target is predicted with Stenotrophomonas *nitritireducens, Stenotrophomonas daejeonensis, Stenotrophomonas acidaminiphila, Stenotrophomonas koreensis* and *Stenotrophomonas rhizophila.* All *Stenotrophomonas* species predicted to be cross-reactive are not associated with human respiratory infection.

Based on *in-silico* analysis, no cross-reactivity is predicted for the following LRT panel targets: *Acinetobacter* spp., *C. pneumoniae*, *K. pneumoniae*, *K. variicola*, *L. pneumophila*, *M. catarrhalis*, *M. morganii*, *M. pneumoniae*, *Proteus* spp., *P. aeruginosa*, *S. marcescens*, *ctx*-M, *kpc*, *ndm*, *oxa*-23, *oxa*-24, *oxa*-48, *oxa*-58, *tem*, *vim*, and *mec*A.

The following limitation regarding cross-reactivity of the Unyvero LRT Application is include in device labeling:

- Based on *in-silico* analysis and exclusivity wet testing, the following LRT panel microorganism assays are expected to cross-react with closely related clinically relevant species: *Citrobacter freundii* (cross-reactive to *C. braakii*, *Kluyvera georgiana;*), *Escherichia coli* (cross-reactive to *E. albertii*, *E. fergusonii* and *Shigella* spp. (*S. dysenteriae*, *S. sonnei*, *S. flexneri*, *S. boydii*)), *Haemophilus influenzae* (cross-reactive to *H. haemolyticus* and *H. parainfluenzae*), *Klebsiella oxytoca* (cross-reactive to *K. michiganensis*).

Results of *in-silico* analysis and wet-testing for near-neighbor microorganism strains is shown in Table 13.

No cross reactivity was observed in testing of the commensal microorganism strains listed in Table 14.

Table 13: Exclusivity testing: *In-silico* prediction, wet-testing of exclusivity samples, and cross-reactivity observed in the clinical study

Close Neighbor Strain	Cross-Reactivity Prediction (*in-silico* analysis)	Wet Testing Result at 10^7 CFU/mL, Strain ID	Cross-Reactions observed in Clinical Study (N = number of specimens)
Citrobacter freundii			
Citrobacter braakii	Detection predicted at higher than LoD concentrations [b]	-	-
Kluyvera georgiana	Detection predicted at higher than LoD concentrations	-	-
Citrobacter koseri	Detection not predicted	negative ATCC 27156	-
Enterobacter cloacae complex			
Enterobacter soli [a]	Detection predicted at LoD	-	-
Enterobacter mori [a]	Detection predicted at LoD	-	-
Enterobacter nickellidurans [a]	Detection predicted at LoD	-	-
Escherichia coli			
Shigella dysenteriae [a]	Detection predicted at LoD	-	-
Shigella boydii [a]	Detection predicted at LoD	-	-
Shigella flexneri [a]	Detection predicted at LoD	-	-
Shigella sonnei [a]	Detection predicted at LoD	-	-
Escherichia albertii	Detection predicted at LoD	-	-
Escherichia fergusonii	Detection predicted at LoD	-	-
Haemophilus influenzae			
Haemophilus haemolyticus	Detection predicted at higher than LoD concentrations	positive ATCC 33390	2
Haemophilus parahaemolyticus	Detection not predicted	negative ATCC 10014	-
Haemophilus parainfluenzae	Detection not predicted	negative ATCC 33392	1
Aggregatibacter actino-mycetemcomitans	Detection not predicted	negative ATCC 33384	-

Close Neighbor Strain	Cross-Reactivity Prediction (*in-silico* analysis)	Wet Testing Result at 10^7 CFU/mL, Strain ID	Cross-Reactions observed in Clinical Study (N = number of specimens)
Aggregatibacter aphrophilus	Detection not predicted	negative ATCC 19415	-
Klebsiella oxytoca			
Klebsiella michiganensis	Detection predicted at LoD/ Detection predicted at higher than LoD concentrations [c]	-	-
Staphylococcus aureus			
Staphylococcus argenteus [a]	Detection predicted at LoD	-	-
Staphylococcus simiae [a]	Detection predicted at LoD		-
CNS: *S. epidermidis* *S. capitis* *S. lugdunensis* *S. haemolyticus* *S. saprophyticus*	Detection not predicted	negative ATCC 51625 ATCC 27840 ATCC 43809 ATCC 29970 ATCC 15305	-
Stenotrophomonas maltophilia			
Stenotrophomonas spp., (environmental/soil microorganisms): [a] *S. nitritireducens* *S. daejeonensis* *S. acidaminiphila* *S. koreensis* *S. rhizophila*	Detection predicted at LoD	-	-
Xanthomonas spp. [a]	Detection predicted at higher than LoD concentrations-	-	-
Pseudoxanthomonas spp. [a]	Detection predicted at higher than LoD concentrations-	-	-
Streptococcus pneumoniae			
other *Streptococcus* sp.: *S. agalactiae* *S. anginosus* *S. dysgalactiae* *S. gordonii* *S. intermedius* *S. mitis* *S. mutans* *S. oralis* *S. parasanguinis* *S. pseudopneumoniae* *S. pyogenes* *S. salivarius* *S. sanguinis* *S. vestibularis*	Detection not predicted	negative ATCC 13813 ATCC 33397 ATCC 43078 ATCC 10558 ATCC 27335 ATCC 49456 ATCC 25175 ATCC 35037 ATCC 15912 ATCC BAA-960 ATCC 12344 ATCC 7073 ATCC 10556 ATCC 49124	-

[a] No clinical relevance for respiratory infections

[b] Strains are predicted to be detected at higher than LoD concentrations due to primer and probe mismatches

[c] Few strains are predicted to be detected at higher than LoD concentrations

Table 14: Exclusivity testing, Commensal respiratory flora (No cross reactivity observed)

respiratory flora strain	strain ID	respiratory flora strain	strain ID
Actinomyces odontolyticus	ATCC 17929	*Granulicatella adiacens*	ATCC 49175
Aspergillus fumigatus	ATCC 204305	*Kingella kingae*	ATCC 23330
Candida albicans	ATCC 90028	*Lactobacillus acidophilus*	ATCC 4356
Candida dubliniensis	DSM-13268	*Micrococcus luteus*	ATCC 4698
Candida glabrata	ATCC 2001	*Mycobacterium bovis*	clinical isolate
Candida krusei	ATCC 24210	*Mycoplasma orale*	ATCC 23714
Candida parapsilosis	ATCC 22019	*Neisseria lactamica*	ATCC 23970
Candida tropicalis	ATCC 750	*Neisseria sicca*	ATCC 29193
Cardiobacterium hominis	ATCC 15826	*Pantoea agglomerans*	ATCC 27155
Eikenella corrodens	ATCC 23834	*Peptostreptococcus stomatis*	DSM-17678
Enterococcus faecalis	ATCC 29212	*Porphyromonas gingivalis*	ATCC 33277
Enterococcus faecium	ATCC 35667	*Prevotella buccalis*	ATCC 35310
Fusobacterium nucleatum	ATCC 25586	*Raoultella planticola*	ATCC 33531

g. *Assay Cut-off*

The Unyvero LRT Application is comprised of eight individual multiplex PCR assays and hybridization arrays located in separate reaction chambers in the LRT Cartridge. After hybridization of PCR products, fluorescent array signals are captured with a fluorescent camera system over a defined temperature range. After correction for background values, a signal threshold is applied for probes of individual analytes. In addition to the signal thresholds, individual cutoffs are defined to reduce background signals for certain analytes that are commonly part of the host flora of healthy individuals.

h. *Interfering substances:*

An interfering substances study was conducted to evaluate potential inhibitory effects on the performance of the Unyvero LRT Application with substances that may be present in lower respiratory specimens. The substances evaluated included respiratory medications, antibiotics, sample storage media, sample liquefying agent (LRT lysis buffer reagent), blood, mucin and human DNA. Each substance was evaluated in samples at a (b) (4) concentration as recommended in the CLSI guideline, 'Interference Testing in Clinical Chemistry'. Samples were prepared with pools of six representative microorganisms targeted by the LRT assay: *E. coli*, *K. pneumoniae*, *M. morganii*, *P. aeruginosa*, *S. aureus*/*mec*A+, and *S. maltophilia*. Each microorganism was spiked into test samples at concentrations close to LoD. Test samples were evaluated in duplicate and compared to results from control samples prepared with microorganisms only.

A qualitative analysis of study results was performed. A substance was considered as a potential interferent if reduced positivity was observed in samples spiked with the

substance in comparison to control samples. A quantitative analysis was also conducted for which trends in assay signal were assessed as compared to control samples.

No interference was observed for any substance evaluated at the tested concentrations. Substances evaluated and their respective test concentrations are presented in Table 15.

Table 15: Interfering substances study

	Interfering Substance	Test concentration	Interference
Reference/Control	PBS (no interferents added)	N/A	N/A
respiratory drugs	Guaifenesin	1.5×10^{-2} M	no
	Dextromethorphan	3.7×10^{-6} M	no
	Acetyl-Cysteine	1×10^{-2} M	no
	Salbutamol	4×10^{-6} M	no
	Carbocystein	2.8×10^{-3} M	no
	Ambroxol	8×10^{-4} M	no
	Beclomethason	7×10^{-4} M	no
	Theophyllin	2.2×10^{-4} M	no
antibiotics	Ampicillin	1.5×10^{-4} M	no
	Cefuroxime	1.4×10^{-3} M	no
	Erythromycin	8.2×10^{-5} M	no
	Ciprofloxacin	3×10^{-5} M	no
	Amikacin	1.4×10^{-4} M	no
	Imipenem	5×10^{-4} M	no
	Clindamycin	8.9×10^{-5} M	no
	Trimethoprim	1.4×10^{-4} M	no
	Sulfamethoxazole	1.6×10^{-3} M	no
inhalation agent (NaCl)	sodium chloride	5% w/v	no
lysis buffer	lysis buffer DTT, 90% v/v	lysis buffer: 90% v/v (final conc. in lysis tube) or 80% v/v (for added sample), DTT: 40 mM (final conc. in lysis tube) or 35 mM (for added sample)	no
sample components/ARM matrix components	EDTA blood	100% v/v	no
	human placenta DNA	1 µg/µL	no
	fish sperm DNA	4 µg/µL	no
	mucin (pig stomach, type II)	20 mg/mL	no

h. *Competitive Interference:*

To evaluate the potential for competitive inhibition between targeted microorganisms, various combinations of microorganisms were evaluated in contrived samples. Study samples were prepared with low positive analytes at near LoD concentrations together with high positive analytes at ~10^7 CFU or higher. Control samples containing analytes at low concentrations were also tested.

The following analytes were selected for evaluation for competitive inhibition based on the prevalence of each microorganism in tracheal aspirate specimens. Two sample pools were prepared with multiple targeted microorganisms at either low or high concentrations in each sample as shown in Table 16 and 17.

Table 16: Pool 1 (High positive/Low positive microorganism pool)

Low Positive Analytes (tested near LoD)	High Positive Analytes
E. coli/*tem*	*P. aeruginosa*
K. pneumoniae	*S. aureus*/mecA
S. maltophilia	*A. baumanii*
Proteus spp.	

Table 17: Pool 2 (High positive/Low positive microorganism pool)

Low Positive Analytes	High Positive Analytes
P. aeruginosa	*E. coli*/*tem*
S. aureus/*mec*A	*K. pneumoniae*
A. baumanii	*S. maltophilia*
	P. mirabilis

Six replicates were evaluated for each of the two test samples and each of the two controls.

Except for *Proteus* spp., all low positive microorganisms listed in Tables 14 and 15 were detected in all test replicates. For *Proteus* spp., only 4/6 replicates were positive for samples containing low positive Proteus concentrations combined with other microorganisms in high concentrations. These results are acceptable as *Proteus* spp. was present in the sample pool at a concentration of 0.5x LoD. In addition, the low positive control was positive for only 2/6 replicates, indicating that the false negative results for *Proteus* spp. were not likely due to competitive inhibition.

i. Carry-over:

A study was conducted to evaluate the potential for sample to sample carry-over during the Unyvero LRT Application testing process. The study comprised alternating high positive and negative contrived samples tested on the same Unyvero test system. The system setup included 1 Unyvero cockpit, 1 Lysator and 2 Analyzers. The test set included a total of 20 (5 on each analyzer slot) positive and negative cartridge runs. Prior to test initiation, additional negative cartridge runs per slot were performed to confirm the absence of any contaminants in test materials and devices. Samples were prepared in ARM matrix with positive samples containing seven representative microorganisms at

high concentrations (~10^7 CFU/mL). Results for all 48 test runs were fully valid with no false positive results observed for negative ARM samples or for negative controls.

j. *Fresh versus frozen study*:

A fresh versus frozen study was conducted to assess the impact of freezing tracheal aspirate specimen on the performance of the LRT assay. Aspirate specimens that were tested prospectively (fresh) during the LRT clinical study were re-tested after having been exposed to prolonged storage since initial testing. Testing included a total of 30 specimens containing low and high concentrations of representative analytes.

Specimens were chosen to include specimens with initial positive results covering multiple different pathogen and resistance markers as well specimen results covering a representative signal range as observed during prospective LRT clinical testing. Specimens included in the study were positive for both single and multiple target analytes. Due to multi-detections, the set of 30 samples was initially positive for a total of 73 LRT analytes (pathogens and antibiotic resistance markers combined).

Of the 30 test runs performed, 29 runs were completely valid and one was partially valid. Results of the study showed overall positivity of 97% for all analytes (71 of 73 expected positives) and no trend toward higher or lower assay signals between fresh and frozen test results.

Results of the study demonstrated that the freeze-thaw process and prolonged frozen storage did have a significant effect on analyte positivity or on average assay signal intensities.

2. Comparison studies:

a. Method comparison

N/A

b. Matrix comparison:

Due to the challenge of obtaining large volumes of natural negative tracheal aspirate matrix, Artificial Respiratory Matrix (ARM) was used to prepare samples for LoD, reproducibility and other analytical studies. A separate matrix equivalency study was performed to compare assay performance for samples prepared in ARM and samples prepared in pooled natural tracheal aspirate matrix. Tracheal aspirate test samples were prepared using pools of 4-6 individual tracheal aspirates determined to be negative for all LRT panel analytes. Both ARM and natural aspirate matrices were spiked with microorganism concentrations near the LoD for nine representative LRT analytes (between 10^4 – 10^6 CFU/mL). Matrix equivalence was evaluated based on qualitative analysis (percent positivity) and quantitative analyses (comparison of mean assay signal).

As shown in Table 18 below, positivity rates for ARM Matrix were slightly lower for all analytes overall than for the natural aspirate matrix, suggesting that the ARM matrix may be somewhat more challenging (i.e., for some analytes, the LoD may be slightly higher in ARM than in natural tracheal aspirate matrix). It was noted however that Unyvero LRT assay signal levels for positive Unyvero LRT samples were equivalent between the two matrix types for each LRT microorganisms. The study results support the use of ARM for preparation of samples for the reproducibility study and other analytical studies.

Table 18: Results from Matrix Comparison

Target Analyte	ARM Matrix	Aspirate Matrix
S. marcescens	4/6	6/6
E. coli	6/6	6/6
K. pneumoniae	6/6	5/6
vim	6/6	6/6
M. morganii	5/6	6/6
S. aureus	5/6	6/6
tem	6/6	6/6
P. aeruginosa	4/6	6/6
S. maltophilia	5/6	6/6
Total Positive/Expected	**47/54 = 87%**	**53/54 = 98%**

3. Clinical Studies:

Prospective Study:

Clinical performance of the Unyvero LRT Application performed on the Unyvero System was evaluated in a multi-center study at nine clinical sites in the United States. A total of 860 tracheal aspirate specimens were prospectively collected from patients with signs and symptoms of lower respiratory infection and were tested with the LRT assay within 24 hours of specimen collection. Specimens excluded from the performance analyses included 38 specimens that did not meet the specimen inclusion/exclusion criteria, 161 specimens that generated non-reportable results (e.g., fully invalid results or instrument failures) and 58 specimens that generated partially invalid results (invalid results in one or more PCR chambers). Altogether, a total number of 603 evaluable prospectively tested specimens were included in the performance analyses. Gram stains (quality screening) were performed for the majority of specimens tested in the study.

Reference/comparator methods used in the prospective study (Table 19) included standard of care (SoC) tracheal aspirate culture and validated comparator PCR assays. All positive comparator PCR results were followed with bi-directional sequencing. Validation of each comparator PCR assay included demonstration of similar LoDs and inclusivity to the Unyvero LRT Application.

Table 19: Prospective Study Reference/Comparator Methods

Prospective Study Reference/Comparator Methods
a) **SoC** (culture)
b) **Composite Comparator** for 'typical'[1] microorganisms • SoC (culture) • Independent and validated multiplexed PCR assay for which any positive PCR result is followed by bi-directional sequencing. One comparator PCR per microorganism target for 'atypical'[2] microorganisms • Two independent and validated multiplex PCR assays for which any positive PCR result is followed by bi-directional sequencing
c) Multiplexed PCR assays followed by bi-directional sequencing (for antibiotic resistance markers). One comparator PCR target per resistance marker.

