U.S. FOOD & DRUG ADMINISTRATION

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December 20, 2019

Curetis GmbH
% Gail Radcliffe
President
Radcliffe Consulting, Inc.
231 Fairbanks Street
West Boylston, Massachusetts 01583

Re: K191967
Trade/Device Name: Unyvero LRT BAL Application
Regulation Number: 21 CFR 866.3985
Regulation Name: Device To Detect And Identify Microorganisms And Associated Resistance Marker Nucleic Acids Directly In Respiratory Specimens
Regulatory Class: Class II
Product Code: QBH
Dated: July 22, 2019
Received: July 23, 2019

Dear Gail Radcliffe:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal

statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Kristian Roth, Ph.D.
Branch Chief
Bacterial Multiples and Medical Countermeasures
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K191967

Device Name
Unyvero LRT BAL Application

Indications for Use *(Describe)*
The Unyvero LRT BAL Application is a qualitative nucleic acid multiplex test intended for the simultaneous detection and identification of nucleic acid sequences from the following microorganisms (N = 20) and antibiotic resistance markers (N = 10) in bronchoalveolar lavage (BAL)-like specimens (BAL or mini-BAL) from adult hospitalized patients with suspected lower respiratory tract infections.

[continued on page 2]

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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[*continued from Form FDA 3881, page 1*]

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	-
Pneumocystis jirovecii	-
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	*mecA*
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 BAL Application) and *A. ursingii* (not detected by LRT BAL Application).

[b] *ctx*-M1 subgroup.

[c] *Enterobacter cloacae* complex includes: *E. asburiae, E. chengduensis, E. chuandaensis, E. cloacae, E. hormaechei* (incl. ssp. *xiangfangensis*), *E. kobei, E. ludwigii*, *E. roggenkampii, E. sichuanensis* as well as *E. bugandensis* (not yet recognized as member of the *E. cloacae* complex).

[d] *Klebsiella pneumoniae* includes two variants: *K. pneumoniae* (variant 1), and *K. quasipneumoniae* (variant 2).

[e] *Proteus* spp. includes *P. cibarius, P. hauseri, P. mirabilis*, *P. penneri* and *P. vulgaris.*

The Unyvero LRT BAL 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 BAL specimens may contain colonizing microorganisms, detection of Unyvero LRT BAL microbial targets does not indicate that the microorganism is the cause of the disease. Unyvero positive results do not rule out co-infection with other microorganisms. 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 antibiotic resistance markers cannot be definitively linked to specific microorganisms, and may be present in organisms that are not detected by the Unyvero LRT BAL Application.

Microbiology cultures of BALs should be performed to obtain isolates for species identification and antimicrobial susceptibility testing and to identify potential microorganisms not targeted by the Unyvero LRT BAL Application.

510(k) SUMMARY – LRT BAL Application (K191967)

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92. The assigned 510(k) number is K191967.

807.92 (a)(1):

Name: Curetis GmbH
Address: Max-Eyth-Strasse 42
Holzgerlingen, Germany 71088

Phone: +49 7031 49195-24
Email: regulatory@curetis.com
Contact: Karsten Mueller, Head of Quality and Regulatory Affairs

807.92 (a)(2): Device name - trade name and common name, and classification

Trade name:
Unyvero LRT BAL Application

Common Name: Detection and identification of microorganisms and associated resistance marker nucleic acids directly in respiratory specimens

Classification / Product Code: 21 CFR Part 866.3985 / QBH

807.92 (a)(3): Identification of the legally marketed predicate devices

The Unyvero LRT BAL Application is substantially equivalent to its predecessor test system, the Unyvero LRT Application (Curetis, GmbH, Germany), granted *de novo* status under DEN170047 on April 3rd, 2018.

807.92 (a)(4): Device Description

The Unyvero LRT BAL Application automates and integrates DNA purification and eight parallel multiplex endpoint PCR reactions. It provides qualitative detection of nucleic acids from multiple lower respiratory pathogens using hybridization on PCR chamber arrays in a single use cartridge from a single bronchoalveolar lavage (BAL)-like specimen (BAL or mini-BAL).

The Unyvero LRT BAL Application identifies 20 microorganisms and 10 antibiotic resistance markers as listed in the Intended Use Statement, below.

Overview

The Unyvero LRT BAL Application uses a multiplex PCR approach following array hybridization which targets 30 individual analytes (microorganisms (N = 20) and antibiotic resistance markers (N = 10) divided into eight separate PCR reactions that are performed in individual reaction chambers simultaneously on a Unyvero LRT BAL Application cartridge. Multiplex compositions are designed to avoid any expected common occurrence of certain analytes within the same multiplex to largely reduce competitive PCR inhibition. Individual analyte assays of the Unyvero LRT BAL panel are designed to exhibit low or absent cross-reactivity with the relevant bronchoalveolar lavage (BAL)-like specimens (BAL or mini-BAL) sample matrix or 'close neighbor' strains. Array oligonucleotides are designed for similar hybridization and melting temperatures (approx. 65 – 80 °C, varying by amplicon). Hybridization and melting temperatures are used to exclude non-specific hybridization signals for improved signal specificity.

Instrument, Cartridge, and Other Consumables

The instrumentation consists of one (or more) Unyvero L4 Lysator, one (or more) Unyvero A50 Analyzer, a Unyvero C8 Cockpit, and four single-use consumables: the Unyvero LRT BAL Cartridge, the Unyvero Sample Tube, Sample Tube Cap and the Unyvero Master Mix. A Unyvero Sample Tube Holder is supplied as accessory to simplify the sample filling step.

- Unyvero LRT BAL Cartridge contains DNA isolation and purification reagents, primers, hybridization and wash buffers, and oligonucleotides for detection.

- Unyvero T1 Sample Tube and Transport Cap contains glass beads and buffers to lyse microorganisms and liquefy the sample.

- Unyvero T1 Sample Tube Cap seals the Unyvero Sample Tube and contains Proteinase K and a synthetic control gene for process monitoring.

- Unyvero M1 Master Mix Tube contains reagents for DNA amplification.

Controls

An internal control (a synthetic gene without any homology to known sequences) is processed in every chamber in order to verify the DNA purification, amplification, array hybridization, and detection.

Other than the built-in controls, no external materials are supplied with Unyvero LRT BAL Application devices and consumables. Good laboratory practice recommends running external positive and negative controls using samples cultured in the laboratory.

Reagents

No additional reagents are required to perform the Unyvero LRT BAL Application; all reagents are supplied within the cartridge or within the other consumables with the exception of the polymerase Master Mix, which is provided separately (frozen).

Assay Procedures

How to perform a Unyvero LRT BAL test:

1. Remove the Unyvero Sample Tube from its packaging and slide it in the Unyvero Sample Tube Holder in the upright position with the barcoded end at the bottom.
2. Remove the Transport Cap from the Sample Tube by pulling it upward.
3. Pipette 180 µL of vortexed patient specimen into the Sample Tube and close it using the Unyvero Cap provided in the LRT BAL kit; align the small nodules on the neck of the Sample Tube with the openings on the Cap and press down to lock in place.
4. Scan the Sample Tube and place it into the Lysator. Close the Lysator lid to start the Lysator.
5. Remove Master Mix from freezer and thaw at room temperature (15 °C - 25 °C) for approximately 30 minutes.
6. When lysis is complete, remove the Sample Tube from the Lysator and place it into the labeled position on the left-hand side of the Unyvero LRT BAL Cartridge.
7. Place the thawed Master Mix into the labeled position on the right-hand side of the Cartridge.
8. Scan the Cartridge on the Cockpit and insert it into the position indicated on the Analyzer. The software provides on-screen instructions to start the test.
9. View results after completion of the run.

During the automated analysis (Step 8), which is entirely controlled by the A50 Analyzer, the sample is mixed with ethanol and then transferred onto the DNA purification column, where buffers purify and elute the DNA. Eluted 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, consisting of one labeled and one non-labeled primer for the respective multiplex end-point PCR. After specific amplification, PCR products are hybridized to the corresponding array probes. Each array has been manufactured with probes corresponding to the amplicons for the targeted microorganism sequences described above. A total of up to 49 spots per array allows for redundant detection with at least four spots per analyte, as well as spots for intensity calibration, and orientation markers for the image processing software. Binding of amplicons to specific probes is detected by analyzing fluorescence images of the arrays. Result data are displayed on the C8 Cockpit for visualization, printout, temporary storage and electronic data export.

A test run is completed after 4 to 5 hrs, and results for panel microorganisms and corresponding antibiotic resistance markers are displayed on the Cockpit screen. Four screens are provided:

- The **Result Summary screen** provides a quick overview of all detected LRT BAL panel microorganisms, together with all detected corresponding antibiotic resistance markers.
- The **Microorganisms screen** provides a list of all panel microorganisms grouped in Gram-positive bacteria, non-fermenting bacteria, Enterobacteriaceae and other microorganisms together with the analysis result (reported as detected, not detected or invalid).
- The **Result Details screen** provides a list of all analyzed microorganisms and the corresponding antibiotic resistance markers together with the analysis result (reported as detected, not detected, invalid or NA).
- **Test Details screen** showing user name, lot numbers of used consumables, expiration dates of the consumables and start and stop times and dates of the test.

Results can be reviewed on the cockpit or, optionally, be printed out. All results are saved in a database on the Unyvero Cockpit for later review and printing.

Software

The Unyvero software is designed to:

- Manage analysis workflow (Cockpit)
- Carry out sample lysis (Lysator)
- Execute the analysis and generate the analytical result (Analyzer)
- Manage communication among units (Cockpit, Lysator, Analyzer)
- Monitor internal mechanical / electrical actuators (Lysator, Analyzer)
- Present analysis results (Cockpit)
- Store analysis results (Cockpit)

Each device (Cockpit, Lysator, Analyzer) is a subsystem within the overall system, and each consists of hardware and software components. The different devices are interconnected by an Ethernet based communication interface, and system functionality is provided by the interaction of all three device types. Only the Cockpit presents a rich user interface and allows interaction with the operator. The Lysator and Analyzer units include a simple display for showing device status. Optional HIS/LIS connectivity allows transferring results to a hospital or laboratory information system.

807.92 (a)(5): Intended Use

The Unyvero LRT BAL Application is a qualitative nucleic acid multiplex test intended for the simultaneous detection and identification of nucleic acid sequences from the following microorganisms (N = 20) and antibiotic resistance markers (N = 10) in bronchoalveolar lavage (BAL)-like specimens (BAL or mini-BAL) from adult hospitalized patients with suspected lower respiratory tract infections.

Microorganism	Associated Antibiotic Resistance Marker(s)
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	-
Pneumocystis jirovecii	-
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 BAL Application), and *A. ursingii* (not detected by LRT BAL Application).
[b] *ctx*-M1 subgroup.
[c] *Enterobacter cloacae* complex includes: *E. asburiae*, *E. chengduensis*, *E. chuandaensis*, *E. cloacae*, *E. hormaechei* (incl. ssp. *xiangfangensis*), *E. kobei*, *E. ludwigii*, *E. roggenkampii*, *E. sichuanensis* and *E. bugandensis* (not yet recognized as member of the *E. cloacae* complex).
[d] *Klebsiella pneumoniae* includes two variants: *K. pneumoniae* (variant 1), and *K. quasipneumoniae* (variant 2).
[e] *Proteus* spp. includes: *P. cibarius, P. hauseri*, *P. mirabilis*, *P. penneri*, and *P. vulgaris.*

The Unyvero LRT BAL 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 BAL specimens may contain colonizing microorganisms, detection of Unyvero LRT BAL

microbial targets does not indicate that the microorganism is the cause of the disease. Unyvero positive results do not rule out co-infection with other microorganisms. 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 antibiotic resistance markers cannot be definitively linked to specific microorganisms, and may be present in organisms that are not detected by the Unyvero LRT BAL Application.

Microbiology cultures of BALs should be performed to obtain isolates for species identification and antimicrobial susceptibility testing and to identify potential microorganisms not targeted by the Unyvero LRT BAL Application.

807.92 (a)(6): Technological Similarities and Differences to the Predicate

Table 1: Comparison of the Unyvero LRT Application and the Unyvero LRT BAL Application

Element	**New Device:** **Curetis Unyvero LRT BAL Application**	**Predicate:** **Curetis Unyvero LRT Application (DEN170047)**
Specimen Type	Bronchoalveolar lavage (BAL)-like specimens from adult hospitalized patients with suspected lower respiratory tract infections	Endotracheal aspirate specimens from adult hospitalized patients with suspected lower respiratory tract infections
Organisms Detected	Bacteria: *Acinetobacter* spp. *Citrobacter freundii* *Enterobacter cloacae* complex *Escherichia coli* *Haemophilus influenzae* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Klebsiella variicola* *Moraxella catarrhalis* *Morganella morganii* *Proteus* spp. *Pseudomonas aeruginosa* *Serratia marcescens* *Staphylococcus aureus* *Stenotrophomonas maltophilia* *Streptococcus pneumoniae* Atypical Bacteria: *Chlamydia pneumoniae* *Legionella pneumophila* *Mycoplasma pneumoniae* Fungus: *Pneumocystis jirovecii* Antimicrobial Resistance Genes:	Bacteria: *Acinetobacter* spp. *Citrobacter freundii* *Enterobacter cloacae* complex *Escherichia coli* *Haemophilus influenzae* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Klebsiella variicola* *Moraxella catarrhalis* *Morganella morganii* *Proteus* spp. *Pseudomonas aeruginosa* *Serratia marcescens* *Staphylococcus aureus* *Stenotrophomonas maltophilia* *Streptococcus pneumoniae* Atypical Bacteria: *Chlamydia pneumoniae* *Legionella pneumophila* *Mycoplasma pneumoniae* Antimicrobial Resistance Genes:

	ctx-M *kpc* *mec*A *ndm* *oxa*-23 *oxa*-24 *oxa*-48 *oxa*-58 *tem* *vim*	*ctx*-M *kpc* *mec*A *ndm* *oxa*-23 *oxa*-24 *oxa*-48, *oxa*-58, *tem* *vim*
Analyte	DNA	DNA
Technological Principles	Multiplex nucleic acid	Multiplex nucleic acid
Result Types	Qualitative for all analytes	Qualitative for all analytes
Instrumentation	Unyvero System	Unyvero System
Time to Result	About 4-5 hrs	About 4-5 hrs
Reagent Storage	Room temperature (below -20 °C, Master Mix Tube only)	Room temperature (below -20 °C, Master Mix Tube only)
Test Interpretation	Automated test interpretation and report generation.	Automated test interpretation and report generation.
Controls	Control included in each test to monitor for the presence of PCR inhibitors and enables the system to detect failures in the testing process.	Control included in each test to monitor for the presence of PCR inhibitors and enables the system to detect failures in the testing process.
User Complexity	Moderate	Moderate

807.92 (b)(1): Brief Description of Nonclinical Data

Limits-of-Detection (LoDs)

Limits-of-Detection were determined for each LRT BAL panel analyte by serial dilutions of reference strains prepared in pooled negative native lavage specimens as test matrix. The lowest test concentration with a positivity rate of 95% or higher was determined as the LoD for each specific panel analyte (e.g., at least 19 of 20 positive results for at least 20 performed test replicates). Tables 2 and 3 summarize LoDs for all LRT BAL panel microorganisms or antibiotic resistance markers, respectively.

Table 2: LoDs for LRT BAL panel microorganisms.

	Reference Strain ID	LoD [CFU/mL]
Acinetobacter spp.	ATCC 19606 (*A. baumannii*)	2.0×10^4
Chlamydia pneumoniae	ATCC VR-2282 [a]	3.2×10^2
Citrobacter freundii	ATCC 8090	8.0×10^4
Enterobacter cloacae complex	ATCC 13047 (*E. cloacae*)	2.0×10^5
Escherichia coli	ATCC 11775	2.0×10^4
Haemophilus influenzae	ATCC 33391	2.0×10^4
Klebsiella oxytoca	ATCC 13182	1.0×10^4
Klebsiella pneumoniae (var. 1)	ATCC 13883	4.0×10^4
Klebsiella quasipneumoniae (*K. pneumoniae*, var. 2)	ATCC 700603	4.0×10^4
Klebsiella variicola	ATCC BAA-830	2.0×10^4
Legionella pneumophila	ATCC 33152	8.0×10^4
Moraxella catarrhalis	ATCC 25238	1.5×10^5
Morganella morganii	ATCC 25830	2.0×10^4
Mycoplasma pneumoniae	ATCC 29085 [b]	1.6×10^3
Pneumocystis jirovecii	36_0314 [c]	5.0×10^5
Proteus spp.	ATCC 29906 (*P. mirabilis*) ATCC 29905 (*P. vulgaris*)	5.0×10^3
Pseudomonas aeruginosa	ATCC 10145	6.3×10^2
Serratia marcescens	ATCC 13880	4.0×10^4
Staphylococcus aureus	ATCC 12600	1.5×10^5
Stenotrophomonas maltophilia	ATCC 13637	5.0×10^3
Streptococcus pneumoniae	ATCC 49619	2.0×10^4

[a] Cell supernatant (in IFU/mL).
[b] Bacterial suspension, quantified by qPCR (in copies/mL).
[c] Positive clinical specimen, quantified by qPCR (in copies/mL). LoD was determined using DNA extracted from a positive clinical specimen, and then confirmed by testing dilutions of this clinical specimen at the LoD concentration (20/20 positive results).

Table 3: LoDs for LRT BAL panel antibiotic resistance markers.

	Reference Strain ID	Host Microorganism	LoD [CFU/mL]
ctx-M	NCTC 13443	*Klebsiella pneumoniae*	1.0×10^4
kpc	NCTC 13438	*Klebsiella pneumoniae*	4.0×10^4
*mec*A	NCTC 12493	*Staphylococcus aureus*	4.0×10^5
ndm	NCTC 13443	*Klebsiella pneumoniae*	2.0×10^4
oxa-23	NCTC 13301	*Acinetobacter baumannii*	1.0×10^7
oxa-24	NCTC 13302	*Acinetobacter baumannii*	5.0×10^3
oxa-48	NCTC 13442	*Klebsiella pneumoniae*	3.0×10^5
oxa-58	NCTC 13305	*Acinetobacter baumannii*	5.0×10^4
tem [a]	NCTC 13443	*Klebsiella pneumoniae*	2.0×10^4
vim	NCTC 13437	*Pseudomonas aeruginosa*	2.0×10^4

[a] Although the LRT BAL Application reports *tem* results only for *H. influenzae* as corresponding host microorganism, LoD was determined with a *tem* positive *E. coli* strain. Note that inclusivity testing was also successfully performed with *tem* positive *H. influenzae* strains.

Inclusivity

LRT BAL Microorganisms

Inclusivity wet testing was performed with reference strains for each microorganism analyte at concentrations near the LoD with contrived samples using pooled native negative lavage specimens as sample matrix (Table 4). Reference strains used for LoD determination are considered inclusive and were used as positive controls. Inclusivity was established near LoD (< 5x LoD) for all but one of the tested reference strains. For one reference strain of *M. pneumoniae*, tested as genomic DNA extract, inclusivity was demonstrated with a reduced analytical sensitivity at 5x LoD, although no primer or probe deviations to the corresponding LRT BAL assay were present.

Table 4: Inclusivity study results for LRT BAL panel microorganisms.

Reference Strain	Strain ID	Test Conc. [CFU/ml]	x-fold LoD	# Pos./ # Exp.
analyte: *Acinetobacter* spp.				
***Acinetobacter baumannii* (pos. control/LoD ref. strain)**	**ATCC 19606**	**4.0×10^4**	**2x**	**6/6**
Acinetobacter baumannii	NCTC 13305	4.0×10^4	2x	2/2
Acinetobacter baumannii	NCTC 13301	4.0×10^4	2x	2/2
Acinetobacter baumannii	NCTC 13302	4.0×10^4	2x	2/2
Acinetobacter baumannii	Micromyx 6148	4.0×10^4	2x	2/2
Acinetobacter baumannii	Micromyx 4410	4.0×10^4	2x	2/2
Acinetobacter baumannii	JMI 49755	4.0×10^4	2x	2/2
Acinetobacter baumannii	NRZ-00449	1.0×10^4	0.5x	2/2
Acinetobacter baumannii	UCLA A4	1.0×10^4	0.5x	2/2
Acinetobacter calcoaceticus	ATCC 23055	4.0×10^4	2x	2/2
Acinetobacter lwoffii	ATCC 15309	4.0×10^4	2x	2/2
Acinetobacter haemolyticus	ATCC 17906	4.0×10^4	2x	2/2
Chlamydia pneumoniae	**ATCC VR-2282**	**6.4×10^2 [a]**	**2x**	**4/4**

Reference Strain	Strain ID	Test Conc. [CFU/ml]	x-fold LoD	# Pos./ # Exp.
(pos. control/LoD ref. strain)				
Chlamydia pneumoniae	ATCC VR-1310	6.4 x 10^{2} [a]	2x	2/2
Chlamydia pneumoniae	ATCC 53592	6.4 x 10^{2} [a]	2x	0/2
		1.0 x 10^{3} [a]	3x	2/2
Citrobacter freundii **(pos. control/LoD ref. strain)**	**ATCC 8090**	**1.6 x 10^{5}**	**2x**	**4/4**
Citrobacter freundii	ATCC 43864	1.6 x 10^{5}	2x	2/2
Citrobacter freundii	NCTC 8581	1.6 x 10^{5}	2x	2/2
Citrobacter freundii	NRZ-00452	1.6 x 10^{5}	2x	2/2
Citrobacter freundii	UCLA C1	1.6 x 10^{5}	2x	2/2
analyte: *Enterobacter cloacae* complex				
Enterobacter cloacae **(pos. control/LoD ref. strain)**	**ATCC 13047**	**4.0 x 10^{5}**	**2x**	**7/7**
Enterobacter cloacae	ATCC 23355	4.0 x 10^{5}	2x	2/2
Enterobacter cloacae	ATCC 49141	4.0 x 10^{5}	2x	2/2
Enterobacter cloacae	ATCC BAA-2468	4.0 x 10^{5}	2x	2/2
Enterobacter cloacae	JMI 46239	4.0 x 10^{5}	2x	2/2
Enterobacter cloacae	NRZ-00239	4.0 x 10^{5}	2x	2/2
Enterobacter cloacae ssp. *dissolvens*	ATCC 23373	4.0 x 10^{5}	2x	2/2
Enterobacter hormaechei	ATCC 49162	4.0 x 10^{5}	2x	2/2
Enterobacter asburiae	ATCC 35953	4.0 x 10^{5}	2x	2/2
Escherichia coli **(pos. control/LoD ref. strain)**	**ATCC 11775**	**4.0 x 10^{4}**	**2x**	**4/4**
Escherichia coli	ATCC 25922	4.0 x 10^{4}	2x	2/2
Escherichia coli	ATCC 35218	4.0 x 10^{4}	2x	4/4
Escherichia coli	ATCC BAA-2523	4.0 x 10^{4}	2x	2/2
Escherichia coli	NCTC 13351	4.0 x 10^{4}	2x	4/4
Escherichia coli	NCTC 13476	4.0 x 10^{4}	2x	2/2
Escherichia coli	JMI 50067	4.0 x 10^{4}	2x	2/2
Haemophilus influenzae **(non-typeable/non-capsulated) (pos. control/LoD ref. strain)**	**ATCC 33391**	**4.0 x 10^{4}**	**2x**	**3/3**
Haemophilus influenzae (serotype a)	ATCC 9006	4.0 x 10^{4}	2x	2/2
Haemophilus influenzae (serotype c)	ATCC 9007	4.0 x 10^{4}	2x	2/2
Haemophilus influenzae (serotype b)	ATCC 10211	4.0 x 10^{4}	2x	1/2
		6.0 x 10^{4}	3x	2/2
Haemophilus influenzae (serotype b)	ATCC 49247	4.0 x 10^{4}	2x	2/2
Haemophilus influenzae (serotype b)	ATCC 49766	4.0 x 10^{4}	2x	2/2
Klebsiella oxytoca **(pos. control/LoD ref. strain)**	**ATCC 13182**	**4.0 x 10^{4}**	**4x**	**3/3**
Klebsiella oxytoca	ATCC 43863	4.0 x 10^{4}	4x	2/2
Klebsiella oxytoca	ATCC 8724	4.0 x 10^{4}	4x	2/2
Klebsiella oxytoca	ATCC 49131	4.0 x 10^{4}	4x	2/2
Klebsiella oxytoca	NCIMB 12819	4.0 x 10^{4}	4x	2/2
Klebsiella oxytoca	NRZ-22060	4.0 x 10^{4}	4x	2/2
analyte: *Klebsiella pneumoniae*				

Reference Strain	Strain ID	Test Conc. [CFU/ml]	x-fold LoD	# Pos./ # Exp.
***Klebsiella pneumoniae*, variant 1 (pos. control/LoD ref. strain)**	**ATCC 13883**	**8.0 x 10^4**	**2x**	**4/4**
***Klebsiella quasipneumoniae* (*K. pneumoniae*, variant 2) (pos. control/LoD ref. strain)**	**ATCC 700603**	**8.0 x 10^4**	**2x**	**3/3**
Klebsiella pneumoniae	NCTC 13439	8.0 x 10^4	2x	2/2
Klebsiella pneumoniae	NCTC 13438	8.0 x 10^4	2x	2/2
Klebsiella pneumoniae	NCTC 13442	8.0 x 10^4	2x	2/2
Klebsiella pneumoniae	NCTC 13443	2.0 x 10^4	0.5x	3/3
Klebsiella pneumoniae	Micromyx 4653	8.0 x 10^4	2x	2/2
Klebsiella pneumoniae	Micromyx 4676	8.0 x 10^4	2x	2/2
Klebsiella pneumoniae	JMI 49767	2.0 x 10^4	0.5x	2/2
Klebsiella pneumoniae	NRZ-00002	2.0 x 10^4	0.5x	2/2
Klebsiella pneumoniae	NRZ-00103	8.0 x 10^4	2x	2/2
***Klebsiella variicola* (pos. control/LoD ref. strain)**	**ATCC BAA-830**	**4.0 x 10^4**	**2x**	**3/3**
Klebsiella variicola	clinical strain 1	4.0 x 10^4	2x	2/2
Klebsiella variicola	clinical strain 2	4.0 x 10^4	2x	2/2
Klebsiella variicola	clinical strain 3	4.0 x 10^4	2x	2/2
Klebsiella variicola	clinical strain 4	4.0 x 10^4	2x	2/2
***Legionella pneumophila* (serotype 1) (pos. control/LoD ref. strain)**	**ATCC 33152**	**1.6 x 10^5**	**2x**	**7/7**
Legionella pneumophila (serotype 2)	ATCC 33154	1.6 x 10^5	2x	2/2
Legionella pneumophila (serotype 3)	ATCC 33155	1.6 x 10^5	2x	2/2
Legionella pneumophila (serotype 6)	ATCC 33215	1.6 x 10^5	2x	2/2
Legionella pneumophila (serotype 8)	ATCC 35096	1.6 x 10^5	2x	2/2
Legionella pneumophila (serotype 10)	ATCC 43283	1.6 x 10^5	2x	2/2
Legionella pneumophila	UCLA L1	1.6 x 10^5	2x	2/2
Legionella pneumophila	UCLA L5	1.6 x 10^5	2x	2/2
Legionella pneumophila	UCLA L6	1.6 x 10^5	2x	2/2
***Moraxella catarrhalis* (pos. control/LoD ref. strain)**	**ATCC 25238**	**3.0 x 105**	**2x**	**4/4**
Moraxella catarrhalis	ATCC 43617	3.0 x 10^5	2x	2/2
Moraxella catarrhalis	ATCC 8176	3.0 x 10^5	2x	2/2
Moraxella catarrhalis	ATCC 25240	3.0 x 10^5	2x	2/2
Moraxella catarrhalis	ATCC 23246	3.0 x 10^5	2x	3/3
Moraxella catarrhalis	ATCC 49143	3.0 x 10^5	2x	2/2
***Morganella morganii* (pos. control/LoD ref. strain)**	**ATCC 25830**	**4.0 x 10^4**	**2x**	**2/3**
Morganella morganii	ATCC 8019	4.0 x 10^4	2x	2/2
Morganella morganii	ATCC 25829	4.0 x 10^4	2x	0/1
		6.0 x 10^4	3x	2/2
Morganella morganii	DSM-46262	4.0 x 10^4	2x	1/2
		6.0 x 10^4	3x	2/2
Morganella morganii ssp. *sibonii*	ATCC 49948	4.0 x 10^4	2x	2/2
***Mycoplasma pneumoniae* (type 1) (pos. control/LoD ref. strain)**	**ATCC 29085**	**3.2 x 10^3 [b]**	**2x**	**5/7**

Reference Strain	Strain ID	Test Conc. [CFU/ml]	x-fold LoD	# Pos./ # Exp.
Mycoplasma pneumoniae (type 1)	ATCC 29343	3.2 x 10^{3} [b]	2x	2/2
Mycoplasma pneumoniae (type 2)	ATCC 15531 [c, d]	3.2 x 10^{3} [b]	2x	0/2
		5.0 x 10^{3} [b]	3x	1/2
		6.4 x 10^{3} [b]	4x	1/2
		7.0 x 10^{3} [b]	4.4x	1/2
		8.0 x 10^{3} [b]	5x	2/2
Mycoplasma pneumoniae (type 1)	ATCC 15293	3.2 x 10^{3} [b]	2x	2/2
Mycoplasma pneumoniae (type 2)	ATCC 49894	3.2 x 10^{3} [b]	2x	2/2
***Pneumocystis jirovecii* (pos. control/LoD ref. strain)**	**pos. specimen 1**	**1.0 x 10^{6} [b]**	**2x**	**1/1**
Pneumocystis jirovecii	pos. specimen 2	1.0 x 10^{6} [b]	2x	2/2
Pneumocystis jirovecii	pos. specimen 3	1.0 x 10^{6} [b]	2x	2/2
Pneumocystis jirovecii	pos. specimen 4	1.0 x 10^{6} [b]	2x	2/2
Pneumocystis jirovecii	pos. specimen 5	1.0 x 10^{6} [b]	2x	2/2
analyte: *Proteus* spp.				
***Proteus vulgaris* (pos. control/LoD ref. strain)**	**ATCC 29905**	**1.0 x 10^{4}**	**2x**	**7/7**
***Proteus mirabilis* (pos. control/LoD ref. strain)**	**ATCC 29906**	**1.0 x 10^{4}**	**2x**	**2/2**
Proteus mirabilis	ATCC 12453	1.0 x 10^{4}	2x	2/2
Proteus mirabilis	ATCC 14153	1.0 x 10^{4}	2x	2/2
Proteus mirabilis	ATCC 25933	1.0 x 10^{4}	2x	2/2
Proteus vulgaris	ATCC 6380	1.0 x 10^{4}	2x	2/2
Proteus vulgaris	ATCC 8427	1.0 x 10^{4}	2x	2/2
Proteus hauseri	ATCC 700826	1.0 x 10^{4}	2x	2/2
Proteus penneri	ATCC 33519	1.0 x 10^{4}	2x	2/2
***Pseudomonas aeruginosa* (pos. control/LoD ref. strain)**	**ATCC 10145**	**1.3 x 10^{3}**	**2x**	**3/3**
Pseudomonas aeruginosa	ATCC 27853	1.3 x 10^{3}	2x	2/2
Pseudomonas aeruginosa	NCTC 13437	1.3 x 10^{3}	2x	2/2
Pseudomonas aeruginosa	Micromyx 2562	2.0 x 10^{3}	3x	2/2
Pseudomonas aeruginosa	NRZ-00196	1.3 x 10^{3}	2x	2/2
Pseudomonas aeruginosa	UCLA P20	1.3 x 10^{3}	2x	2/2
***Serratia marcescens* (pos. control/LoD ref. strain)**	**ATCC 13880**	**8.0 x 10^{4}**	**2x**	**3/3**
Serratia marcescens	ATCC 14756	8.0 x 10^{4}	2x	2/2
Serratia marcescens	ATCC 15365	8.0 x 10^{4}	2x	2/2
Serratia marcescens	ATCC 27117	8.0 x 10^{4}	2x	2/2
Serratia marcescens	ATCC 43861	8.0 x 10^{4}	2x	2/2
Serratia marcescens ssp. *sakuensis*	DSM-17174	8.0 x 10^{4}	2x	2/2
***Staphylococcus aureus* (pos. control/LoD ref. strain)**	**ATCC 12600**	**3.0 x 10^{5}**	**2x**	**7/7**
Staphylococcus aureus	ATCC BAA-2312	3.0 x 10^{5}	2x	2/2
Staphylococcus aureus	NCTC 12493	3.0 x 10^{5}	2x	2/2
Staphylococcus aureus	ATCC 33591	3.0 x 10^{5}	2x	2/2
Staphylococcus aureus	DSM-17091	3.0 x 10^{5}	2x	2/2

Reference Strain	Strain ID	Test Conc. [CFU/ml]	x-fold LoD	# Pos./ # Exp.
Staphylococcus aureus	ATCC 29213	3.0 x 10^5	2x	2/2
Staphylococcus aureus	ATCC 43300	3.0 x 10^5	2x	2/2
Stenotrophomonas maltophilia **(pos. control/LoD ref. strain)**	**ATCC 13637**	**1.0 x 10^4**	**2x**	**4/4**
Stenotrophomonas maltophilia	ATCC 13636	1.0 x 10^4	2x	0/2
		1.5 x 10^4	3x	2/2
Stenotrophomonas maltophilia	ATCC 17666	1.0 x 10^4	2x	2/2
Stenotrophomonas maltophilia	ATCC 49130	1.0 x 10^4	2x	2/2
Stenotrophomonas maltophilia	DSM-50173	1.0 x 10^4	2x	2/2
Stenotrophomonas maltophilia	DSM-21874 [e] [NCIMB 9528]	1.0 x 10^4	2x	0/2
		1.5 x 10^4	3x	0/2
		2.0 x 10^4	4x	2/2
Streptococcus pneumoniae **serotype 19F (pos. control/LoD ref. strain)**	**ATCC 49619**	**4.0 x 10^4**	**2x**	**4/7 [f]**
Streptococcus pneumoniae serotype 3	ATCC 6303	4.0 x 10^4	2x	2/2
Streptococcus pneumoniae serotype 5	ATCC 6305	4.0 x 10^4	2x	2/2
Streptococcus pneumoniae	ATCC 49150 [g]	4.0 x 10^4	2x	0/2
		8.0 x 10^4	4x	2/2
Streptococcus pneumoniae	ATCC 33400 [h]	4.0 x 10^4	2x	1/2
		6.0 x 10^4	3x	1/2
		8.0 x 10^4	4x	2/2
Streptococcus pneumoniae serotype 2	ATCC 27336	4.0 x 10^4	2x	2/2
Streptococcus pneumoniae serotype 1	ATCC 6301	4.0 x 10^4	2x	2/2
Streptococcus pneumoniae serotype 9V	DSM-11865	4.0 x 10^4	2x	2/2
Streptococcus pneumoniae serotype 23F	DSM-11866	4.0 x 10^4	2x	2/2
		6.0 x 10^4	3x	1/2
		8.0 x 10^4	4x	2/2
Streptococcus pneumoniae serotype 6B	DSM-11867	4.0 x 10^4	2x	2/2

[a] In IFU/mL for *C. pneumoniae*.
[b] In copies/mL for *M. pneumoniae* and *P. jirovecii*.
[c] Quantified DNA extract (reference material) from a commercial provider.
[d] For *M. pneumoniae* ATCC 15531, a positivity rate of 2/2 was obtained at a 5x LoD concentration. Sequencing did not reveal any mismatches to primer and probe binding sites and the observed slightly reduced sensitivity is likely due to the different source material (DNA extract instead of a cell suspension).
[e] *S. maltophilia* strain DSM-21874 was positive only at a 4x LoD concentration. Sequencing did not reveal any mismatches to primer or probe sequences.
[f] *S. pneumoniae* LoD reference strain ATCC 46916 did not show consistently positive signals at 2x LoD. ATCC 46916 controls were repeated using a freshly prepared counted culture stock. This time, a positivity rate of 8/8 was obtained at a 2x LoD concentration, as expected.
[g] *S. pneumoniae* strain ATCC 49150 was only positive at 4x LoD. Sequencing did not reveal any mismatches to primer or probe sequences.
[h] *S. pneumoniae* strain ATCC 33400 was only consistently positive at 4x LoD. Sequencing did not reveal any mismatches to primer or probe sequences.

To supplement inclusivity testing, in silico GenBank BLAST analyses of LRT BAL panel primer and probe sequences were performed for each LRT BAL panel microorganism (search performed July 2019) for all applicable strain entries. BLAST analyses identified strain entries for which detection by LRT BAL is predicted at LoD (match of relevant primer and probe sequences), only with reduced sensitivity (typically, single relevant mismatches of primer or probe sequences; detection likely at higher than LoD concentrations only), or is not predicted (multiple relevant mismatches in primer and probe sequences).

Table 5 lists microorganisms for which inclusivity was demonstrated by wet testing and are supported by *in silico* analysis (predicted at LoD or predicted with reduced sensitivity). Note that additional *in silico* analysis results are provided for strains that have not been wet tested. Such *in silico* results are provided as supplementary data only. Results are not intended to be a surrogate for wet testing and do not assure that specific strains will be detected.

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

Table 5: Inclusive LRT BAL panel microorganisms as demonstrated by inclusivity wet testing and/or as predicted by *in silico* analysis (at LoD concentration or with reduced sensitivity). For strains for which *in silico* analysis predicts higher than LoD concentrations for one or more applicable entries, numbers of GenBank entries are additionally listed.

	Wet Testing	*In Silico*: at LoD	*In Silico*: Reduced Sensitivity	Comment
***Acinetobacter* spp.**				
A. baumannii	x	x	-	
A. calcoaceticus	x	x	-	
A. lwoffii	x	x	-	
A. haemolyticus	x	x	-	
A. nosocomialis	-	x	-	
A. pittii	-	x	-	
A. junii	-	x	-	
A. parvus	-	x	-	
A. lactucae	-	x	-	
A. oleivorans	-	x	-	
A. schindleri	-	x	-	
A. guillouiae	-	-	x 1 entry	
A. radioresistens	-	-	x 3 entries	
A. soli	-	-	x 1 entry	
A. ursingii	-	-	x 1 entry	reference strain DSM-16037 tested negative at 1.5 x 10^7 CFU/mL
Chlamydia pneumoniae	x	x	-	
Citrobacter freundii	x	x 26 entries	x 4 entries	
***Enterobacter cloacae* complex**				
E. cloacae	x	x	-	including ssp. *dissolvens*

	Wet Testing	*In Silico*: at LoD	*In Silico*: Reduced Sensitivity	Comment
E. hormaechei	x	x	-	including ssp. *oharae*, *steigerwaltii*, *hoffmannii*
E. hormaechei ssp. xiangfangensis	-	x 7 entries	x 1 entry	
E. kobei	-	x	-	
E. ludwigii	-	x	-	
E. sichuanensis	-	x	-	
E. chengduensis	-	x	-	
E. chuandaensis	-	x	-	
E. roggenkampii	-	x	-	
E. asburiae	x	x 11 entries	x 1 entry	
E. bugandensis	-	x	-	not yet recognized as member of the *E. cloacae* complex
Escherichia coli	x	x	-	
Haemophilus influenzae	x	x 65 entries	x 1 entry	
Klebsiella oxytoca	x	x 16 entries	x 6 entries	
Klebsiella pneumoniae				
variant 1 (*K. pneumoniae*)	x	x	-	
variant 2 (*K. quasipneumoniae*)	x	x 11 entries	x 10 entries	
Klebsiella variicola	x	x	-	
Legionella pneumophila	x	x	-	some entries predict detection at LoD, other entries predict a possible slight performance reduction; strains for both variants were included in inclusivity wet testing and were detected at concentrations near LoD.
Moraxella catarrhalis	x	x	-	
Morganella morganii	x	x	-	
Mycoplasma pneumoniae	x	x	-	
Pneumocystis jirovecii	x	x	-	
***Proteus* spp.**				
P. mirabilis	x	x	-	
P. vulgaris	x	x	-	
P. hauseri	x	x	-	
P. penneri	x	x	-	
P. cibarius	-	x	-	
Pseudomonas aeruginosa	x	x	-	
Serratia marcescens	x	x	-	
Staphylococcus aureus	x	x > 450 entries	x 1 entry	
Stenotrophomonas maltophilia	x	x	-	
Streptococcus pneumoniae	x	x > 240 entries	x 1 entry	including serotype 7F

LRT BAL Antibiotic Resistance Markers

Similar to microorganisms, inclusivity wet testing was performed with reference strains carrying antibiotic resistance markers of the LRT BAL panel at concentrations near LoD with contrived samples using pooled negative native lavage specimens as sample matrix (Table 6). Subgroups of antibiotic resistance markers are added, if known (NA: unknown). Where phenotypic antimicrobial susceptibility testing (AST) results are available, this information is also shown (e.g., carbapenem^R = resistant to carbapenems, carbapenem^S = susceptible to carbapenems).

Inclusivity was established near LoD for all tested reference strains.

Table 6: Inclusivity study results for LRT BAL panel antibiotic resistance markers.

Analyte	Subgroup	Reference Strain	Strain ID	Test Conc. [CFU/ml]	x-fold LoD	# Pos./ # Exp.
***ctx*-M**	NA	***Klebsiella pneumoniae* (pos. control/LoD ref. strain)**	**NCTC 13443**	**2.0 x 10^4**	**2x**	**3/3**
	ctx-M3	*Klebsiella pneumoniae*	NRZ-00751	2.0 x 10^4	2x	2/2
	ctx-M15	*Klebsiella pneumoniae*	NRZ-00249	2.0 x 10^4	2x	2/2
	NA	*Enterobacter cloacae*	JMI 46239	2.0 x 10^4	2x	1/2
				3.0 x 10^4	3x	2/2
	NA	*Escherichia coli*	JMI 50067	2.0 x 10^4	2x	2/2
	NA	*Klebsiella pneumoniae*	NRZ-00002	2.0 x 10^4	2x	1/2
				3.0 x 10^4	3x	2/2
	NA	*Enterobacter cloacae*	ATCC BAA-2468	4.0 x 10^4	4x	2/2
	NA	*Klebsiella pneumoniae*	JMI 49831	4.0 x 10^4	4x	2/2
	NA	*Klebsiella pneumoniae*	JMI 49767	2.0 x 10^4	2x	2/2
kpc	*kpc*-3	***Klebsiella pneumoniae* (pos. control/LoD ref. strain)**	**NCTC 13438**	**8.0 x 10^4**	**2x**	**5/5**
	kpc-2	*Escherichia coli*	NRZ-00281	8.0 x 10^4	2x	2/2
	kpc-2	*Klebsiella pneumoniae*	NRZ-00103	8.0 x 10^4	2x	2/2
	kpc-3	*Escherichia coli*	NRZ-00222	8.0 x 10^4	2x	1/2
				1.2 x 10^5	3x	2/2
	kpc-3	*Klebsiella pneumoniae* (carbapenem^R)	Micromyx 4653	8.0 x 10^4	2x	4/4
	kpc-3	*Klebsiella pneumoniae* (carbapenem^R)	Micromyx 4676	8.0 x 10^4	2x	4/4
***mec*A**	NA	***Staphylococcus aureus* (methicillin^R, cefoxitin^R) (pos. control/LoD ref. strain)**	**NCTC 12493**	**8.0 x 10^5**	**2x**	**3/3**
	SCCmecI	*Staphylococcus aureus*	RKI 07-03165	8.0 x 10^5	2x	2/2
	SCCmecII	*Staphylococcus aureus*	RKI 01-00694	8.0 x 10^5	2x	2/2
	SCCmecIII	*Staphylococcus aureus* (methicillin^R)	ATCC 33591	8.0 x 10^5	2x	2/2
	SCCmecIV	*Staphylococcus aureus*	RKI 09-00187	8.0 x 10^5	2x	2/2
	SCCmecV	*Staphylococcus aureus*	RKI 08-02492	8.0 x 10^5	2x	2/2
	NA	*Staphylococcus aureus* (methicillin^R)	DSM-17091	8.0 x 10^5	2x	2/2
	SCCmecII	*Staphylococcus aureus* (methicillin^R)	ATCC 43300	3.0 x 10^5	0.8x	2/2

Analyte	Subgroup	Reference Strain	Strain ID	Test Conc. [CFU/ml]	x-fold LoD	# Pos./ # Exp.
ndm	*ndm*-1	***Klebsiella pneumoniae* (pos. control/LoD ref. strain)**	**NCTC 13443**	**4.0×10^4**	**2x**	**5/5**
	ndm-1	*Acinetobacter baumannii*	JMI 49755	4.0×10^4	2x	4/4
	ndm-1	*Enterobacter cloacae* (imipenem^R, ertapenem^R)	ATCC BAA-2468	4.0×10^4	2x	1/2
				6.0×10^4	3x	2/2
	ndm-1	*Enterobacter cloacae*	JMI 46239	4.0×10^4	2x	4/4
	ndm-1	*Escherichia coli*	JMI 50067	4.0×10^4	2x	2/2
	ndm-1	*Klebsiella pneumoniae*	JMI 49767	2.0×10^4	1x	2/2
	ndm-1	*Klebsiella pneumoniae*	JMI 49831	4.0×10^4	2x	2/2
***oxa*-23**	oxa-23	***Acinetobacter baumannii* (pos. control/LoD ref. strain)**	**NCTC 13301**	**2.0×10^7**	**2x**	**3/3**
	NA	*Acinetobacter baumannii* (carbapenem^R)	Micromyx 4410	2.0×10^7	2x	2/2
	NA	*Acinetobacter baumannii* (carbapenem^R)	Micromyx 6148	2.0×10^7	2x	2/2
	NA	*Acinetobacter baumannii* (carbapenem^R)	Micromyx 6149	2.0×10^7	2x	2/2
	NA	*Acinetobacter baumannii* (carbapenem^R)	Micromyx 6153	2.0×10^7	2x	2/2
	NA	*Acinetobacter baumannii* (imipenem^R, meropenem^R)	UCLA A5	2.0×10^7	2x	2/2
***oxa*-24**	*oxa*-25	***Acinetobacter baumannii* (pos. control/LoD ref. strain)**	**NCTC 13302**	**1.0×10^4**	**2x**	**3/3**
	oxa-72	*Acinetobacter baumannii*	NRZ-00449	1.0×10^4	2x	2/2
	NA	*Acinetobacter baumannii* (imipenem^R, meropenem^R)	UCLA A4	1.0×10^4	2x	2/2
	NA	*Acinetobacter baumannii* (imipenem^R, meropenem^R)	clinical strain 1	1.0×10^4	2x	2/2
	NA	*Acinetobacter baumannii* (imipenem^R, meropenem^R)	clinical strain 2	1.0×10^4	2x	2/2
***oxa*-48**	*oxa*-48	***Klebsiella pneumoniae* (pos. control/LoD ref. strain)**	**NCTC 13442**	**6.0×10^5**	**2x**	**3/3**
	oxa-48	*Escherichia coli* (ertapenem^R)	ATCC BAA-2523	6.0×10^5	2x	2/2
	oxa-48	*Escherichia coli*	NRZ-00176	6.0×10^5	2x	1/2
				9.0×10^5	3x	2/2
	oxa-162	*Escherichia coli*	NRZ-00361	6.0×10^5	2x	2/2
	oxa-162	*Klebsiella pneumoniae*	NRZ-00472	6.0×10^5	2x	2/2
	oxa-232	*Klebsiella oxytoca*	NRZ-22060	6.0×10^5	2x	2/2
***oxa*-58**	*oxa*-58	***Acinetobacter baumannii* (pos. control/LoD ref. strain)**	**NCTC 13305**	**1.0×10^5**	**2x**	**2/3**
	oxa-58	*Acinetobacter baumannii*	NRZ-00518	1.0×10^5	2x	3/3
tem	NA	***Klebsiella pneumoniae* (pos. control/LoD ref. strain)**	**NCTC 13443**	**4.0×10^4**	**2x**	**2/2**
	tem-1	*Escherichia coli*	ATCC 35218	4.0×10^4	2x	2/2
	tem-3	*Escherichia coli* (ESBL)	NCTC 13351	4.0×10^4	2x	2/2
	NA	*Citrobacter freundii*	ATCC 43864	4.0×10^4	2x	2/2
	NA	*Enterobacter cloacae*	JMI 46239	4.0×10^4	2x	2/2
	NA	*Escherichia coli*	JMI 50067	2.0×10^4	1x	2/2
	NA	*Klebsiella pneumoniae*	NRZ-00751	2.0×10^4	1x	2/2
	NA	*Escherichia coli*	ATCC BAA-2523	4.0×10^4	2x	2/2

Analyte	Subgroup	Reference Strain	Strain ID	Test Conc. [CFU/ml]	x-fold LoD	# Pos./ # Exp.
	NA	*Acinetobacter baumannii*	JMI 49755	4.0×10^4	2x	2/2
	NA	*Haemophilus influenzae* (ampicillin^R, cefinase^R)	clinical strain 1	4.0×10^4	2x	2/2
	NA	*Haemophilus influenzae* (cefinase^R)	clinical strain 2	4.0×10^4	2x	2/2
	NA	*Klebsiella pneumoniae*	Micromyx 4676	8.0×10^4	4x	2/2
vim	*vim*-10	***Pseudomonas aeruginosa* (pos. control/LoD ref. strain)**	**NCTC 13437**	**4.0×10^4**	**2x**	**3/3**
	vim-1	*Citrobacter freundii*	NRZ-00452	4.0×10^4	2x	2/2
	vim-1	*Pseudomonas aeruginosa* (ceftazidime^R, imipenem^R)	DSM-24600	4.0×10^4	2x	2/2
	vim-1	*Enterobacter cloacae*	NRZ-00239	4.0×10^4	2x	2/2
	vim-1	*Klebsiella pneumoniae*	NCTC 13439	4.0×10^4	2x	2/2
	vim-1	*Klebsiella pneumoniae*	NCTC 13440	4.0×10^4	2x	2/2
	NA	*Pseudomonas aeruginosa*	UCLA P20	1.3×10^3	0.1x	2/2
	NA	*Pseudomonas aeruginosa* (carbapenem^R)	Micromyx 2562	4.0×10^4	2x	2/2

To supplement inclusivity testing for further specific antibiotic resistance marker variants, reference sequences for all available variants belonging to individual antibiotic resistance markers or marker subgroups were compiled and analyzed *in silico*.

Subgroups or variants for applicable LRT BAL panel antibiotic resistance markers for which detection is predicted at LoD (match of relevant primer and probe sequences), with reduced sensitivity (typically, single relevant mismatches of primer or probe sequences; detection likely at higher than LoD concentrations only), or is not predicted (multiple relevant mismatches in primer and probe sequences) are listed in Table 7. Such predictions are provided as supplementary data only. Results are not intended to be a surrogate for wet testing and do not assure that specific variant will be detected.

NOTE: The performance of the Unyvero LRT BAL Application has not been established for antibiotic resistance marker variants other than those listed in the inclusivity test results table above.

Table 7: *In silico* prediction for LRT BAL panel antibiotic resistance markers for applicable subgroups or variants.

Antibiotic Resistance Marker: Subgroup	Detection Predicted at LoD: Variant No.	Detection Predicted with Reduced Sensitivity: Variant No.	Detection Not Predicted: Variant No.
***ctx*-M:** *ctx*-M1 subgroup	1, 3, 10, 11, 12, 15, 22, 23, 28 - 30, 32 - 34, 36, 37, 42, 52 - 55, 57, 58, 60 - 62, 66, 68, 69, 71, 72, 79, 80, 82, 83, 88, 96, 101, 103, 107, 108, 109, 114, 116, 117, 132, 133, 136, 138, 139, 142, 144, 150, 155 - 158, 162 - 164, 166, 167, 169, 170, 172, 173, 175 - 177, 179 - 184, 186, 188 - 190, 193, 194, 197, 202 - 204, 206 - 212, 216, 218, 222, 224	64	-
***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, 192, 195, 196, 198, 199, 201, 214, 215, 217, 219, 221, 223
kpc	1 - 39	-	-
ndm	1 - 24, 27	-	-
***oxa*:** *oxa*-23	23, 27, 49, 73, 134, 146, 165 - 171, 225, 239, 366, 398, 422, 423, 435, 440, 469, 481 - 483, 565, 657, 806 - 808, 810, 813 - 815, 816, 818	103, 133, 809, 811, 812, 816	-
***oxa*:** *oxa*-24	24 - 26, 33, 40, 72, 139, 160, 207, 437, 653	-	-
***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, 547, 566, 567, 731, 788, 793	204	54, 436, 535
***oxa*:** *oxa*-58	58, 96, 97, 164, 397, 467, 512	-	420
tem	1 - 4, 6, 8 - 12, 15 - 17, 19 - 22, 24, 26, 28 - 30, 32 - 36, 40, 43, 45, 47 - 49, 52 - 55, 57, 60, 63, 67, 68, 70 - 72, 76 - 88, 90 - 99, 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, 229 - 235, 237	151	178
vim	1 - 6, 8 - 12, 14 - 20, 23 - 46, 48 - 54, 56, 58, 60, 62	57, 59	7, 13, 47, 61

NOTE: *H. influenzae* commonly hosts *tem*-1. *tem* will only be reported by the LRT BAL Application if *H. influenzae* is concurrently detected.