[1]'Typical' analytes: *Acinetobacter* spp., *C. freundii*, *E. cloacae* complex, *E. coli*, *H. influenzae*, *K. oxytoca*, *K. pneumoniae*, *K. variicola*, *M. catarrhalis*, *M. morganii*, *Proteus* sp., *P. aeruginosa*, *S. marcescens*, *S. aureus*, *S. maltophilia*, *S. pneumoniae*.
[2]'Atypical' analytes: *C. pneumoniae, L. pneumophila*, *M. pneumoniae*

For each 'typical' microorganism target, clinical performance of the Unyvero LRT Application was evaluated in comparison to culture. In addition, clinical performance was evaluated as compared to a composite comparator of culture and PCR/bi-directional sequencing. For the composite comparator, the specimen was considered positive for a microorganism target if culture was positive or if the validated comparator PCR and follow-up bi-directional sequencing was positive. Any specimen that was negative by both culture and PCR was considered negative for the microorganism.

For each 'atypical' microorganism target (*Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and *Legionella pneumophila*), clinical performance of the Unyvero LRT Application was evaluated in comparison to a composite of two validated PCR assays for which all positive PCR results were followed by bi-directional sequencing. Specimens that were positive for a targeted microorganism by either of the two PCR/sequencing assays were considered positive by the composite comparator. Specimens that were negative for both PCR assays were considered negative for the analyte.

Clinical performance of the Unyvero LRT Application for 'typical' microorganism targets as compared to culture are shown in Table 20. Performance for both 'typical' and 'atypical' microorganism targets as compared to their respective composite comparator methods are shown in Table 21.

Table 20: Prospective study, comparison to reference culture

	TP	FN	FP [b]	TN	PPA [%] (95 % CI)	NPA [%] (95 % CI)	PPV [%] (95 % CI)	NPV [%] (95 % CI)
Acinetobacter spp.	10	0	17	576	**100.0** (72.3 - 100.0)	**97.1** (95.5 - 98.2)	**37.0** (21.5 - 55.8)	**100.0** (99.3 - 100.0)
Citrobacter freundii	1	3	2	597	**25.0** (4.6 - 69.9)	**99.7** (98.8 - 99.9)	**33.3** (6.1 - 79.2)	**99.5** (98.5 - 99.8)
Enterobacter cloacae complex	14	1	6	582	**93.3** (70.2 - 98.8)	**99.0** (97.8 - 99.5)	**70.0** (48.1 - 85.5)	**99.8** (99.0 - 100.0)
Escherichia coli	23	1	22	557	**95.8** (79.8 - 99.3)	**96.2** (94.3 - 97.5)	**51.1** (37.0 - 65.0)	**99.8** (99.0 - 100.0)
Haemophilus influenzae	8	0	16	579	**100.0** (67.6 - 100.0)	**97.3** (95.7 - 98.3)	**33.3** (18.0 - 53.3)	**100.0** (99.3 - 100.0)
Klebsiella oxytoca	4	2	11	586	**66.7** (30.0 - 90.3)	**98.2** (96.7 - 99.0)	**26.7** (10.9 - 52.0)	**99.7** (98.8 - 99.9)
Klebsiella pneumoniae [a]	21	3	17	562	**87.5** (69.0 - 95.7)	**97.1** (95.3 – 98.2)	**55.3** (39.7 - 69.9)	**99.5** (98.5 - 99.8)
Klebsiella variicola [a]	2	0	2	599	**100.0** (34.2 - 100.0)	**99.7** (98.8 – 99.9)	**50.0** (15.0 – 85.0)	**100.0** (99.4 – 100.0)
Moraxella catarrhalis	1	2	12 [c]	588	**33.3** (6.1 - 79.2)	**98.0** (96.5 - 98.9)	**7.7** (1.4 - 33.3)	**99.7** (98.8 - 99.9)
Morganella morganii	1	0	9	593	**100.0** (20.7 - 100.0)	**98.5** (97.2 - 99.2)	**10.0** (1.8 - 40.4)	**100.0** (99.4 - 100.0)
Proteus spp.	10	1	19	573	**90.9** (62.3 - 98.4)	**96.8** (95.0 - 97.9)	**34.5** (19.9 - 52.7)	**99.8** (99.0 - 100.0)
Pseudomonas aeruginosa	64	5	20	514	**92.8** (84.1 - 96.9)	**96.3** (94.3 - 97.6)	**76.2** (66.1 - 84.0)	**99.0** (97.8 - 99.6)
Serratia marcescens	11	0	14	578	**100.0** (74.1 - 100.0)	**97.6** (96.1 - 98.6)	**44.0** (26.7 - 62.9)	**100.0** (99.3 - 100.0)
Staphylococcus aureus	81	2	39	481	**97.6** (91.6 - 99.3)	**92.5** (89.9 - 94.5)	**67.5** (58.7 - 75.2)	**99.6** (98.5 - 99.9)
Stenotrophomonas maltophilia	25	1	27	550	**96.2** (81.1 - 99.3)	**95.3** (93.3 - 96.8)	**48.1** (35.1 - 61.3)	**99.8** (99.0 - 100.0)
Streptococcus pneumoniae	7	2	6	588	**77.8** (45.3 - 93.7)	**99.0** (97.8 - 99.5)	**53.8** (29.1 - 76.8)	**99.7** (98.8 - 99.9)

[a] As *K. variicola* is often reported by culture as *K. pneumoniae*, DNA extracts for culture positive *K. pneumoniae* samples were sequenced. For two of 26 *K. pneumoniae* positive samples a sequencing result for *K. variicola* was obtained. Strains identified for both samples were confirmed by sequencing of provided isolates and *K. variicola* was assigned as reference identity.

[b] Specimens with false positive LRT results were analyzed with molecular assays (PCR/bi-directional sequencing) using sample DNA extracts for presence or absence of microorganisms: presence of microorganisms was confirmed in 16 of 17 cases for *Acinetobacter* spp., 0 of 2 cases for *C. freundii*, 5 of 6 cases for *E. cloacae* complex, 21 of 22 cases for *E. coli*, 14 of 16 cases for *H. influenzae*, 7 of 11 cases for *K. oxytoca*, 15 of 17 cases for *K. pneumoniae*, 2 of 2 cases for *K. variicola*, 12 of 12 cases for *M. catarrhalis*, 6 of 9 cases for *M. morganii*, 18 of 19 cases for *Proteus* spp., 16 of 20 cases for *P. aeruginosa*, 13 of 14 cases for *S. marcescens*, 36 of 39 cases for *S. aureus*, 27 of 27 cases for *S. maltophilia*, and 6 of 6 cases for *S. pneumoniae*.

[c] 11/12 FP results for *M. catarrhalis* when compared to culture were confirmed by the molecular assay (PCR/bi-directional sequencing). Clinical relevance of such findings however has not been established.

Table 21: Prospective study, comparison to composite comparator

	TP	FN	FP	TN	**PPA [%]** (95 % CI)	**NPA [%]** (95 % CI)	**PPV [%]** (95 % CI)	**NPV [%]** (95 % CI)
Acinetobacter spp.	23	1	4	575	**95.8** (79.8 - 99.3)	**99.3** (98.2 - 99.7)	**85.2** (67.6 - 94.1)	**99.8** (99.0 - 100.0)
Chlamydia pneumoniae [b]	0	0	0	603	**na**	**100.0** (99.4 - 100.0)	**na**	**100.0** (99.4 - 100.0)
Citrobacter freundii	1	5	2	595	**16.7** (3.0 - 56.3)	**99.7** (98.8 - 99.9)	**33.3** (6.1 - 79.2)	**99.2** (98.1 - 99.6)
Enterobacter cloacae complex	17	1	3	582	**94.4** (74.2 - 99.0)	**99.5** (98.5 - 99.8)	**85.0** (64.0 - 94.8)	**99.8** (99.0 - 100.0)
Escherichia coli	37	1	8	557	**97.4** (86.5 - 99.5)	**98.6** (97.2 - 99.3)	**82.2** (68.7 - 90.7)	**99.8** (99.0 - 100.0)
Haemophilus influenzae	15	2	8	577	**88.2** (65.7 - 96.7)	**98.6** (97.3 - 99.3)	**65.2** (44.9 - 81.2)	**99.7** (98.7 - 99.9)
Klebsiella oxytoca	7	2	8	586	**77.8** (45.3 - 93.7)	**98.7** (97.4 - 99.3)	**46.7** (24.8 - 69.9)	**99.7** (98.8 - 99.9)
Klebsiella pneumoniae [a]	30	3	7	562	**90.9** (76.4 - 96.9)	**98.8** (97.5 - 99.4)	**81.1** (65.8 - 90.5)	**99.5** (98.5 - 99.8)
Klebsiella variicola [a]	2	0	2	599	**100.0** (34.2 - 100.0)	**99.7** (98.8 - 99.9)	**50.0** (15.0 - 85.0)	**100.0** (99.4 - 100.0)
Legionella pneumophila [b]	2	0	0	601	**100.0** (34.2 - 100.0)	**100.0** (99.4 - 100.0)	**100.0** (34.2 - 100.0)	**100.0** (99.4 - 100.0)
Moraxella catarrhalis	12	11 [c]	1	579	**52.2** (33.0 - 70.8)	**99.8** (99.0 - 100.0)	**92.3** (66.7 - 98.6)	**98.1** (96.7 - 99.0)
Morganella morganii	6	1	4	592	**85.7** (48.7 - 97.4)	**99.3** (98.3 - 99.7)	**60.0** (31.3 - 83.2)	**99.8** (99.1 - 100.0)
Mycoplasma pneumoniae [b]	2	0	1	600	**100.0** (34.2 - 100.0)	**99.8** (99.1 - 100.0)	**66.7** (20.8 - 93.9)	**100.0** (99.4 - 100.0)
Proteus spp.	24	1	5	573	**96.0** (80.5 - 99.3)	**99.1** (98.0 - 99.6)	**82.8** (65.5 - 92.4)	**99.8** (99.0 - 100.0)
Pseudomonas aeruginosa	76	9	8	510	**89.4** (81.1 - 94.3)	**98.5** (97.0 - 99.2)	**90.5** (82.3 - 95.1)	**98.3** (96.7 - 99.1)
Serratia marcescens	21	3	4	575	**87.5** (69.0 - 95.7)	**99.3** (98.2 - 99.7)	**84.0** (65.3 - 93.6)	**99.5** (98.5 - 99.8)
Staphylococcus aureus	109	10	11	473	**91.6** (85.2 - 95.4)	**97.7** (96.0 - 98.7)	**90.8** (84.3 - 94.8)	**97.9** (96.2 - 98.9)
Stenotrophomonas maltophilia	50	6	2	545	**89.3** (78.5 - 95.0)	**99.6** (98.7 - 99.9)	**96.2** (87.0 - 98.9)	**98.9** (97.6 - 99.5)
Streptococcus pneumoniae	10	6	3	584	**62.5** (38.6 - 81.5)	**99.5** (98.5 - 99.8)	**76.9** (49.7 - 91.8)	**99.0** (97.8 - 99.5)

[a] As *K. variicola* is often reported by culture as *K. pneumoniae*, DNA extracts for culture positive *K. pneumoniae* samples were sequenced. For two of 26 *K. pneumoniae* positive samples a sequencing result for *K. variicola* was obtained. Strain identities for both samples were confirmed by sequencing of provided isolates and *K. variicola* was assigned as reference identity.

[b] 'Atypical' microorganisms *C. pneumoniae*, *L. pneumophila* and *M. pneumoniae* were compared to two independent molecular tests (PCR/bi-directional sequencing) as composite comparator.

[c] 9/11 FN results when compared to the composite comparator were only reported positive by the molecular comparator assay (PCR/bi-directional sequencing) for *M. catarrhalis*.

For prospective specimens with positive culture results, positive percent agreement (PPA) for the Unyvero LRT Application was calculated based on semi-quantitative reference culture results as shown in Table 22.

Table 22: Prospective study, comparison to semi-quantitative culture results

	Semi-quantitative culture result	TP	FN	PPA [%]
Acinetobacter spp.	rare	2	0	100.0
	few	0	0	na
	moderate	6	0	100.0
	numerous	2	0	100.0
Citrobacter freundii	rare	0	1	0.0
	few	0	1	0.0
	moderate	1	0	100.0
	numerous	0	1	0.0
Enterobacter cloacae complex	rare	0	0	na
	few	6	0	100.0
	moderate	6	1	85.7
	numerous	2	0	100.0
Escherichia coli	rare	3	0	100.0
	few	4	1	80.0
	moderate	8	0	100.0
	numerous	8	0	100.0
Haemophilus influenzae	rare	0	0	na
	few	1	0	100.0
	moderate	2	0	100.0
	numerous	5	0	100.0
Klebsiella oxytoca	rare	0	2	0.0
	few	3	0	100.0
	moderate	0	0	na
	numerous	1	0	100.0
Klebsiella pneumoniae	rare	0	1	0.0
	few	8	0	100.0
	moderate	9	1	90.0
	numerous	4	1	80.0
Klebsiella variicola	rare	0	0	na
	few	1	0	100.0
	moderate	1	0	100.0
	numerous	0	0	na
Moraxella catarrhalis	rare	0	0	na
	few	0	0	na
	moderate	0	2	0.0
	numerous	1	0	100.0
Morganella morganii	rare	0	0	na
	few	0	0	na
	moderate	1	0	100.0
	numerous	0	0	na

	Semi-quantitative culture result	TP	FN	PPA [%]
Proteus spp.	rare	1	1	50.0
	few	3	0	100.0
	moderate	3	0	100.0
	numerous	3	0	100.0
Pseudomonas aeruginosa	rare	7	2	77.8
	few	13	2	86.7
	moderate	28	0	100.0
	numerous	16	0	100.0
Serratia marcescens	rare	1	0	100.0
	few	4	0	100.0
	moderate	4	0	100.0
	numerous	2	0	100.0
Staphylococcus aureus	rare	2	2	50.0
	few	13	0	100.0
	moderate	35	0	100.0
	numerous	30	0	100.0
Stenotrophomonas maltophilia	rare	0	0	na
	few	6	0	100.0
	moderate	11	0	100.0
	numerous	8	1	88.9
Streptococcus pneumoniae	rare	0	0	na
	few	0	0	na
	moderate	6	1	85.7
	numerous	1	1	50.0

For the 603 prospectively tested specimens included in the performance analyses, the LRT assay detected at least one microorganism in 312 specimens (51.7%) and culture reported at least one microorganism in 236 specimens (39.1%).

Multi-detections were reported by the LRT assay for 125 specimens (20.7%) and reported by culture for 62 specimens (10.3%) (Table 23).

Table 23: Numbers of targeted microorganisms as reported by LRT or Culture

Numbers of Detected Microorganisms	Aspirate Specimens			
	LRT		SoC (Culture)	
	# specimens	[%]	# specimens	[%]
0	**291**	**48.3**	**367**	**60.9**
any positive	**312**	**51.7**	**236**	**39.1**
1	187	31.0	174	28.9
2	75	12.4	55	9.1
3	25	4.1	6	1.0
4	17	2.8	1	0.2
5	3	0.5	0	0.0
6	4	0.7	0	0.0
8	1	0.2	0	0.0

For 227 specimens, both the LRT Application and culture reported at least one LRT panel microorganism (Table 24). The LRT Application was negative for nine specimens with positive culture results. For 282 specimens, both the LRT Application and culture reported a negative result (no growth or normal/mixed flora result). For 85 specimens, the LRT Application reported a positive result while culture was negative. Of the 85 LRT-positive/culture negative specimens, culture results were reported as normal flora (65), no growth (18) or presence/absence of flora not reported (2).

Table 24: Comparison of positive and negative culture results to LRT

	Positive Culture	Negative Culture		
LRT Result	Microorganism(s) Reported	No Growth	Normal/mixed Flora	NA[a]
any positive by LRT	227	18	65	2
negative by LRT	9	105	169	8

[a] presence or absence of flora not reported

Tables 25 and Table 26 include details of multi-detections observed during the prospective clinical study for 'typical' LRT analytes. Multi-detections reported by the LRT Application (N=122) and compared to culture are shown in Table 23. Multi-detections reported by culture (N=62) and compared to Unyvero LRT are shown in Table 24.

Table 23 lists the multi-detection results generated by the LRT Application and identifies any discordant results (microorganism targets identified by the LRT application and negative by culture). For example, both *E. coli* and *S. aureus* were detected by LRT in five specimens and both *E. coli* and *S. aureus* were reported by culture in only four of the five cases. Similarly, four specimens were positive for both *P. aeruginosa* and *S. aureus* by LRT; two of the corresponding cultures were negative for *P. aeruginosa* and three were negative for *S. aureus*.

In Table 24, multi-detections generated by culture are compared to LRT assay results and identifies any discordant results (culture positive/LRT negative). For example, culture identified five specimens that grew both *S. aureus* and *K. pneumoniae*. In four cases the LRT assay also identified both microorganisms; however, in one case, the LRT assay detected only *S. aureus*, but not *K. pneumoniae*.