Exclusivity and Cross-Reactivity

For each of the LRT BAL panel analytes, cross-reactive species (clinically relevant close neighbor strains) were predicted by GenBank BLAST search. Predictions were complemented with exclusivity wet testing for close-neighbor strains or common respiratory flora microorganisms performed in duplicate at a worst-case concentration (typically: 1.5 x 10^7 CFU/mL for bacterial microorganisms, or as indicated) by spiking into 0.25x ARM (artificial respiratory matrix) surrogate as sample matrix.

Exclusivity study results are listed in
Table 8. For *Haemophilus haemolyticus* (ATCC 33390) and *Aggregatibacter aphrophilus* (ATCC 19415), cross-reactivity to *Haemophilus influenzae* close to LoD concentrations (for ATCC 33390) or with reduced sensitivity (for ATCC 19415), respectively, was observed. Another strain, Haemophilus parainfluenzae (ATCC 33392) cross-reacted to Haemophilus influenzae only at very high concentrations in some of the tests (> 100x LoD, > 10^7 CFU/mL).

Table 8: Exclusivity study results.

Strain	Test Concentration	Cross-Reactivity
respiratory flora microorganisms	[in CFU/mL]	
Actinomyces odontolyticus	1.5 x 10^7	no
Aspergillus fumigatus	1.5 x 10^7	no
Candida albicans	1.5 x 10^7	no
Candida dubliniensis	1.5 x 10^7	no
Candida glabrata	1.5 x 10^7	no
Candida krusei	1.5 x 10^7	no
Candida parapsilosis	1.5 x 10^7	no
Candida tropicalis	1.5 x 10^7	no
Cardiobacterium hominis	1.5 x 10^7	no
Eikenella corrodens	1.5 x 10^7	no
Enterococcus faecalis	1.5 x 10^7	no
Enterococcus faecium	1.5 x 10^7	no
Fusobacterium nucleatum	5.0 x 10^6 [d]	no
Granulicatella adiacens	1.5 x 10^7	no
Kingella kingae	1.5 x 10^7	no
Lactobacillus acidophilus	1.5 x 10^7	no
Micrococcus luteus	1.5 x 10^7	no
Mycobacterium bovis	1.5 x 10^7 [d]	no
Mycoplasma orale	1.5 x 10^7	no
Neisseria lactamica	1.5 x 10^7 [d]	no
Neisseria sicca	1.5 x 10^7	no
Pantoae agglomerans	1.5 x 10^7	no
Peptostreptococcus stomatis	5.0 x 10^6 [d]	no
Porphyromonas gingivalis	1.5 x 10^7 [d]	no
Prevotella buccalis	1.5 x 10^7	no
Raoultella planticola	1.5 x 10^7	no

close neighbor microorganisms	[in CFU/mL]	
Acinetobacter ursingii	1.5 x 10^7	no
Aggregatibacter actinomycetemcomitans	1.5 x 10^7 [d]	no
Aggregatibacter aphrophilus	1.5 x 10^7	yes (*H. influenzae*) [a]
Citrobacter koseri	1.5 x 10^7	no
Haemophilus haemolyticus	1.5 x 10^7	yes (*H. influenzae*) [b]
Haemophilus parahaemolyticus	1.5 x 10^7	no
Haemophilus parainfluenzae	1.5 x 10^7	yes (*H. influenzae*) [c]
Legionella longbeachae	1.5 x 10^7	no
Legionella/Tatlockia micdadei	1.5 x 10^7	no
Staphylococcus capitis	1.5 x 10^7	no
Staphylococcus epidermidis	1.5 x 10^7	no
Staphylococcus haemolyticus	1.5 x 10^7	no
Staphylococcus lugdunensis	1.5 x 10^7	no
Staphylococcus saprophyticus	1.5 x 10^7	no
Streptococcus agalactiae	1.5 x 10^7	no
Streptococcus anginosus	1.5 x 10^7	no
Streptococcus dysgalactiae	1.5 x 10^7	no
Streptococcus gordonii	1.5 x 10^7	no
Streptococcus intermedius	1.5 x 10^7	no
Streptococcus mitis	1.5 x 10^7	no
Streptococcus mutans	1.5 x 10^7	no
Streptococcus oralis	1.5 x 10^7	no
Streptococcus parasanguinis	1.5 x 10^7	no
Streptococcus pseudopneumoniae	1.5 x 10^7	no
Streptococcus pyogenes	1.5 x 10^7	no
Streptococcus salivarius	1.5 x 10^7	no
Streptococcus sanguinis	1.5 x 10^7	no
Streptococcus vestibularis	1.5 x 10^7	no
respiratory viruses [e]	[in copies/mL]	
Adenovirus 41	1.0 x 10^5	no
Enterovirus 68	1.0 x 10^5	no
Enterovirus 71	1.0 x 10^5	no
Parainfluenzae Virus 1	1.0 x 10^5	no
Parainfluenzae Virus 2	1.0 x 10^5	no
Parainfluenzae Virus 3	1.0 x 10^5	no
Parainfluenzae Virus 4	1.0 x 10^5	no
Rhinovirus	1.0 x 10^5	no
RSV A	1.0 x 10^5	no
RSV B	1.0 x 10^5	no

[a] For *A. aphrophilus*, cross-reactivity to *H. influenzae* was observed down to a concentration of 2.0 x 10^5 CFU/mL (1/2 tests positive). For a concentration of 1.0 x 10^5 CFU/mL (equivalent to 5x LoD of *H. influenzae*), cross-reactivity was not observed any more. According to BLAST analysis, cross-reactivity of *A. aphrophilus* to *H. influenzae* is only predicted for some strains (including the tested reference strain ATCC 19415), but not for other strains.
[b] For *H. haemolyticus*, cross-reactivity to *H. influenzae* was observed down to a concentration of 4.0 x 10^5 CFU/mL (2/2 tests positive, equivalent to 2x LoD of *H. influenzae*). Sequence comparison by BLAST for *H. haemolyticus* to *H. influenzae* shows a 3' mismatch to

one of the assay primers, while the second primer and all internal array probes show a full match. Therefore, the observed cross-reactivity is supported by BLAST analysis, although only predicted with reduced sensitivity.
[c] For *H. parainfluenzae*, cross-reactivity to *H. influenzae* was observed only at the tested worst-case concentration of 1.5 x 10^7 CFU/mL (2/4 tests positive, equivalent to 750x LoD of *H. influenzae*). Additional tests at 2.0 x 10^6 CFU/mL (equivalent to 100x LoD of *H. influenzae*), cross-reactivity was not observed any more.
[d] Tested as DNA extract (in copies/mL).
[e] Tested as DNA or RNA extract (in copies/mL).

Table 9 lists predicted possible cross-reactivity of applicable LRT BAL panel microorganisms based on *in silico* analysis, exclusivity wet testing and cases observed during the prospective or archived study.

Table 9: *In silico* cross-reactivity prediction, results of exclusivity wet testing and cross-reactive specimens observed for the prospective and archived study.

Close Neighbor Strain [a]	**Cross-Reactivity Prediction** (*in-silico* analysis)	**Wet Testing Result**	**Cross-Reactions Observed in Clinical Study** (N = 1,408 prosp. or arch. specimens)
Citrobacter freundii			
Citrobacter braakii	Detection predicted at higher than LoD concentrations (certain strains) / Detection not predicted (other strains) [c]	-	-
Citrobacter pasteurii	Detection predicted at higher than LoD concentrations	-	-
Citrobacter werkmannii	Detection predicted at higher than LoD concentrations (certain strains) / Detection not predicted (other strains) [c]	-	-
Citrobacter youngae	Detection predicted at LoD (certain strains) / Detection predicted at higher than LoD concentrations (other strains) [c]	-	1
Kluyvera georgiana	Detection predicted at higher than LoD concentrations	-	-
Citrobacter koseri	Detection not predicted	negative ATCC 27156	-
Escherichia coli			
Shigella dysenteriae [b]	Detection predicted at LoD	-	-
Shigella boydii [b]	Detection predicted at LoD	-	-
Shigella flexneri [b]	Detection predicted at LoD	-	-
Shigella sonnei [b]	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	5
Haemophilus parahaemolyticus	Detection not predicted	negative ATCC 10014	-
Haemophilus parainfluenzae	Detection not predicted	positive at worst-case concentration only ATCC 33392	1
Haemophilus aegypticus [b]	Detection predicted at LoD	-	-

Aggregatibacter actino-mycetemcomitans	Detection not predicted	negative ATCC 33384	-
Aggregatibacter aphrophilus	Detection predicted at higher than LoD concentrations (certain strains) / Detection not predicted (other strains) [c]	positive ATCC 19415	3
Aggregatibacter segnis	Detection predicted at higher than LoD concentrations	-	-
Klebsiella oxytoca			
Klebsiella michiganensis	Detection predicted at LoD (certain strains) / Detection predicted at higher than LoD concentrations (other strains) [c]	-	-
Klebsiella pneumoniae			
Klebsiella quasivariicola	Detection predicted at higher than LoD concentrations	-	-
Staphylococcus aureus			
Staphylococcus argenteus [b]	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	-
Streptococcus pneumoniae			
other *Streptococcus* spp.: *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] For several analyte assays, soil, environmental, plant or animal derived close neighbor strains are also predicted at LoD or at higher than LoD concentrations (*Citrobacter freundii*:, *C. portucalensis*, *Enterobacter cloacae* complex: *E. soli*, *E. mori*, *E. nickellidurans*; *Escherichia coli*: *E. marmotae*; *Staphylococcus aureus*: *S. schweitzeri*, *S. simiae*; *Stenotrophomonas maltophilia*: *S. nitritireducens*, *S. daejeonensis*, *S. acidaminiphila*, *S. koreensis*, *S. rhizophila*, *Xanthomonas* spp., *Pseudoxanthomonas* spp.).
[b] Clinical relevance unlikely for respiratory infections.
[c] Predictions for available strains distribute into different categories.

Interference Testing

Interference testing had already been established with the Unyvero LRT Application (predicate device), which relies on the same DNA extraction procedures and chemistry as the LRT BAL Application. Results are included below for reference. Possible interfering substances, such as respiratory medications, antibiotics, sample storage media, lavage media, sample liquefying agent (DTT), lysis buffer and endogenous substances blood and human DNA were tested. Pools of panel analytes were analyzed with and without interfering substances added at concentrations recommended in the CLSI guideline 'Interference Testing in Clinical Chemistry'. Representative analytes were pooled and added to PBS as surrogate matrix containing potentially interfering substances at concentrations as indicated in Table 10. No interference was observed at tested concentrations.

Table 10: Tested possible interfering substances (M: test concentration in mol/L).

	Interferent	Test Concentration	Interference Observed
respiratory drugs	Guaifenesin	1.5×10^{-2} M	no
	Dextromethorphan	3.7×10^{-6} M	no
	Acetyl-Cystein	1.0×10^{-2} M	no
	Salbutamol	4.0×10^{-6} M	no
	Carbocystein	2.8×10^{-3} M	no
	Ambroxol	8.0×10^{-4} M	no
	Beclomethason	7.0×10^{-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	3.0×10^{-5} M	no
	Amikacin	1.4×10^{-4} M	no
	Imipenem	5.0×10^{-4} M	no
	Clindamycin	8.9×10^{-5} M	no
	Trimethoprim	1.4×10^{-4} M	no
	Sulfamethoxazole	1.6×10^{-3} M	no
lavage sample media	Ringer-Lactate solution	100%	no
	Ringer solution	100%	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	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

Reproducibility

Assay reproducibility was established with artificial samples (viable strains spiked in ARM surrogate matrix) with a representative strain panel (incl. Gram-positive, Gram-negative and 'atypical' microorganisms) covering the following LRT BAL panel analytes: *Acinetobacter* spp., *C. pneumoniae*, *C. freundii*, *H. influenzae*, *K. pneumoniae* (comprising LRT BAL panel antibiotic resistance markers *ctx*-M, *ndm*, and *tem*), *M. morganii*, *Proteus* spp. and, *S. aureus* (comprising LRT BAL panel antibiotic resistance marker *mec*A). Reproducibility testing was performed at a moderate concentration (2.5x - 10x LoD of the corresponding target analyte), at a low concentration (1x - 4x LoD of the corresponding target analyte), and with all analytes negative (surrogate matrix only) on three different Unyvero systems. Each Unyvero system mimicked a different clinical setting, consisted of one Unyvero Cockpit, three Unyvero Lysators, up to six Unyvero Analyzers, and was operated by one of three dedicated operators with different levels of work experience.

Each operator performed 3 'moderate', 3 'low', and 3 'negative' replicates per test shift on his/her Unyvero system (maximum: 18 replicates per test day). Each operator performed a minimum of 30 replicates per concentration level (90 replicates in total per Unyvero system, 270 replicates in total for all three Unyvero systems with 90 replicates for each concentration level) distributed over a minimum of five test days with sample identities blinded to the operator. Samples on individual Analyzer slots were rotated (e.g., 'moderate' followed by 'low', followed by 'negative' etc.) to achieve a non-consecutive testing schedule for each sample type between test shifts. Failed runs as well as runs with single invalid analyte results were repeated to achieve a minimum number of at least 30 valid results for each of the target analytes at all test concentration levels.

Tables 11 to 13 summarize the reproducibility results for each concentration level, along with the agreement rates with the expected result and 95% confidence intervals (95% CI, calculated using the Wilson score method).

Table 11: Reproducibility study results (agreement with expected result) for concentration level 'moderate', 5x LoD per test analyte or as indicated.

moderate	x-fold LoD	Unyvero System/ Operator	Agreement with Expected Result		
			# Pos. /# Exp.	%	95% CI
Acinetobacter baumannii ATCC 19606	5	operator 1	30/31	96.8	83.8 - 99.4
		operator 2	31/31	100.0	89.0 - 100.0
		operator 3	30/30	100.0	88.7 - 100.0
		total	**91/92**	**98.9**	**94.1 - 99.8**
Chlamydia pneumoniae ATCC VR-2282	5	operator 1	31/31	100.0	89.0 - 100.0
		operator 2	31/31	100.0	89.0 - 100.0
		operator 3	30/30	100.0	88.7 - 100.0
		total	**92/92**	**100.0**	**96.0 - 100.0**
Citrobacter freundii ATCC 8090	5	operator 1	29/30 [a]	96.7	83.3 - 99.4
		operator 2	31/31	100.0	89.0 - 100.0
		operator 3	30/30	100.0	88.7 - 100.0
		total	**90/91**	**98.9**	**94.0 - 99.8**
Haemophilus influenzae ATCC 33391	5	operator 1	29/31	93.5	79.3 - 98.2
		operator 2	31/31	100.0	89.0 - 100.0
		operator 3	30/30	100.0	88.7 - 100.0
		total	**90/92**	**97.8**	**92.4 - 99.4**
Klebsiella pneumoniae NCTC 13443	2.5	operator 1	31/31	100.0	89.0 - 100.0
		operator 2	31/31	100.0	89.0 - 100.0
		operator 3	30/30	100.0	88.7 - 100.0
		total	**92/92**	**100.0**	**96.0 - 100.0**
***ctx*-M** NCTC 13443	10	operator 1	31/31	100.0	89.0 - 100.0
		operator 2	31/31	100.0	89.0 - 100.0
		operator 3	30/30	100.0	88.7 - 100.0
		total	**92/92**	**100.0**	**96.0 - 100.0**
ndm NCTC 13443	5	operator 1	29/30 [a]	96.7	83.3 - 99.4
		operator 2	31/31	100.0	89.0 - 100.0
		operator 3	30/30	100.0	88.7 - 100.0
		total	**90/91**	**98.9**	**94.0 - 99.8**
tem NCTC 13443	5	operator 1	28/31	90.3	75.1 - 96.7
		operator 2	31/31	100.0	89.0 - 100.0
		operator 3	30/30	100.0	88.7 - 100.0
		total	**89/92**	**96.7**	**90.8 - 98.9**
Morganella morganii ATCC 25830	5	operator 1	31/31	100.0	89.0 - 100.0
		operator 2	31/31	100.0	89.0 - 100.0
		operator 3	30/30	100.0	88.7 - 100.0
		total	**92/92**	**100.0**	**96.0 - 100.0**
Proteus vulgaris ATCC 29905	5	operator 1	31/31	100.0	89.0 - 100.0
		operator 2	30/30 [a]	100.0	88.7 - 100.0
		operator 3	30/30	100.0	88.7 - 100.0
		total	**91/91**	**100.0**	**96.0 - 100.0**
Staphylococcus aureus NCTC 12493	8.3	operator 1	31/31	100.0	89.0 - 100.0
		operator 2	31/31	100.0	89.0 - 100.0
		operator 3	30/30	100.0	88.7 - 100.0
		total	**92/92**	**100.0**	**96.0 - 100.0**
***mec*A** NCTC 12493	3	operator 1	31/31	100.0	89.0 - 100.0
		operator 2	31/31	100.0	89.0 - 100.0
		operator 3	30/30	100.0	88.7 - 100.0
		total	**92/92**	**100.0**	**96.0 - 100.0**

[a] Reduced number of available results due to invalid analyte results.

Table 12: Reproducibility study results (agreement with expected result) for concentration level 'low', 2x LoD per analyte or as indicated.

low	x-fold LoD	Unyvero System/ Operator	Agreement with Expected Result		
			# Pos. /# Exp.	%	95% CI
Acinetobacter baumannii ATCC 19606	2	operator 1	29/30	96.7	83.3 - 99.4
		operator 2	31/31	100.0	89.0 - 100.0
		operator 3	29/30	96.7	83.3 - 99.4
		total	**89/91**	**97.8**	**92.3 - 99.4**
Chlamydia pneumoniae ATCC VR-2282	2	operator 1	30/30	100.0	88.7 - 100.0
		operator 2	31/31	100.0	89.0 - 100.0
		operator 3	30/30	100.0	88.7 - 100.0
		total	**91/91**	**100.0**	**96.0 - 100.0**
Citrobacter freundii ATCC 8090	2	operator 1	28/30	93.3	78.7 - 98.2
		operator 2	26/31	83.9	67.4 - 92.9
		operator 3	29/30	96.7	83.3 - 99.4
		total	**83/91**	**91.2**	**83.6 - 95.5**
Haemophilus influenzae ATCC 33391	2	operator 1	27/30	90.0	74.4 - 96.5
		operator 2	31/31	100.0	89.0 - 100.0
		operator 3	29/30	96.7	83.3 - 99.4
		total	**87/91**	**95.6**	**89.2 - 98.3**
Klebsiella pneumoniae NCTC 13443	1	operator 1	30/30	100.0	88.7 - 100.0
		operator 2	31/31	100.0	89.0 - 100.0
		operator 3	28/30	93.3	78.7 - 98.2
		total	**89/91**	**97.8**	**92.3 - 99.4**
***ctx*-M** NCTC 13443	4	operator 1	29/30	96.7	83.3 - 99.4
		operator 2	30/31	96.8	83.8 - 99.4
		operator 3	30/30	100.0	88.7 - 100.0
		total	**89/91**	**97.8**	**92.3 - 99.4**
ndm NCTC 13443	2	operator 1	29/30	96.7	83.3 - 99.4
		operator 2	30/31	96.8	83.8 - 99.4
		operator 3	30/30	100.0	88.7 - 100.0
		total	**89/91**	**97.8**	**92.3 - 99.4**
tem NCTC 13443	2	operator 1	27/30	90.0	74.4 - 96.5
		operator 2	31/31	100.0	89.0 - 100.0
		operator 3	29/30	96.7	83.3 - 99.4
		total	**87/91**	**95.6**	**89.2 - 98.3**
Morganella morganii ATCC 25830	2	operator 1	30/30	100.0	88.7 - 100.0
		operator 2	30/31	96.8	83.8 - 99.4
		operator 3	30/30	100.0	88.7 - 100.0
		total	**90/91**	**98.9**	**94.0 - 99.8**
Proteus vulgaris [a] ATCC 29905	2	operator 1	25/30	83.3	66.4 - 92.7
		operator 2	24/31	77.4	60.2 - 88.6
		operator 3	27/30	90.0	74.4 - 96.5
		total	**76/91**	**83.5**	**74.6 - 89.7**
Staphylococcus aureus NCTC 12493	3	operator 1	29/30	96.7	83.3 - 99.4
		operator 2	30/30 [b]	100.0	88.7 - 100.0
		operator 3	30/30	100.0	88.7 - 100.0
		total	**89/90**	**98.9**	**94.0 - 99.8**
mecA NCTC 12493	1.3	operator 1	30/30	100.0	88.7 - 100.0
		operator 2	31/31	100.0	89.0 - 100.0
		operator 3	29/30	96.7	83.3 - 99.4
		total	**90/91**	**98.9**	**94.0 - 99.8**

[a] *Proteus* spp. demonstrated a slightly lower than expected positivity rate for the 2x LoD concentration with a positivity rate (for all operators combined) of 83.5% with a 95% CI of 74.6% - 89.7%. However, for an alternative test series performed in parallel for the sample stability study using the same strain and the identical cartridge lot with pooled negative BAL matrix the expected positivity rate at a 2x LoD concentration for *Proteus* spp. was confirmed (positivity rate: 92/93, 98.9%).
[b] Reduced number of available results due to invalid analyte results.

Table 13: Reproducibility study results (agreement with expected result) for negative samples.

negative	Unyvero System/ Operator	Agreement with Expected Result		
		# Neg. /# Exp.	%	95% CI
Acinetobacter baumannii ATCC 19606	operator 1	31/31	100.0	89.0 - 100.0
	operator 2	31/31	100.0	89.0 - 100.0
	operator 3	30/30	100.0	88.7 - 100.0
	total	**92/92**	**100.0**	**96.0 - 100.0**
Chlamydia pneumoniae ATCC VR-2282	operator 1	31/31	100.0	89.0 - 100.0
	operator 2	31/31	100.0	89.0 - 100.0
	operator 3	30/30	100.0	88.7 - 100.0
	total	**92/92**	**100.0**	**96.0 - 100.0**
Citrobacter freundii ATCC 8090	operator 1	31/31	100.0	89.0 - 100.0
	operator 2	31/31	100.0	89.0 - 100.0
	operator 3	30/30	100.0	88.7 - 100.0
	total	**92/92**	**100.0**	**96.0 - 100.0**
Haemophilus influenzae ATCC 33391	operator 1	31/31	100.0	89.0 - 100.0
	operator 2	31/31	100.0	89.0 - 100.0
	operator 3	30/30	100.0	88.7 - 100.0
	total	**92/92**	**100.0**	**96.0 - 100.0**
Klebsiella pneumoniae NCTC 13443	operator 1	31/31	100.0	89.0 - 100.0
	operator 2	30/30 [a]	100.0	88.7 - 100.0
	operator 3	30/30	100.0	88.7 - 100.0
	total	**91/91**	**100.0**	**96.0 - 100.0**
***ctx*-M** NCTC 13443	operator 1	31/31	100.0	89.0 - 100.0
	operator 2	30/30 [a]	100.0	88.7 - 100.0
	operator 3	30/30	100.0	88.7 - 100.0
	total	**91/91**	**100.0**	**96.0 - 100.0**
ndm NCTC 13443	operator 1	31/31	100.0	89.0 - 100.0
	operator 2	31/31	100.0	89.0 - 100.0
	operator 3	30/30	100.0	88.7 - 100.0
	total	**92/92**	**100.0**	**96.0 - 100.0**
tem NCTC 13443	operator 1	31/31	100.0	89.0 - 100.0
	operator 2	31/31	100.0	89.0 - 100.0
	operator 3	30/30	100.0	88.7 - 100.0
	total	**92/92**	**100.0**	**96.0 - 100.0**
Morganella morganii ATCC 25830	operator 1	31/31	100.0	89.0 - 100.0
	operator 2	30/30 [a]	100.0	88.7 - 100.0
	operator 3	30/30	100.0	88.7 - 100.0
	total	**91/91**	**100.0**	**96.0 - 100.0**
Proteus vulgaris ATCC 29905	operator 1	30/30 [a]	100.0	88.7 - 100.0
	operator 2	31/31	100.0	89.0 - 100.0
	operator 3	30/30	100.0	88.7 - 100.0
	total	**91/91**	**100.0**	**96.0 - 100.0**
Staphylococcus aureus NCTC 12493	operator 1	31/31	100.0	89.0 - 100.0
	operator 2	31/31	100.0	89.0 - 100.0
	operator 3	30/30	100.0	88.7 - 100.0
	total	**92/92**	**100.0**	**96.0 - 100.0**
***mec*A** NCTC 12493	operator 1	31/31	100.0	89.0 - 100.0
	operator 2	30/30 [a]	100.0	88.7 - 100.0
	operator 3	30/30	100.0	88.7 - 100.0
	total	**91/91**	**100.0**	**96.0 - 100.0**

[a] Reduced number of available results due to invalid analyte results.

For three cartridge runs, unexpected positive results were reported with weak signals close to the assay thresholds on different test days and different operators/test systems (2x *E. coli*, for one 'moderate' and one 'low' test sample, 1x *oxa*-23, for one 'moderate' test sample).

Fresh-Frozen Study

Assay reproducibility between fresh samples and samples stored frozen was established with artificial samples (viable strains spiked in pooled native negative BAL specimens as test matrix) with the representative strain panel as described for the reproducibility study above. Testing was performed at a moderate concentration (2.5x - 10x LoD of the corresponding target analyte, at least 10 replicates per condition), at a low concentration (1x - 4x LoD of the corresponding target analyte, at least 30 replicates per condition) and with all target analytes negative (at least 10 replicates per condition).

Pair-wise tests with the LRT BAL Application were performed for samples tested within 2 hrs after test sample preparation (Time 0) and samples tested after freezing below -70 °C for at least 1 day and thawing immediately before testing.