Table 25: Multi-detections of 'typical' microorganisms detected by LRT Application (N=122) as compared to culture (excludes three specimens that were positive for 'atypical' microorganisms -see table footnote)

Multi-detections in Aspirate Specimens by LRT Application [a]	Number of Specimens with Multi-Detections by LRT	Number of Specimens with Discordant Results	False Positive Analytes: Microorganisms negative by SoC culture (LRT Positive/SoC Negative) (N) = Number of Specimens
P. aeruginosa, *S. maltophilia*	9	7	*S. maltophilia* (7)
E. coli, *S. aureus*	5	1	*E. coli* (1); *S. aureus* (1)
P. aeruginosa, *S. aureus*	4	3	*P. aeruginosa* (2); *S. aureus* (3)
S. aureus, *S. maltophilia*	4	2	*S. aureus* (1); *S. maltophilia* (2)
H. influenzae, *S. aureus*	4	3	*H. influenzae* (3)
K. pneumoniae, *S. aureus*	3	0	
E. cloacae complex, *E. coli*	3	1	*E. cloacae* complex (1)
E. coli, *P. aeruginosa*	3	2	*E. coli* (1); *P. aeruginosa* (1)
Proteus spp., *S. aureus*	3	2	*Proteus* spp. (2)
M. catarrhalis, *S. aureus*	3	2	*M. catarrhalis* (2); *S. aureus* (1)
H. influenzae, *Proteus* spp., *S. aureus*	2	2	*H. influenzae* (2); *Proteus* spp. (2)
E. coli, *K. pneumoniae*	2	2	*E. coli* (2); *K. pneumoniae* (2)
K. pneumoniae, *S. maltophilia*	2	1	*S. maltophilia* (1)
Proteus spp., *P. aeruginosa*, *S. aureus*	2	2	*Proteus* spp. (2); *P. aeruginosa* (1); *S. aureus* (2)
E. cloacae complex, *K. oxytoca*, *S. maltophilia*	2	2	*E. cloacae* complex (2); *K. oxytoca* (2); *S. maltophilia* (2)
K. oxytoca, *S. maltophilia*	2	1	*K. oxytoca* (1); *S. maltophilia* (1)
Proteus spp., *P. aeruginosa*	2	2	*Proteus* spp. (2); *P. aeruginosa* (1)
Acinetobacter spp., *S. aureus*	2	2	*Acinetobacter* spp. (2)
S. marcescens, *S. aureus*	2	2	*S. marcescens* (2); *S. aureus* (1)
E. coli, *K. pneumoniae*, *Proteus* spp.	2	1	*E. coli* (1)
E. cloacae complex, *E. coli*, *S. aureus*	1	1	*E. cloacae* complex (1); *E. coli* (1)
H. influenzae, *P. aeruginosa*, *S. marcescens*	1	1	*H. influenzae* (1); *P. aeruginosa* (1); *S. marcescens* (1)
Acinetobacter spp., *Proteus* spp., *P. aeruginosa*, *S. marcescens*, *S. maltophilia*	1	1	*Acinetobacter* spp. (1); *Proteus* spp. (1); *S. marcescens* (1); *S. maltophilia* (1)
E. cloacae complex, *K. variicola*, *M. catarrhalis*	1	1	*M. catarrhalis* (1)
E. coli, *H. influenzae*, *K. pneumoniae*, *M. morganii*	1	1	*H. influenzae* (1); *M. morganii* (1)
H. influenzae, *K. pneumoniae*	1	1	*H. influenzae* (1)
Acinetobacter spp., *E. coli*, *H. influenzae*, *P. aeruginosa*, *S. aureus*, *S. maltophilia*	1	1	*Acinetobacter* spp. (1); *E. coli* (1); *H. influenzae* (1); *S. maltophilia* (1)
E. cloacae complex, *E. coli*, *K. oxytoca*, *P. aeruginosa*	1	1	*E. cloacae* complex (1); *E. coli* (1); *K. oxytoca* (1); *P. aeruginosa* (1)
Acinetobacter spp., *K. pneumoniae*	1	1	*Acinetobacter* spp. (1); *K. pneumoniae* (1)

Multi-detections in Aspirate Specimens by LRT Application [a]	Number of Specimens with Multi-Detections by LRT	Number of Specimens with Discordant Results	False Positive Analytes: Microorganisms negative by SoC culture (LRT Positive/SoC Negative) (N) = Number of Specimens
M. morganii, *Proteus* spp., *P. aeruginosa*, *S. aureus*	1	1	*M. morganii* (1); *S. aureus* (1)
M. morganii, *S. marcescens*	1	1	*S. marcescens* (1)
Acinetobacter spp., *E. cloacae* complex, *P. aeruginosa*	1	1	*Acinetobacter* spp. (1)
E. coli, *P. aeruginosa*, *S. marcescens*	1	1	*E. coli* (1)
K. variicola, *S. maltophilia*	1	1	*K. variicola* (1); *S. maltophilia* (1)
K. pneumoniae, *S. marcescens*, *S. aureus*	1	1	*K. pneumoniae* (1); *S. marcescens* (1); *S. aureus* (1)
Acinetobacter spp., *P. aeruginosa*, *S. aureus*, *S. maltophilia*	1	1	*Acinetobacter* spp. (1); *S. aureus* (1)
E. coli, *H. influenzae*, *S. aureus*	1	1	*H. influenzae* (1)
Acinetobacter spp., *K. pneumoniae*, *P. aeruginosa*, *S. maltophilia*	1	1	*Acinetobacter* spp. (1); *K. pneumoniae* (1)
Acinetobacter spp., *M. morganii*, *Proteus* spp., *P. aeruginosa*	1	1	*Acinetobacter* spp. (1); *M. morganii* (1)
K. pneumoniae, *M. morganii*, *Proteus* spp., *P. aeruginosa*, *S. marcescens*, *S. maltophilia*	1	1	*K. pneumoniae* (1); *M. morganii* (1); *S. maltophilia* (1)
Acinetobacter spp., *M. catarrhalis*, *M. morganii*, *Proteus* spp., *P. aeruginosa*, *S. marcescens*, *S. maltophilia*, *S. pneumoniae*	1	1	*M. catarrhalis* (1); *M. morganii* (1); *Proteus* spp. (1); *S. marcescens* (1)
E. cloacae complex, *Proteus* spp.	1	1	*Proteus* spp. (1)
E. coli, *S. maltophilia*	1	0	
M. morganii, *P. aeruginosa*	1	1	*M. morganii* (1)
E. coli, *M. catarrhalis*, *P. aeruginosa*	1	1	*E. coli* (1); *M. catarrhalis* (1); *P. aeruginosa* (1)
M. catarrhalis, *P. aeruginosa*, *S. aureus*	1	1	*M. catarrhalis* (1); *P. aeruginosa* (1)
S. marcescens, *S. maltophilia*	1	0	
K. pneumoniae, *P. aeruginosa*, *S. aureus*	1	1	*S. aureus* (1)
E. coli, *S. marcescens*, *S. aureus*, *S. maltophilia*	1	1	*E. coli* (1)
K. pneumoniae, *M. morganii*, *S. aureus*	1	1	*M. morganii* (1)
Acinetobacter spp., *Proteus* spp., *P. aeruginosa*, *S. maltophilia*	1	1	*Proteus* spp. (1); *P. aeruginosa* (1); *S. maltophilia* (1)
C. freundii, *K. oxytoca*	1	1	*C. freundii* (1); *K. oxytoca* (1)
P. aeruginosa, *S. marcescens*, *S. maltophilia*	1	1	*S. marcescens* (1); *S. maltophilia* (1)
E. coli, *P. aeruginosa*, *S. maltophilia*	1	1	*S. maltophilia* (1)
P. aeruginosa, *S. aureus*, *S. maltophilia*	1	1	*S. aureus* (1); *S. maltophilia* (1)
E. cloacae complex, *S. aureus*	1	0	
Acinetobacter spp., *E. coli*, *P. aeruginosa*, *S. marcescens*, *S. aureus*, *S. maltophilia*	1	1	*Acinetobacter* spp. (1); *E. coli* (1); *S. aureus* (1); *S. maltophilia* (1)

Multi-detections in Aspirate Specimens by LRT Application [a]	Number of Specimens with Multi-Detections by LRT	Number of Specimens with Discordant Results	False Positive Analytes: Microorganisms negative by SoC culture (LRT Positive/SoC Negative) (N) = Number of Specimens
Acinetobacter spp., *Proteus* spp., *P. aeruginosa*, *S. aureus*	1	1	*Acinetobacter* spp. (1); *Proteus* spp. (1); *P. aeruginosa* (1)
K. oxytoca, *P. aeruginosa*	1	1	*K. oxytoca* (1); *P. aeruginosa* (1)
E. coli, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*	1	1	*E. coli* (1); *K. pneumoniae* (1)
Acinetobacter spp., *E. coli*, *K. pneumoniae*, *Proteus* spp., *P. aeruginosa*, *S. aureus*	1	1	*Acinetobacter* spp. (1); *E. coli* (1); *Proteus* spp. (1); *S. aureus* (1)
Acinetobacter spp., *S. marcescens*, *S. maltophilia*	1	1	*Acinetobacter* spp. (1)
E. coli, *Proteus* spp., *P. aeruginosa*, *S. marcescens*	1	1	*E. coli* (1)
K. oxytoca, *S. aureus*	1	1	*K. oxytoca* (1)
K. oxytoca, *P. aeruginosa*, *S. maltophilia*	1	0	
Proteus spp., *P. aeruginosa*, *S. marcescens*, *S. maltophilia*	1	1	*Proteus* spp. (1); *S. marcescens* (1); *S. maltophilia* (1)
E. coli, *H. influenzae*	1	0	
Acinetobacter spp., *K. pneumoniae*, *S. aureus*, *S. maltophilia*	1	1	*K. pneumoniae* (1)
Acinetobacter spp., *M. morganii*, *Proteus* spp., *S. aureus*	1	1	*Acinetobacter* spp. (1); *M. morganii* (1)
H. influenzae, *S. pneumoniae*	1	0	
Acinetobacter spp., *E. coli*, *K. pneumoniae*, *P. aeruginosa*	1	1	*Acinetobacter* spp. (1); *E. coli* (1); *K. pneumoniae* (1)
K. pneumoniae, *K. variicola*	1	1	*K. pneumoniae* (1); *K. variicola* (1)
P. aeruginosa, *S. marcescens*	1	0	
K. oxytoca, *K. pneumoniae*, *M. morganii*, *P. aeruginosa*, *S. marcescens*	1	1	*K. oxytoca* (1); *M. morganii* (1); *P. aeruginosa* (1); *S. marcescens* (1)
Acinetobacter spp., *E. coli*, *P. aeruginosa*, *S. marcescens*, *S. maltophilia*	1	1	*Acinetobacter* spp. (1); *E. coli* (1); *S. marcescens* (1); *S. maltophilia* (1)
M. catarrhalis, *Proteus* spp., *P. aeruginosa*, *S. marcescens*	1	1	*M. catarrhalis* (1); *Proteus* spp. (1); *P. aeruginosa* (1); *S. marcescens* (1)
H. influenzae, *M. catarrhalis*	1	1	*M. catarrhalis* (1)
K. pneumoniae, *P. aeruginosa*	1	1	*K. pneumoniae* (1); *P. aeruginosa* (1)
C. freundii, *K. oxytoca*, *K. pneumoniae*, *S. aureus*	1	1	*C. freundii* (1); *K. pneumoniae* (1)
Acinetobacter spp., *Proteus* spp., *S. aureus*	1	1	*Proteus* spp. (1)
K. variicola, *S. aureus*	1	1	*S. aureus* (1)

[a] Multidetections/'atypical' analytes: This table does not include three specimens with multi-detections including 'atypical' microorganisms:

- *M. pneumoniae* – two specimens positive for *M. pneumoniae* with another analyte (one *H. influenzae*, and one *S. pneumoniae*), of which one specimen was FP for *H. influenzae* and one specimen was FP for *S. pneumoniae* as compared to culture. Both specimens were concordant for *M. pneumoniae* as compared to the composite comparator
- *L. pneumophila* – one specimen positive for *L. pneumophila*/*S. aureus* (*S. aureus* detection concordant with culture and *L. pneumophila* concordant with the composite comparator)

Table 26: Multiple 'typical' microorganisms reported by culture (N=62), as compared to LRT Application

Multi-Detections in Aspirate Specimens reported by SoC culture	Number of Specimens with Multi-Detections by SoC culture	Total Number of Specimens with Discordant Results	False Negative Analytes: Microorganisms negative by LRT (SoC positive/LRT negative) (N) = number of specimens
K. pneumoniae, *S. aureus*	5	1	*K. pneumoniae* (1)
E. coli, *S. aureus*	5	0	
P. aeruginosa, *S. maltophilia*	4	0	
P. aeruginosa, *S. aureus*	3	0	
E. coli, *P. aeruginosa*	3	1	*P. aeruginosa* (1)
P. aeruginosa, *S. marcescens*	3	0	
Proteus spp., *P. aeruginosa*, *S. marcescens*	2	0	
Proteus spp., *S. aureus*	2	0	
S. marcescens, *S. maltophilia*	2	0	
K. oxytoca, *S. aureus*	2	1	*K. oxytoca* (1)
Proteus spp., *P. aeruginosa*	2	0	
K. pneumoniae, *P. aeruginosa*	2	0	
S. aureus, *S. maltophilia*	2	0	
E. cloacae complex, *E. coli*	2	0	
E. cloacae complex, *K. pneumoniae*	1	1	*K. pneumoniae* (1)
C. freundii, *Proteus* spp.	1	1	*C. freundii* (1); *Proteus* spp. (1)
K. oxytoca, *S. maltophilia*	1	0	
Acinetobacter spp., *P. aeruginosa*, *S. maltophilia*, *S. pneumoniae*	1	0	
H. influenzae, *S. aureus*	1	0	
E. coli, *K. pneumoniae*, *Proteus* spp.	1	0	
Acinetobacter spp., *S. aureus*, *S. maltophilia*	1	0	
E. cloacae complex, *S. aureus*	1	0	
Acinetobacter spp., *P. aeruginosa*	1	1	*P. aeruginosa* (1)
K. pneumoniae, *Proteus* spp.	1	0	
M. catarrhalis, *S. aureus*	1	0	
S. marcescens, *S. aureus*, *S. maltophilia*	1	0	
E. coli, *H. influenzae*	1	0	
H. influenzae, *S. pneumoniae*	1	0	
Acinetobacter spp., *S. aureus*	1	0	
E. cloacae complex, *P. aeruginosa*	1	0	
C. freundii, *P. aeruginosa*	1	1	*C. freundii* (1)
K. oxytoca, *P. aeruginosa*, *S. maltophilia*	1	0	
E. coli, *K. pneumoniae*	1	0	

Multi-Detections in Aspirate Specimens reported by SoC culture	Number of Specimens with Multi-Detections by SoC culture	Total Number of Specimens with Discordant Results	False Negative Analytes: Microorganisms negative by LRT (SoC positive/LRT negative) (N) = number of specimens
K. pneumoniae, *S. maltophilia*	1	0	
E. coli, *S. maltophilia*	1	0	
C. freundii, *S. pneumoniae*	1	1	*C. freundii* (1)
M. catarrhalis, *S. pneumoniae*	1	1	*M. catarrhalis* (1); *S. pneumoniae* (1)

Retrospective Clinical Study, Archived Specimens

For the retrospective clinical study, 266 previously frozen tracheal aspirate specimens were tested at US study sites with the Unyvero LRT Application. Four specimens were excluded for not meeting specimen inclusion criteria, 32 specimens were excluded due to non-reportable results and 19 specimens with reportable results were excluded due to partially valid results. The remaining 211 evaluable US study specimens were supplemented with 158 specimens collected at other US or European sites and tested in-house at Curetis. Altogether, results from a total number of 369 evaluable archived specimens were included in the performance analyses. All specimens were selected based on positive standard of care results which included culture for 'typical' analytes and other test methods for 'atypical' analytes. All historical positive results were confirmed with validated PCR/bi-directional sequencing assays prior to inclusion in the study.

Tables 27 and 28 include results for the retrospective clinical study for 'typical' and 'atypical' analytes respectively. Note that a standard reference method for the 'atypical' analytes was not applied to all archived specimens; therefore, only positive percent agreement could be calculated for these analytes.

Table 27: Archived study performance, 'typical' microorganisms

	Positivity TP/(TP+FN)	PPA [%] (95 % CI)	Negativity [b] TN/(TN+FP)	NPA [%] (95 % CI)
Acinetobacter spp.	18/18	**100.0** (82.4 - 100.0)	344/350 [c]	**98.3** (96.3 - 99.2)
Citrobacter freundii	2/2	**100.0** (34.2 - 100.0)	354/360	**98.3** (96.4 - 99.2)
Enterobacter cloacae complex	24/25	**96.0** (80.5 - 99.3)	329/336	**97.9** (95.8 - 99.0)
Escherichia coli	37/38	**97.4** (86.5 - 99.5)	311/329	**94.5** (91.5 - 96.5)
Haemophilus influenzae	21/24	**87.5** (69.0 - 95.7)	326/337	**96.7** (94.3 - 98.2)
Klebsiella oxytoca	17/17	**100.0** (81.6 - 100.0)	337/347	**97.1** (94.8 - 98.4)
Klebsiella pneumoniae [a]	28/29	**96.6** (82.8 - 99.4)	321/338	**95.0** (92.1 - 96.8)
Klebsiella variicola [a]	9/9	**100.0** (70.1 - 100.0)	356/358	**99.4** (98.0 - 99.8)
Moraxella catarrhalis	9/9	**100.0** (70.1 - 100.0)	336/345	**97.4** (95.1 - 98.6)
Morganella morganii	1/1	**100.0** (20.7 - 100.0)	346/353	**98.0** (96.0 - 99.0)
Proteus spp.	29/30	**96.7** (83.3 - 99.4)	325/338	**96.2** (93.5 - 97.7)
Pseudomonas aeruginosa	48/52	**92.3** (81.8 - 97.0)	286/310	**92.3** (88.7 - 94.7)
Serratia marcescens	34/35	**97.1** (85.5 - 99.5)	326/333	**97.9** (95.7 - 99.0)
Staphylococcus aureus	72/78	**92.3** (84.2 - 96.4)	259/286	**90.6** (86.6 - 93.4)
Stenotrophomonas maltophilia	33/34	**97.1** (85.1 - 99.5)	298/328	**90.9** (87.2 - 93.5)
Streptococcus pneumoniae	21/23	**91.3** (73.2 - 97.6)	335/341	**98.2** (96.2 - 99.2)

[a] As *K. variicola* is often reported by culture as *K. pneumoniae*, DNA extracts for culture positive *K. pneumoniae* specimens were sequenced. For nine of 38 *K. pneumoniae* positive specimens a sequencing result for *K. variicola* was obtained and *K. variicola* was assigned as reference identity.

[b] Specimens with FP results obtained in 211 US study specimens were analyzed by molecular tests (PCR/bi-directional sequencing). Sequencing confirmed the presence of microorganisms in FP specimens as follows: *Acinetobacter* spp. 6 of 6, *C. freundii* 3 of 5, *E. cloacae* complex 6 of 7, *E. coli* 13 of 13, *H. influenzae* 4 of 6 (2 non-confirmed specimens were identified as *H. haemolyticus)*, *K. oxytoca* 8 of 8, *K. pneumoniae* 13 of 13, *K. variicola* 2 of 2, *M. catarrhalis* 3 of 3, *M. morganii* 6 of 7, *M. pneumoniae* 0 of 1, *Proteus* spp. 10 of 10, *P. aeruginosa* 15 of 16, *S. marcescens* 5 of 5, *S. aureus* 13 of 13, *S. maltophilia* 19 of 19, *S. pneumoniae* 1 of 1.

[c] Note that for supplementary specimens partially valid results were included, therefore, the total number of data points for each microorganism analyte may differ.

Table 28: Archived study performance, 'atypical' microorganisms

	Positivity TP/(TP+FN)	PPA [%] (95 % CI)
Chlamydia pneumoniae	0/0	na
Legionella pneumophila	2/2	100.0 (34.2 - 100.0)
Mycoplasma pneumoniae	0/0	na

Clinical Study, Contrived Specimens:

For low prevalence microorganism and resistance marker analytes, the prospective and archived studies were supplemented with contrived specimens. Each contrived specimen was prepared in a unique natural tracheal aspirate specimen matrix. All aspirate specimen matrices were prescreened to ensure that they were negative for all Unyvero LRT analytes prior to use in the study. Specimens were spiked with pools of microorganisms at two concentrations; low positive (2x LoD or lower) and moderate positive (typically 3-10x LoD). Microorganisms evaluated in the contrived study were *C. pneumoniae*, *C. freundii*, *K. oxytoca*, *K. variicola*, *L. pneumophila*, *M. catarrhalis*, *M. morganii,* and *M. pneumoniae*. LRT Application resistance markers evaluated in the contrived study were *ctx*-M, *oxa*-23, *oxa*-24, *oxa*-48, *oxa*-58, *kpc*, *vim*, and *ndm*. Testing of contrived specimens was performed at three sites in the United States as well as in-house at Curetis.

Up to five different strains for each analyte were used to prepared contrived specimens with the total number of specimens ranging from 21 to 50 for each microorganism or resistance marker.

Table 29 below shows positivity rates (number of positive LRT results / number of expected positive results, PPA) and negativity rates (number of negative LRT results / number of expected negative results, NPA) observed in the study.

For other LRT panel microorganisms not evaluated in the contrived study, the following false positive results were observed: 1 of 292 for *Proteus* spp. (NPA: 99.7%, 95% CI: 98.1% – 99.9%); 4 of 216 for *P. aeruginosa* (NPA: 98.1%, 95% CI: 95.3% – 99.3%); 2 of 297 for *S. marcescens* (NPA: 99.3%, 95% CI: 97.6% – 99.8%); 2 of 258 for *S. aureus* (NPA: 99.2%, 95% CI: 97.2% – 99.8%); and 1 of 291 for *S. pneumoniae* (NPA: 99.7%, 95% CI: 98.1% – 99.9%). Additional false positive results were observed for *E. cloacae* complex: (21 of 266, NPA: 92.1% 95% CI: 88.2% – 94.8%); for *E. coli* (6 of 205, NPA: 97.1%, 95% CI: 93.8% – 98.7%); and *S. maltophilia* (10 of 317, NPA: 96.8%, 95% CI: 94.3% – 98.3%) with false positive results linked to contamination of test materials for 19 of 21 (*E. cloacae* complex), 5 of 6 (*E. coli*), and 10 of 10 (*S. maltophilia*) positive results.

Results from the contrived study are presented in Table 29.

Table 29: Contrived specimen testing

Analyte strain IDs (# tests) [a]	**Concentration [CFU/mL], (x LoD)**	**Positivity (# positive/ # expected)**	**PPA [%] (95 % CI)**	**Negativity (# negative/ # not expected)**	**NPA [%] (95 % CI)**
Chlamydia pneumoniae ATCC VR2282 (in IFU/ml) (21) [b, c]	1.5×10^4 (1x)	14/14	100.0 (78.5 - 100.0)		
	4.5×10^4 (3x)	7/7	100.0 (64.6 - 100.0)		
	total	**21/21**	**100.0** (84.5 - 100.0)	**303/303**	**100.0** (98.7 - 100.0)
Citrobacter freundii ATCC 8090 (20), ATCC 43864 (20), NRZ-00452 (10)	4×10^5 (2x)	21/25	84.0 (65.3 - 93.6)		
	1×10^6 (5x)	22/25	88.0 (70.0 - 95.8)		
	total	**43/50**	**86.0** (73.8 - 93.1)	**236/237**	**99.6** (97.6 - 99.9)
Klebsiella oxytoca ATCC 13182 (6), ATCC 49131 (6), ATCC 43863 (6), NCIMB 12819 (5), NRZ-22060 (5)	8×10^4 (0.4x)	12/14	85.7 (60.1 - 96.0)		
	4×10^5 (2x)	13/14	92.9 (68.5 - 98.7)		
	total	**25/28**	**89.3** (72.8 - 96.3)	**294/295**	**99.7** (98.1 - 99.9)
Klebsiella variicola ATCC BAA-830 (28)	2×10^5 (2x)	12/13	92.3 (66.7 - 98.6)		
	1×10^6 (10x)	15/15	100.0 (79.6 - 100.0)		
	total	**27/28**	**96.4** (82.3 - 99.4)	**295/296**	**99.7** (98.1 - 99.9)
Legionella pneumophila ATCC 33154 (9), ATCC 33215 (10), ATCC 35096 (10), ATCC 43283 (10), ATCC 33155 (10)	4×10^6 (2x)	24/24	100.0 (86.2 - 100.0)		
	1×10^7 (5x)	25/25	100.0 (86.7 - 100.0)		
	total	**49/49**	**100.0** (92.7 - 100.0)	**247/247**	**100.0** (98.5 - 100.0)
Moraxella catarrhalis ATCC 25238 (10), ATCC 43617 (20), ATCC 8176 (10), ATCC 25240 (10)	1.6×10^6 (2x)	24/25	96.0 (80.5 - 99.3)		
	5×10^6 (6x)	24/25	96.0 (80.5 - 99.3)		
	total	**48/50**	**96.0** (86.5 - 98.9)	**245/245**	**100.0** (98.5 - 100.0)
Morganella morganii ATCC 25830 (20), ATCC8019 (10), ATCC 25829 (9), DSM-46262 (10)	1×10^6 (2x)	24/24	100.0 (86.2 - 100.0)		
	3×10^6 (6x)	23/25	92.0 (75.0 - 97.8)		
	total	**47/49**	**95.6** (83.3 - 98.9)	**245/245**	**100.0** (98.5 - 100.0)
Mycoplasma pneumoniae ATCC 29085 (20), ATCC 29343 (20), (in CCU/mL), ATCC 15492 (10) (in CFU/mL) [d]	2×10^5 (2x)	25/25	100.0 (86.7 - 100.0)		
	5×10^5 (5x)	25/25	100.0 (86.7 - 100.0)		
	total	**50/50**	**100.0** (92.9 - 100.0)	**274/274**	**100.0** (98.6 - 100.0)
	2×10^5 (2x)	23/25	92.0		

Analyte strain IDs (# tests) [a]	**Concentration [CFU/mL], (x LoD)**	**Positivity (# positive/ # expected)**	**PPA [%] (95 % CI)**	**Negativity (# negative/ # not expected)**	**NPA [%] (95 % CI)**
***ctx*-M** NRZ-00751, NRZ-00002, NRZ-00249, (*K. pneumoniae*), JMI 46239 (*E. cloacae*), JMI 50067 (*E. coli*) (10 each)			(75.0 - 97.8)		
	6 x 10^5 (6x)	24/25	96.0 (80.5 - 99.3)		
	total	**47/50**	**94.0** (83.8 - 97.9)	**183/192** [e]	**95.3** (91.3 - 97.5)
kpc NRZ-00222 (10), NRZ-00281 (10) (*E. coli*), NRZ-00223 (9), Micromyx 4653 (10), Micromyx 4676 (*K. pneumoniae*) (10)	1 x 10^6 (2x)	23/24	95.8 (79.8 - 99.3)		
	3 x 10^6 (6x)	25/25	100.0 (86.7 - 100.0)		
	total	**48/49**	**98.0** (89.3 - 99.6)	**245/245**	**100.0** (98.5 - 100.0)
ndm JMI 50067 (6) (*E. coli*), NCTC 13443 (6), JMI 49831 (6) (*K. pneumoniae*), JMI 49755 (5) (*A. baumannii*), JMI 46239 (6) (*E. cloacae*)	1 x 10^5 (2x)	13/14	92.9 (68.5 - 98.7)		
	5 x 10^5 (10x)	15/15	100.0 (79.6 - 100.0)		
	total	**28/29**	**96.6** (82.8 - 99.4)	**237/263** [e]	**90.1** (85.9 - 93.2)
***oxa*-23** Micromyx 4410 (6) ,Micromyx 6148 (6), Micromyx 6149 (4) , Micromyx 6153 (6), UCLA A5 (6) (*A. baumannii*) (6 each)	1 x 10^7 (0,5x)	13/14	92.9 (68.5 - 98.7)		
	2 x 10^7 (1x)	14/14	100.0 (78.5 - 100.0)		
	total	**27/28**	**96.4** (82.3 - 99.4)	**139/140**	**99.3** (96.1 - 99.9)
***oxa*-24** NCTC 13302, NRZ-00449, UCLA A4, two clinical isolates (*A. baumannii*) (10 each)	6 x 10^4 (0,1x)	25/25	100.0 (86.7 - 100.0)		
	3 x 10^5 (0,6x)	25/25	100.0 (86.7 - 100.0)		
	total	**50/50**	**100.0** (92.9 - 100.0)	**90/90**	**100.0** (95.9 - 100.0)
***oxa*-48** NRZ-00176 (19), ATCC BAA-2523 (10) (*E. coli*), NCTC 13442 (10), NRZ-00002 (10) (*K. pneumoniae*)	4 x 10^6 (2x)	24/24	100.0 (86.2 - 100.0)		
	1 x 10^7 (5x)	24/25	96.0 (80.5 - 99.3)		
	total	**48/49**	**98.0** (89.3 - 99.6)	**205/205**	**100.0** (98.2 - 100.0)
***oxa*-58** NCTC 13305 (18), NRZ-00518 (12) (*A. baumannii*)	4 x 10^5 (0,5x)	15/15	100.0 (79.6 - 100.0)		
	1 x 10^6 (1.3x)	15/15	100.0 (79.6 - 100.0)		
	total	**30/30**	**100.0** (88.7 - 100.0)	**138/138**	**100.0** (97.3 - 100.0)
vim NRZ-00452 (10) (C. freundii), NRZ-00239 (20) (*E. cloacae*),DSM-24600 (19) (*P. aeruginosa*)	1 x 10^5 (2x)	22/24	91.7 (74.2 - 97.7)		
	3 x 10^5 (6x)	25/25	100.0 (86.7 - 100.0)		
	total	**47/49**	**95.9** (86.3 - 98.9)	**234/234**	**100.0** (98.4 - 100.0)

[a] A total of 50 tests (for *C. freundii, L. pneumophila, M. catarrhalis, M. pneumoniae, ctx*-M, *kpc*, *oxa*-24, *oxa*-48. *vim*) or 30 tests (for *K. oxytoca, K. variicola, M. morganii, ndm*, *oxa*-23, *oxa*-58) or 22 tests (for *C. pneumoniae*) was performed. Missing results were due to invalid test results.

[b] Numbers in parentheses indicate number of tests performed for an individual strain

[c] IFU: inclusion-forming units

[d] CCU: color-changing units, concentration used for ATCC 15492: 1 x 10^6 and 3 x 10^6 CFU/mL (1 CCU/mL was estimated to be equivalent to 10 CFU/mL)

[e] Eight positive *ctx*-M results and 24 positive *ndm* results were linked to contamination in test materials

Clinical Performance, Resistance Marker Targets

Performance characteristics for LRT Application antibiotic resistance marker targets were evaluated in the prospective study (603 aspirate specimens) and supplemented with results from contrived specimens (results shown in Table 29 above).

To assess the performance of the Unyvero LRT Application for detection of each resistance marker target, positive and negative percent agreement was calculated as compared to results of validated multiplex PCR assays followed by bi-directional sequencing.

It is noted that antibiotic resistance marker results are only reported by the LRT Application if at least one corresponding host microorganism is simultaneously detected. If an applicable microorganism is not detected in the specimen, positive resistance marker results are masked on the results screen (i.e., the result is masked and the report indicates N/A regardless whether the resistance marker is detected or not detected). Evaluation of assay performance for detection of resistance markers by the LRT Application was performed both with and without application of masking/reporting rules.

Table 30 includes performance of the LRT assay for detection of resistance marker targets as observed in the prospective study and compared to PCR/bi-directional sequencing. Analysis includes all positive resistance marker results (i.e., without software masking).

Table 30: Prospective Study, Resistance marker performance as compared to molecular comparator assays (multiplex PCR followed by bi-directional sequencing), without application of software masking

	Positivity TP / (TP+FN)	**PPA [%]** (95 % CI)	**Negativity TN / (TN+FP)**	**NPA [%]** (95 % CI)
ctx-M	15/16	**93.8** (71.7 - 98.9)	584/587	**99.5** (98.5 - 99.8)
kpc	7/7	**100.0** (64.6 - 100.0)	596/596	**100.0** (99.4 - 100.0)
ndm	0/0	na	603/603	**100.0** (99.4 - 100.0)
oxa-23	6/7	**85.7** (48.7 - 97.4)	594/596	**99.7** (98.8 - 99.9)
oxa-24	2/3	**66.7** (20.8 - 93.9)	600/600	**100.0** (99.4 - 100.0)
oxa-48	0/0	na	602/603	**99.8** (99.1 - 100.0)
oxa-58	0/0	na	603/603	**100.0** (99.4 - 100.0)
tem	54/54	**100.0** (93.4 - 100.0)	537/549	**97.8** (96.2 - 98.7)
vim	2/2	**100.0** (34.2 - 100.0)	601/601	**100.0** (99.4 - 100.0)
*mec*A	108/124	**87.1** (80.1 - 91.9)	453/479	**94.6** (92.2 - 96.3)

Table 31 shows LRT performance in the prospective study for each targeted resistance marker based on comparison to molecular comparator assays (PCR/bi-directional sequencing) for only those specimens in which an applicable LRT microorganism target was detected by the LRT assay (i.e., results shown are after application of software masking). Based on the reporting rules for the Unyvero LRT assay, performance for *ctx*-M, *bla*KPC, *bla*NDM and *bla*VIM includes specimens that were positive by the Unyvero LRT Application for a targeted microorganism of the Enterobacteriaceae, *Acinetobacter* spp. and/or *P. aeruginosa*. For *oxa*-48, performance includes only those specimens that were positive for one or more of the Enterobacteriaceae targets. For *oxa*-23, *oxa*-24 and *oxa*-58, the performance includes only those specimens that were positive for *Acinetobacter* spp. For *bla*TEM, performance includes only those specimens that were positive for *H. influenzae*. For *mec*A, performance includes only those specimens that were positive for *S. aureus*.

Table 31: Prospective Study, Resistance marker performance as compared to molecular comparator assays (multiplex PCR followed by bi-directional sequencing), with application of masking.

	Positivity TP / (TP+FN)	**PPA [%]** (95 % CI)	**Negativity TN / (TN+FP)**	**NPA [%]** (95 % CI)	**Not reported (masked)**
ctx-M	15/16	**93.8** (71.7 - 98.9)	177/179	**98.9** (96.0 - 99.7)	408
kpc	6/6	**100.0** (61.0 - 100.0)	189/189	**100.0** (98.0 - 100.0)	408
ndm	0/0	na	195/195	**100.0** (98.1 - 100.0)	408
oxa-23	6/7	**85.7** (48.7 - 97.4)	18/20	**90.0** (69.9 - 97.2)	576
oxa-24	2/2	**100.0** (34.2 - 100.0)	25/25	**100.0** (86.7 - 100.0)	576
oxa-48	0/0	na	138/139	**99.3** (96.0 - 99.9)	464
oxa-58	0/0	na	27/27	**100.0** (87.5 - 100.0)	576
tem	8/8	**100.0** (67.6 - 100.0)	16/16	**100.0** (80.6 - 100.0)	579
vim	2/2	**100.0** (34.2 - 100.0)	193/193	**100.0** (98.0 - 100.0)	408
mecA	54/59	**91.5** (81.6 - 96.3)	53/61	**86.9** (76.2 - 93.2)	483

Tables 32-41 include performance for resistance marker targets for each applicable microorganism detected by the LRT assay. Performance for each resistance marker target is evaluated as compared to PCR/bi-directional sequencing. Each table includes only the subset of specimens that are positive by LRT for the specified microorganism target; therefore, the results shown are after application of software masking.