Tables 14 to 16 summarize the fresh-frozen test results for each of the tested concentration levels (moderate, low, negative) for both storage conditions (tested freshly, tested frozen), along with the agreement rates with the expected result and 95% confidence intervals (95% CI, calculated using the Wilson score method).

Comparable agreement rates with the expected result were obtained for all test analytes for both test conditions.

Table 14: Fresh-frozen study test results for concentration level 'moderate', approx. 5x LoD per test analyte.

moderate	x-fold LoD	Test Condition	Agreement with Expected Result		
			# Pos. /# Exp.	%	95% CI
Acinetobacter baumannii	5	fresh	11/11	100.0	74.1 - 100.0
ATCC 19606		frozen	11/11	100.0	74.1 - 100.0
Chlamydia pneumoniae	5	fresh	11/11	100.0	74.1 - 100.0
ATCC VR-2282		frozen	11/11	100.0	74.1 - 100.0
Citrobacter freundii	5	fresh	11/11	100.0	74.1 - 100.0
ATCC 8090		frozen	11/11	100.0	74.1 - 100.0
Haemophilus influenzae	5	fresh	10/11	90.9	62.3 - 98.4
ATCC 33391		frozen	11/11	100.0	74.1 - 100.0
Klebsiella pneumoniae	2.5	fresh	11/11	100.0	74.1 - 100.0
NCTC 13443		frozen	11/11	100.0	74.1 - 100.0
***ctx*-M**	10	fresh	11/11	100.0	74.1 - 100.0
NCTC 13443		frozen	11/11	100.0	74.1 - 100.0
ndm	5	fresh	11/11	100.0	74.1 - 100.0
NCTC 13443		frozen	11/11	100.0	74.1 - 100.0
tem	5	fresh	10/11	90.9	62.3 - 98.4
NCTC 13443		frozen	11/11	100.0	74.1 - 100.0
Morganella morganii	5	fresh	11/11	100.0	74.1 - 100.0
ATCC 25830		frozen	11/11	100.0	74.1 - 100.0
Proteus vulgaris	5	fresh	11/11	100.0	74.1 - 100.0
ATCC 29905		frozen	11/11	100.0	74.1 - 100.0
Staphylococcus aureus	8.3	fresh	10/10 [a]	100.0	72.3 - 100.0
NCTC 12493		frozen	11/11	100.0	74.1 - 100.0
***mec*A**	3	fresh	11/11	100.0	74.1 - 100.0
NCTC 12493		frozen	11/11	100.0	74.1 - 100.0
total (all analytes)		**fresh**	**129/131**	**98.5**	**94.6 - 99.6**
		frozen	**132/132**	**100.0**	**97.2 - 100.0**

[a] Reduced number of available results due to invalid analyte results.

Table 15: Fresh-frozen study test results for concentration level 'low', approx. 2x LoD per test analyte.

low	x-fold LoD	Test Condition	Agreement with Expected Result		
			# Pos. /# Exp.	%	95% CI
Acinetobacter baumannii	2	fresh	31/32	96.9	84.3 - 99.4
ATCC 19606		frozen	31/31	100.0	89.0 - 100.0
Chlamydia pneumoniae	2	fresh	32/32	100.0	89.3 - 100.0
ATCC VR-2282		frozen	31/31	100.0	89.0 - 100.0
Citrobacter freundii	2	fresh	31/32	96.9	84.3 - 99.4
ATCC 8090		frozen	30/31	96.8	83.8 - 99.4
Haemophilus influenzae	2	fresh	29/31	93.5	79.3 - 98.2
ATCC 33391		frozen	28/30 [a]	93.3	78.7 - 98.2
Klebsiella pneumoniae	1	fresh	31/32	96.9	84.3 - 99.4
NCTC 13443		frozen	31/31	100.0	89.0 - 100.0
***ctx*-M**	4	fresh	32/32	100.0	89.3 - 100.0
NCTC 13443		frozen	31/31	100.0	89.0 - 100.0
ndm	2	fresh	31/32	96.9	84.3 - 99.4
NCTC 13443		frozen	31/31	100.0	89.0 - 100.0
tem	2	fresh	30/31	96.8	83.8 - 99.4
NCTC 13443		frozen	29/30 [a]	96.7	83.3 - 99.4
Morganella morganii	2	fresh	32/32	100.0	89.3 - 100.0
ATCC 25830		frozen	31/31	100.0	89.0 - 100.0
Proteus vulgaris	2	fresh	32/32	100.0	89.3 - 100.0
ATCC 29905		frozen	30/31	96.8	83.8 - 99.4
Staphylococcus aureus	3	fresh	32/32	100.0	89.3 - 100.0
NCTC 12493		frozen	31/31	100.0	89.0 - 100.0
***mec*A**	1.3	fresh	32/32	100.0	89.3 - 100.0
NCTC 12493		frozen	31/31	100.0	89.0 - 100.0
total (all analytes)		**fresh**	**375/382**	**98.2**	**96.3 - 99.1**
		frozen	**365/370**	**98.6**	**96.9 - 99.4**

[a] Reduced number of available results due to invalid analyte results.

Table 16: Fresh-frozen study test results for samples negative for all analytes.

negative	Test Condition	Agreement with Expected Result		
		# Neg. /# Exp.	%	95% CI
Acinetobacter baumannii	fresh	10/10	100.0	72.3 - 100.0
ATCC 19606	frozen	11/11	100.0	74.1 - 100.0
Chlamydia pneumoniae	fresh	10/10	100.0	72.3 - 100.0
ATCC VR-2282	frozen	11/11	100.0	74.1 - 100.0
Citrobacter freundii	fresh	10/10	100.0	72.3 - 100.0
ATCC 8090	frozen	11/11	100.0	74.1 - 100.0
Haemophilus influenzae	fresh	10/10	100.0	72.3 - 100.0
ATCC 33391	frozen	11/11	100.0	74.1 - 100.0
Klebsiella pneumoniae	fresh	10/10	100.0	72.3 - 100.0
NCTC 13443	frozen	11/11	100.0	74.1 - 100.0
***ctx*-M**	fresh	10/10	100.0	72.3 - 100.0
NCTC 13443	frozen	11/11	100.0	74.1 - 100.0
ndm	fresh	10/10	100.0	72.3 - 100.0
NCTC 13443	frozen	11/11	100.0	74.1 - 100.0
tem	fresh	10/10	100.0	72.3 - 100.0
NCTC 13443	frozen	11/11	100.0	74.1 - 100.0
Morganella morganii	fresh	10/10	100.0	72.3 - 100.0
ATCC 25830	frozen	11/11	100.0	74.1 - 100.0
Proteus vulgaris	fresh	10/10	100.0	72.3 - 100.0
ATCC 29905	frozen	11/11	100.0	74.1 - 100.0
Staphylococcus aureus	fresh	9/9 [a]	100.0	70.1 - 100.0
NCTC 12493	frozen	11/11	100.0	74.1 - 100.0
***mec*A**	fresh	10/10	100.0	72.3 - 100.0
NCTC 12493	frozen	11/11	100.0	74.1 - 100.0
total (all analytes)	**fresh**	**119/119**	**100.0**	**96.9 - 100.0**
	frozen	**132/132**	**100.0**	**97.2 - 100.0**

[a] Reduced number of available results due to invalid analyte results.

Sample Stability Study

Assay reproducibility between fresh samples and samples stored refrigerated for at least 24 hrs was established with artificial samples using the same experimental approach as for the fresh-frozen study.

Pair-wise tests with the LRT BAL Application were performed for samples tested within 2 hrs after test sample preparation (Time 0) and samples tested after at total storage of at least 24 hrs including at least 2 hrs at a worst-case ambient temperature of 30 °C and at least 22 hrs stored in a refrigerator.

Tables 17 to 19 summarize the sample stability test results for each of the tested concentration levels (moderate, low, negative) for both test conditions (tested freshly, tested after storage), along with the agreement rates with the expected result and 95% confidence intervals (95% CI, calculated using the

Wilson score method). Comparable agreement rates with the expected result were obtained for all test analytes for both test conditions.

Table 17: Sample stability study test results for concentration level 'moderate', approx. 5x LoD per test analyte, and a storage time of at least 24 hrs.

moderate	x-fold LoD	Test Condition	Agreement with Expected Result		
			# Pos. /# Exp.	%	95% CI
Acinetobacter baumannii	5	fresh	10/10	100.0	72.3 - 100.0
ATCC 19606		24 hrs	11/11	100.0	74.1 - 100.0
Chlamydia pneumoniae	5	fresh	10/10	100.0	72.3 - 100.0
ATCC VR-2282		24 hrs	11/11	100.0	74.1 - 100.0
Citrobacter freundii	5	fresh	10/10	100.0	72.3 - 100.0
ATCC 8090		24 hrs	11/11	100.0	74.1 - 100.0
Haemophilus influenzae	5	fresh	10/10	100.0	72.3 - 100.0
ATCC 33391		24 hrs	11/11	100.0	74.1 - 100.0
Klebsiella pneumoniae	2.5	fresh	10/10	100.0	72.3 - 100.0
NCTC 13443		24 hrs	11/11	100.0	74.1 - 100.0
***ctx*-M**	10	fresh	10/10	100.0	72.3 - 100.0
NCTC 13443		24 hrs	11/11	100.0	74.1 - 100.0
ndm	5	fresh	9/10	90.0	59.6 - 98.2
NCTC 13443		24 hrs	11/11	100.0	74.1 - 100.0
tem	5	fresh	10/10	100.0	72.3 - 100.0
NCTC 13443		24 hrs	11/11	100.0	74.1 - 100.0
Morganella morganii	5	fresh	10/10	100.0	72.3 - 100.0
ATCC 25830		24 hrs	11/11	100.0	74.1 - 100.0
Proteus vulgaris	5	fresh	10/10	100.0	72.3 - 100.0
ATCC 29905		24 hrs	11/11	100.0	74.1 - 100.0
Staphylococcus aureus	8.3	fresh	9/9 [a]	100.0	70.1 - 100.0
NCTC 12493		24 hrs	11/11	100.0	74.1 - 100.0
***mec*A**	3	fresh	10/10	100.0	72.3 - 100.0
NCTC 12493		24 hrs	11/11	100.0	74.1 - 100.0
total (all analytes)		**fresh**	**118/119**	**99.2**	**95.4 - 99.9**
		24 hrs	**132/132**	**100.0**	**97.2 - 100.0**

[a] Reduced number of available results due to invalid analyte results.

Table 18: Sample stability study test results for concentration level 'low', approx. 2x LoD per test analyte, and a storage time of at least 24 hrs.

low	x-fold LoD	Test Condition	Agreement with Expected Result		
			# Pos. /# Exp.	%	95% CI
Acinetobacter baumannii	2	fresh	31/32	96.9	84.3 - 99.4
ATCC 19606		24 hrs	31/32	96.9	84.3 - 99.4
Chlamydia pneumoniae	2	fresh	32/32	100.0	89.3 - 100.0
ATCC VR-2282		24 hrs	32/32	100.0	89.3 - 100.0
Citrobacter freundii	2	fresh	31/32	96.9	84.3 - 99.4
ATCC 8090		24 hrs	29/32	90.6	75.8 - 96.8
Haemophilus influenzae	2	fresh	29/31	93.5	79.3 - 98.2
ATCC 33391		24 hrs	31/31 [a]	100.0	89.0 - 100.0
Klebsiella pneumoniae	1	fresh	31/32	96.9	84.3 - 99.4
NCTC 13443		24 hrs	32/32	100.0	89.3 - 100.0
***ctx*-M**	4	fresh	32/32	100.0	89.3 - 100.0
NCTC 13443		24 hrs	32/32	100.0	89.3 - 100.0
ndm	2	fresh	31/32	96.9	84.3 - 99.4
NCTC 13443		24 hrs	31/32	96.9	84.3 - 99.4
tem	2	fresh	30/31	96.8	83.8 - 99.4
NCTC 13443		24 hrs	30/31 [a]	96.8	83.8 - 99.4
Morganella morganii	2	fresh	32/32	100.0	89.3 - 100.0
ATCC 25830		24 hrs	31/32	96.9	84.3 - 99.4
Proteus vulgaris	2	fresh	32/32	100.0	89.3 - 100.0
ATCC 29905		24 hrs	30/30	100.0	88.7 - 100.0
Staphylococcus aureus	3	fresh	32/32	100.0	89.3 - 100.0
NCTC 12493		24 hrs	32/32	100.0	89.3 - 100.0
***mec*A**	1.3	fresh	32/32	100.0	89.3 - 100.0
NCTC 12493		24 hrs	32/32	100.0	89.3 - 100.0
total (all analytes)		**fresh**	**375/382**	**98.2**	**96.3 - 99.1**
		24 hrs	**373/380**	**98.2**	**96.2 - 99.1**

[a] Reduced number of available results due to invalid analyte results.

Table 19: Sample stability study test results for samples negative for all analytes for a total storage time of at least 24 hrs.

negative	Test Condition	Agreement with Expected Result		
		# Neg. /# Exp.	%	95% CI
Acinetobacter baumannii	fresh	11/11	100.0	74.1 - 100.0
ATCC 19606	24 hrs	11/11	100.0	74.1 - 100.0
Chlamydia pneumoniae	fresh	11/11	100.0	74.1 - 100.0
ATCC VR-2282	24 hrs	11/11	100.0	74.1 - 100.0
Citrobacter freundii	fresh	11/11	100.0	74.1 - 100.0
ATCC 8090	24 hrs	11/11	100.0	74.1 - 100.0
Haemophilus influenzae	fresh	11/11	100.0	74.1 - 100.0
ATCC 33391	24 hrs	11/11	100.0	74.1 - 100.0
Klebsiella pneumoniae	fresh	11/11	100.0	74.1 - 100.0
NCTC 13443	24 hrs	11/11	100.0	74.1 - 100.0
***ctx*-M**	fresh	10/10 [a]	100.0	72.3 - 100.0
NCTC 13443	24 hrs	11/11	100.0	74.1 - 100.0
ndm	fresh	11/11	100.0	74.1 - 100.0
NCTC 13443	24 hrs	11/11	100.0	74.1 - 100.0
tem	fresh	11/11	100.0	74.1 - 100.0
NCTC 13443	24 hrs	11/11	100.0	74.1 - 100.0
Morganella morganii	fresh	10/10 [a]	100.0	72.3 - 100.0
ATCC 25830	24 hrs	11/11	100.0	74.1 - 100.0
Proteus vulgaris	fresh	11/11	100.0	74.1 - 100.0
ATCC 29905	24 hrs	11/11	100.0	74.1 - 100.0
Staphylococcus aureus	fresh	11/11	100.0	74.1 - 100.0
NCTC 12493	24 hrs	11/11	100.0	74.1 - 100.0
***mec*A**	fresh	11/11	100.0	74.1 - 100.0
NCTC 12493	24 hrs	11/11	100.0	74.1 - 100.0
total (all analytes)	**fresh**	**130/130**	**100.0**	**97.1 - 100.0**
	24 hrs	**132/132**	**100.0**	**97.2 - 100.0**

807.92 (b)(2): Brief Description of Clinical Data

Introduction

Clinical performance of the LRT BAL Application was established by a prospective study cohort using 1,016 lavage specimens that had been collected at nine US clinical sites from hospitalized patients suspected for lower respiratory infections, by an archived study comprising 392 confirmed positive lavage specimens predominantly collected at US clinical sites, as well as by a contrived study using artificial positive samples to augment for rare LRT BAL panel analytes.

Performance characteristics of LRT BAL antibiotic resistance markers was evaluated for the prospective study cohort. Furthermore, antibiotic resistance markers detected by LRT BAL were correlated to resistance phenotypes of corresponding strain isolates using antimicrobial susceptibility test (AST) results collected for the prospective and archived study cohorts. Finally, rare antibiotic resistance markers were also included for contrived study testing.

A. Prospective Study with Frozen Specimens

Specimen Cohort: Inclusion/Exclusion Criteria, Demographics

BAL and mini-BAL specimens from hospitalized patients, age 18 or older, suspected of a lower respiratory infection, were eligible for the prospective study. Specimens had been collected at nine US clinical sites between June 2015 and July 2016 for a previous clinical study. Specimens were stored frozen within 24 hrs after arrival of the specimen in the lab.

Demographic information for prospective specimens included into the LRT BAL prospective study is described in Table 20.

Table 20: Demographic patient data for the LRT BAL prospective study.

Included Specimens	**1,016**
Sex	
male	598
female	418
Age	
18 - 30 years	69
31 - 60 years	367
> 60 years	580
Clinical Setting	
ICU	505
ward	511

Reference Methods – Microorganisms

For 'typical' microorganisms, LRT BAL Application results were compared to standard-of-care (SoC) culture results as the reference. SoC culture results had been reported either qualitatively or quantitatively. For quantitative cultures, a reporting threshold of 10^3 CFU/mL or higher for mini-BAL specimens and of 10^4 CFU/mL or higher for BAL specimens was applied, following recommendations by the Infectious Diseases Society of America (IDSA) and the American Thoracic Society (ATS). For 'atypical' microorganisms *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Legionella pneumophila* and *Pneumocystis jirovecii*, no common SoC reference test was performed. Instead, SoC testing had only been performed on special request by the physician for a limited subset of patients.

In addition, LRT BAL Application results for 'typical' microorganisms were compared to a composite comparator as a second reference method. This composite comparator consists of the results of SoC culture combined with a molecular multiplex PCR comparator assay for which all positive PCR results were followed by bi-directional sequencing. For the composite comparator, any specimen that was positive by either SoC culture and/or positive by PCR and bi-directional sequencing was considered positive for the respective microorganism. Any specimen that was negative for both SoC culture and PCR was considered negative (Table 21A). Validated comparator PCR assays with analytical sensitivities in a similar range to the corresponding LRT BAL assays were included into the multiplex PCR comparator assay. These primers target different genetic loci than those used for the LRT BAL Application.

For 'atypical' microorganisms, a combination of two different validated PCR comparator assays was used as composite comparator, each targeting different genetic loci compared to the corresponding LRT BAL assays. As shown in Table 21B, specimens that were positive for either of the PCR/sequencing comparator assays were considered positive and specimens that were negative for both PCR/sequencing assays were considered negative.

Table 21: Result determination algorithms for the composite comparator: (A) for 'typical' microorganisms, (B) for 'atypical' microorganisms.

A **composite comparator result ('typical' microorganisms)**		Comparator 2: **PCR assay** (included in multiplex PCR)	
		positive result	**negative** result
Comparator 1: **SoC (culture)**	**positive** result	composite result: **positive**	composite result: **positive**
	negative result	composite results: **positive**	composite result: **negative**

B **composite comparator result ('atypical' microorganisms)**		Comparator 2: **PCR assay 2** (included in multiplex PCR)	
		positive result	**negative** result
Comparator 1: **PCR assay 1** (included in multiplex PCR)	**positive** result	composite result: **positive**	composite result: **positive**
	negative result	composite results: **positive**	composite result: **negative**

Reference Methods – Antibiotic Resistance Markers

PCR assays corresponding to each LRT BAL antibiotic resistance marker assay were included into the multiplex PCR comparator assay as a (single) molecular reference. Positive PCRs were followed by bi-directional sequencing.

Cultured isolates had been collected for positive prospective specimens whenever possible. Isolates were regrown and evaluated by MALDI-TOF to confirm strain identities and by whole genome sequencing using a next generation sequencing (NGS) approach to screen for presence or absence of LRT BAL panel antibiotic resistance markers. A few isolates that failed to grow were evaluated by PCR/bi-directional sequencing from frozen isolate stocks to confirm identity and to screen for presence or absence of such markers.

Phenotypic AST results for positive specimens as reported by SoC culture were collected to correlate antibiotic resistance markers detected by the LRT BAL Application to resistance phenotypes.

Discrepant Result Analysis

False positive discrepant results were analyzed by performing singleplex PCRs/bi-directional sequencing using primer pairs targeting different genetic loci compared to the corresponding LRT BAL assays on specimen DNA extracts for each discrepant LRT BAL analyte.

Microorganism Results – Comparison to SoC Culture Reference, PPA/NPA

For the prospective clinical study cohort, 1,016 previously collected specimens were tested with the LRT BAL Application. For 'typical' microorganisms, LRT BAL results were compared to the SoC culture result as reference (Table 22) to determine true positives (TP), false negatives (FN), false

positives (FP) and true negatives (TN), as well as positive percent agreements (PPA, TP / (TP + FN)), negative percent agreements (NPA, TN / (TN + FP)), positive predictive values (PPV, TP / (TP + FP)) and negative predictive values (NPV, TN / (TN + FN)), calculated together with their two-sided 95% confidence intervals (95% CI), determined according to the Wilson score method.

Table 22: Prospective study (N = 1,016 specimens [a]), comparison to SoC microbiology culture results.

	TP	FN	FP [c, d]	TN	**PPA [%]** (95% CI)	**NPA [%]** (95% CI)	**PPV [%]** (95% CI)	**NPV [%]** (95% CI)
Acinetobacter spp.	10	1	11	993	**90.9** (62.3 - 98.4)	**98.9** (98.0 - 99.4)	**47.6** (28.3 - 67.6)	**99.9** (99.4 - 100.0)
Citrobacter freundii	1	0	3	1,011	**100.0** (20.7 - 100.0)	**99.7** (99.1 - 99.9)	**25.0** (4.6 - 69.9)	**100.0** (99.6 - 100.0)
Enterobacter cloacae complex	13	4 [e]	7	991	**76.5** (52.7 - 90.4)	**99.3** (98.6 - 99.7)	**65.0** (43.3 - 81.9)	**99.6** (99.0 - 99.8)
Escherichia coli	17	1	30	968	**94.4** (74.2 - 99.0)	**97.0** (95.7 - 97.9)	**36.2** (24.0 - 50.5)	**99.9** (99.4 - 100.0)
Haemophilus influenzae	8	1	48	958	**88.9** (56.5 - 98.0)	**95.2** (93.7 - 96.4)	**14.3** (7.4 - 25.7)	**99.9** (99.4 - 100.0)
Klebsiella oxytoca	6	1	8	1,001	**85.7** (48.7 - 97.4)	**99.2** (98.4 - 99.6)	**42.9** (21.4 - 67.4)	**99.9** (99.4 - 100.0)
Klebsiella pneumoniae [b]	20	4 [f]	10	982	**83.3** (64.1 - 93.3)	**99.0** (98.2 - 99.5)	**66.7** (48.8 - 80.8)	**99.6** (99.0 - 99.8)
Klebsiella variicola [b]	0	2	2	1,012	**0.0** (0.0 - 65.8)	**99.8** (99.3 - 99.9)	**0.0** (0.0 - 65.8)	**99.8** (99.3 - 99.9)
Moraxella catarrhalis	2	0	13	997	**100.0** (34.2 - 100.0)	**98.7** (97.8 - 99.2)	**13.3** (3.7 - 37.9)	**100.0** (99.6 - 100.0)
Morganella morganii	0	0	3	1,009	NA	**99.7** (99.1 - 99.9)	**0.0** (0.0 - 56.1)	**100.0** (99.6 - 100.0)
Proteus spp.	4	0	6	1,006	**100.0** (51.0 - 100.0)	**99.4** (98.7 - 99.7)	**40.0** (16.8 - 68.7)	**100.0** (99.6 - 100.0)
Pseudomonas aeruginosa	69	3	43	900	**95.8** (88.5 - 98.6)	**95.4** (93.9 - 96.6)	**61.6** (52.4 - 70.1)	**99.7** (99.0 - 99.9)
Serratia marcescens	12	0	5	998	**100.0** (75.8 - 100.0)	**99.5** (98.8 - 99.8)	**70.6** (46.9 - 86.7)	**100.0** (99.6 - 100.0)
Staphylococcus aureus	63	8	41	904	**88.7** (79.3 - 94.2)	**95.7** (94.2 - 96.8)	**60.6** (51.0 - 69.4)	**99.1** (98.3 - 99.6)
Stenotrophomonas maltophilia	19	2	22	972	**90.5** (71.1 - 97.4)	**97.8** (96.7 - 98.5)	**46.3** (32.1 - 61.3)	**99.8** (99.3 - 99.9)
Streptococcus pneumoniae	3	0	10	1,003	**100.0** (43.9 - 100.0)	**99.0** (98.2 - 99.5)	**23.1** (8.2 - 50.3)	**100.0** (99.6 - 100.0)

[a] Observed invalid LRT BAL analyte results: *Acinetobacter* spp. (1), *C. freundii* (1), *E. cloacae* complex (1), *H. influenzae* (1), *M. catarrhalis* (4), *M. morganii* (4), *P. aeruginosa* (1), *S. marcescens* (1) and *S. maltophilia* (1).

[b] *K. variicola* is typically reported by culture as *K. pneumoniae*. To discriminate *K. variicola* from *K. pneumoniae*, *K. pneumoniae* strain isolates were sequenced. For a few cases, for which no isolates were provided, sequencing was performed from specimen DNA extracts instead. Two of 26 cases were identified as *K. variicola* and confirmed by both isolate and DNA extract sequencing. Results for *K. pneumoniae* and *K. variicola* performance are calculated based on the species identified by sequencing.

[c] Note that many of the FP detections are confirmed when comparing against the composite comparator reference that includes molecular reference assays (see section 'Comparison to Composite Comparator Reference').

[d] Specimens with false positive LRT BAL results were analyzed with molecular assays (PCR/bi-directional sequencing) using specimen DNA extracts for presence or absence of the corresponding microorganism: presence was confirmed in 11 of 11 cases for *Acinetobacter* spp., 3 of 3 cases for *C. freundii*, 7 of 7 cases for *E. cloacae* complex, 18 of 30 cases for *E. coli*, 42 of 48 cases for *H. influenzae*, 7 of 8 cases for *K. oxytoca*, 5 of 10 cases for *K. pneumoniae*, 2 of 2 cases for *K. variicola*, 13 of 13 cases for *M. catarrhalis*, 3 of 3 cases for *M. morganii*, 6 of 6 cases for *Proteus* spp., 41 of 43 cases for *P. aeruginosa*, 4 of 5 cases for *S. marcescens*, 31 of 41 cases for *S. aureus*, 21 of 22 cases for *S. maltophilia*, and 10 of 10 cases for *S. pneumoniae*. For cases that were not confirmed for *H. influenzae*, sequencing

results identified *H. haemolyticus* (3x), *H. parainfluenzae* (1x) and *Aggregatibacter aphrophilus* (1x). For all other cases, PCRs did not amplify sufficient amounts for sequencing.
[e] Two of the four specimens that were FN for *E. cloacae* complex contained multiple host microorganisms identified by culture, and/or LRT BAL. For one specimen, *Acinetobacter* spp. was additionally reported by both LRT BAL and culture. For the other specimen, *S. maltophilia* was additionally reported by LRT BAL only.
[f] Two of the four specimens that were FN for *K. pneumoniae* contained multiple host microorganisms identified by culture and/or the molecular reference testing. For one FN specimen, *P. aeruginosa* was additionally reported by both LRT BAL and culture. For the other specimen, *E. cloacae* complex was additionally reported by both LRT BAL and culture and *S. maltophilia* was additionally reported by LRT BAL only.

As no common SoC reference method was performed for the 'atypical' microorganisms reported by the LRT BAL panel, Table 23 lists positive and negative detections by the LRT BAL Application, compared to the subset of cases for which a SoC test was performed on special request by the physician. For *C. pneumoniae*, only one SoC test with a negative result was performed. For the three other 'atypical' microorganisms, the following PPA/NPA estimates when compared against SoC tests were observed: *L. pneumophila* PPA 0/1 (0%), NPA 237/237 (100%); *M. pneumoniae* PPA 0/0, NPA 28/28 (100%); *P. jirovecii* PPA 5/5 (100%), NPA 99/100 (99%).

Table 23: Summary of LRT BAL results for 'atypical' microorganisms for the prospective study.

	SOC Test (tested on request only)		**LRT BAL result**	
			positive	**negative**
Chlamydia pneumoniae	**not tested**	**1,015 (99.9%)**	**0**	**1,015**
	tested	**1 (0.1%)**	**0**	**1**
	positive result	0	0	0
	negative result	1	0	1
Legionella pneumophila	**not tested**	**778 (76.6%)**	**1**	**776 [a]**
	tested	**238 (23.4%)**	**0**	**238**
	positive result	1	0	1 [b]
	negative result	237	0	237
Mycoplasma pneumoniae	**not tested**	**988 (97.2%)**	**6**	**982**
	tested	**28 (2.8%)**	**0**	**28**
	positive result	0	0	0
	negative result	28	0	28
Pneumocystis jirovecii	**not tested**	**911 (89.7%)**	**16**	**895**
	tested [c]	**105 (10.3%)**	**6**	**99**
	positive result	5	5 [d]	0
	negative result	100	1 [e]	99

[a] Reduced number of available LRT BAL results due to one invalid analyte result.
[b] *L. pneumophila* was only reported positive by culture after 13 days.
[c] Applied *Pneumocystis* SoC methods for 105 specimens were DFA (63 specimens), IFA (29 specimens), and PCR (13 specimens).
[d] Positive by DFA (three specimens) or IFA (two specimens).
[e] Negative by DFA, confirmed positive by molecular reference tests.

Microorganisms - Stratification by Qualitative and Quantitative Culture Result

For all prospective specimens with positive culture results, true positive and false negative LRT BAL results were stratified by the semi-quantitative result reported by qualitative SoC culture (categories: rare, few, moderate, numerous) or by quantitative SoC culture (categories: 103 - < 104, 104 - < 105, 105 - < 106, > 106, in CFU/mL) as shown in Table 24.

Table 24: Prospective study, comparison to SoC culture, stratification by qualitative or quantitative culture result.

	Qualitative Culture Result					Quantitative Culture Result				
	Category	Total	TP	FN	PPA [%]	Category	Total	TP	FN	PPA [%]
Acinetobacter spp.	rare	**0**	0	0	NA	10^3 - < 10^4	**1**	1	0	100.0
	few	**2**	2	0	100.0	10^4 - < 10^5	**5**	5	0	100.0
	moderate	**1**	0	1	0.0	10^5 - < 10^6	**1**	1	0	100.0
	numerous	**1**	1	0	100.0	> 10^6	**0**	0	0	NA
Citrobacter freundii	rare	**0**	0	0	NA	10^3 - < 10^4	**0**	0	0	NA
	few	**1**	1	0	100.0	10^4 - < 10^5	**0**	0	0	NA
	moderate	**0**	0	0	NA	10^5 - < 10^6	**0**	0	0	NA
	numerous	**0**	0	0	NA	> 10^6	**0**	0	0	NA
Enterobacter cloacae complex	rare	**1**	0	1	0.0	10^3 - < 10^4	**1**	0	1	0.0
	few	**1**	1	0	100.0	10^4 - < 10^5	**6**	5	1	83.3
	moderate	**6**	5	1	83.3	10^5 - < 10^6	**1**	1	0	100.0
	numerous	**1**	1	0	100.0	> 10^6	**0**	0	0	NA
Escherichia coli	rare	**4**	3	1	75.0	10^3 - < 10^4	**0**	0	0	NA
	few	**2**	2	0	100.0	10^4 - < 10^5	**5**	5	0	100.0
	moderate	**1**	1	0	100.0	10^5 - < 10^6	**3**	3	0	100.0
	numerous	**3**	3	0	100.0	> 10^6	**0**	0	0	NA
Haemophilus influenzae	rare	**0**	0	0	NA	10^3 - < 10^4	**0**	0	0	NA
	few	**0**	0	0	NA	10^4 - < 10^5	**6**	5	1	83.3
	moderate	**2**	2	0	100.0	10^5 - < 10^6	**1**	1	0	100.0
	numerous	**0**	0	0	NA	> 10^6	**0**	0	0	NA
Klebsiella oxytoca	rare	**0**	0	0	NA	10^3 - < 10^4	**1**	1	0	100.0
	few	**2**	2	0	100.0	10^4 - < 10^5	**3**	2	1	66.7
	moderate	**1**	1	0	100.0	10^5 - < 10^6	**0**	0	0	NA
	numerous	**0**	0	0	NA	> 10^6	**0**	0	0	NA
Klebsiella pneumoniae	rare	**1**	0	1	0.0	10^3 - < 10^4	**4**	3	1	75.0
	few	**4**	3	1	75.0	10^4 - < 10^5	**11**	11	0	100.0
	moderate	**1**	0	1	0.0	10^5 - < 10^6	**2**	2	0	100.0
	numerous	**1**	1	0	100.0	> 10^6	**0**	0	0	NA
Klebsiella variicola	rare	**0**	0	0	NA	10^3 - < 10^4	**0**	0	0	NA
	few	**0**	0	0	NA	10^4 - < 10^5	**1**	0	1	0.0
	moderate	**0**	0	0	NA	10^5 - < 10^6	**0**	0	0	NA
	numerous	**1**	0	1	0.0	> 10^6	**0**	0	0	NA
Moraxella catarrhalis	rare	**0**	0	0	NA	10^3 - < 10^4	**0**	0	0	NA
	few	**0**	0	0	NA	10^4 - < 10^5	**0**	0	0	NA
	moderate	**0**	0	0	NA	10^5 - < 10^6	**0**	0	0	NA
	numerous	**2**	2	0	100.0	> 10^6	**0**	0	0	NA
Morganella morganii	rare	**0**	0	0	NA	10^3 - < 10^4	**0**	0	0	NA
	few	**0**	0	0	NA	10^4 - < 10^5	**0**	0	0	NA
	moderate	**0**	0	0	NA	10^5 - < 10^6	**0**	0	0	NA
	numerous	**0**	0	0	NA	> 10^6	**0**	0	0	NA
Proteus spp.	rare	**0**	0	0	NA	10^3 - < 10^4	**1**	1	0	100.0

	Qualitative Culture Result					Quantitative Culture Result				
	Category	Total	TP	FN	PPA [%]	Category	Total	TP	FN	PPA [%]
	few	**0**	0	0	NA	10^4 - < 10^5	**0**	0	0	NA
	moderate	**3**	3	0	100.0	10^5 - < 10^6	**0**	0	0	NA
	numerous	**0**	0	0	NA	> 10^6	**0**	0	0	NA
Pseudomonas aeruginosa [a]	rare	**7**	5	2	71.4	10^3 - < 10^4	**5**	5	0	100.0
	few	**13**	12	1	92.3	10^4 - < 10^5	**19**	19	0	100.0
	moderate	**16**	16	0	100.0	10^5 - < 10^6	**4**	4	0	100.0
	numerous	**7**	7	0	100.0	> 10^6	**0**	0	0	NA
Serratia marcescens	rare	**0**	0	0	NA	10^3 - < 10^4	**4**	4	0	100.0
	few	**1**	1	0	100.0	10^4 - < 10^5	**4**	4	0	100.0
	moderate	**2**	2	0	100.0	10^5 - < 10^6	**1**	1	0	100.0
	numerous	**0**	0	0	NA	> 10^6	**0**	0	0	NA
Staphylococcus aureus [a]	rare	**8**	4	4	50.0	10^3 - < 10^4	**5**	4	1	80.0
	few	**11**	10	1	90.9	10^4 - < 10^5	**18**	17	1	94.4
	moderate	**11**	10	1	90.9	10^5 - < 10^6	**10**	10	0	100.0
	numerous	**7**	7	0	100.0	> 10^6	**0**	0	0	NA
Stenotrophomonas maltophilia	rare	**1**	1	0	100.0	10^3 - < 10^4	**1**	0	1	0.0
	few	**1**	1	0	100.0	10^4 - < 10^5	**6**	6	0	100.0
	moderate	**6**	6	0	100.0	10^5 - < 10^6	**0**	0	0	NA
	numerous	**6**	5	1	83.3	> 10^6	**0**	0	0	NA
Streptococcus pneumoniae	rare	**0**	0	0	NA	10^3 - < 10^4	**0**	0	0	NA
	few	**0**	0	0	NA	10^4 - < 10^5	**0**	0	0	NA
	moderate	**2**	2	0	100.0	10^5 - < 10^6	**1**	1	0	100.0
	numerous	**0**	0	0	NA	> 10^6	**0**	0	0	NA

[A] One *P. aeruginosa* and one *S. aureus* case were reported positive by SoC culture without any qualitative or quantitative information, and were therefore not included in this table.

Microorganism Results – Comparison to SoC Culture Reference, Concordance Rates for the Prospective Study

For 212 specimens, at least one LRT BAL panel microorganism was reported by both the LRT BAL Application and SoC; for 21 specimens, a positive SoC result was missed by LRT BAL. For 632 specimens, both the LRT BAL Application and SoC reported a negative result (no growth or normal/mixed flora result). For 151 specimens, the LRT BAL Application reported a positive result while SoC was negative. For 65 of these 151 specimens, SoC reported normal/mixed flora, while for 81 of 151 specimens, no growth was reported (Table 25).

Table 25: Comparison of positive and negative SoC results to LRT BAL.

	Positive SoC Result (N = 233)	**Negative SoC Results (N = 783)**			
LRT BAL Result	**Microorganism(s) Reported**	No Growth	Normal/Mixed Flora	NA [a]	**Total**
any positive (N = 363)	**212**	81	65	5	**151**
all negative (N = 653)	**21**	358	258	16	**632**

[a] Presence or absence of flora not reported.

For 1,016 prospective specimens, the LRT BAL Application reported 363 specimens with at least one LRT BAL panel microorganism. For 113 specimens, multi-detections with two or more LRT BAL panel microorganisms were reported. SoC culture reported 233 specimens with at least one LRT BAL panel microorganism. For 40 specimens, multi-detections with two or more LRT BAL panel microorganisms were reported (Table 26).

Table 26: Numbers of detected LRT BAL panel microorganisms as reported by LRT BAL or SoC.

Number of Detected Microorganisms	**LRT BAL**		**SoC**	
	# specimens	[%]	# specimens	[%]
0	**653**	**64.3**	**783**	**77.1**
any positive	**363**	**35.7**	**233**	**22.9**
1	250	24.6	194	19.1
2	78	7.7	31	3.1
3	19	1.9	5	0.5
4	7	0.7	3	0.3
5	7	0.7	0	0.0
6	2	0.2	0	0.0

Table 27 lists all LRT BAL results compared to SoC culture results stratified into the following categories:

category 1: concordant result for both LRT BAL and SoC

- category 1a: both negative,
- category 1b: both positive with a fully concordant result,

category 2: LRT BAL reported additional microorganisms compared to SoC

- category 2a: for specimens with a negative SoC result,
- category 2b: for specimens with a positive SoC result,

category 3: LRT BAL reported fewer microorganisms compared to SoC

- category 3a: for specimens with a negative LRT BAL result,
- category 3b: for specimens with a positive LRT BAL result,

category 4: LRT BAL and SoC report discordant results with different microorganisms

- category 4a: LRT BAL and SoC reportings share at least one concordant microorganism,
- category 4b: LRT BAL and SoC results are fully discordant.

When comparing LRT BAL to SoC culture results, an overall concordance rate of 75.9% (632 negative specimens (62.2%), 139 positive specimens (13.7%)) was observed; discrepancies were mostly due to LRT BAL detection of additional microorganisms. For 214 of all specimens (21.1%), LRT BAL reported one or more additional microorganisms compared to SoC. 151 of these specimens (14.9%) were reported fully negative by SoC, while for 63 specimens (6.2%) at least one LRT BAL panel microorganism was reported by SoC. For 25 of all specimens (2.5%), one or more positive SoC results were not reported by LRT BAL. 21 of these specimens (2.1%) were reported fully negative by LRT BAL, while four specimens (0.4%) were reported with at least one positive LRT BAL result. For 0.4% of all specimens, LRT BAL and SoC reported different microorganisms. Two of these specimens (0.2%) shared at least one concordant microorganism result, while for four specimens (0.4%) results were fully discordant.

Table 27: Comparison of LRT BAL and SoC results, full listing.

LRT BAL Result	SoC Result	# Cases	%
Category 1: concordant LRT BAL and SoC results		**concordant results: 75.9%**	
Category 1a: both negative		**632**	**62.2**
Category 1b: concordant positive results		**139**	**13.7**
Acinetobacter spp.	*Acinetobacter* spp.	4	
Enterobacter cloacae complex	*Enterobacter cloacae* complex	4	
Escherichia coli	*Escherichia coli*	10	
Haemophilus influenzae	*Haemophilus influenzae*	5	
Klebsiella pneumoniae	*Klebsiella pneumoniae*	6	
Moraxella catarrhalis	*Moraxella catarrhalis*	1	
Pneumocystis jirovecii	*Pneumocystis jirovecii*	5	
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	31	
Serratia marcescens	*Serratia marcescens*	5	
Staphylococcus aureus	*Staphylococcus aureus*	39	
Stenotrophomonas maltophilia	*Stenotrophomonas maltophilia*	8	
Streptococcus pneumoniae	*Streptococcus pneumoniae*	1	

LRT BAL Result	SoC Result	# Cases	%
Acinetobacter spp., *Citrobacter freundii*	*Acinetobacter* spp., *Citrobacter freundii*	1	
Acinetobacter spp., *Stenotrophomonas maltophilia*	*Acinetobacter* spp., *Stenotrophomonas maltophilia*	1	
Enterobacter cloacae complex, *Staphylococcus aureus*	*Enterobacter cloacae* complex, *Staphylococcus aureus*	1	
Escherichia coli, Klebsiella oxytoca	*Escherichia coli, Klebsiella oxytoca*	1	
Klebsiella oxytoca, Staphylococcus aureus	*Klebsiella oxytoca, Staphylococcus aureus*	1	
Klebsiella pneumoniae, Pseudomonas aeruginosa	*Klebsiella pneumoniae, Pseudomonas aeruginosa*	1	
Proteus spp., *Pseudomonas aeruginosa*	*Proteus* spp., *Pseudomonas aeruginosa*	1	
Pseudomonas aeruginosa, Staphylococcus aureus	*Pseudomonas aeruginosa, Staphylococcus aureus*	5	
Pseudomonas aeruginosa, Stenotrophomonas maltophilia	*Pseudomonas aeruginosa, Stenotrophomonas maltophilia*	3	
Serratia marcescens, Staphylococcus aureus	*Serratia marcescens, Staphylococcus aureus*	1	
Staphylococcus aureus, Stenotrophomonas maltophilia	*Staphylococcus aureus, Stenotrophomonas maltophilia*	3	
Proteus spp., *Pseudomonas aeruginosa, Staphylococcus aureus*	*Proteus* spp., *Pseudomonas aeruginosa, Staphylococcus aureus*	1	
Category 2: LRT BAL detects additional microorganisms		**additional detections: 21.1 %**	
Category 2a: negative SoC result		**151**	**14.9**
Acinetobacter spp.	negative	3	
Citrobacter freundii	negative	1	
Enterobacter cloacae complex	negative	4	
Escherichia coli	negative	14	
Haemophilus influenzae	negative	24	
Klebsiella oxytoca	negative	2	
Klebsiella pneumoniae	negative	3	
Legionella pneumophila	negative	1	
Moraxella catarrhalis	negative	3	
Mycoplasma pneumoniae	negative	4	
Pneumocystis jirovecii	negative	9	
Pseudomonas aeruginosa	negative	27	
Staphylococcus aureus	negative	21	
Stenotrophomonas maltophilia	negative	2	
Streptococcus pneumoniae	negative	6	
Enterobacter cloacae complex, *Staphylococcus aureus*	negative	1	
Escherichia coli, Klebsiella pneumoniae	negative	1	
Escherichia coli, Serratia marcescens	negative	1	
Haemophilus influenzae, Moraxella catarrhalis	negative	3	
Haemophilus influenzae, Mycoplasma pneumoniae	negative	1	
Haemophilus influenzae, Staphylococcus aureus	negative	1	
Haemophilus influenzae, Streptococcus pneumoniae	negative	3	
Klebsiella oxytoca,	negative	1	

LRT BAL Result	SoC Result	# Cases	%
Proteus spp.			
Klebsiella pneumoniae, *Staphylococcus aureus*	negative	1	
Moraxella catarrhalis, *Pneumocystis jirovecii*	negative	1	
Moraxella catarrhalis, *Streptococcus pneumoniae*	negative	1	
Pneumocystis jirovecii, *Staphylococcus aureus*	negative	1	
Pseudomonas aeruginosa, *Staphylococcus aureus*	negative	6	
Staphylococcus aureus, *Stenotrophomonas maltophilia*	negative	1	
Enterobacter cloacae complex, *Haemophilus influenzae*, *Moraxella catarrhalis*	negative	1	
Escherichia coli, *Haemophilus influenzae*, *Staphylococcus aureus*	negative	1	
Klebsiella oxytoca, *Pseudomonas aeruginosa*, *Staphylococcus aureus*	negative	1	
Enterobacter cloacae complex, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Morganella morganii*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*	negative	1	
Category 2b: positive result for both LRT BAL and SoC		**63**	**6.2**
Acinetobacter spp., *Klebsiella pneumoniae*	*Klebsiella pneumoniae*	1	
Acinetobacter spp., *Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	1	
Enterobacter cloacae complex, *Mycoplasma pneumoniae*	*Enterobacter cloacae* complex	1	
Escherichia coli, *Haemophilus influenzae*	*Escherichia coli*	1	
Escherichia coli, *Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	1	
Escherichia coli, *Serratia marcescens*	*Escherichia coli*	1	
Escherichia coli, *Staphylococcus aureus*	*Escherichia coli*	1	
Escherichia coli, *Staphylococcus aureus*	*Staphylococcus aureus*	1	
Haemophilus influenzae, *Klebsiella oxytoca*	*Klebsiella oxytoca*	1	
Haemophilus influenzae, *Moraxella catarrhalis*	*Haemophilus influenzae*	1	
Haemophilus influenzae, *Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	3	
Haemophilus influenzae, *Staphylococcus aureus*	*Haemophilus influenzae*	1	
Haemophilus influenzae, *Staphylococcus aureus*	*Staphylococcus aureus*	2	
Klebsiella oxytoca, *Klebsiella pneumoniae*	*Klebsiella pneumoniae*	1	
Klebsiella oxytoca, *Serratia marcescens*	*Klebsiella oxytoca*	1	

LRT BAL Result	SoC Result	# Cases	%
Klebsiella oxytoca, *Stenotrophomonas maltophilia*	*Klebsiella oxytoca*	1	
Klebsiella pneumoniae, *Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	1	
Klebsiella pneumoniae, *Staphylococcus aureus*	*Klebsiella pneumoniae*	1	
Klebsiella pneumoniae, *Staphylococcus aureus*	*Staphylococcus aureus*	1	
Pneumocystis jirovecii, *Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	1	
Pneumocystis jirovecii, *Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*	2	
Proteus spp., *Staphylococcus aureus*	*Proteus* spp.	1	
Pseudomonas aeruginosa, *Serratia marcescens*	*Pseudomonas aeruginosa*	1	
Pseudomonas aeruginosa, *Staphylococcus aureus*	*Pseudomonas aeruginosa*	1	
Pseudomonas aeruginosa, *Staphylococcus aureus*	*Staphylococcus aureus*	2	
Pseudomonas aeruginosa, *Stenotrophomonas maltophilia*	*Pseudomonas aeruginosa*	3	
Staphylococcus aureus, *Stenotrophomonas maltophilia*	*Staphylococcus aureus*	1	
Acinetobacter spp., *Klebsiella pneumoniae*, *Staphylococcus aureus*	*Klebsiella pneumoniae*, *Staphylococcus aureus*	1	
Acinetobacter spp., *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia*	*Klebsiella pneumoniae*	1	
Acinetobacter spp., *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*	*Acinetobacter* spp., *Pseudomonas aeruginosa*	1	
Enterobacter cloacae complex, *Klebsiella pneumoniae*, *Moraxella catarrhalis*	*Enterobacter cloacae* complex	1	
Enterobacter cloacae complex, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*	*Enterobacter cloacae* complex	1	
Escherichia coli, *Morganella morganii*, *Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	1	
Escherichia coli, *Pneumocystis jirovecii*, *Pseudomonas aeruginosa*	*Escherichia coli*	1	
Escherichia coli, *Pseudomonas aeruginosa*, *Serratia marcescens*	*Pseudomonas aeruginosa*, *Serratia marcescens*	1	
Escherichia coli, *Pseudomonas aeruginosa*, *Staphylococcus aureus*	*Staphylococcus aureus*	1	
Haemophilus influenzae, *Moraxella catarrhalis*, *Streptococcus pneumoniae*	*Moraxella catarrhalis*, *Streptococcus pneumoniae*	1	
Klebsiella oxytoca, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*	*Pseudomonas aeruginosa*	1	
Klebsiella pneumoniae, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*	*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*	1	

LRT BAL Result	SoC Result	# Cases	%
Pseudomonas aeruginosa, *Serratia marcescens*, *Stenotrophomonas maltophilia*	*Pseudomonas aeruginosa*	1	
Pseudomonas aeruginosa, *Serratia marcescens*, *Stenotrophomonas maltophilia*	*Pseudomonas aeruginosa*, *Serratia marcescens*	1	
Acinetobacter spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*	*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*	1	
Acinetobacter spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia*	*Acinetobacter* spp.	1	
Acinetobacter spp., *Escherichia coli*, *Proteus* spp., *Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	1	
Enterobacter cloacae complex, *Escherichia coli*, *Klebsiella oxytoca*, *Stenotrophomonas maltophilia*	*Enterobacter cloacae* complex, *Escherichia coli*, *Klebsiella oxytoca*	1	
Escherichia coli, *Haemophilus influenzae*, *Pneumocystis jirovecii*, *Staphylococcus aureus*	*Staphylococcus aureus*	1	
Haemophilus influenzae, *Proteus* spp., *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*	*Pseudomonas aeruginosa*	1	
Pseudomonas aeruginosa, *Serratia marcescens*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*	*Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*	1	
Acinetobacter spp., *Enterobacter cloacae* complex, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*	*Enterobacter cloacae* complex, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*	1	
Citrobacter freundii, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcescens*	*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcescens*	1	
Enterobacter cloacae complex, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella variicola*, *Proteus* spp.	*Enterobacter cloacae* complex	1	
Escherichia coli, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Stenotrophomonas maltophilia*	*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Stenotrophomonas maltophilia*	1	
Escherichia coli, *Morganella morganii*, *Proteus* spp., *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*	*Pseudomonas aeruginosa*	1	
Haemophilus influenzae, *Klebsiella pneumoniae*,	*Haemophilus influenzae*, *Klebsiella pneumoniae*,	1	

LRT BAL Result	SoC Result	# Cases	%
Klebsiella variicola, *Proteus* spp., *Staphylococcus aureus*	*Proteus* spp., *Staphylococcus aureus*		
Haemophilus influenzae, *Klebsiella pneumoniae*, *Moraxella catarrhalis*, *Pneumocystis jirovecii*, *Serratia marcescens*	*Serratia marcescens*	1	
Acinetobacter spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*	*Klebsiella pneumoniae*	1	
Category 3: LRT BAL detects fewer microorganisms		**fewer detections: 2.5 %**	
Category 3a: LRT BAL negative		**21**	**2.1**
negative	*Enterobacter cloacae* complex	2	
negative	*Escherichia coli*	1	
negative	*Klebsiella oxytoca*	1	
negative	*Klebsiella pneumoniae*	2	
negative	*Klebsiella variicola*	1	
negative	*Legionella pneumophila*	1	
negative	*Pneumocystis jirovecii*	3	
negative	*Pseudomonas aeruginosa*	3	
negative	*Staphylococcus aureus*	5	
negative	*Stenotrophomonas maltophilia*	2	
Category 3b: positive result for both LRT BAL and SoC		**4**	**0.4**
Acinetobacter spp.	*Acinetobacter* spp., *Enterobacter cloacae* complex	1	
Pseudomonas aeruginosa	*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*	1	
Stenotrophomonas maltophilia	*Haemophilus influenzae*, *Stenotrophomonas maltophilia*	1	
Acinetobacter spp., *Enterobacter cloacae* complex	*Acinetobacter* spp., *Enterobacter cloacae* complex, *Klebsiella variicola*	1	
Category 4 LRT BAL and SoC detect different microorganisms		**discordant microorganism results: 0.6 %**	
Category 4a: partially concordant positive results		**2**	**0.2**
Citrobacter freundii, *Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*, *Staphylococcus aureus*	1	
Enterobacter cloacae complex, *Klebsiella oxytoca*, *Stenotrophomonas maltophilia*	*Enterobacter cloacae* complex, *Klebsiella pneumoniae*	1	
Category 4b: fully discordant results		**4**	**0.4**
Haemophilus influenzae	*Staphylococcus aureus*	1	
Pseudomonas aeruginosa	*Acinetobacter* spp.	1	
Pseudomonas aeruginosa	*Staphylococcus aureus*	1	
Stenotrophomonas maltophilia	*Enterobacter cloacae* complex	1	

Microorganism Results – Comparison to Composite Comparator Reference

LRT BAL results from the prospective clinical study were also compared to a composite comparator reference consisting of SoC culture results combined with a validated molecular test (PCR/sequencing, being part of a multiplex PCR assay). For 'atypical' microorganisms (for which SoC testing was not performed for all specimens), the composite comparator reference consists of two validated molecular tests for each microorganism (PCR/sequencing, also being part of the multiplex PCR assay). Table 28 summarizes comparison results together with their 95% confidence intervals.