Table 32: Prospective Study, Resistance marker performance as compared molecular comparator assays, stratified for LRT positive samples for *Acinetobacter* spp. (N=27). Note that detection of each resistance marker cannot be definitively linked to *Acinetobacter* spp.

Acinetobacter spp.	Positivity TP / (TP+FN)	PPA [%] (95 % CI)	Negativity TN / (TN+FP)	NPA [%] (95 % CI)
ctx-M	5/5	**100.0** (56.6 - 100.0)	21/22	**95.5** (78.2 - 99.2)
kpc	2/2	**100.0** (34.2 - 100.0)	25/25	**100.0** (86.7 - 100.0)
ndm	0/0	**na**	27/27	**100.0** (87.5 - 100.0)
oxa-23	6/7	**85.7** (48.7 - 97.4)	18/20	**90.0** (69.9 - 97.2)
oxa-24	2/2	**100.0** (34.2 - 100.0)	25/25	**100.0** (86.7 - 100.0)
oxa-58	0/0	**na**	27/27	**100.0** (87.5 - 100.0)
vim	1/1	**100.0** (20.7 - 100.0)	26/26	**100.0** (87.1 - 100.0)

Table 33: Prospective Study, Resistance marker performance as compared to molecular comparator assays, stratified for LRT positive samples for *Citrobacter freundii* (N=3)

Citrobacter freundii	Positivity TP / (TP+FN)	PPA [%] (95 % CI)	Negativity TN / (TN+FP)	NPA [%] (95 % CI)
ctx-M	0/0	**na**	3/3	**100.0** (43.9 - 100.0)
kpc	0/0	**na**	3/3	**100.0** (43.9 - 100.0)
ndm	0/0	**na**	3/3	**100.0** (43.9 - 100.0)
oxa-48	0/0	**na**	3/3	**100.0** (43.9 - 100.0)
vim	0/0	**na**	3/3	**100.0** (43.9 - 100.0)

Table 34: Prospective Study, Resistance marker performance as compared to molecular comparator assays, stratified for LRT positive samples for *Enterobacter cloacae* complex (N=20). Note that detection of each resistance marker cannot be definitively linked to *E. cloacae* complex.

***Enterobacter cloacae* complex**	**Positivity TP / (TP+FN)**	**PPA [%]** (95 % CI)	**Negativity TN / (TN+FP)**	**NPA [%]** (95 % CI)
ctx-M	0/0	**na**	20/20	**100.0** (83.9 - 100.0)
kpc	1/1	**100.0** (20.7 - 100.0)	19/19	**100.0** (83.2 - 100.0)
ndm	0/0	**na**	20/20	**100.0** (83.9 - 100.0)
oxa-48	0/0	**na**	20/20	**100.0** (83.9 - 100.0)
vim	0/0	**na**	20/20	**100.0** (83.9 - 100.0)

Table 35: Prospective Study, Resistance marker performance as compared to molecular comparator assays, stratified for LRT positive samples for *K. oxytoca* (N=15). Note that detection of each resistance marker cannot be definitively linked to *K. oxytoca*.

Klebsiella oxytoca	**Positivity TP / (TP+FN)**	**PPA [%]** (95 % CI)	**Negativity TN / (TN+FP)**	**NPA [%]** (95 % CI)
ctx-M	0/0	**na**	14/15	**93.3** (70.2 - 98.8)
kpc	0/0	**na**	15/15	**100.0** (79.6 - 100.0)
ndm	0/0	**na**	15/15	**100.0** (79.6 - 100.0)
oxa-48	0/0	**na**	15/15	**100.0** (79.6 - 100.0)
vim	0/0	**na**	15/15	**100.0** (79.6 - 100.0)

Table 36: Prospective Study, Resistance marker performance as compared to molecular comparator assays, stratified for LRT positive samples for *K. pneumoniae* (N=38). Note that detection of each resistance marker cannot be definitively linked to *K. pneumoniae*.

Klebsiella pneumoniae	**Positivity TP / (TP+FN)**	**PPA [%]** (95 % CI)	**Negativity TN / (TN+FP)**	**NPA [%]** (95 % CI)
ctx-M	8/8	**100.0** (67.6 - 100.0)	29/30	**96.7** (83.3 - 99.4)
kpc	2/2	**100.0** (34.2 - 100.0)	36/36	**100.0** (90.4 - 100.0)
ndm	0/0	**na**	38/38	**100.0** (90.8 - 100.0)
oxa-48	0/0	**na**	38/38	**100.0** (90.8 - 100.0)
vim	0/0	**na**	38/38	**100.0** (90.8 - 100.0)

Table 37: Prospective Study, Resistance marker performance as compared to molecular comparator assays, stratified for LRT positive samples for *K. variicola* (N=4). Note that detection of each resistance marker cannot be definitively linked to *K. variicola*.

Klebsiella variicola	**Positivity TP / (TP+FN)**	**PPA [%]** (95 % CI)	**Negativity TN / (TN+FP)**	**NPA [%]** (95 % CI)
ctx-M	0/0	**na**	4/4	**100.0** (51.0 - 100.0)
kpc	0/0	**na**	4/4	**100.0** (51.0 - 100.0)
ndm	0/0	**na**	4/4	**100.0** (51.0 - 100.0)
oxa-48	0/0	**na**	4/4	**100.0** (51.0 - 100.0)
vim	0/0	**na**	4/4	**100.0** (51.0 - 100.0)

Table 38: Prospective Study, Resistance marker performance as compared to molecular comparator assays, stratified for LRT positive samples for *Morganella morganii* (N=10). Note that detection of each resistance marker cannot be definitively linked to *Morganella morganii*.

Morganella morganii	**Positivity TP / (TP+FN)**	**PPA [%]** (95 % CI)	**Negativity TN / (TN+FP)**	**NPA [%]** (95 % CI)
ctx-M	2/2	**100.0** (34.2 – 100.0)	8/8	**100.0** (76.6- 100.0)
kpc	2/2	**100.0** (34.2 – 100.0)	8/8	**100.0** (67.6 - 100.0)
ndm	0/0	**na**	10/10	**100.0** (72.3 - 100.0)
oxa-48	0/0	**na**	9/10	**90.0** (59.6 - 98.2)
vim	1/1	**100.0** (20.7 – 100.0)	9/9	**100.0** (70.1 - 100.0)

Table 39: Prospective Study, Resistance marker performance as compared to molecular comparator assays, stratified for LRT positive samples for *Proteus spp.* (N=29). Note that detection of each resistance marker cannot be definitively linked to *Proteus spp.*

***Proteus* spp.**	**Positivity TP / (TP+FN)**	**PPA [%]** (95 % CI)	**Negativity TN / (TN+FP)**	**NPA [%]** (95 % CI)
ctx-M	3/3	**100.0** (43.9 - 100.0)	26/26	**100.0** (87.1 - 100.0)
kpc	1/1	**100.0** (20.7 - 100.0)	28/28	**100.0** (87.9 - 100.0)
ndm	0/0	**na**	29/29	**100.0** (88.3 - 100.0)
oxa-48	0/0	**na**	29/29	**100.0** (88.3 - 100.0)
vim	1/1	**100.0** (20.7 - 100.0)	28/28	**100.0** (87.9 - 100.0)

Table 40: Prospective Study, Resistance marker performance as compared to molecular comparator assays, stratified for LRT positive samples for *Pseudomonas aeruginosa* (N=84). Note that detection of each resistance marker cannot be definitively linked to *Pseudomonas aeruginosa*.

Pseudomonas aeruginosa	**Positivity TP / (TP+FN)**	**PPA [%]** (95 % CI)	**Negativity TN / (TN+FP)**	**NPA [%]** (95 % CI)
ctx-M	9/9	**100.0** (70.1 - 100.0)	75/75	**100.0** (95.1 - 100.0)
kpc	3/3	**100.0** (43.9 - 100.0)	81/81	**100.0** (95.5 - 100.0)
ndm	0/0	**na**	84/84	**100.0** (95.6 - 100.0)
oxa-48	0/0	**na**	83/84	**98.8** (93.6 - 99.8)
vim	2/2	**100.0** (34.2 - 100.0)	82/82	**100.0** (95.5 - 100.0)

Table 41: Prospective Study, Resistance marker performance as compared to molecular comparator assays, stratified for LRT positive samples for *Serratia marcescens* (N=25). Note that detection of each resistance marker cannot be definitively linked *Serratia marcescens*.

Serratia marcescens	**Positivity TP / (TP+FN)**	**PPA [%]** (95 % CI)	**Negativity TN / (TN+FP)**	**NPA [%]** (95 % CI)
ctx-M	1/1	**100.0** (20.7 - 100.0)	24/24	**100.0** (86.2 - 100.0)
kpc	4/4	**100.0** (51.0 - 100.0)	21/21	**100.0** (84.5 - 100.0)
ndm	0/0	**na**	25/25	**100.0** (86.7 - 100.0)
oxa-48	0/0	**na**	25/25	**100.0** (86.7 - 100.0)
vim	0/0	**na**	25/25	**100.0** (86.7 - 100.0)

For the prospective study, additional analyses of clinical performance for detection of resistance marker targets was conducted in combination with results from microorganism detection. For each marker, two different 3x3 tables were generated; one 3x3 table with Comparator 'A', reflecting culture as the reference method for LRT microorganism targets and one 3x3 table with Comparator 'B', reflecting the composite comparator method (culture plus PCR/bi-directional sequencing) for LRT microorganism targets. For the resistance markers, the comparator method was PCR/bi-directional sequencing for all analyses.

Agreement rates were determined for the following resistance marker/microorganism combinations following the Unyvero LRT reporting rules:

- *tem*: *H. influenzae*
- *ctx*-M, *kpc*, *vim*: All Enterobacteriaceae targets, *Acinetobacter* spp., and *P. aeruginosa* combined
- *oxa*-48: all Enterobacteriaceae targets combined
- *oxa*-23, *oxa*-24: *Acinetobacter* spp.
- *mec*A: *S. aureus*

Numbers of available culture isolates, results of linkage analysis (confirmation that the host microorganism strain is the source of the antibiotic resistance marker determined by PCR/bi-directional sequencing of culture isolates), and the number of multi-detection samples (more than one host microorganism detected by the composite comparator (comparator A) or SoC/culture (comparator B) are indicated as footnotes to each of the agreement tables. Agreement tables are not presented for *ndm* and *oxa*-58 as there were no positive results for these analytes by either the LRT or comparator assays.

Results are shown in Tables 42 through 49, with data presented in two tables for each resistance marker (one with Comparator A and one with Comparator B).

Table 42: Agreement rates for *tem* between LRT and comparator methods A and B, (single) host microorganism: *H. influenzae*

A

H. influenzae [c] ***tem***		**Composite Comparator Result for Host Microorganism + PCR/Seq for Antibiotic Resistance Marker**			
		Org+/Res+ [b]	Org+/Res-	Org-	total
Unyvero Result	Org+/Res+ [a]	**6**	0	2	8
	Org+/Res-	0	**9**	6	15
	Org-	1	1	**577**	579
	total	7	**10**	**585**	602
Haemophilus influenzae/tem			rate [%]	positivity	95 % CI
Agreement (Org+/Res+)			85.7	6/7	48.7 - 97.4
Agreement (Org+/Res-)			90.0	9/10	59.6 - 98.2
Agreement (Org-)			98.6	577/585	97.3 - 99.3

[a] isolate linkage: 3 culture positive cases, 2 isolates [2 of 2 confirmed]
[b] multi-detection specimens: not applicable (*tem* reported only with *H. influenzae*)
[c] note that other LRT panel microorganisms (Enterobacteriaceae, *Acinetobacter* spp., *P. aeruginosa)* could be the source of *tem*

B

H. influenzae [c] ***tem***		**Culture Result for Host Microorganism + PCR/Seq for Antibiotic Resistance Marker**			
		Org+/Res+ [b]	Org+/Res-	Org-	total
Unyvero Result	Org+/Res+ [a]	**3**	0	5	8
	Org+/Res-	0	**5**	11	16
	Org-	0	0	**579**	579
	total	**3**	**5**	**595**	603
Haemophilus influenzae/tem			rate [%]	positivity	95 % CI
Agreement (Org+/Res+)			100	3/3	43.9 - 100.0
Agreement (Org+/Res-)			100	5/5	56.6 - 100.0
Agreement (Org-)			97.3	579/595	95.7 - 98.3

[a] Isolate linkage: 2 isolates [2 of 2 confirmed]
[b] Multi-detection specimens: not applicable (*tem* reported only with *H. influenzae*)
[c] Note that other LRT panel microorganisms (Enterobacteriaceae, *Acinetobacter* spp., *P. aeruginosa* could be the source of *tem*

Table 43: Agreement rates for *ctx*-M between LRT and comparator methods A and B, host microorganisms: Enterobacteriaceae, *Acinetobacter* spp., *P. aeruginosa.*

A

Corr. Host Microorganism *ctx*-M		Composite Comparator Result for Host Microorganism + PCR/Seq for Antibiotic Resistance Marker			
		Org+/Res+ [b]	Org+/Res-	Org-	total
Unyvero Result	Org+/Res+ [a]	**15**	1	1	17
	Org+/Res-	1	**156**	20	177
	Org-	0	16	**392**	408
	total	**16**	**173**	**413**	602
Corr. host microorganism /*ctx*-M		rate [%]	positivity	95 % CI	
Agreement (Org+/Res+)		93.8	15/16	71.7 - 98.9	
Agreement (Org+/Res-)		90.2	156/173	84.8 - 93.8	
Agreement (Org-)		94.9	392/413	92.4 - 96.7	

[a] At least one isolate available for 10 specimens [linkage confirmed for 5 of 10 specimens]
[b] Multi-detection specimens (two or more corresponding LRT host microorganisms): 9 of 16

B

Corr. Host Microorganism *ctx*-M		Culture Result for Host Microorganism + PCR/Seq for Antibiotic Resistance Marker			
		Org+/Res+ [b]	Org+/Res-	Org-	total
Unyvero Result	Org+/Res+ [a]	**11**	0	6	17
	Org+/Res-	1	**128**	49	178
	Org-	0	10	**398**	408
	total	**12**	**138**	**453**	**603**
Corr. host microorganism /*ctx*-M		rate [%]	positivity	95 % CI	
Agreement (Org+/Res+)		91.7	11/12	64.6 - 98.5	
Agreement (Org+/Res-)		92.8	128/138	87.2 - 96.0	
Agreement (Org-)		87.9	398/453	84.5 - 90.6	

[a] At least one isolate available for 10 specimens [linkage confirmed for 5 of 10 specimens]
[b] Multi-detection specimens (two or more corresponding LRT host microorganisms): 6 of 12

Table 44: Agreement rates for *kpc* between LRT and comparator methods A and B, host microorganisms: Enterobacteriaceae, *Acinetobacter* spp., *P. aeruginosa*.

A

Corr. Host Microorganism *kpc*		Composite Comparator Result for Host Microorganism + PCR/Seq for Antibiotic Resistance Marker			
		Org+/Res+ [b]	Org+/Res-	Org-	total
Unyvero Result	Org+/Res+ [a]	**6**	0	0	6
	Org+/Res-	0	**167**	21	188
	Org-	0	16	**392**	408
	total	**6**	**183**	**413**	602
Corr. host microorganism /*kpc*		rate [%]	positivity	95 % CI	
Agreement (Org+/Res+)		100.0	6/6	61.0 - 100.0	
Agreement (Org+/Res-)		91.3	167/183	86.3 - 94.5	
Agreement (Org-)		94.9	392/413	92.4 - 96.7	

[a] Sample linkage: at least one isolate available for 5 specimens [linkage confirmed for 4 of 5 specimens]
[b] Multi-detection specimens (two or more corresponding LRT host microorganisms): 4 of 6

B

Corr. Host Microorganism *kpc*		Culture Result for Host Microorganism + PCR/Seq for Antibiotic Resistance Marker			
		Org+/Res+ [b]	Org+/Res-	Org-	total
Unyvero Result	Org+/Res+ [a]	**6**	0	0	6
	Org+/Res-	0	**134**	55	189
	Org-	0	10	**398**	408
	total	**6**	**144**	**453**	603
Corr. host microorganism /*kpc*		rate [%]	positivity	95 % CI	
Agreement (Org+/Res+)		100	6/6	61.0 - 100.0	
Agreement (Org+/Res-)		93.1	134/144	87.7 - 96.2	
Agreement (Org-)		87.9	398/453	84.5 - 90.6	

[a] Sample linkage: at least one isolate available for 5 specimens [linkage confirmed for 4 of 5 specimens]
[b] Multi-detection specimens (two or more corresponding LRT host microorganisms): 2 of 6

Table 45: Agreement rates for *vim* between LRT and comparator methods A and B, host microorganisms: Enterobacteriaceae, *Acinetobacter* spp., *P. aeruginosa*.