Table 28: Prospective study (N = 1,016 specimens [a]), comparison to composite comparator.

	TP	FN	FP	TN	**PPA [%]** (95% CI)	**NPA [%]** (95% CI)	**PPV [%]** (95% CI)	**NPV [%]** (95% CI)
Acinetobacter spp.	16	2	5	992	**88.9** (67.2 - 96.9)	**99.5** (98.8 - 99.8)	**76.2** (54.9 - 89.4)	**99.8** (99.3 - 99.9)
Chlamydia pneumoniae [b]	0	0	0	1,016	NA	**100.0** (99.6 - 100.0)	NA	**100.0** (99.6 - 100.0)
Citrobacter freundii	1	0	3	1,011	**100.0** (20.7 - 100.0)	**99.7** (99.1 - 99.9)	**25.0** (4.6 - 69.9)	**100.0** (99.6 - 100.0)
Enterobacter cloacae complex	18	4	2	991	**81.8** (61.5 - 92.7)	**99.8** (99.3 - 99.9)	**90.0** (69.9 - 97.2)	**99.6** (99.0 - 99.8)
Escherichia coli	28	6	19	963	**82.4** (66.5 - 91.7)	**98.1** (97.0 - 98.8)	**59.6** (45.3 - 72.4)	**99.4** (98.7 - 99.7)
Haemophilus influenzae	20	2	36	957	**90.9** (72.2 - 97.5)	**96.4** (95.0 - 97.4)	**35.7** (24.5 - 48.8)	**99.8** (99.2 - 99.9)
Klebsiella oxytoca	8	2	6	1,000	**80.0** (49.0 - 94.3)	**99.4** (98.7 - 99.7)	**57.1** (32.6 - 78.6)	**99.8** (99.3 - 99.9)
Klebsiella pneumoniae [a, c]	21	4	9	981	**84.0** (65.3 - 93.6)	**99.1** (98.3 - 99.5)	**70.0** (52.1 - 83.3)	**99.6** (99.0 - 99.8)
Klebsiella variicola [a, c]	0	2	1	1,012	**0.0** (0.0 - 65.8)	**99.9** (99.4 - 100.0)	**0.0** (0.0 - 79.3)	**99.8** (99.3 - 99.9)
Legionella pneumophila [b]	1	0	0	1,014	**100.0** (20.7 - 100.0)	**100.0** (99.6 - 100.0)	**100.0** (20.7 - 100.0)	**100.0** (99.6 - 100.0)
Moraxella catarrhalis	12	4	3	993	**75.0** (50.5 - 89.8)	**99.7** (99.1 - 99.9)	**80.0** (54.8 - 93.0)	**99.6** (99.0 - 99.8)
Morganella morganii	3	0	0	1,009	**100.0** (43.9 - 100.0)	**100.0** (99.6 - 100.0)	**100.0** (43.9 - 100.0)	**100.0** (99.6 - 100.0)
Mycoplasma pneumoniae [b]	3	0	3	1,010	**100.0** (43.9 - 100.0)	**99.7** (99.1 - 99.9)	**50.0** (18.8 - 81.2)	**100.0** (99.6 - 100.0)
Pneumocystis jirovecii [b]	20	5 [d]	2	989	**80.0** (60.9 - 91.1)	**99.8** (99.3 - 99.9)	**90.9** (72.2 - 97.5)	**99.5** (98.8 - 99.8)
Proteus spp.	10	0	0	1,006	**100.0** (72.3 - 100.0)	**100.0** (99.6 - 100.0)	**100.0** (72.3 - 100.0)	**100.0** (99.6 - 100.0)
Pseudomonas aeruginosa	100	7	12	896	**93.5** (87.1 - 96.8)	**98.7** (97.7 - 99.2)	**89.3** (82.2 - 93.8)	**99.2** (98.4 - 99.6)
Serratia marcescens	15	1	2	997	**93.8** (71.7 - 98.9)	**99.8** (99.3 - 99.9)	**88.2** (65.7 - 96.7)	**99.9** (99.4 - 100.0)
Staphylococcus aureus	80	16	24	896	**83.3** (74.6 - 89.5)	**97.4** (96.1 - 98.2)	**76.9** (68.0 - 84.0)	**98.2** (97.2 - 98.9)
Stenotrophomonas maltophilia	34	7	7	967	**82.9** (68.7 - 91.5)	**99.3** (98.5 - 99.7)	**82.9** (68.7 - 91.5)	**99.3** (98.5 - 99.7)
Streptococcus pneumoniae	11	0	2	1,003	**100.0** (74.1 - 100.0)	**99.8** (99.3 - 99.9)	**84.6** (57.8 - 95.7)	**100.0** (99.6 - 100.0)

[a] Observed invalid LRT BAL analyte results: *Acinetobacter* spp. (1), *C. freundii* (1), *E. cloacae* complex (1), *H. influenzae* (1), *M. catarrhalis* (4), *M. morganii* (4), *P. aeruginosa* (1), *S. marcescens* (1) and *S. maltophilia* (1). For one SoC negative specimen, *K. pneumoniae* and *K. variicola* were simultaneously reported by the molecular test of the composite comparator, but were negative by SoC culture. As it was not feasible to resolve whether these analytes exceeded the molecular reporting threshold, both were set to 'invalid'. For this specimen, LRT BAL had reported a positive result for *K. variicola* and a negative result for *K. pneumoniae*.
[b] 'Atypical' microorganisms *C. pneumoniae*, *L. pneumophila*, *M. pneumoniae*, and *P. jirovecii* were compared to two independent molecular tests (PCR/bi-directional sequencing) as composite comparator.
[c] *K. variicola* is typically reported by culture as *K. pneumoniae*. To discriminate *K. variicola* from *K. pneumoniae*, provided *K. pneumoniae* strain isolates were sequenced. For a few cases, no isolate was provided and sequencing was performed from specimen DNA extracts instead. Two of 27 cases were identified as *K. variicola* and confirmed by both isolate and DNA extract sequencing. Results for *K. pneumoniae* and *K. variicola* performance are calculated based on the species identified by sequencing.
[d] For two of five FN *P. jirovecii* cases, repeat comparator testing results indicating specimen degradation during prolonged storage as likely reason for observed failures.

Antibiotic Resistance Marker Results – Comparison to Molecular Reference

Performance characteristics of LRT BAL panel antibiotic resistance markers were evaluated for the prospective study (1,016 specimens) by comparing to corresponding molecular reference assays to determine PPAs (TP / (TP + FN)) and NPAs (FN / (FN + FP)) together with 95% confidence intervals. For each antibiotic resistance marker, one PCR assay was performed followed by bi-directional sequencing.

Antibiotic resistance marker results are only reported by the LRT BAL Application if at least one corresponding host microorganism is simultaneously detected. Results for antibiotic resistance markers detected without a positive corresponding host microorganism result are masked on the results screen. Table 29 reports antibiotic resistance markers as displayed on the LRT BAL results screen, i.e., all antibiotic resistance markers for which a corresponding host microorganism was simultaneously detected. Note that PPA/NPA performance data are driven by LRT BAL microorganism results.

Table 29: Comparison of antibiotic resistance markers to corresponding molecular reference assays (PCR/sequencing) for the prospective study (N = 1,016 specimens) for markers as displayed and reported on the LRT BAL results screen (number of detected host microorganisms for each marker in brackets).

	TP	FN	FP [c, d]	TN	**PPA [%]** (95% CI)	**NPA [%]** (95% CI)
ctx-M (N = 208)	8	1 [b]	1	197	**88.9** (56.5 - 98.0)	**99.5** (97.2 - 99.9)
kpc (N = 208)	3	0	1	204	**100.0** (43.9 - 100.0)	**99.5** (97.3 - 99.9)
*mec*A (N = 104)	22	0	25 [e]	57	**100.0** (85.1 - 100.0)	**69.5** (58.9 - 78.4)
ndm (N = 208)	1	0	0	207	**100.0** (20.7 - 100.0)	**100.0** (98.2 - 100.0)
oxa-23 (N = 21)	3	0	0	18	**100.0** (43.9 - 100.0)	**100.0** (82.4 - 100.0)
oxa-24 (N = 21)	3	0	1	16	**100.0** (43.9 - 100.0)	**94.1** (73.0 - 99.0)
oxa-48 (N = 112)	1	0	0	111	**100.0** (20.7 - 100.0)	**100.0** (96.7 - 100.0)
oxa-58 (N = 21)	0	0	0	21	NA	**100.0** (84.5 - 100.0)
tem (N = 56)	9	0	7 [f]	40	**100.0** (70.1 - 100.0)	**85.1** (72.3 - 92.6)
vim (N = 208)	0	0	1	207	NA	**99.5** (97.3 - 99.9)

[a] Observed invalid LRT BAL analyte results: *ctx*-M (1) and *oxa*-24 (1).
[b] One false negative result belongs to the *ctx*-M9 subgroup that is not covered by the *ctx*-M assay of the LRT BAL Application (targeted against *ctx*-M1 subgroup).
[c] Note that molecular reference assays use cutoffs to achieve analytical sensitivities in a similar range to LRT BAL. Negative results by molecular reference assays do not preclude the presence of the antibiotic resistance marker in the specimen at a low concentration, nor its possible clinical relevance. Please refer to section 'Correlation of Detected Antibiotic Resistance Markers to Strain Genotypes and Phenotypes', p. 68 for genotypic and phenotypic correlation of reported antibiotic resistance markers.
[d] Specimens with false positive LRT BAL results were analyzed with additional molecular assays (PCR/bi-directional sequencing) using specimen DNA extracts for presence or absence of antibiotic resistance markers. Presence of antibiotic resistance markers was confirmed in 0 of 1 case for *ctx*-M, 1 of 1 case of *kpc*, 10 of 25 cases for *mec*A, 1 of 1 case for *oxa*-24, 6 of 7 cases for *tem* and 1 of 1 case for *vim*. Cross-reactivity to other off-panel analytes has not been observed. For all not confirmed cases, PCRs did not amplify sufficient amounts for sequencing.
[e] Note that Staphylococci other than *S. aureus* commonly harbor *mec*A. Positive *mec*A results can, therefore, be due to the presence of this marker in such microorganisms.
[f] Although *tem* is reported by LRT BAL for *H. influenzae* only, other Gram-negative microorganisms (e.g., Enterobacteriaceae, *Acinetobacter* spp., *P. aeruginosa*) can harbor *tem*. Positive *tem* results can, therefore, be due to the presence of this marker in such microorganisms.

Antibiotic Resistance Marker Results – Agreement Rates to Positive and Negative Microorganisms as Detected by Culture/Isolate Sequencing or Composite Comparator Testing

In order to determine agreement rates of antibiotic resistance markers reported by LRT BAL to positive and negative reference results, a comparison to the following references was performed: (**A**) SoC culture result for microorganisms, combined with sequencing results/marker screenings of provided strain isolates, and (**B**) composite comparator result for microorganisms and molecular reference results (PCR/sequencing) for antibiotic resistance markers. Note that an isolate was not provided for all SoC positive microorganisms, thereby limiting the dataset for comparison (**A**). Comparison (**B**) evaluates the entire specimen cohort, including all positive LRT BAL results that were negative by

SoC culture. Molecular reference assays use cutoffs that were set to achieve an analytical assay sensitivity in a comparable range to LRT BAL.

For both comparisons, the following agreement rates are evaluated for *ctx*-M, *kpc*, *ndm*, and *vim* (for Enterobacteriaceae, *Acinetobacter* spp. and/or *P. aeruginosa* as possible host microorganisms), for *oxa*-48 (for Enterobacteriaceae), for *tem* (for *H. influenzae*), and for *mec*A (for *S. aureus*) in Tables 30 - 38:

1. Agreement of one (or more) detected host microorganisms in combination with an antibiotic resistance marker (**Org+/Res+**) between LRT BAL and reference methods (**A**) or (**B**).

2. Agreement of one (or more) detected host microorganisms without reported antibiotic resistance marker (**Org+/Res-**) between LRT BAL and reference methods (**A**) or (**B**).

3. Agreement of a negative result for one (or more) host microorganisms (Org-) between LRT BAL and reference methods (**A**) and (**B**).

As presence of mecA is expected to directly correlate with cefoxitin/oxacillin resistance in *S. aureus* strains (MRSA, methicillin-resistant *S. aureus*), while the absence of *mec*A is expected to correlate with susceptible *S. aureus* strains (MSSA, methicillin-sensitive *S. aureus*), *mec*A was furthermore compared to the MRSA/MSSA status of the isolate as reported by SoC culture as a third reference (C) in Table 38:

1. Agreement of a detected *S. aureus* in combination with a detected *mec*A as reported by LRT BAL (**Org+/Res+**) to *S. aureus* reported with an MRSA phenotype by SoC culture (**Org+/MRSA**).

2. Agreement of a detected *S. aureus* together with a negative result for *mec*A (**Org+/Res-**) as reported by LRT BAL to *S. aureus* reported with an MSSA phenotype by SoC culture (**Org+/MSSA**).

3. Agreement of a negative result for *S. aureus* between LRT BAL and SoC culture.

No agreement tables are included for *oxa*-58 for which positive results were reported neither by the LRT BAL Application, nor by the reference methods.

Table 30: Agreement rates for *oxa*-23 between LRT BAL and reference methods A (culture) and B (composite comparator) for *Acinetobacter* spp. as host microorganism.

A

***Acinetobacter* spp. *oxa*-23**		**Culture Result for *Acinetobacter* spp. + Isolate Sequencing/Marker Screening for *oxa*-23**				
		Org+/Res+	Org+/Res-	Org+/ No Isolate	Org-	Total
LRT BAL Result	Org+/Res+	**1**	0	1	1	3
	Org+/Res-	0	**7**	1	10	18
	Org-	0	0	1	**993**	994
	Total	**1**	**7**	3	**1,004**	1,015 [a]
***Acinetobacter* spp./*oxa*-23**			rate [%]		positivity	95% CI
Agreement (Org+/Res+)			100.0		1/1	20.7 - 100.0
Agreement (Org+/Res-)			100.0		7/7	64.6 - 100.0
Agreement (Org-)			98.9		993/1,004	98.0 - 99.4

B

***Acinetobacter* spp. *oxa*-23**		**Composite Comparator Result for *Acinetobacter* spp. + Mol. Reference (PCR/Seq.) for *oxa*-23**			
		Org+/Res+	Org+/Res-	Org-	Total
LRT BAL Result	Org+/Res+	**3**	0	0	3
	Org+/Res-	0	**13**	5	18
	Org-	0	2	**992**	994
	Total	**3**	**15**	**997**	1,015 [a]
***Acinetobacter* spp./*oxa*-23**			rate [%]	positivity	95% CI
Agreement (Org+/Res+)			100.0	3/3	43.9 - 100.0
Agreement (Org+/Res-)			86.7	13/15	62.1 - 96.3
Agreement (Org-)			99.5	992/997	98.8 - 99.8

[a] One invalid LRT BAL result for *Acinetobacter* spp.

Table 31: Agreement rates for *oxa*-24 between LRT BAL and reference methods A (culture) and B (composite comparator) for *Acinetobacter* spp. as host microorganism.

A

***Acinetobacter* spp. *oxa*-24**		**Culture Result for *Acinetobacter* spp. + Isolate Sequencing/Marker Screening for *oxa*-24**				
		Org+/Res+	Org+/Res-	Org+/ No Isolate	Org-	Total
LRT BAL Result	Org+/Res+	**3**	0	0	1	4
	Org+/Res-	0	**4**	2	10	16
	Org-	0	0	1	**993**	994
	Total	**3**	**4**	3	**1,004**	1,014 [a]
***Acinetobacter* spp./*oxa*-24**			rate [%]		positivity	95% CI
Agreement (Org+/Res+)			100.0		3/3	43.9 - 100.0
Agreement (Org+/Res-)			100.0		4/4	51.0 - 100.0
Agreement (Org-)			98.9		993/1,004	98.0 - 99.4

B

***Acinetobacter* spp. *oxa*-24**		**Composite Comparator Result for *Acinetobacter* spp. + Mol. Reference (PCR/Seq.) for *oxa*-24**			
		Org+/Res+	Org+/Res-	Org-	Total
LRT BAL Result	Org+/Res+	**3**	0	1	4
	Org+/Res-	0	**12**	4	16
	Org-	1	1	**992**	994
	Total	**4**	**13**	**997**	1,014 [a]
***Acinetobacter* spp./*oxa*-24**		rate [%]		positivity	95% CI
Agreement (Org+/Res+)		75.0		3/4	30.1 - 95.4
Agreement (Org+/Res-)		92.3		12/13	66.7 - 98.6
Agreement (Org-)		99.5		992/997	98.8 - 99.8

[a] One invalid LRT BAL result for *Acinetobacter* spp. and one for *oxa*-24.

Table 32: Agreement rates for *ctx*-M between LRT BAL and reference methods A (culture) and B (composite comparator) for combined possible host microorganisms: Enterobacteriaceae, *Acinetobacter* spp., *P. aeruginosa*.

A

Combined Hosts *ctx*-M		**Culture Result for Host Microorganism(s) + Isolate Sequencing/Marker Screening for *ctx*-M**				
		Org+/Res+	Org+/Res-	Org+/ No Isolate	Org-	Total
LRT BAL Result	Org+/Res+	**3** [d]	4 [b, e]	1 [b, f]	1	9
	Org+/Res-	0	**92**	32	74	198
	Org-	0	3	8	**794**	805
	Total	**3** [a]	**99**	41	**869**	1,012 [c]
Combined Hosts/*ctx*-M			rate [%]		positivity	95% CI
Agreement (Org+/Res+)			100.0		3/3	43.9 - 100.0
Agreement (Org+/Res-)			92.9		92/99	86.1 - 96.5
Agreement (Org-)			91.4		794/869	89.3 - 93.1

B

Combined Hosts *ctx*-M		**Composite Comparator Result for Host Microorganism(s) + Mol. Reference (PCR/Seq.) for *ctx*-M**			
		Org+/Res+	Org+/Res-	Org-	total
LRT BAL Result	Org+/Res+	**8**	1	0	9
	Org+/Res-	1	**161**	36	198
	Org-	0	20	**785**	805
	total	**9** [a]	**182**	**821**	1,012 [c]
Combined Hosts/*ctx*-M			rate [%]	positivity	95% CI
Agreement (Org+/Res+)			88.9	8/9	56.5 - 98.0
Agreement (Org+/Res-)			88.5	161/182	83.0 - 92.3
Agreement (Org-)			95.6	785/821	94.0 - 96.8

[a] Specimens with multiple detected possible host microorganisms by culture: 1 of 3, by the composite comparator: 6 of 9.
[b] For one 'Org+/Res-' and for one 'Org+/No Isolate' case, presence of the *ctx*-M gene was confirmed for an off-panel isolate of *Providencia stuartii*.
[c] Four invalid LRT BAL *ctx*-M results.
[d] SoC reported the following possible corresponding host microorganisms for three Org+/Res+ cases: *E. coli*, *K. pneumoniae*, *K. pneumoniae*/*P. aeruginosa*.
[e] SoC reported the following possible corresponding host microorganisms for four Org+/Res- cases: *E. coli*, *P. aeruginosa* (2x), *K. pneumoniae*/*P. aeruginosa*/*S. marcescens*.
[f] SoC reported the following possible corresponding host microorganisms for one Org+/No Isolate case: *K. pneumoniae*.

Table 33: Agreement rates for *kpc* between LRT BAL and reference methods A (culture) and B (composite comparator) for combined possible host microorganisms: Enterobacteriaceae, *Acinetobacter* spp., *P. aeruginosa*.

A

Combined Hosts *kpc*		**Culture Result for Host Microorganism(s) + Isolate Sequencing/Marker Screening for *kpc***				
		Org+/Res+	Org+/Res-	Org+/ No Isolate	Org-	Total
LRT BAL Result	Org+/Res+	**1** [b]	2 [c]	1 [d]	0	4
	Org+/Res-	0	**96**	32	76	204
	Org-	0	3	8	**797**	808
	Total	**1** [a]	**101**	41	**873**	1,016
Combined Hosts/*kpc*			rate [%]		positivity	95% CI
Agreement (Org+/Res+)			100.0		1/1	20.7 - 100.0
Agreement (Org+/Res-)			95.0		96/101	88.9 - 97.9
Agreement (Org-)			91.3		797/873	89.2 - 93.0

B

Combined Hosts *kpc*		**Composite Comparator Result for Host Microorganism(s) + Mol. Reference (PCR/Seq.) for *kpc***			
		Org+/Res+	Org+/Res-	Org-	Total
LRT BAL Result	Org+/Res+	**3**	1	0	4
	Org+/Res-	0	**167**	37	204
	Org-	0	20	**788**	808
	Total	**3** [a]	**188**	**825**	1,016
Combined Hosts/*kpc*			rate [%]	positivity	95% CI
Agreement (Org+/Res+)			100.0	3/3	43.9 - 100.0
Agreement (Org+/Res-)			88.8	167/188	83.5 - 92.6
Agreement (Org-)			95.5	788/825	93.9 - 96.7

[a] Specimens with multiple detected possible host microorganisms by culture: 0 of 1, by the composite comparator: 2 of 3.
[b] SoC reported the following possible corresponding host microorganisms for one Org+/Res+ case: *E. cloacae* complex.
[c] SoC reported the following possible corresponding host microorganisms for two Org+/Res- cases: *Acinetobacter* spp., *K. pneumoniae/P. aeruginosa.*
[d] SoC reported the following possible corresponding host microorganisms for one Org+/No Isolate case: *K. pneumoniae.*

Table 34: Agreement rates for *ndm* between LRT BAL and reference methods A (culture) and B (composite comparator) for combined possible host microorganisms: Enterobacteriaceae, *Acinetobacter* spp., *P. aeruginosa*.

A

Combined Hosts *ndm*		**Culture Result for Host Microorganism(s) + Isolate Sequencing/Marker Screening for *ndm***				
		Org+/Res+	Org+/Res-	Org+/ No Isolate	Org-	Total
LRT BAL Result	Org+/Res+	**1** [c]	0	0	0	1
	Org+/Res-	0	**98**	33	76	207
	Org-	0	3	8	**796**	807
	Total	**1** [a]	**101**	41	**872**	1,015 [b]
Combined Hosts/*ndm*			rate [%]		positivity	95% CI
Agreement (Org+/Res+)			100.0		1/1	20.7 - 100.0
Agreement (Org+/Res-)			97.0		98/101	91.6 - 99.0
Agreement (Org-)			91.3		796/872	89.2 - 93.0

B

Combined Hosts *ndm*		**Composite Comparator Result for Host Microorganism(s) + Mol. Reference (PCR/Seq.) for *ndm***			
		Org+/Res+	Org+/Res-	Org-	Total
LRT BAL Result	Org+/Res+	**1**	0	0	1
	Org+/Res-	0	**170**	37	207
	Org-	0	20	**787**	807
	Total	**1** [a]	**190**	**824**	1,015 [b]
Combined Hosts/*ndm*			rate [%]	positivity	95% CI
Agreement (Org+/Res+)			100.0	1/1	20.7 - 100.0
Agreement (Org+/Res-)			89.5	170/190	84.3 - 93.1
Agreement (Org-)			95.5	787/824	93.9 - 96.7

[a] Specimens with multiple detected possible host microorganisms by culture: 1 of 1, by the composite comparator: 1 of 1.
[b] One invalid LRT BAL result for *ndm*.
[c] SoC reported the following possible corresponding host microorganisms for one Org+/Res+ case: *K. pneumoniae/P. aeruginosa*.

Table 35: Agreement rates for *vim* between LRT BAL and reference methods A (culture) and B (composite comparator) for combined possible host microorganisms: Enterobacteriaceae, *Acinetobacter* spp., *P. aeruginosa*.

A

Combined Hosts *vim*		**Culture Result for Host Microorganism(s) + Isolate Sequencing/Marker Screening for *vim***				
		Org+/Res+	Org+/Res-	Org+/ No Isolate	Org-	Total
LRT BAL Result	Org+/Res+	**0**	1 [a]	0	0	1
	Org+/Res-	0	**98**	33	76	207
	Org-	0	3	8	**797**	808
	Total	**0**	**102**	41	**873**	1,016
Combined Hosts/*vim*			rate [%]		positivity	95% CI
Agreement (Org+/Res+)			NA		0/0	NA
Agreement (Org+/Res-)			96.1		98/102	90.3 - 98.5
Agreement (Org-)			91.3		797/873	89.2 - 93.0

B

Combined Hosts *vim*		**Composite Comparator Result for Host Microorganism(s) + Mol. Reference (PCR/Seq.) for *vim***			
		Org+/Res+	Org+/Res-	Org-	Total
LRT BAL Result	Org+/Res+	**0**	1	0	1
	Org+/Res-	0	**170**	37	207
	Org-	0	20	**788**	808
	Total	**0**	**191**	**825**	1,016
Combined Hosts/*vim*			rate [%]	positivity	95% CI
Agreement (Org+/Res+)			NA	0/0	NA
Agreement (Org+/Res-)			89.0	170/191	83.8 - 92.7
Agreement (Org-)			95.5	788/825	93.9 - 96.7

[a] SoC reported the following possible corresponding host microorganisms for one Org+/Res- case: *P. aeruginosa*.

Table 36: Agreement rates for oxa-48 between LRT BAL and reference methods A (culture) and B (composite comparator) for combined possible host microorganisms: Enterobacteriaceae.

A

Combined Hosts *oxa*-48		**Culture Result for Host Microorganism(s) + Isolate Sequencing/Marker Screening for *oxa*-48**				
		Org+/Res+	Org+/Res-	Org+/ No Isolate	Org-	Total
LRT BAL Result	Org+/Res+	**1** [c]	0	0	0	1
	Org+/Res-	0	**51**	13	47	111
	Org-	0	4	6	**890**	900
	Total	**1** [a]	**55**	19	**937**	1,012 [b]
Combined Hosts/*oxa*-48			rate [%]		positivity	95% CI
Agreement (Org+/Res+)			100.0		1/1	20.7 - 100.0
Agreement (Org+/Res-)			92.7		51/55	82.7 - 97.1
Agreement (Org-)			95.0		890/937	93.4 - 96.2

B

Combined Hosts *oxa*-48		**Composite Comparator Result for Host Microorganism(s) + Mol. Reference (PCR/Seq.) for *oxa*-48**			
		Org+/Res+	Org+/Res-	Org-	Total
LRT BAL Result	Org+/Res+	**1**	0	0	1
	Org+/Res-	0	**84**	27	111
	Org-	0	14	**886**	900
	Total	**1** [a]	**98**	**913**	1,012 [b]
Combined Hosts/*oxa*-48			rate [%]	positivity	95% CI
Agreement (Org+/Res+)			100.0	1/1	20.7 - 100.0
Agreement (Org+/Res-)			85.7	84/98	77.4 - 91.3
Agreement (Org-)			97.0	886/913	95.7 - 98.0

[a] Specimens with multiple detected possible host microorganisms by culture: 0 of 1, by the composite comparator: 0 of 1.
[b] Four invalid LRT BAL results for *oxa*-48.
[c] SoC reported the following possible corresponding host microorganisms for one Org+/Res+ case: *K. pneumoniae*/*P. aeruginosa*.

Table 37: Agreement rates for *tem* between LRT BAL and reference methods A (culture) and B (composite comparator) for *H. influenzae* as host microorganism.

A

H. influenzae* *tem		**Culture Result for *H. influenzae* + Isolate Sequencing/Marker Screening for *tem***				
		Org+/Res+	Org+/Res-	Org+/ No Isolate	Org-	Total
LRT BAL Result	Org+/Res+	**2**	0	1	13	16
	Org+/Res-	0	**3**	2	35	40
	Org-	0	0	1	**958**	959
	Total	**2**	**3**	4	**1,006**	1,015 [a]
H. influenzae/tem			rate [%]		positivity	95% CI
Agreement (Org+/Res+)			100.0		2/2	34.2 - 100.0
Agreement (Org+/Res-)			100.0		3/3	43.9 - 100.0
Agreement (Org-)			95.2		958/1,006	93.7 - 96.4

B

H. influenzae* *tem		**Composite Comparator Result for *H. influenzae* + Mol. Reference (PCR/Seq.) for *tem***			
		Org+/Res+	Org+/Res-	Org-	Total
LRT BAL Result	Org+/Res+	**6**	0	10	16
	Org+/Res-	0	**14**	26	40
	Org-	0	2	**957**	959
	Total	**6**	**16**	**993**	1,015 [a]
H. influenzae/tem			rate [%]	positivity	95% CI
Agreement (Org+/Res+)			100.0	6/6	61.0 - 100.0
Agreement (Org+/Res-)			87.5	14/16	64.0 - 96.5
Agreement (Org-)			96.4	957/993	95.0 - 97.4

[a] One LRT BAL run with an invalid result for both *H. influenzae* and *tem*.

Table 38: Agreement rates for *mec*A between LRT BAL and reference methods A (culture) and B (composite comparator) and C (phenotypic resistance as reported by AST for the culture isolate) for *S. aureus* as host microorganism.

A

***S. aureus* *mec*A**		**Culture Result for *S. aureus* + Isolate Sequencing/Marker Screening for *mec*A**				
		Org+/Res+	Org+/Res-	Org+/ No Isolate	Org-	Total
LRT BAL Result	Org+/Res+	**17**	4	9	17	47
	Org+/Res-	1	**23**	9	24	57
	Org-	3	2	3	**904**	912
	Total	**21**	**29**	21	**945**	1,016
***S. aureus/mec*A**			rate [%]		positivity	95% CI
Agreement (Org+/Res+)			81.0		17/21	60.0 - 92.3
Agreement (Org+/Res-)			79.3		23/29	61.6 - 90.2
Agreement (Org-)			95.7		904/945	94.2 - 96.8

B

***S. aureus* *mec*A**		**Composite Comparator Result for *S. aureus* + Mol. Reference (PCR/Seq.) for *mec*A**			
		Org+/Res+	Org+/Res-	Org-	Total
LRT BAL Result	Org+/Res+	**20**	18 [a]	9	47
	Org+/Res-	0	**42**	15	57
	Org-	5	11	**896**	912
	Total	**25**	**71**	**920**	1,016
***S. aureus/mec*A**			rate [%]	positivity	95% CI
Agreement (Org+/Res+)			80.0	20/25	60.9 - 91.1
Agreement (Org+/Res-)			59.2	42/71	47.5 - 69.8
Agreement (Org-)			97.4	896/920	96.1 - 98.2

C

***S. aureus* *mec*A**		**Culture Result for *S. aureus* + and Cefoxitin/Oxacillin AST Results**				
		Org+/ MRSA	Org+/ MSSA	Org+/ No AST	Org-	Total
LRT BAL Result	Org+/Res+	**23** [b]	6	1	17	47
	Org+/Res-	1	**27**	5	24	57
	Org-	3	4	1	**904**	912
	Total	**27**	**37**	7	**945**	1,016
***S. aureus*/MRSA status**			rate [%]		positivity	95% CI
Agreement (Org+/Res+)			85.1		23/27	67.5 - 94.1
Agreement (Org+/Res-)			73.0		27/37	57.0 - 84.6
Agreement (Org-)			95.7		904/945	94.2 - 96.8

[a] 18 cases were determined as Org+/Res- negative by the molecular reference assay using cutoffs set to achieve an analytical sensitivity in a comparable range to LRT BAL. For 10 of these 18 cases, presence of *mec*A was confirmed by alternate molecular tests (PCR/sequencing).
[b] One strain was reported by the clinical site as MRSA, although this was not supported by the provided oxacillin Kirby-Bauer zone diameter. Independent cefoxitin AST confirmed the MRSA phenotype for this strain.

B. Archived Study

Specimen Cohort: Inclusion/Exclusion Criteria, Demographics

Lavage specimens from patients, age 18 or older, suspected of a lower respiratory infection, with at least one LRT BAL panel microorganism reported positive by standard-of-care (SoC) were eligible for the archived study. Specimens were collected at 11 US clinical sites between 2015 and 2019, complemented with few specimens collected from other sites. In contrast to the prospective study, where specimens were only from hospitalized patients (inpatients), specimens from outpatients or from patients with an unknown status were also accepted. Confirmatory testing (PCR/bi-directional sequencing using two independent PCR assays per analyte) to exclude specimens that may have degraded during storage was performed for the reported SoC results prior to the study. Only specimens with at least one confirmed SoC result were included.

The archived cohort included 197 specimens from a previous study. These specimens were supplemented with 195 specimens meeting inclusion criteria that had been collected over time to achieve a total set of 392 archived study specimens.

Demographic information for specimens included into the LRT BAL archived study is described in Table 39.

Table 39: Demographic patient data for the LRT BAL archived study.

Included Specimens	**392**
Sex [a]	
male	198
female	122
Age [b]	
18 - 30 years	18
31 - 60 years	106
> 60 years	190
Clinical Setting [c]	
ICU	112
ward	101
in-patient (not specified)	69
out-patient	32

[a] Not reported: 72 patients.
[b] Not reported: 78 patients.
[c] Not reported: 78 patients.

Reference Methods

For all archived specimens, LRT BAL Application microorganism results were compared to confirmed positive results from SoC microbiology culture (for 'typical' microorganism) or other SoC methods (for 'atypical' microorganisms) as reference. SoC culture results were reported either qualitatively or quantitatively. For quantitative cultures, a reporting threshold of 10^3 CFU/mL or higher for mini-BAL specimens and of 10^4 CFU/mL or higher for BAL specimens was applied, following recommendations by the Infectious Diseases Society of America (IDSA) and the American Thoracic Society (ATS).

Evaluation of collected isolates and AST results, as well as discrepant result resolution was performed as for the prospective study.

Microorganism Results – Comparison to confirmed SoC reference, PPA/NPA

LRT BAL Application results were compared to confirmed SoC results as reference (Tables 40 and 41) to determine true positives (TP), false negatives (FN), false positives (FP) and true negatives (TN) and to calculate positive percent agreements (PPA, TP / (TP + FN)) and negative percent agreements (NPA, TN / (TN + FP)), together with their two-sided 95% confidence intervals (95% CI), determined according to the Wilson score method.

Note that SoC testing for 'atypical' microorganisms was only performed on special request by the physician. Other specimens besides the ones collected because of a positive SoC result for any of the 'atypical' microorganisms, were typically not tested, and, therefore, the NPA cannot be determined.

Table 40: Archived study (N = 392), comparison to confirmed SoC results, 'typical' microorganisms.

	TP	FN	FP [b]	TN	**PPA [%]** (95% CI)	**NPA [%]** (95% CI)
Acinetobacter spp.	18	0	3	371	**100.0** (82.4 - 100.0)	**99.2** (97.7 - 99.7)
Citrobacter freundii	5	0	5	382	**100.0** (56.6 - 100.0)	**98.7** (97.0 - 99.4)
Enterobacter cloacae complex	15	4 [c]	0	373	**78.9** (56.7 - 91.5)	**100.0** (99.0 - 100.0)
Escherichia coli	46	3	17	326	**93.9** (83.5 - 97.9)	**95.0** (92.2 - 96.9)
Haemophilus influenzae	50	0	21	321	**100.0** (92.9 - 100.0)	**93.9** (90.8 - 95.9)
Klebsiella oxytoca	16	1	6	369	**94.1** (73.0 - 99.0)	**98.4** (96.6 - 99.3)
Klebsiella pneumoniae [a]	29	2	10	351	**93.5** (79.3 - 98.2)	**97.2** (95.0 - 98.5)
Klebsiella variicola [a]	2	0	4	386	**100.0** (34.2 - 100.0)	**99.0** (97.4 - 99.6)
Moraxella catarrhalis	21	0	9	362	**100.0** (84.5 - 100.0)	**97.6** (95.5 - 98.7)
Morganella morganii	1	0	0	391	**100.0** (20.7 - 100.0)	**100.0** (99.0 - 100.0)
Proteus spp.	15	0	7	370	**100.0** (79.6 - 100.0)	**98.1** (96.2 - 99.1)

Pseudomonas aeruginosa	54	2	2	334	**96.4** (87.9 - 99.0)	**99.4** (97.9 - 99.8)
Serratia marcescens	23	2	3	364	**92.0** (75.0 - 97.8)	**99.2** (97.6 - 99.7)
Staphylococcus aureus	56	2	21	313	**96.6** (88.3 - 99.1)	**93.7** (90.6 - 95.9)
Stenotrophomonas maltophilia	37	3	10	342	**92.5** (80.1 - 97.4)	**97.2** (94.9 - 98.4)
Streptococcus pneumoniae	34	1	5	352	**97.1** (85.5 - 99.5)	**98.6** (96.8 - 99.4)

[a] *K. variicola* is typically reported by culture as *K. pneumoniae*. To discriminate *K. variicola* from *K. pneumoniae*, *K. pneumoniae* strain isolates, if available, were sequenced. If no isolate was provided, sequencing was performed from specimen DNA extracts instead. Results for *K. pneumoniae* and *K. variicola* performance are calculated based on the species identified by sequencing.
[b] Specimens with false positive LRT BAL results were analyzed with molecular assays (PCR/bi-directional sequencing) using specimen DNA extracts for presence or absence of microorganisms. Presence of microorganisms was confirmed in 3 of 3 cases for *Acinetobacter* spp., 4 of 5 cases for *C. freundii* (one confirmed case was reported as *C. youngae* by SoC), 15 of 17 cases for *E. coli*, 17 of 21 cases for *H. influenzae*, 6 of 6 cases for *K. oxytoca*, 7 of 10 cases for *K. pneumoniae*, 2 of 4 cases for *K. variicola*, 8 of 9 cases for *M. catarrhalis*, 6 of 7 cases for *Proteus* spp., 2 of 2 cases for *P. aeruginosa*, 1 of 3 cases for *S. marcescens*, 18 of 21 cases for *S. aureus*, 10 of 10 cases for *S. maltophilia*, and 4 of 5 cases for *S. pneumoniae*. For cases that were not confirmed for *H. influenzae*, sequencing results identified *H. haemolyticus (2x)*, *A. aphropilus* (2x). For all other cases, PCRs did not amplify sufficient amounts for sequencing.
[c] Three of the four specimens that were FN for *E. cloacae* complex contained multiple host microorganisms identified by culture, and/or LRT BAL. For one specimen, *Klebsiella oxytoca* was additionally reported by both LRT BAL and culture. For the two other specimens, *Proteus* spp. or *E. coli*, respectively, were additionally reported by LRT BAL only.

Table 41: Archived study (N = 392), comparison to confirmed SoC results, 'atypical' microorganisms.

	TP	FN	add. pos. [a]	add. neg.	**PPA [%]** (95% CI)
Chlamydia pneumoniae	0	0	0	392	**NA**
Legionella pneumophila	17	2	0	373	**89.5** (68.6 - 97.1)
Mycoplasma pneumoniae	5	1	3	383	**83.3** (43.7 - 97.0)
Pneumocystis jirovecii	16	3	13	360	**84.2** (62.4 - 94.5)

[a] Specimens with additional positive ('add. pos.') LRT BAL results were analyzed with molecular assays (PCR/bi-directional sequencing) using specimen DNA extracts for presence or absence of microorganisms: presence was confirmed in 2 of 3 cases for *M. pneumoniae*, and 10 of 13 cases for *P. jirovecii*. For all other cases, PCRs did not amplify sufficient amounts for sequencing.

For all archived specimens, true positive and false negative LRT BAL results were stratified by the semi-quantitative result reported by qualitative SoC culture (categories: rare, few, moderate, numerous) or by quantitative SoC culture (categories: 103 - < 104, 104 - < 105, 105 - < 106, > 106, in CFU/mL) as shown in Table 42.

Table 42: Archived study, comparison to confirmed SoC results, stratification by qualitative or quantitative SoC culture result.

	Qualitative Culture Result [a]					Quantitative Culture Result [a]				
	Category	**Total**	**TP**	**FN**	**PPA [%]**	**Category**	**Total**	**TP**	**FN**	**PPA [%]**
Acinetobacter spp.	rare	0	0	0	NA	10^3 - < 10^4	3	3	0	100.0
	few	3	3	0	100.0	10^4 - < 10^5	4	4	0	100.0
	moderate	2	2	0	100.0	10^5 - < 10^6	2	2	0	100.0
	numerous	3	3	0	100.0	> 10^6	0	0	0	NA
Citrobacter freundii	rare	0	0	0	NA	10^3 - < 10^4	0	0	0	NA
	few	0	0	0	NA	10^4 - < 10^5	3	3	0	100.0
	moderate	1	1	0	100.0	10^5 - < 10^6	1	1	0	100.0
	numerous	0	0	0	NA	> 10^6	0	0	0	NA
Enterobacter cloacae complex	rare	0	0	0	NA	10^3 - < 10^4	1	0	1	0.0
	few	2	2	0	100.0	10^4 - < 10^5	2	1	1	50.0
	moderate	3	2	1	66.7	10^5 - < 10^6	1	1	0	100.0
	numerous	6	6	0	100.0	> 10^6	0	0	0	NA
Escherichia coli	rare	7	6	1	85.7	10^3 - < 10^4	0	0	0	NA
	few	12	12	0	100.0	10^4 - < 10^5	4	4	0	100.0
	moderate	6	6	0	100.0	10^5 - < 10^6	3	3	0	100.0
	numerous	10	10	0	100.0	> 10^6	0	0	0	NA
Haemophilus influenzae	rare	1	1	0	100.0	10^3 - < 10^4	0	0	0	NA
	few	8	8	0	100.0	10^4 - < 10^5	8	8	0	100.0
	moderate	7	7	0	100.0	10^5 - < 10^6	6	6	0	100.0
	numerous	13	13	0	100.0	> 10^6	3	3	0	100.0
Klebsiella oxytoca	rare	2	2	0	100.0	10^3 - < 10^4	1	1	0	100.0
	few	5	4	1	80.0	10^4 - < 10^5	1	1	0	100.0
	moderate	0	0	0	NA	10^5 - < 10^6	2	2	0	100.0
	numerous	2	2	0	100.0	> 10^6	0	0	0	NA
Klebsiella pneumoniae	rare	1	0	1	0.0	10^3 - < 10^4	0	0	0	NA
	few	10	10	0	100.0	10^4 - < 10^5	3	3	0	100.0
	moderate	9	8	1	88.9	10^5 - < 10^6	2	2	0	100.0
	numerous	1	1	0	100.0	> 10^6	0	0	0	NA
Klebsiella variicola	rare	0	0	0	NA	10^3 - < 10^4	0	0	0	NA
	few	1	1	0	100.0	10^4 - < 10^5	0	0	0	NA
	moderate	1	1	0	100.0	10^5 - < 10^6	0	0	0	NA
	numerous	0	0	0	NA	> 10^6	0	0	0	NA
Moraxella catarrhalis	rare	0	0	0	NA	10^3 - < 10^4	1	1	0	100.0
	few	3	3	0	100.0	10^4 - < 10^5	2	2	0	100.0
	moderate	5	5	0	100.0	10^5 - < 10^6	2	2	0	100.0
	numerous	6	6	0	100.0	> 10^6	1	1	0	100.0
Morganella morganii	rare	0	0	0	NA	10^3 - < 10^4	1	1	0	100.0
	few	0	0	0	NA	10^4 - < 10^5	0	0	0	NA
	moderate	0	0	0	NA	10^5 - < 10^6	0	0	0	NA
	numerous	0	0	0	NA	> 10^6	0	0	0	NA
Proteus spp.	rare	4	4	0	100.0	10^3 - < 10^4	0	0	0	NA
	few	2	2	0	100.0	10^4 - < 10^5	1	1	0	100.0
	moderate	2	2	0	100.0	10^5 - < 10^6	2	2	0	100.0
	numerous	2	2	0	100.0	> 10^6	0	0	0	NA
Pseudomonas aeruginosa	rare	6	4	2	66.7	10^3 - < 10^4	2	2	0	100.0
	few	7	7	0	100.0	10^4 - < 10^5	4	4	0	100.0
	moderate	12	12	0	100.0	10^5 - < 10^6	7	7	0	100.0
	numerous	7	7	0	100.0	> 10^6	0	0	0	NA
Serratia marcescens	rare	3	3	0	100.0	10^3 - < 10^4	1	1	0	100.0

	Qualitative Culture Result [a]					Quantitative Culture Result [a]				
	Category	**Total**	**TP**	**FN**	**PPA [%]**	**Category**	**Total**	**TP**	**FN**	**PPA [%]**
	few	4	3	1	75.0	10^4 - < 10^5	2	2	0	100.0
	moderate	1	1	0	100.0	10^5 - < 10^6	8	8	0	100.0
	numerous	2	2	0	100.0	> 10^6	0	0	0	NA
Staphylococcus aureus	rare	6	5	1	83.3	10^3 - < 10^4	0	0	0	NA
	few	14	13	1	92.9	10^4 - < 10^5	6	6	0	100.0
	moderate	7	7	0	100.0	10^5 - < 10^6	6	6	0	100.0
	numerous	9	9	0	100.0	> 10^6	1	1	0	100.0
Stenotrophomonas maltophilia	rare	3	2	1	66.7	10^3 - < 10^4	1	1	0	100.0
	few	18	16	2	88.9	10^4 - < 10^5	4	4	0	100.0
	moderate	6	6	0	100.0	10^5 - < 10^6	1	1	0	100.0
	numerous	4	4	0	100.0	> 10^6	0	0	0	NA
Streptococcus pneumoniae	rare	2	1	1	50.0	10^3 - < 10^4	0	0	0	NA
	few	4	4	0	100.0	10^4 - < 10^5	4	4	0	100.0
	moderate	5	5	0	100.0	10^5 - < 10^6	6	6	0	100.0
	numerous	11	11	0	100.0	> 10^6	2	2	0	100.0

[a] One *Acinetobacter* spp., 4 *E. cloacae* complex, 7. *E. coli*, 4 *H. influenzae*, 4 *K. oxytoca*, 5 *K. pneumoniae*, 1 *M. catarrhalis*, 2 *Proteus* spp., 11 *P. aeruginosa*, 4 *S. marcescens*, 9 *S. aureus*, 3 *S. maltophilia* and 1 *S. pneumoniae* case were reported as positive by SoC culture without qualitative or quantitative results, and were therefore not included in this table.

Antibiotic Resistance Markers

Antibiotic resistance markers reported by LRT BAL for the archived study are listed in Table 43. Note that antibiotic resistance markers are only reported and displayed on the Unyvero results screen, if a corresponding host microorganism was simultaneously detected. Results for *ctx*-M, *kpc*, *ndm*, and *vim* are reported for LRT BAL positive specimens for Enterobacteriaceae, *Acinetobacter* spp., and *P. aeruginosa* (N = 212); results for *oxa*-48 are reported for LRT BAL positive specimens for Enterobacteriaceae (N = 166); results for *oxa*-23, *oxa*-24, and *oxa*-58 are reported for LRT BAL positive specimens for *Acinetobacter* spp. (N = 21); results for *tem* are reported for LRT BAL positive specimens for *H. influenzae* (N = 71); and results for *mec*A are reported for LRT BAL positive specimens for *S. aureus* (N = 77). Only specimens with antibiotic resistance markers reported positive by LRT BAL were tested for such markers by molecular reference assays (PCR/sequencing).

Table 43: Archived study (N = 392), listing of antibiotic resistance marker results as reported by LRT BAL.

	Positive [a]	Negative
ctx-M (N = 212)	14	198
kpc (N = 212)	2	210
*mec*A (N = 77)	44	33
ndm (N = 212)	0	212
oxa-23 (N = 21)	4	17
oxa-24 (N = 21)	3	18
oxa-48 (N = 166)	0	166
oxa-58 (N = 21)	1	20
tem (N = 71)	24	47
vim (N = 212)	0	212

[a] Specimens with false positive LRT BAL results were analyzed with molecular assays (PCR/bi-directional sequencing) using specimen DNA extracts for presence or absence of microorganisms: presence of microorganisms was confirmed in 14 of 14 cases for *ctx*-M, 2 of 2 cases for *kpc*, 34 of 44 cases for *mec*A, 4 of 4 cases for *oxa*-23, 3 of 3 cases for *oxa*-24, 1 of 1 case for *oxa*-58, and 22 of 24 cases for *tem*. For all other cases, PCRs did not amplify sufficient amounts for sequencing.

Correlation of Detected Antibiotic Resistance Markers to Strain Genotypes and Phenotypes

As described in the previous sections, isolates that had been collected for most of the prospective and part of archived study specimens were screened for presence or absence of antibiotic resistance markers of the LRT BAL panel. Furthermore, corresponding AST results were collected to predict resistance phenotypes of such isolates using one or more of the drug assays listed in Table 44. AST results were reported as MIC values or zone diameters (for Kirby-Bauer tests). Strain phenotypes across all test sites were standardized according to breakpoints listed in CLSI guidance M100S (Performance Standards for Antimicrobial Susceptibility Testing, 26th Edition 2016). 'Intermediate' calls were regarded as 'resistant' calls for this study. For the analysis, any strain was regarded as 'resistant' if at least one of the corresponding drugs resulted in an 'intermediate' or 'resistant' call. Any strain was regarded as 'susceptible' if a 'sensitive' call was obtained for all applicable tested drugs.

Table 44: AST assays used for evaluation of antibiotic resistance markers detected by LRT BAL.

Antibiotic Resistance Marker(s)	Associated Resistance	AST Assay
tem	penicillins	*H. influenzae*: ampicillin, cefinase
ctx-M	3rd generation cephalosporins, cefepime	Enterobacteriaceae: cefotaxime, ceftazidime, ceftriaxone *Acinetobacter* spp.: cefotaxime, ceftazidime, ceftriaxone, cefepime *P. aeruginosa*: ceftazidime, cefepime
kpc, *ndm*, *oxa*-48, *vim*	carbapenems	Enterobacteriaceae: meropenem, ertapenem, imipenem
kpc, *ndm*, *oxa*-23, *oxa*-24, *oxa*-58, *vim*	carbapenems	*Acinetobacter* spp. meropenem, imipenem
kpc, *ndm, vim*	carbapenems	*P. aeruginosa*: meropenem, imipenem
*mec*A	oxacillin/cefoxitin	*S. aureus*: oxacillin, cefoxitin

Antibiotic resistance markers reported by LRT BAL were correlated to available isolates for:

1. Genotypic agreement of reported antibiotic resistance markers to presence of such markers in cultured isolates for a specific specimen.

2. Phenotypic agreement of reported antibiotic resistance markers with associated phenotypic culture results to provided isolates as determined by antimicrobial susceptibility tests (AST).

Note that antibiotic resistance marker results are only reported by the LRT BAL Application if a corresponding host microorganism is simultaneously reported. Otherwise, such results are masked on the Unyvero results screen. Antibiotic resistance marker results reported together with a corresponding host microorganism may not be linked to this organism, but may have originated from other on-panel or off-panel microorganisms, e.g., *mec*A may not only originate from *S. aureus*, but also originate from coagulase-negative Staphylococci present in respiratory flora; tem may not only originate from *H. influenzae*, but also from other on-panel or off-panel Enterobacteriaceae. If multiple corresponding microorganisms are simultaneously reported for a certain antibiotic resistance marker, one or more than one of these microorganisms could be the host of such marker.

Genotypic and phenotypic agreements were evaluated for all relevant resistance phenotypes including all applicable specimens from the prospective and archived study combined (Tables 45 to 49).

Oxacillin/Cefoxitin Resistance for S. aureus (mecA)

Table 45: Correlation of positive *mec*A results reported by LRT BAL for the prospective ('p') or archived ('a') study to available *S. aureus* isolates (genotypic agreement, 'yes': presence of *mec*A confirmed by sequencing of isolate, 'no': *mec*A not confirmed, 'not prov.': no isolate provided) and to AST results indicating oxacillin/cefoxitin resistance for isolates with confirmed *mec*A (phenotypic agreement; R: resistant phenotype, S: sensitive phenotype, NA: AST results not reported).