A

Corr. Host Microorganism *vim*		Composite Comparator Result for Host Microorganism + PCR/Seq for Antibiotic Resistance Marker			
		Org+/Res+ [b]	Org+/Res-	Org-	total
Unyvero Result	Org+/Res+ [a]	**2**	0	0	2
	Org+/Res-	0	**171**	21	192
	Org-	0	16	**392**	408
	total	**2**	**187**	**413**	602
Corr. host microorganism/*vim*			rate [%]	positivity	95 % CI
Agreement (Org+/Res+)			100.0	2/2	34.2 - 100.0
Agreement (Org+/Res-)			91.4	171/187	86.6 - 94.7
Agreement (Org-)			94.9	392/413	92.4 - 96.7

[a] Sample linkage: at least 1 isolate available for 2 specimens [linkage confirmed for 2 of 2 specimens]
[b] Multi-detection specimens (two or more corresponding LRT host microorganisms): 1 of 2

B

Corr. Host Microorganism *vim*		Culture Result for Host Microorganism + PCR/Seq for Antibiotic Resistance Marker			
		Org+/Res+ [b]	Org+/Res-	Org-	total
Unyvero Result	Org+/Res+ [a]	**2**	0	0	2
	Org+/Res-	0	**138**	55	193
	Org-	0	10	**398**	408
	total	**2**	**148**	**453**	603
Corr. host microorganism/*vim*			rate [%]	positivity	95 % CI
Agreement (Org+/Res+)			100.0	2/2	34.2 - 100.0
Agreement (Org+/Res-)			93.2	138/148	88.0 - 96.3
Agreement (Org-)			87.9	398/453	84.5 - 90.6

[a] Sample linkage: at least 1 isolate available for 2 specimens [linkage confirmed for 2 of 2 specimens]
[b] Multi-detection specimens (two or more corresponding LRT host microorganisms): 1 of 2

Table 46: Agreement rates for *oxa*-48 between LRT and comparator methods A and B, host microorganisms: Enterobacteriaceae.

A

Corr. Host Microorganism *oxa*-48		Composite Comparator Result for Host Microorganism + PCR/Seq for Antibiotic Resistance Marker			
		Org+/Res+ [b]	Org+/Res-	Org-	total
Unyvero Result	Org+/Res+ [a]	**0**	0	1	1
	Org+/Res-	0	**119**	19	138
	Org-	0	10	**453**	463
	total	**0**	**129**	**473**	602
Corr. host microorganism/*oxa*-48			rate [%]	positivity	95 % CI
Agreement (Org+/Res+)			na	0/0	na
Agreement (Org+/Res-)			92.2	119/129	86.3 - 95.7
Agreement (Org-)			95.8	453/473	93.6 - 97.2

[a] Sample linkage: N/A, no culture positive specimens, no isolates
[b] Multi-detection specimens (two or more corresponding host microorganisms): none

B

Corr. Host Microorganism *oxa*-48		Culture Result for Host Microorganism + PCR/Seq for Antibiotic Resistance Marker			
		Org+/Res+ [b]	Org+/Res-	Org-	total
Unyvero Result	Org+/Res+ [a]	**0**	0	1	1
	Org+/Res-	0	**80**	59	139
	Org-	0	8	**455**	463
	total	**0**	**88**	**515**	603
Corr. host microorganism/*oxa*-48			rate [%]	positivity	95 % CI
Agreement (Org+/Res+)			na	0/0	na
Agreement (Org+/Res-)			90.9	80/88	83.1 - 95.3
Agreement (Org-)			88.3	455/515	85.3 - 90.8

[a] Sample linkage: N/A, no culture positive specimens, no isolates
[b] Multi-detection specimens (two or more corresponding host microorganisms): none

Table 47: Agreement rates for *oxa*-23 between LRT and comparator methods A and B, (single) host microorganism: *Acinetobacter* spp.

A

***Acinetobacter* spp. *oxa*-23**		**Composite Comparator Result for Host Microorganism + PCR/Seq for Antibiotic Resistance Marker**			
		Org+/Res+ [b]	Org+/Res-	Org-	total
Unyvero Result	Org+/Res+ [a]	**6**	2	0	8
	Org+/Res-	1	**13**	5	19
	Org-	0	1	**575**	576
	total	7	**16**	**580**	603
***Acinetobacter* spp./*oxa*-23**			rate [%]	positivity	95 % CI
Agreement (Org+/Res+)			85.7	6/7	48.7 - 97.4
Agreement (Org+/Res-)			81.3	13/16	57.0 - 93.4
Agreement (Org-)			99.1	575/580	98.0 - 99.6

[a] Isolate linkage: 2 culture positive specimens, 2 isolates [2 of 2 confirmed]
[b] Multi-detection specimens: not applicable (*oxa*-23 reported only with *Acinetobacter* spp.)

B

***Acinetobacter* spp. *oxa*-23**		**Culture Result for Host Microorganism + PCR/Seq for Antibiotic Resistance Marker**			
		Org+/Res+ [b]	Org+/Res-	Org-	total
Unyvero Result	Org+/Res+ [a]	**2**	0	6	8
	Org+/Res-	1	7	11	19
	Org-	0	0	**576**	576
	total	**3**	7	**593**	603
***Acinetobacter* spp./*oxa*-23**			rate [%]	positivity	95 % CI
Agreement (Org+/Res+)			66.7	2/3	20.8 - 93.9
Agreement (Org+/Res-)			100.0	7/7	64.6 - 100.0
Agreement (Org-)			97.1	576/593	95.5 - 98.2

[a] Isolate linkage: 2 isolates [2 of 2 confirmed]
[b] Multi-detection specimens: not applicable (*oxa*-23 reported only with *Acinetobacter* spp.)

Table 48: Agreement rates for *oxa*-24 between LRT and comparator methods A and B, host microorganisms: Enterobacteriaceae, *Acinetobacter* spp., *P. aeruginosa.*

A

***Acinetobacter* spp. *oxa*-24**		**Composite Comparator Result for Host Microorganism + PCR/Seq for Antibiotic Resistance Marker**			
		Org+/Res+ [b]	Org+/Res-	Org-	total
Unyvero Result	Org+/Res+ [a]	**2**	0	0	2
	Org+/Res-	0	**20**	5	25
	Org-	0	1	**575**	576
	total	**2**	**21**	**580**	603
***Acinetobacter* spp./*oxa*-24**			rate [%]	positivity	95 % CI
Agreement (Org+/Res+)			100.0	2/2	34.2 - 100.0
Agreement (Org+/Res-)			95.2	20/21	77.3 - 99.2
Agreement (Org-)			99.1	575/580	98.0 - 99.6

[a] Isolate linkage: 1 culture positive specimen, 1 isolate [1 of 1 confirmed]
[b] Multi-detection specimens: not applicable (*oxa*-24 reported only with *Acinetobacter* spp.)

B

***Acinetobacter* spp. *oxa*-24**		**Culture Result for Host Microorganism + PCR/Seq for Antibiotic Resistance Marker**			
		Org+/Res+ [b]	Org+/Res-	Org-	total
Unyvero Result	Org+/Res+ [a]	**1**	0	1	**2**
	Org+/Res-	0	**9**	**16**	**25**
	Org-	0	0	**576**	**576**
	total	**1**	**9**	**593**	**603**
***Acinetobacter* spp./*oxa*-24**			rate [%]	positivity	95 % CI
Agreement (Org+/Res+)			100.0	1/1	20.7 - 100.0
Agreement (Org+/Res-)			100.0	9/9	70.1 - 100.0
Agreement (Org-)			97.1	576/593	95.5 - 98.2

[a] Isolate linkage: 1 isolate [1 of 1 confirmed]
[b] Multi-detection specimens: not applicable (*oxa*-24 reported only with *Acinetobacter* spp.)

Table 49. Agreement rates for *mec*A between LRT and comparator methods A and B, (single) host microorganism: *S. aureus*.

A

***S. aureus* *mec*A**		**Composite Comparator Result for Host Microorganism + PCR/Seq for Antibiotic Resistance Marker**			
		Org+/Res+ [b]	Org+/Res-	Org-	total
Unyvero Result	Org+/Res+ [a]	**53**	7	2	62
	Org+/Res-	5	**44**	9	58
	Org-	4	6	**473**	483
	total	**62**	**57**	**484**	603
		***S. aureus*/*mec*A**	rate [%]	positivity	95 % CI
		Agreement (Org+/Res+)	85.5	53/62	74.7 - 92.2
		Agreement (Org+/Res-)	77.2	44/57	64.8 - 86.2
		Agreement (Org-)	97.7	473/484	96.0 - 98.7

[a] Isolate linkage: 48 culture positive specimens, 36 isolates [29 of 36 confirmed]
[b] Multi-detection specimens: not applicable (*mec*A only reported with *S. aureus*)

B

***S. aureus* *mec*A**		**Culture Result for Host Microorganism + PCR/Seq for Antibiotic Resistance Marker**			
		Org+/Res+ [b]	Org+/Res-	Org-	total
Unyvero Result	Org+/Res+ [a]	**44**	4	14	62
	Org+/Res-	4	**29**	25	58
	Org-	0	2	**481**	483
	total	**48**	**35**	**520**	603
		***S. aureus*/*mec*A**	rate [%]	positivity	95 % CI
		Agreement (Org+/Res+)	91.7	44/48	80.4 - 96.7
		Agreement (Org+/Res-)	82.9	29/35	67.3 - 91.9
		Agreement (Org-)	92.5	481/520	89.9 - 94.5

[a] Isolate linkage: 36 isolates [29 of 36 confirmed]
[b] Multi-detection specimens: not applicable (*mec*A reported only with *S. aureus*)

For the prospective study, clinical performance of the Unyvero LRT Application for detection of *mec*A was also compared to phenotypic antimicrobial susceptibility (AST) testing results for all *S. aureus* isolates recovered from the reference culture. Culture results were the reference method *S. aureus* detection and cefoxitin and/or oxacillin AST results were used as the phenotypic reference method for *mec*A. Results are shown in Table 50 below.

Table 50: Agreement rates for *S. aureus*/*mec*A between LRT and phenotypes of corresponding *S. aureus* strains.

***S. aureus mec*A**		**Culture Result for *S. aureus* and Cefoxitin/Oxacillin AST results**			
		SA+/Res+ (MRSA)	SA+/Res- (MSSA)	SA-	total
Unyvero Result	SA+/mecA+	**40**	7	14	61
	SA+/mecA--	1	**31**	25	57
	SA-	2	0	**481**	483
	total	**43**	**38**	**520**	601
		***S. aureus/mec*A**	rate [%]	positivity	95 % CI
		Agreement (SA+/Res+)	93.0	40/43	81.4 - 97.6
		Agreement (SA+/Res-)	81.6	31/38	66.6 - 90.8
		Agreement (SA-)	92.5	481/520	89.9 - 94.5

A known limitation for detection of LRT resistance marker targets directly from tracheal aspirate specimens is that detection of markers cannot be definitively linked to corresponding detected microorganisms. For example, on-panel microorganisms present in the specimen that are not detected by LRT or off-panel microorganisms could serve as the source of a detected marker. In addition, tracheal aspirate specimens commonly contain multiple microorganisms, of which more than one microorganism could be the source of a detected resistance marker.

For the prospective and archived U.S. specimen cohorts, an analysis of genotypic 'linkage' as well as phenotypic agreement was performed to evaluate the relationship between detection of resistance markers by the LRT Application directly from tracheal aspirate specimens, the presence of the marker in corresponding culture isolates (based on PCR/bi-directional sequencing), and agreement with phenotypic AST results of reference culture isolates. Included in the analysis were specimens that were positive for targeted resistance markers by the LRT assay and were also 'true positive' for applicable LRT microorganism targets (i.e., positive by LRT and positive by culture for applicable microorganism targets). The analysis also included only those specimens for which isolates were available for confirmatory genotypic testing. For 'true positive' specimens, positive results for antibiotic resistance marker targets were compared to individual available isolates from SoC/culture positive specimens for:

1. Genotypic Linkage: Genotypic linkage of positive LRT antibiotic resistance marker results to sequencing of cultured isolates (presence of the antibiotic resistance marker in the genome of one (or more) host microorganism strains isolated from a specific specimen, determined by PCR/bi-directional sequencing). Note that only a subset of isolates was available for this analysis. Confirmed genotypic linkage is defined as a positive LRT result for a resistance marker where a corresponding culture isolate is also positive by sequencing).

2. Phenotypic Analysis: Agreement of positive LRT resistance marker results to associated phenotypic antimicrobial susceptibility testing (AST) results. AST results were collected for the antimicrobials listed in Table 51. Analysis of AST agreement with positive LRT results is presented in Tables 53-59 only for the subset of specimens to include those LRT positive specimens demonstrating genotypic linkage of the detected resistance marker.

For evaluation of phenotypic agreement of positive LRT resistance marker results, AST results for applicable antibiotics were collected and evaluated for all applicable reference culture isolates (Table 51). AST results were reported as MIC values or zone diameters (for Kirby-Bauer tests). AST results were determined using breakpoints listed in CLSI guidance M100S (Performance Standards for Antimicrobial Susceptibility Testing, 26th Edition 2016). “Intermediate” AST results were regarded as “resistant” and any strain was regarded as “resistant” if at least one of the corresponding drug AST results were “intermediate” or “resistant”. Any strain was regarded as “susceptible” if AST results were susceptible for all applicable tested antibiotics.

Table 51: AST assays used for evaluation of antibiotic resistance markers detected by LRT

Antibiotic resistance marker	Associated resistance	AST assay
tem	Penicillins	Ampicillin, Cefinase
ctx-M	3rd Generation Cephalosporins and Cefepime	Enterobacteriaceae: Cefotaxime, Ceftazidime, Ceftriaxone *Acinetobacter* spp.: Cefotaxime, Ceftazidime, Ceftriaxone, Cefepime *P. aeruginosa*: Ceftazidime, Cefepime
kpc, *ndm*	Carbapenems	Enterobacteriaceae: Meropenem, Ertapenem, Imipenem *Acinetobacter* spp./ *P. aeruginosa*: Meropenem, Imipenem
oxa-48		Enterobacteriaceae: Meropenem, Ertapenem, Imipenem
oxa-23, oxa-24, and *oxa-58*		*Acinetobacter* spp.: Meropenem, Imipenem
*mec*A	Oxacillin	*S. aureus*: Oxacillin, Cefoxitin

Evaluation of genotypic linkage and phenotypic agreement for resistance markers detected by the LRT Application was performed on a per specimen basis. For many specimens, more than one isolate with the potential to carry a detected resistance marker was recovered from the reference culture.

For specimens that were positive for LRT antibiotic resistance markers, the overall number and percent of specimens with confirmed genotypic linkage as observed in the archived (N=185) and prospective (N=603) studies combined is shown in Table 52. Results are not presented for *ndm* and *oxa*-58 (no positive LRT results) and for *oxa*-48 (one positive LRT result, no isolate available).

Table 52: Performance, Linkage Analysis

Resistance Marker Detected	LRT Microorganism Target	Number of specimens with demonstrated linkage/Number of True positive LRT Microorganism Results	Percentage of Specimens with Confirmed Linkage
tem	*H. influenzae*	1/2	50%
ctx-M	*Enterobacteriaceae, P. aeruginosa*, and/or *Acinetobacter spp.*	9/16	56.3%
kpc	*Enterobacteriaceae, P. aeruginosa*, and/or *Acinetobacter spp.*	4/5	80%
vim	*Enterobacteriaceae, P. aeruginosa,* and/or *Acinetobacter* spp.	2/3	66.7%
oxa-23	*Acinetobacter* spp.	4/4	100%
oxa-24	*Acinetobacter* spp.	1/1	100%
*mec*A	*S. aureus*	37/47	78.7%

Further details of the genotypic linkage and phenotypic agreement analyses are shown in Tables 53-59 with 'true positive' specimens presented in individual table rows. An additional column is added to each table listing specimens with positive resistance markers results and corresponding microorganism targets detected by LRT but not recovered by culture. It is noted that these microorganisms may be the source of the detected resistance marker; however, due to the lack of isolates, linkage and agreement with AST results cannot be evaluated.

Analysis of phenotypic agreement for the subset of specimens with positive LRT Application resistance marker results showed 100% agreement (i.e., resistance) for all positive specimens with culture isolates demonstrating genotypic linkage (i.e., corresponding culture isolate is positive for the resistance marker).

The following microorganism abbreviations are used in Tables 53-59: *Acinetobacter* spp. (Aci), *Enterobacter cloacae* complex (Ecl), *Escherichia coli* (Eco), *Haemophilus influenzae* (Hae), *Klebsiella oxytoca* (Kox), *Klebsiella pneumoniae* (Kpn), *Morganella morganii* (Mor), *Proteus* spp. (Pro), *Pseudomonas aeruginosa* (Pse), *Serratia marcescens* (Ser), *Staphylococcus aureus* (Sau).

Table 53: Linkage analysis for LRT positive specimens for *tem* that are true positive for *H. influenzae* and have available isolates. Agreement of AST results for specimens with confirmed linkage

True Positive Host Microorganisms [a]	# Specimens			Isolate Linkage		**Linkage [%] (95 % CI)**	**Agreement of AST result for linked microorganisms [%] [b] (95 % CI)**
	all	arch.	prosp.	not conf.	conf.		
tem	2 [d]	0	2	1	1	**1/2 50.0** (9.4-90.6)	**1/1 100.0** (20.7-100.0)
Hae*	1	0	1	0	1		**R**
Hae, [Eco*] [c]	1	0	1	1	0		-

[a] True positive: SoC positive *H. influenzae* detected by LRT, *: indicates microorganisms for a certain specimen with confirmed presence of *tem* ("linkage") by PCR/bi-directional sequencing, "[Org.]": indicates other true positive microorganisms (Enterobacteriaceae, *Acinetobacter* spp., *P. aeruginosa*) that could potentially be the source of *tem*.

[b] R: resistant to Penicillin, S: susceptible to Penicillin, na: no AST data available.

[c] Note that *tem* was linked to an *E. coli* isolate isolated from this specimen and the *H. influenzae* isolate also isolated from this specimen was susceptible to cefinase.

[d] In total, four *H. influenzae* true positive specimens (one archived, three prospective) were reported positive for *tem* by LRT; *H. influenzae* isolates were available for two cases.

Table 54: Linkage analysis for LRT positive specimens for *ctx-M* that are true positive for applicable LRT microorganisms and have available isolates. Agreement of AST results for specimens with confirmed linkage

True Positive Host Microorganisms [a]	# Specimens			Isolate Linkage		**Linkage [%] (95 % CI)**	**Agreement of AST result for linked microorganisms [%] [b] (95 % CI)**	Specimens that are LRT positive/culture negative for additional applicable microorganisms [c]
	all	arch.	prosp.	not conf.	conf.			
***ctx*-M**	**16** [d]	**6**	**10**	**7**	**9**	**9/16 56.3** (33.2-76.9)	7/7 **100.0** (64.6-100.0)	
Eco*, Pse	2	0	2	0	2		**R (1), na (1)**	0 of 2
Kpn*	2	1	1	0	2		**R (1), na (1)**	1 of 2
Eco*	1	0	1	0	1		**R**	0 of 1
Kpn*, Pse	1	1	0	0	1		**R**	1 of 1
(Kpn), Pse, Pro*	1	1	0	0	1		**R**	0 of 1
Kpn, Pro*	1	1	0	0	1		**R**	1 of 1
Pse, Pro*	1	0	1	0	1		**R**	1 of 1
Pse	4	1	3	4	0		-	4 of 4
(Kpn), Pse	1	0	1	1	0		-	0 of 1
Mor, Pse, Ser	1	1	0	1	0		-	1 of 1
(Pse), (Pro), Ser	1	0	1	1	0		-	1 of 1

[a] True positive: SoC positive microorganisms detected by LRT, *: indicates microorganisms for a certain specimen with confirmed presence of *ctx*-M ("linkage") by PCR/bi-directional sequencing, "(Org.)": indicates microorganisms for which no isolate was collected and linkage analysis could not be performed.

[b] R: resistant to Third Generation Cephalosporins, na: no AST data

[c] Additional LRT positive/culture negative results for applicable targeted microorganisms that could potentially be the source of detected antibiotic resistance markers.

[d] In total, 23 specimens were reported positive for *ctx*-M by LRT (12 archived, 11 prospective); isolates were available for 16 cases.

Table 55: Linkage analysis for LRT positive specimens for *kpc* that are true positive for applicable LRT microorganisms and have available isolates. Agreement of AST results for specimens with confirmed linkage

True Positive Host Microorganisms [a]	# Specimens			Isolate Linkage		**Linkage [%] (95 % CI)**	**Agreement of AST result for linked microorganisms [%] [b] (95 % CI)**	Specimens that are LRT positive/culture negative for additional applicable microorganisms [c]
	all	arch.	prosp.	not conf.	conf.			
kpc	**5 [d]**	**0**	**5**	**1**	**4**	**4/5 80.0** (37.6-96.4)	**3/3 100.0** (43.9-100.0)	
Ecl*	1	0	1	0	1		**na**	0 of 1
Kpn*	1	0	1	0	1		**R**	0 of 1
Mor*	1	0	1	0	1		**R**	1 of 1
Pse*	1	0	1	0	1		**R**	1 of 1
(Pse), (Pro), Ser	1	0	1	1	0		-	1 of 1

[a] True positive: SoC positive microorganisms detected by LRT, *: indicates microorganisms for a certain specimen with confirmed presence of *kpc* ("linkage") by PCR/bi-directional sequencing, "(Org.)": indicates microorganisms for which no isolate was collected and linkage analysis could not be performed.

[b] R: resistant to Carbapenems, na: no AST data available

[c] Additional LRT positive/culture negative results for applicable targeted microorganisms that could potentially be the source of detected antibiotic resistance markers.

[d] In total, nine specimens were reported positive for *kpc* by LRT (three archived, six prospective); isolates were available for five cases.

Table 56: Linkage analysis for LRT positive specimens for vim that are true positive for applicable LRT microorganisms and have available isolates. Agreement of AST results for specimens with confirmed linkage

True Positive Host Microorganisms [a]	# Specimens			Isolate Linkage		**Linkage [%] (95 % CI)**	**Agreement of AST result for linked microorganisms [%] [b] (95 % CI)**	Specimens that are LRT positive/culture negative for additional applicable microorganisms [c]
	all	arch.	prosp.	not conf.	conf.			
vim	**3 [d]**	**1**	**2**	**1**	**2**	**2/3 66.7 %** (20.8-93.9)	**2/2 100.0** (34.2-100.0)	
Pse*	1	0	1	0	1		**R**	0 of 1
Pse*, (Pro)	1	0	1	0	1		**R**	1 of 1
Kpn, (Pro)	1	1	0	1	0		-	1 of 1

[a] True positive: SoC positive microorganisms detected by LRT, *: indicates microorganisms for a certain specimen with confirmed

presence of *vim* ("linkage") by PCR/bi-directional sequencing, "(Org.)": indicates microorganisms for which no isolate was collected and linkage analysis could not be performed.

[b] R: resistant to Carbapenems, na: no AST data available.

[c] Additional LRT positive/culture negative results for applicable targeted microorganisms that could potentially be source of detected antibiotic resistance markers.

[d] In total, three specimens (one archived, two prospective) were reported positive for *vim* by LRT, isolates were available for all cases.

Table 57: Linkage analysis for LRT positive specimens for *oxa*-23 that are true positive for Acinetobacter spp. and have available isolates. Agreement of AST results for specimens with confirmed linkage

True Positive Host Microorganisms [a]	# Specimens			Isolate Linkage		**Linkage [%] (95 % CI)**	**Agreement of AST result for linked microorganisms [%] [b] (95 % CI)**
	all	arch.	prosp.	not conf.	conf.		
***oxa*-23**	**4 [c]**	**2**	**2**	**0**	**4**	**4/4 100.0** (51.0-100.0)	**3/3 100.0** (43.9-100.0)
Aci*	4	2	2	0	4		R (3), na (1)

[a] True positive: SoC positive *Acinetobacter* spp. detected by LRT, *: indicates microorganisms for a certain specimen with confirmed presence of *oxa*-23 ("linkage") by PCR/bi-directional sequencing.

[b] R: resistant to Carbapenems, S: susceptible to Carbapenems, na: no AST data available

[c] In total, eight specimens were reported positive for *oxa*-23 by LRT (six archived, two prospective); isolates were available for four cases.

Table 58: Linkage analysis for LRT positive specimens for *oxa*-24 that are true positive for *Acinetobacter* spp. and have available isolates. Agreement of AST results for specimens with confirmed linkage

True Positive Host Microorganisms [a]	# Specimens			Isolate Linkage		**Linkage [%] 95 % CI**	**Agreement of AST result for linked microorganisms [%] [b] (95 % CI)**
	all	arch.	prosp.	not conf.	conf.		
***oxa*-24**	**1 [c]**	**0**	**1**	**0**	**1**	**1/1 100.0** (20.7-100.0)	**1/1 100.0** (20.7-100.0)
Aci*	1	0	1	0	1		**R**

[a] True positive: SoC positive *Acinetobacter* spp. detected by LRT, *: indicates microorganisms for a certain specimen with confirmed presence of *oxa*-24 ("linkage") by PCR/bi-directional sequencing.

[b] R: resistant to Carbapenems, S: susceptible to Carbapenems, na: no AST data available.

[c] In total, one prospective specimen with available isolate was reported positive for *oxa*-24 by LRT.

Table 59: Linkage analysis for LRT positive specimens for *mec*A that are true positive for *S. aureus* and have available isolates. Agreement of AST results for specimens with confirmed linkage.

True Positive Host Microorganisms [a]	# Specimens			Isolate Linkage		**Linkage [%] (95 % CI)**	**Agreement of AST result for linked microorganisms [%] [b] (95 % CI)**
	all	arch.	prosp.	not conf.	conf.		
***mec*A**	**47 [c]**	**11**	**36**	**10**	**37**	**37/47 78.7** (65.1-88.0)	**36/36 100.0** (90.4-100.0)
Sau*	37	8	29	0	37		R (36), na (1)
Sau	10	3	7	10	0		-

[a] True positive: SoC positive *S. aureus* detected by LRT, *: indicates microorganisms for a certain specimen with confirmed presence of *mec*A ("linkage") by PCR/bi-directional sequencing.
[b] R: resistant to oxacillin, S: susceptible to oxacillin, na: no AST data available.
[c] In total, 81 specimens were reported positive for *mec*A by LRT (33 archived, 48 prospective); isolates were available for 47 cases.

Summary of Clinical Study:

The clinical study design for evaluation of the Unyvero LRT assay did not include evaluation of patient outcome and therefore the potential impact of test results on patient care is unknown. The assay labeling and assay report include multiple limitations and warnings for the laboratory and clinician, most importantly stating that results from this assay must be used in conjunction with results from culture.

Microorganism Targets ('typical' bacteria): Clinical performance of the Unyvero LRT Application for detection of 'typical' microorganisms in tracheal aspirate specimens was evaluated by comparing LRT results to traditional semi-quantitative culture as well as to a composite comparator consisting of culture plus a molecular comparator (PCR followed by bi-directional sequencing). For most microorganism targets, assay sensitivity and specificity compared to culture were greater than 90% and 95% respectively.

The analysis and interpretation of tracheal aspirate cultures can be somewhat subjective, with the judgement of the laboratory technologist playing a critical role in determining the final culture results. Current recommendations for reporting semi-quantitative results for tracheal aspirate cultures are based on the relative quantities of all potential pathogens as well as the amount of normal respiratory flora that grow on culture plates.

LRT false positive results when compared to culture are not unexpected as the Unyvero LRT assay does not distinguish between viable and non-viable organisms, does not quantify the amount of DNA present and reports detected microbial DNA without providing information on the presence or amount of normal respiratory flora.

Although the LRT assay generated a significant number of false positive results compared to culture, most of these results were confirmed to be positive by PCR and sequencing, demonstrating that the targeted bacterial DNA was present in the specimen and the Unyvero

LRT assay correctly detected the presence of microbial DNA targets. For many false positive results, culture results were reported as mixed flora or normal respiratory flora and many specimens were positive by the LRT assay for two or more targeted microorganisms as shown in tables 25 above.

False positive results for 'typical' bacterial analytes can be mitigated by limitations provided in the assay labeling and test report informing the laboratory and clinician that detected microorganisms may be from colonizing flora and may not be the causative agent of disease. Most importantly, false positive and false negative Unyvero results can be mitigated by the requirement that concomitant culture is performed for all tracheal aspirate specimens tested with the Unyvero LRT Application.

Microorganism targets ('atypical' bacteria): Performance for the 'atypical' analytes was demonstrated primarily with contrived specimens with Unyvero LRT results showing acceptable agreement to expected results. The following limitation is included in the package insert:

- A negative result for the 'atypical' microorganisms (*C. pneumoniae, L. pneumophila, and M. pneumoniae*) does not exclude the presence of this microorganism in the patient specimen. A positive result should be evaluated in the overall context of the patient's clinical condition and other laboratory results being part of the standard-of-care routine.

Resistance marker targets: The Unyvero LRT assay demonstrated acceptable performance for detection of targeted resistance markers when compared to validated PCR and sequencing assays. The assay masking rules applied by the assay software allow reporting of positive resistance marker results only for specimens with concurrently detected microorganisms that have the potential to carry the detected marker. However, despite these rules, detected resistance markers cannot always be definitively be linked to the concurrently detected microorganism as shown in Table 52 above. False positive results for resistance markers can be mitigated by the requirement for concomitant culture and subsequent AST testing. False negative results for resistance markers are mitigated by the following limitation included in the assay report:

- Antimicrobial resistance may occur via multiple mechanisms other than the resistance markers detected by the Unyvero LRT assay. Negative results for LRT resistance markers do not indicate antimicrobial susceptibility of detected organisms.

Note: Greater than (b) additional prospectively collected specimens were evaluated in the pivotal clinical study for the Unyvero LRT Application and results from this testing were submitted in the *De Novo* application. Results for this specimen cohort were reviewed and considered as additional supportive evidence in the assessment of Unyvero LRT Application performance.

4. Clinical cut-off:

   Not applicable

5. Expected values/Reference range:

Of the 603 evaluable tracheal aspirate specimens in the prospective study, the LRT Application reported 312 specimens with at least one positive LRT panel microorganism, including 125 specimens with multi-detections for two or more microorganisms. Reference culture testing reported 236 specimens with at least one LRT panel microorganism, including 62 specimens with two or more LRT panel microorganisms reported. Expected values (number of positive results for each microorganism reported by the Unyvero LRT Application) are presented in Table 60 for all prospectively tested specimens as well as for specimens positive by LRT for multiple microorganisms. Table 61 includes the numbers of positive resistance marker results reported by the LRT assay in the prospective study.

Table 60: Expected values of LRT panel microorganism targets, prospective study

	Expected Values for all Specimens (N= 603)		**Expected Values for Multi-detection Specimens (N= 125)**	
	# specimens	[%]	# specimens	[%]
Acinetobacter spp.	27	4.5	20	16.0
Chlamydia pneumoniae	0	0.0	0	0.0
Citrobacter freundii	3	0.5	2	1.6
Enterobacter cloacae complex	20	3.3	11	8.8
Escherichia coli	45	7.5	32	25.6
Haemophilus influenzae	24	4.0	15	12.0
Klebsiella oxytoca	15	2.5	11	8.8
Klebsiella pneumoniae	38	6.3	25	20.0
Klebsiella variicola	4	0.7	4	3.2
Legionella pneumophila	2	0.3	1	0.8
Moraxella catarrhalis	13	2.2	9	7.2
Morganella morganii	10	1.7	10	8.0
Mycoplasma pneumoniae	3	0.5	2	1.6
Proteus spp.	29	4.8	25	20.0
Pseudomonas aeruginosa	84	13.9	54	43.2
Serratia marcescens	25	4.1	20	16.0
Staphylococcus aureus	120	19.9	57	45.6
Stenotrophomonas maltophilia	52	8.6	39	31.2
Streptococcus pneumoniae	13	2.2	3	2.4

Table 61: Expected values of LRT panel antibiotic resistance markers as determined by the LRT Application for the prospective aspirate study

	Expected Values (N= 603)	
	# specimens	[%]
ctx-M	17	2.8
kpc	6	1.0
ndm	0	0.0
oxa-23	8	1.3
oxa-24	2	0.3
oxa-48	1	0.2
oxa-58	0	0.0
tem	8	1.3
vim	2	0.3
mecA	62	10.3

Table 62 includes the numbers of positive results for resistance markers and concurrently detected microorganism targets as observed in the prospective clinical study. Results are stratified by the number of resistance marker targets detected.

Table 62: Expected Values of resistance markers and microorganism targets, prospective study

Microorganism	Resistance Marker	# Specimens
No resistance marker reported		**513**
negative (no microorganisms detected)	-	291
Pseudomonas aeruginosa	-	30
Staphylococcus aureus	-	29
Stenotrophomonas maltophilia	-	13
Escherichia coli	-	11
Streptococcus pneumoniae	-	10
Klebsiella pneumoniae	-	9
Pseudomonas aeruginosa, Stenotrophomonas maltophilia	-	9
Enterobacter cloacae complex	-	8
Haemophilus influenzae	-	7
Acinetobacter spp.	-	6
Serratia marcescens	-	5
Moraxella catarrhalis	-	4
Proteus spp.	-	4
Enterobacter cloacae complex, *Escherichia coli*	-	3
Klebsiella oxytoca	-	3
Moraxella catarrhalis, Staphylococcus aureus	-	3
Pseudomonas aeruginosa, Staphylococcus aureus	-	3
Staphylococcus aureus, Stenotrophomonas maltophilia	-	3
Enterobacter cloacae complex, *Klebsiella oxytoca, Stenotrophomonas maltophilia*	-	2
Escherichia coli, Klebsiella pneumoniae	-	2

Microorganism	Resistance Marker	# Specimens
Escherichia coli, Klebsiella pneumoniae, Proteus spp.	-	2
Escherichia coli, Pseudomonas aeruginosa	-	2
Escherichia coli, Staphylococcus aureus	-	2
Haemophilus influenzae, Proteus spp., *Staphylococcus aureus*	-	2
Haemophilus influenzae, Staphylococcus aureus	-	2
Klebsiella oxytoca, Stenotrophomonas maltophilia	-	2
Klebsiella pneumoniae, Stenotrophomonas maltophilia	-	2
Proteus spp., *Pseudomonas aeruginosa*	-	2
Proteus spp., *Staphylococcus aureus*	-	2
Acinetobacter spp., *Enterobacter cloacae* complex, *Pseudomonas aeruginosa*	-	1
Acinetobacter spp., *Klebsiella pneumoniae, Saureus, Stenotrophomonas maltophilia*	-	1
Acinetobacter spp., *Moraxella catarrhalis, Morganella morganii, Proteus* spp., *Pseudomonas aeruginosa, Serrratia marcescens, Stenotrophomonas maltophilia, Streptococcus pneumoniae*	-	1
Acinetobacter spp., *Proteus* spp., *Pseudomonas aeruginosa, Serrratia marcescens, Stenotrophomonas maltophilia*	-	1
Acinetobacter spp., *Serrratia marcescens, Stenotrophomonas maltophilia*	-	1
Citrobacter freundii	-	1
Citrobacter freundii, Klebsiella oxytoca	-	1
Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae, Morganella morganii	-	1
Escherichia coli, Haemophilus influenzae, Staphylococcus aureus	-	1
Escherichia coli, Moraxella catarrhalis, Pseudomonas aeruginosa	-	1
Escherichia coli, Proteus spp., *Pseudomonas aeruginosa, Serratia marcescens*	-	1
Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens	-	1
Escherichia coli, Stenotrophomonas maltophilia	-	1
Enterobacter cloacae complex, *Escherichia coli, Klebsiella oxytoca, Pseudomonas aeruginosa*	-	1
Enterobacter cloacae complex, *Klebsiella variicola, Moraxella catarrhalis*	-	1
Enterobacter cloacae complex, *Proteus* spp.	-	1
Enterobacter cloacae complex, *Staphylococcus aureus*	-	1
Haemophilus influenzae, Klebsiella pneumoniae	-	1
Haemophilus influenzae, Moraxella catarrhalis	-	1
Haemophilus influenzae, Pseudomonas aeruginosa, Serratia marcescens	-	1
Klebsiella oxytoca, Klebsiella pneumoniae, Morganella morganii, Pseudomonas aeruginosa, Serratia marcescens	-	1
Klebsiella oxytoca, Pseudomonas aeruginosa	-	1
Klebsiella oxytoca, Pseudomonas aeruginosa, Stenotrophomonas maltophilia	-	1
Klebsiella oxytoca, Staphylococcus aureus	-	1

Microorganism	Resistance Marker	# Specimens
Klebsiella pneumoniae, Klebsiella variicola	-	1
Klebsiella pneumoniae, Morganella morganii, Staphylococcus aureus	-	1
Klebsiella pneumoniae, Pseudomonas aeruginosa	-	1
Klebsiella pneumoniae, Serratia marcescens, Staphylococcus aureus	-	1
Klebsiella variicola, Stenotrophomonas maltophilia	-	1
Legionella pneumophila	-	1
Legionella pneumophila, Staphylococcus aureus	-	1
Moraxella catarrhalis, Proteus spp., *Pseudomonas aeruginosa, Serratia marcescens*	-	1
Mycoplasma pneumoniae	-	1
Mycoplasma pneumoniae, Streptococcus pneumoniae	-	1
Proteus spp., *Pseudomonas aeruginosa, Serratia marcescens, Stenotrophomonas maltophilia*	-	1
Proteus spp., *Pseudomonas aeruginosa, Staphylococcus aureus*	-	1
Pseudomonas aeruginosa, Serratia marcescens	-	1
Pseudomonas aeruginosa, Serratia marcescens, Stenotrophomonas maltophilia	-	1
Serratia marcescens, Staphylococcus aureus	-	1
Serratia marcescens, Stenotrophomonas maltophilia	-	1
One resistance marker reported		**78**
Staphylococcus aureus	*mec*A	34
Klebsiella pneumoniae, Staphylococcus aureus	*mec*A	3
Acinetobacter spp., *Staphylococcus aureus*	*mec*A	2
Escherichia coli, Staphylococcus aureus	*mec*A	2
Acinetobacter spp., *Morganella morganii, Proteus* spp., *Staphylococcus aureus*	*mec*A	1
Citrobacter freundii, Klebsiella oxytoca, Klebsiella pneumoniae, Staphylococcus aureus	*mec*A	1
Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus	*mec*A	1
Escherichia coli, Serratia marcescens, Staphylococcus aureus, Stenotrophomonas maltophilia	*mec*A	1
Enterobacter cloacae complex, *Escherichia coli, Staphylococcus aureus*	*mec*A	1
Klebsiella variicola, Staphylococcus aureus	*mec*A	1
Moraxella catarrhalis, Pseudomonas aeruginosa, Staphylococcus aureus	*mec*A	1
Morganella morganii, Proteus spp., *Pseudomonas aeruginosa, Staphylococcus aureus*	*mec*A	1
Proteus spp., *Pseudomonas aeruginosa, Staphylococcus aureus*	*mec*A	1
Proteus spp., *Staphylococcus aureus*	*mec*A	1
Pseudomonas aeruginosa, Staphylococcus aureus	*mec*A	1
Serratia marcescens, Staphylococcus aureus	*mec*A	1
Staphylococcus aureus, Stenotrophomonas maltophilia	*mec*A	1
Klebsiella pneumoniae	*ctx*-M	3
Escherichia coli	*ctx*-M	2
Acinetobacter spp., *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*	*ctx*-M	1

Microorganism	Resistance Marker	# Specimens
Acinetobacter spp., *Klebsiella pneumoniae*	*ctx*-M	1
Escherichia coli, *Pseudomonas aeruginosa*	*ctx*-M	1
Escherichia coli, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*	*ctx*-M	1
Klebsiella oxytoca	*ctx*-M	1
Enterobacter cloacae complex	*kpc*	1
Klebsiella pneumoniae	*kpc*	1
Morganella morganii, *Serratia marcescens*	*kpc*	1
Acinetobacter spp., *Proteus* spp., *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*	*oxa*-23	1
Acinetobacter spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*	*oxa*-23	1
Acinetobacter spp.	*oxa*-24	1
Morganella morganii, *Pseudomonas aeruginosa*	*oxa*-48	1
Haemophilus influenzae	*tem*	2
Haemophilus influenzae, *Streptococcus pneumoniae*	*tem*	1
Haemophilus influenzae, *Mycoplasma pneumoniae*	*tem*	1
Escherichia coli, *Haemophilus influenzae*	*tem*	1
Haemophilus influenzae, *Staphylococcus aureus*	*tem*	1
Pseudomonas aeruginosa, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*	*vim*	1
Two resistance markers reported		**8**
Escherichia coli, *Staphylococcus aureus*	*ctx*-M, *mec*A	1
Klebsiella pneumoniae, *Pseudomonas aeruginosa*, *Staphylococcus aureus*	*ctx*-M, *mec*A	1
Klebsiella pneumoniae, *Morganella morganii*, *Proteus* spp., *Pseudomonas aeruginosa*, *Serratia marcescens*, *Stenotrophomonas maltophilia*	*ctx*-M, *kpc*	1
Acinetobacter spp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*	*ctx*-M, *oxa*-24	1
Acinetobacter spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Stenotrophomonas maltophilia*	*kpc*, *oxa*-23	1
Acinetobacter spp., *Proteus* spp., *Staphylococcus aureus*	*mec*A, *oxa*-23	1
Acinetobacter spp., *Proteus* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*	*mec*A, *oxa*-23	1
Haemophilus influenzae, *Staphylococcus aureus*	*mec*A, *tem*	1
Three resistance markers reported		**4**
Acinetobacter spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*	*ctx*-M, *mec*A, *oxa*-23	1
Acinetobacter spp., *Escherichia coli*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*	*ctx*-M, *mec*A, *tem*	1
Acinetobacter spp., *Morganella morganii*, *Proteus* spp., *Pseudomonas aeruginosa*	*ctx*-M, *oxa*-23, *vim*	1
Acinetobacter spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*	*kpc*, *mec*A, *oxa*-23	1

M. Instrument Name:

Unyvero System

N. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes _____ or No ___X_____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No ____X____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ___X_____ or No _____

   Level of Concern
   Moderate

   Software Description

   A detailed description of the Unyvero System software was provided and included the function of each Unyvero System software component. A summary of the features of the System component software is shown in Table 63.

Table 63: Unyvero System

Component of Unyvero System	Description and Function of System Component
Unyvero Lysator	The Lysator software is responsible for specimen lysis and communicates with the Cockpit software. The Lysator software manages internal mechanical/electrical function of the Lysator instrument.
Unyvero Analyzer	The Analyzer software controls the integrated mechanical, electronic and optical elements to execute a test run within the Unyvero LRT Cartridge. The Analyzer software is responsible for managing run analysis workflow, generation of test results and communicating with the Unyvero Cockpit software. The Analyzer software monitors internal mechanical/electrical functions of the Analyzer instrument.
Unyvero Cockpit	The Unyvero Cockpit software provides the main user interface for the overall Unyvero System. The Cockpit software is responsible for managing communication between all Unyvero System components (Cockpit, Lysator, Analyzer), management of analysis workflow, and presentation and storage of test results.
Entire Unyvero System	Software for each component is involved in management of communication between components.

Device Hazard Analysis:
A risk analysis and corresponding risk management plan was provided for the Unyvero LRT Application (i.e., assay) and Unyvero System. The risk analysis included potential risks to the patient. Risks identified for the patient were mitigated to Low/Moderate risk levels with assay and instrument controls, use of barcodes for process control, verification and validation procedures and detailed instructions for use. The risk analysis also included potential risks to the operator. Risks identified for the operator were mitigated to Low/Moderate risk levels through labeling, verification and validation, manufacturing quality control measures, and EMC testing. The Device Hazard Analysis for the Unyvero LRT Application and Unyvero System was acceptable.

Architecture Design Chart: A detailed structure of the software used in the Unyvero System was provided.

Software Requirements Specification (SRS): SRS documentation was provided describing requirements and specifications for each of the software components of the Unyvero System was described.

Traceability Analysis: Documentation of traceability matrix that links all product requirements, functional specifications, and verification and validation testing for the complete Unyvero system was provided.

Software Development Environment Description: A description of Unyvero software development environment was provided and was acceptable.

Verification and Validation Testing:
The sponsor provided adequate documentation of verification and validation (V & V) testing covering all software/instrument components of the Unyvero System. V &V

testing of Unyvero System software was successfully completed at the individual component and system integration levels. The normal operation and user interface of all Unyvero software components were also tested and verified.

Revision Level History:

The firm provided a software revision level history that detailed the updates to the system software corresponding to each version.

Unresolved Anomalies: All major residual risks and unresolved anomalies were properly mitigated. Any remaining anomalies did not present major concerns for safety and efficacy for either the user or the patient.

EMC Testing: The Lysator, Analyzer, and Cockpit components of the Unyvero System were subjected to EMC testing. Testing was conducted according to acceptable standards and no EMC issues were observed.

3. Specimen Identification:

   Specimen Identification information can be manually entered or automatically entered using the integrated barcode reader.

4. Specimen Sampling and Handling:

   Before starting the test, the user scans the clinical identification (barcode) from the primary specimen container using the built-in barcode reader of the Unyvero Cockpit or the information may be entered manually on the cockpit on-screen keyboard.

   The specimen is initially vortexed and then manually pipetted into the Unyvero Sample Tube. If the specimen is viscous, a Unyvero T1 Sample Transfer Tool may be used to facilitate pipetting the specimen into the Sample Tube.

   After the specimen is placed in the Sample Tube, the user then places the Unyvero Cap on the Sample Tube, scans the Sample Tube barcode and places it in the Unyvero Lysator. After processing on the Lysator is finished, the user places the Sample Tube and thawed Mastermix into the Unyvero LRT Cartridge, scans the Cartridge barcode and places it into the indicated position in the Unyvero Analyzer as per Unyvero software instructions. The Unyvero software then instructs the user to start the test which is fully automated until completion.

5. Calibration:

   Calibration is not required by the user.

6. Quality Control:

See section L1(c) for information on internal and external controls.

O. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable

P. Proposed Labeling: The labeling is sufficient and satisfies the requirements of 21 CFR parts 801 and 809 as well as the Special Controls for this type of device.

Q. Identified Risks to Health and Mitigation Measures:

Identified Risks	**Mitigation Measures**
Incorrect identification or lack of identification of a pathogenic microorganism by the device can lead to improper patient management	General Controls and Special Controls (1), (2), (3) and (4)
Failure to correctly interpret test results	General Controls and Special Controls (1), (2)(iii), (2)(iv), (2)(v), (2)(vi), (2)(vii), (2)(viii), and (3)
Failure to correctly operate the instrument	General Controls and Special Controls (1), (2)(i), (4)(ii), (4)(iii) and (4)(iv)

R. Benefit/Risk Analysis:

Summary	
Summary of the Benefit(s)	• The Unyvero LRT Application is the first multiplex PCR assay to detect and identify nucleic acids from bacteria and antimicrobial resistance markers directly from tracheal aspirate specimens. • The Unyvero LRT Application can provide bacterial identification results and antimicrobial resistance marker results in approximately 4.5 hours compared to traditional bacterial culture for which final bacterial identification and antimicrobial susceptibility testing can take several days. • The performance of the Unyvero LRT Application demonstrated acceptable performance for detection of assay targets in clinical specimens. Although sensitivity/PPA did not exceed 95% for many analytes for the primary efficacy endpoint and specificity/NPA demonstrated a relatively high rate of false positive results, clinical performance is mitigated by use of the assay in conjunction with traditional culture and interpretation by healthcare providers. • Detection of resistance markers by the Unyvero LRT assay correlated with phenotypic antimicrobial resistance in cultured isolates that carried each antimicrobial resistance marker. Positive antimicrobial resistance marker results from the LRT assay may identify patients for which broad empiric therapy may be necessary.
Summary of the Risk(s)	• False positive results and false negative results are the primary risks associated with use of the Unyvero LRT Application. • A false positive result may lead to unnecessary antimicrobial therapy, and potential adverse drug reactions, such as allergic reactions, *C. difficile* colitis and/or increased antimicrobial resistance. • A false negative result may result in a delay of effective antimicrobial therapy, with subsequent worsening of infection and associated increase in morbidity or mortality. • Misinterpretation of antimicrobial resistance genes could result in unnecessary broad-spectrum antimicrobial therapy or a delay to effective therapy, which could lead to potential adverse drug reactions or increased morbidity or mortality.
Summary of Other Factors	None.

Conclusions Do the probable benefits outweigh the probable risks?	The probable benefits of the Unyvero LRT assay outweigh the potential risks in light of the listed special controls and applicable general controls. The Unyvero LRT assay is the first multiplex PCR diagnostic device to detect and identify bacterial nucleic acids and antimicrobial resistance genes directly from tracheal specimens and is likely to benefit patients by more rapidly diagnosing tracheitis or ventilator-associated pneumonia and identifying highly resistance bacterial infections. The clinical performance observed in comparison to traditional bacterial culture and/or validated PCR assays indicated that the Unyvero LRT assay could provide potential benefits to patients by rapid and accurate diagnosis of lower respiratory tract infections. The proposed special controls will ensure that errors will be uncommon, and potential errors are further mitigated by current laboratory practices, which include standard of care bacterial culture, other diagnostics, and product labeling.

S. Patient Perspectives

This submission did not include specific information on patient perspectives for this device.

T. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.3985. FDA believes that the stated special controls, and applicable general controls, including design controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: QBH

Device Type: Device to detect and identify microorganisms and associated resistance marker nucleic acids directly in respiratory specimens

Class: II (special controls)

Regulation: 21 CFR 866.3985

(a) Identification. A device to detect and identify microorganisms and associated resistance marker nucleic acids directly in respiratory specimens is an *in vitro* diagnostic device intended for the detection and identification of microorganisms and associated resistance markers in respiratory specimens collected from patients with signs or symptoms of respiratory infection. The device is intended to aid in the diagnosis of respiratory infection in conjunction with clinical signs and symptoms and other laboratory findings. These devices do not provide confirmation of antibiotic susceptibility since mechanisms of resistance may exist other than those detected by the device.

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(b) Classification. Class II (special controls). The special controls for this device are:

(1) The intended use for the 21 CFR 809.10 labeling must include a detailed description of what the device detects, the type of results provided to the user, the clinical indications appropriate for test use, and the specific population(s) for which the device is intended.

(2) The 21 CFR 809.10(b) labeling must include:

(i) A detailed device description, including all device components, control elements incorporated into the test procedure, instrument requirements, ancillary reagents required but not provided, and a detailed explanation of the methodology, including all pre-analytical methods for processing of specimens.

(ii) Performance characteristics from analytical studies, including but not limited to limit of detection, inclusivity, reproducibility, cross reactivity, interfering substances, competitive inhibition, carryover/cross contamination, specimen stability, and linearity, as applicable.

(iii) A limiting statement that the device is intended to be used in conjunction with clinical history, signs and symptoms, and results of other diagnostic tests, including culture and antimicrobial susceptibility testing.

(iv) A detailed explanation of the interpretation of test results for clinical specimens and acceptance criteria for any quality control testing.

(v) A limiting statement that negative results for microorganisms do not preclude the possibility of infection, and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

(vi) If applicable, a limiting statement that detected microorganisms may not be the cause of lower respiratory tract infection and may be indicative of colonizing or normal respiratory flora.

(vii) If applicable, a limiting statement that detection of resistance markers cannot be definitively linked to specific microorganisms and that the source of a detected resistance marker may be an organism not detected by the assay, including colonizing flora.

(viii) If applicable, a limiting statement that detection of antibiotic resistance markers may not correlate with phenotypic gene expression.

(3) The 21 CFR 809.10(b) labeling and any test report generated by the device must include a limiting statement that negative results for resistance markers do not indicate susceptibility of detected microorganisms.

(4) Design verification and validation must include:

(i) Performance characteristics from clinical studies that include prospective (sequential) samples and, if appropriate, additional characterized samples. The study must be performed on a study population consistent with the intended use population and compare the device performance to results obtained from an FDA accepted reference method and/or FDA accepted comparator method, as appropriate. Results from the clinical studies must include the clinical study protocol (including predefined statistical analysis plan, if applicable), clinical study report, and results of all statistical analyses.

(ii) A detailed device description including the following:

(A) Thorough description of the assay methodology including, but not limited to, primer/probe sequences, primer/probe design, and rationale for target sequence selection, as applicable.

(B) Algorithm used to generate a final result from raw data (e.g., how raw signals are converted into a reported result).

(iii) A detailed description of device software, including, but not limited to, validation activities and outcomes.

(iv) As part of the risk management activities, an appropriate end user device training program must be offered as an effort to mitigate the risk of failure from user error.