Genotypic Agreement for Specimens with Available Isolate: **21/25**, **84.0%** (95% CI: 65.3 - 93.6)							
Phenotypic Agreement for Isolates with Confirmed Marker: **21/21, 100.0%** (95% CI: 84.5 - 100.0)							
# Cases			**LRT BAL Result** (relevant hosts only)	**SoC Result** (relevant hosts only)	**Genotypic Agreement** (for available isolates)	**Phenotypic Agreement** (for isolates with conf. *mec*A)	**Comment**
total	p	a					
21	17	4	*S. aureus*	*S. aureus*	**yes**	**R** [a]	-
4	4	-	*S. aureus*	*S. aureus*	**no**	-	-

[a] One prospective strain was reported by the clinical site as MRSA, although this was not supported by the provided oxacillin Kirby-Bauer zone diameter. An independent cefoxitin AST confirmed the MRSA phenotype for this strain. For another archived strain, no AST results were reported by the clinical site. The MRSA phenotype was determined by an independent cefoxitin AST instead.

Penicillin Resistance for H. influenzae (tem)

Table 46: Correlation of positive *tem* results reported by LRT BAL for the prospective ('p') or archived ('a') study to available *H. influenzae* isolates (genotypic agreement, 'yes': presence of *tem* confirmed by sequencing of isolate, 'no': *tem* not confirmed, 'not prov.': no isolate provided) and to AST results indicating penicillin resistance for isolates with confirmed *tem* (phenotypic agreement; R: resistant phenotype, S: sensitive phenotype, NA: AST results not reported).

Genotypic Agreement for Specimens with Available Isolate: **4/4**, **100.0%** (95% CI: 51.0 - 100.0)							
Phenotypic Agreement for Isolates with Confirmed Marker: **4/4, 100.0%** (95% CI: 51.0 - 100.0)							
# Cases			**LRT BAL Result** (relevant hosts only)	**SoC Result** (relevant hosts only)	**Genotypic Agreement** (for available isolates)	**Phenotypic Agreement** (for isolates with conf. *tem*)	**Comment**
total	p	a					
4	2	2	*H. influenzae*	*H. influenzae*	**yes**	**R**	-

Third Generation Cephalosporin Resistance for Enterobacteriaceae, Acinetobacter spp., and P. aeruginosa (ctx-M)

Table 47: Correlation of positive *ctx*-M results reported by LRT BAL for the prospective ('p') or archived ('a') study to available isolates of Enterobacteriaceae, *Acinetobacter* spp., or *P. aeruginosa* (genotypic agreement, 'yes': presence of *ctx*-M confirmed by sequencing of isolate, 'no': *ctx*-M not confirmed, 'not prov.': no isolate provided) and to AST results indicating third generation cephalosporin resistance for isolates with confirmed *ctx*-M (phenotypic agreement; R: resistant phenotype, S: sensitive phenotype, NA: AST results not reported).

Genotypic Agreement for Specimens with at Least One Available Isolate: **5/8**, **62.5%** (95% CI: 30.6 - 86.3)					
Phenotypic Agreement for Isolates with Confirmed Marker: **4/4, 100.0%** (95% CI: 51.0 - 100.0)					
Study	**LRT BAL Result** (relevant hosts only)	**SoC Result** (relevant hosts only)	**Genotypic Agreement** (for available isolates)	**Phenotypic Agreement** (for isolates with conf. *ctx*-M)	**Comment**
p	*Acinetobacter* spp.	-	-	-	-
	E. coli	-	-	-	
	K. pneumoniae	*K. pneumoniae*	**yes**	**R**	
	P. aeruginosa	*P. aeruginosa*	no	-	
p	*K. pneumoniae*	*K. pneumoniae*	**yes**	**R**	-
	P. aeruginosa	-	-	-	
p	*E. coli*	*E. coli*	**yes**	**R**	-
	P. aeruginosa	-	-	-	
p	*E. coli*	*E. coli*	no	-	-
	[off-panel]	*Providencia stuartii*	**yes**	**R**	
p	*Acinetobacter* spp.	-	-	-	-
	E. coli	-	-	-	
	K. pneumoniae	*K. pneumoniae*	(not. prov.)	-	
	Proteus spp.	-	-	-	
	P. aeruginosa	-	-	-	
	[off-panel]	*Providencia stuartii*	**yes**	NA	
p	*E. coli*	-	-	-	-
	K. pneumoniae	*K. pneumoniae*	(not. prov.)	-	
	P. aeruginosa	*P. aeruginosa*	**no**	-	
	S. marcescens	*S. marcescens*	(not. prov.)	-	
p	*E. coli*	-	-	-	-
	P. aeruginosa	*P. aeruginosa*	**no**	-	
p	*E. coli*	-	-	-	-
	M. morganii	-	-	-	
	Proteus spp.	-	-	-	
	P. aeruginosa	*P. aeruginosa*	**no**	-	

Carbapenem Resistance for Enterobacteriaceae, Acinetobacter spp., and P. aeruginosa (kpc, ndm, oxa-48, vim)

Table 48: Correlation of positive results for *kpc, ndm, vim* reported by LRT BAL for the prospective ('p') or archived ('a') study to available isolates of Enterobacteriaceae, *Acinetobacter* spp., or *P. aeruginosa*, or positive results for *oxa*-48 to available Enterobacteriaceae isolates (genotypic agreement, 'yes': presence of any of these markers confirmed by sequencing, 'no': presence not confirmed, 'not prov.': no isolate provided) and to AST results indicating carbapenem resistance for isolates with confirmed marker (phenotypic agreement; R: resistant phenotype, S: sensitive phenotype, NA: AST results not reported).

Genotypic Agreement for Specimens with at Least One Available Isolate: **3/5**, **60.0%** (95% CI: 23.1 - 88.2)						
Phenotypic Agreement for Isolates with Confirmed Marker: **2/2, 100.0%** (95% CI: 34.2 - 100.0)						
Study	**Marker**	**LRT BAL Result** (relevant hosts only)	**SoC Result** (relevant hosts only)	**Genotypic Agreement** (for available isolates)	**Phenotypic Agreement** (for isolates with conf. marker)	**Comment**
p	*kpc*	*E. cloacae* complex	*E. cloacae* complex	**yes**	**R**	-
		K. pneumoniae	-	-	-	
		P. aeruginosa	-	-	-	
p	*ndm*, *oxa*-48	*Acinetobacter* spp.	-	-	-	-
		E. coli	-	-	-	
		K. pneumoniae	*K. pneumoniae*	**yes** (both)	**R**	
		P. aeruginosa	*P. aeruginosa*	**no**	-	
p	*kpc*	*Acinetobacter* spp.	*Acinetobacter* spp.	**no**	-	
		E. coli	-	-	-	
		K. pneumoniae	-	**yes**	NA	subclinical isolate [a]
p	*kpc*	*K. pneumoniae*	*K. pneumoniae*	(not prov.)	-	AST: resistant
		P. aeruginosa	*P. aeruginosa*	**no**	-	
p	*vim*	*Proteus* spp.	-	-	-	-
		P. aeruginosa	*P. aeruginosa*	**no**	-	

[a] For *K. pneumoniae*, SoC reported a concentration < 10^3 CFU/mL (considered a negative, 'subclinical' result below the reporting threshold for mini-BAL specimens).

Carbapenem Resistance for Acinetobacter spp. (oxa-23, oxa-24, oxa-58)

Table 49: Correlation of positive results for *oxa*-23, *oxa*-24, *oxa*-58, reported by LRT BAL for the prospective ('p') or archived ('a') study to available *Acinetobacter* spp. isolates (genotypic agreement, 'yes': presence of any of these markers confirmed by sequencing, 'no': presence not confirmed, 'not prov.': no isolate provided) and to AST results indicating carbapenem resistance for isolates with confirmed marker (phenotypic agreement; R: resistant phenotype, S: sensitive phenotype, NA: AST results not reported).

Genotypic Agreement for Specimens with at Least One Available Isolate: **6/6**, **100.0%** (95% CI: 61.0 - 100.0)						
Phenotypic Agreement for Isolates with Confirmed Marker: **6/6, 100.0%** (95% CI: 61.0 - 100.0)						
Study	**Marker**	**LRT BAL Result** (relevant hosts only)	**SoC Result** (relevant hosts only)	**Genotypic Agreement** (for available isolates)	**Phenotypic Agreement** (isolates with conf. marker)	**Comment**
p	*oxa*-23	*Acinetobacter* spp.	*Acinetobacter* spp.	**yes**	**R**	-
a	*oxa*-23	*Acinetobacter* spp.	*Acinetobacter* spp.	**yes**	**R**	-
p	*oxa*-24	*Acinetobacter* spp.	*Acinetobacter* spp.	**yes**	**R**	-
p	*oxa*-24	*Acinetobacter* spp.	*Acinetobacter* spp.	**yes**	**R** [a]	-
a	*oxa*-23	*Acinetobacter* spp.	*Acinetobacter* spp.	**yes**	**R**	-
p	*oxa*-24	*Acinetobacter* spp.	*Acinetobacter* spp.	**yes**	**R**	-

[a] For one prospective strain, a resistant phenotype to meropenem was reported by the clinical site, although this was not supported by the provided Kirby-Bauer zone diameter. Independent AST for meropenem and imipenem confirmed resistance to carbapenems for this strain.

C. Contrived Study

The clinical performance evaluation of rare LRT BAL analytes was supplemented by a contrived study, i.e., by testing artificial clinical specimens that were prepared by spiking pooled microorganisms at concentrations close to their respective analyte LoD or at clinically relevant concentrations into unique negative lavage specimens. LRT BAL microorganisms, for which, due to limited specimen numbers, a PPA of 90% or higher with a lower bound of the two-sided 95% confidence interval of 80% or higher was not demonstrated for the clinical performance evaluation using archived specimens, were selected for contrived specimen testing (*C. pneumoniae*, *C. freundii*, *E. cloacae* complex, *K. oxytoca*, *K. pneumoniae*, *K. variicola*, *L. pneumophila*, *M. morganii*, *M. pneumoniae*, *P. jirovecii*, *Proteus* spp., *S. marcescens*). In addition, all low prevalence LRT BAL antibiotic resistance markers (*ctx*-M, *kpc*, *ndm*, *oxa*-23, *oxa*-24, *oxa*-48, *oxa*-58, *vim*) were also included. For each target analyte a total of 60 contrived specimens with up to five different spiked reference strains was tested at a low concentration (2x LoD, 30 specimens) and at two moderate concentrations (3.5x LoD and 5x LoD, 15 specimens each) spanning the clinically relevant concentration range. For *P. jirovecii*, a positive clinical specimen was used for spiking, and all 60 contrived specimens were tested at a 2x LoD concentration.

Agreement rates to the expected results were determined for each target analyte and stratified by actual test concentrations Tables 50 and 51 show agreement rates for expected positive results (positive results / expected positive results) and expected negative results (negative results / number of expected negative results) together with two-sided 95% confidence intervals (95% CI) for included LRT BAL microorganisms or antibiotic resistance markers, respectively.

Table 50: Contrived study results for targeted LRT BAL microorganism analytes.

	Agreement with Expected Results					
	# Pos./ # Exp.	**%**	**95% CI**	**# Neg./ # Exp.**	**%**	**95% CI**
Chlamydia pneumoniae	**57/60**	**95.0**	**86.3 - 98.3**	**180/180**	**100.0**	**97.9 - 100.0**
6.4 x 10^2/2x LoD	29/30	96.7	83.3 - 99.4			
ATCC 53592	5/6					
ATCC VR-1310	12/12					
ATCC VR-2282	12/12					
1.1 x 10^3/3.5x LoD	15/15	100.0	79.6 - 100.0			
ATCC 53592	3/3					
ATCC VR-1310	6/6					
ATCC VR-2282	6/6					
1.6 x 10^3/5x LoD	13/15	86.7	62.1 - 96.3			
ATCC 53592	2/3					
ATCC VR-1310	5/6					
ATCC VR-2282	6/6					
Citrobacter freundii	**59/60**	**98.3**	**91.1 - 99.7**	**168/168**	**100.0**	**97.8 - 100.0**
1.6 x 10^5/2x LoD	29/30	96.7	83.3 - 99.4			
ATCC 43864	6/6					
ATCC 8090	6/6					
NCTC 8581	5/6					
NRZ-00452	6/6					
UCLA C1	6/6					
2.8 x 10^5/3.5x LoD	15/15	100.0	79.6 - 100.0			
ATCC 43864	3/3					
ATCC 8090	3/3					
NCTC 8581	3/3					
NRZ-00452	3/3					
UCLA C1	3/3					
4.0 x 10^5/5x LoD	15/15	100.0	79.6 - 100.0			
ATCC 43864	3/3					
ATCC 8090	3/3					
NCTC 8581	3/3					
NRZ-00452	3/3					
UCLA C1	3/3					
***Enterobacter cloacae* complex**	**58/60**	**96.7**	**88.6 - 99.1**	**156/156**	**100.0**	**97.6 - 100.0**
4.0 x 10^5/2x LoD	28/30	93.3	78.7 - 98.2			
ATCC 13047	5/6					
ATCC 23373	6/6					
ATCC 49141	5/6					
ATCC BAA-2468	6/6					
NRZ-00239	6/6					
7.0 x 10^5/3.5x LoD	15/15	100.0	79.6 - 100.0			
ATCC 13047	3/3					
ATCC 23373	3/3					
ATCC 49141	3/3					
ATCC BAA-2468	3/3					
NRZ-00239	3/3					
1.0 x 10^6/5x LoD	15/15	100.0	79.6 - 100.0			
ATCC 13047	3/3					
ATCC 23373	3/3					

	Agreement with Expected Results					
	# Pos./ # Exp.	%	95% CI	# Neg./ # Exp.	%	95% CI
ATCC 49141	3/3					
ATCC BAA-2468	3/3					
NRZ-00239	3/3					
Klebsiella oxytoca	**56/60**	**93.3**	**84.1 - 97.4**	**168/168**	**100.0**	**97.8 - 100.0**
2.0 x 10^4/2x LoD	26/30	86.7	70.3 - 94.7			
ATCC 13182	4/6					
ATCC 43863	6/6					
ATCC 49131	6/6					
ATCC 8724	5/6					
NCIMB 12819	5/6					
3.5 x 10^4/3.5x LoD	15/15	100.0	79.6 - 100.0			
ATCC 13182	3/3					
ATCC 43863	3/3					
ATCC 49131	3/3					
ATCC 8724	3/3					
NCIMB 12819	3/3					
5.0 x 10^4/5x LoD	15/15	100.0	79.6 - 100.0			
ATCC 13182	3/3					
ATCC 43863	3/3					
ATCC 49131	3/3					
ATCC 8724	3/3					
NCIMB 12819	3/3					
Klebsiella pneumoniae	**60/60**	**100.0**	**94.0 - 100.0**	**96/96**	**100.0**	**96.2 - 100.0**
8.0 x 10^4/2x LoD	30/30	100.0	88.7 - 100.0			
ATCC 13883	6/6					
Micromyx 4653	6/6					
NCTC 13438	6/6					
NCTC 13439	6/6					
NCTC 13442	6/6					
1.4 x 10^5/3.5x LoD	15/15	100.0	79.6 - 100.0			
ATCC 13883	3/3					
Micromyx 4653	3/3					
NCTC 13438	3/3					
NCTC 13439	3/3					
NCTC 13442	3/3					
2.0 x 10^5/5x LoD	15/15	100.0	79.6 - 100.0			
ATCC 13883	3/3					
Micromyx 4653	3/3					
NCTC 13438	3/3					
NCTC 13439	3/3					
NCTC 13442	3/3					
Klebsiella variicola	**58/60**	**96.7**	**88.6 - 99.1**	**180/180**	**100.0**	**97.9 - 100.0**
4.0 x 10^4/2x LoD	29/30	96.7	83.3 - 99.4			
ATCC BAA-830	6/6					
clinical isolate 1	6/6					
clinical isolate 2	6/6					
clinical isolate 3	6/6					
clinical isolate 4	5/6					
7.0 x 10^4/3.5x LoD	15/15	100.0	79.6 - 100.0			
ATCC BAA-830	3/3					
clinical isolate 1	3/3					
clinical isolate 2	3/3					
clinical isolate 3	3/3					
clinical isolate 4	3/3					
1.0 x 10^5/5x LoD	14/15	93.3	70.2 - 98.8			
ATCC BAA-830	3/3					
clinical isolate 1	3/3					
clinical isolate 2	2/3					
clinical isolate 3	3/3					

	Agreement with Expected Results					
	# Pos./ # Exp.	%	95% CI	# Neg./ # Exp.	%	95% CI
clinical isolate 4	3/3					
Legionella pneumophila	**57/60**	**95.0**	**86.3 - 98.3**	**180/180**	**100.0**	**97.9 - 100.0**
1.6 x 10^5/2x LoD	28/30	93.3	78.7 - 98.2			
ATCC 33152	6/6					
ATCC 33154	5/6					
ATCC 33155	5/6					
ATCC 33215	6/6					
ATCC 35096	6/6					
2.8 x 10^5/3.5x LoD	15/15	100.0	79.6 - 100.0			
ATCC 33152	3/3					
ATCC 33154	3/3					
ATCC 33155	3/3					
ATCC 33215	3/3					
ATCC 35096	3/3					
4.0 x 10^5/5x LoD	14/15	93.3	70.2 - 98.8			
ATCC 33152	2/3					
ATCC 33154	3/3					
ATCC 33155	3/3					
ATCC 33215	3/3					
ATCC 35096	3/3					
Morganella morganii	**52/60**	**86.7**	**75.8 - 93.1**	**179/179**	**100.0**	**97.9 - 100.0**
4.0 x 10^4/2x LoD	23/30	76.7	59.1 - 88.2			
ATCC 25829 [a]	3/6					
ATCC 25830	6/6					
ATCC 49948	6/6					
ATCC 8019	6/6					
DSM-46262 [a]	2/6					
7.0 x 10^4/3.5x LoD	15/15	100.0	79.6 - 100.0			
ATCC 25829	3/3					
ATCC 25830	3/3					
ATCC 49948	3/3					
ATCC 8019	3/3					
DSM-46262	3/3					
1.0 x 10^5/5x LoD	14/15	93.3	70.2 - 98.8			
ATCC 25829	3/3					
ATCC 25830	3/3					
ATCC 49948	3/3					
ATCC 8019	3/3					
DSM-46262	2/3					
Mycoplasma pneumoniae	**52/60**	**86.7**	**75.8 - 93.1**	**180/180**	**100.0**	**97.9 - 100.0**
3.2 x 10^3/2x LoD	23/30	76.7	59.1 - 88.2			
ATCC 15293	6/6					
ATCC 29085	8/12					
ATCC 49894	9/12					
5.6 x 10^3/3.5x LoD	14/15	93.3	70.2 - 98.8			
ATCC 15293	2/3					
ATCC 29085	6/6					
ATCC 49894	6/6					
8.0 x 10^3/5x LoD	15/15	100.0	79.6 - 100.0			
ATCC 15293	3/3					
ATCC 29085	6/6					
ATCC 49894	6/6					
Pneumocystis jirovecii	**59/60**	**98.3**	**91.1 - 99.7**	**180/180**	**100.0**	**97.9 - 100.0**
1.0 x 10^6/2x LoD	59/60	98.3	91.1 - 99.7			
positive clinical specimen	59/60					
***Proteus* spp.**	**60/60**	**100.0**	**94.0 - 100.0**	**180/180**	**100.0**	**97.9 - 100.0**
1.0 x 10^4/2x LoD	30/30	100.0	88.7 - 100.0			
ATCC 12453	6/6					
ATCC 14153	6/6					

	Agreement with Expected Results					
	# Pos./ # Exp.	%	95% CI	# Neg./ # Exp.	%	95% CI
ATCC 25933	6/6					
ATCC 29906	6/6					
ATCC 6380	6/6					
1.8 x 10^4/3.5x LoD	15/15	100.0	79.6 - 100.0			
ATCC 12453	3/3					
ATCC 14153	3/3					
ATCC 25933	3/3					
ATCC 29906	3/3					
ATCC 6380	3/3					
2.5 x 10^4/5x LoD	15/15	100.0	79.6 - 100.0			
ATCC 12453	3/3					
ATCC 14153	3/3					
ATCC 25933	3/3					
ATCC 29906	3/3					
ATCC 6380	3/3					
Serratia marcescens	**59/60**	**98.3**	**91.1 - 99.7**	**180/180**	**100.0**	**97.9 - 100.0**
8.0 x 10^4/2x LoD	29/30	96.7	83.3 - 99.4			
ATCC 13880	6/6					
ATCC 14756	6/6					
ATCC 15365	6/6					
ATCC 27117	6/6					
DSM-17174	5/6					
1.4 x 10^5/3.5x LoD	15/15	100.0	79.6 - 100.0			
ATCC 13880	3/3					
ATCC 14756	3/3					
ATCC 15365	3/3					
ATCC 27117	3/3					
DSM-17174	3/3					
2.0 x 10^5/5x LoD	15/15	100.0	79.6 - 100.0			
ATCC 13880	3/3					
ATCC 14756	3/3					
ATCC 15365	3/3					
ATCC 27117	3/3					
DSM-17174	3/3					

[a] *Morganella morganii* strains ATCC 25829 and DSM-46262 strains were retested with pooled negative BAL matrix at 2x LoD both showing 10/10 results.

[b] *Mycoplasma pneumoniae* strains ATCC 29085 and ATCC 49894 were retested with pooled negative BAL matrix at 2x LoD showing 19/20 or 18/20 positive results, respectively. For three of four unexpected negative cases of ATCC 29085, used unique matrices correlated with reduced signals or false negative results also for other analytes and therefore such failures may be matrix-related.

Table 51: Contrived study results for targeted LRT BAL antibiotic resistance marker analytes.

	Agreement with Expected Results					
	# Pos./ # Exp.	%	95 %CI	# Neg./ # Exp.	%	95 %CI
***ctx*-M**	**59/60**	**98.3**	**91.1 - 99.7**	**120/120**	**100.0**	**96.9 - 100.0**
2.0 x 10^4/2x LoD	30/30	100.0	88.7 - 100.0			
JMI 49767	6/6					
JMI 50067	6/6					
NCTC 13443	6/6					
NRZ-00249	6/6					
NRZ-00751	6/6					
3.5 x 10^4/3.5x LoD	14/15	93.3	70.2 - 98.8			
JMI 49767	3/3					
JMI 50067	2/3					
NCTC 13443	3/3					
NRZ-00249	3/3					

	Agreement with Expected Results					
	# Pos./ # Exp.	%	95 %CI	# Neg./ # Exp.	%	95 %CI
NRZ-00751	3/3					
5.0 x 10^4/5x LoD	15/15	100.0	79.6 - 100.0			
JMI 49767	3/3					
JMI 50067	3/3					
NCTC 13443	3/3					
NRZ-00249	3/3					
NRZ-00751	3/3					
kpc	**58/60**	**96.7**	**88.6 - 99.1**	**156/156**	**100.0**	**97.6 - 100.0**
8.0 x 10^4/2x LoD	29/30	96.7	83.3 - 99.4			
Micromyx 4653	6/6					
NCTC 13438	6/6					
NRZ-00103	6/6					
NRZ-00222	5/6					
NRZ-00281	6/6					
1.4 x 10^5/3.5x LoD	14/15	93.3	70.2 - 98.8			
Micromyx 4653	3/3					
NCTC 13438	3/3					
NRZ-00103	2/3					
NRZ-00222	3/3					
NRZ-00281	3/3					
2.0 x 10^5/5x LoD	15/15	100.0	79.6 - 100.0			
Micromyx 4653	3/3					
NCTC 13438	3/3					
NRZ-00103	3/3					
NRZ-00222	3/3					
NRZ-00281	3/3					
ndm	**59/60**	**98.3**	**91.1 - 99.7**	**132/132**	**100.0**	**97.2 - 100.0**
4.0 x 10^4/2x LoD	29/30	96.7	83.3 - 99.4			
ATCC BAA-2468	6/6					
JMI 46239	6/6					
JMI 49755	5/6					
JMI 49831	6/6					
NCTC 13443	6/6					
7.0 x 10^4/3.5x LoD	15/15	100.0	79.6 - 100.0			
ATCC BAA-2468	3/3					
JMI 46239	3/3					
JMI 49755	3/3					
JMI 49831	3/3					
NCTC 13443	3/3					
1.0 x 10^5/5x LoD	15/15	100.0	79.6 - 100.0			
ATCC BAA-2468	3/3					
JMI 46239	3/3					
JMI 49755	3/3					
JMI 49831	3/3					
NCTC 13443	3/3					
***oxa*-23**	**60/60**	**100.0**	**94.0 - 100.0**	**180/180**	**100.0**	**97.9 - 100.0**
2.0 x 10^7/2x LoD	30/30	100.0	88.7 - 100.0			
Micromyx 4410	6/6					
Micromyx 6148	6/6					
Micromyx 6149	6/6					
NCTC 13301	6/6					
UCLA A5	6/6					
3.5 x 10^7/3.5x LoD	15/15	100.0	79.6 - 100.0			
Micromyx 4410	3/3					
Micromyx 6148	3/3					
Micromyx 6149	3/3					
NCTC 13301	3/3					
UCLA A5	3/3					
5.0 x 10^7/5x LoD	15/15	100.0	79.6 - 100.0			

	Agreement with Expected Results					
	# Pos./ # Exp.	%	95 %CI	# Neg./ # Exp.	%	95 %CI
Micromyx 4410	3/3					
Micromyx 6148	3/3					
Micromyx 6149	3/3					
NCTC 13301	3/3					
UCLA A5	3/3					
***oxa*-24**	**59/60**	**98.3**	**91.1 - 99.7**	**180/180**	**100.0**	**97.9 - 100.0**
1.0 x 10^4/2x LOD	29/30	96.7	83.3 - 99.4			
clinical isolate 1	6/6					
clinical isolate 2	6/6					
NCTC 13302	6/6					
NRZ-00449	5/6					
UCLA A4	6/6					
1.8 x 10^4/3.5x LOD	15/15	100.0	79.6 - 100.0			
clinical isolate 1	3/3					
clinical isolate 2	3/3					
NCTC 13302	3/3					
NRZ-00449	3/3					
UCLA A4	3/3					
2.5 x 10^4/5x LOD	15/15	100.0	79.6 - 100.0			
clinical isolate 1	3/3					
clinical isolate 2	3/3					
NCTC 13302	3/3					
NRZ-00449	3/3					
UCLA A4	3/3					
***oxa*-48**	**59/60**	**98.3**	**91.1 - 99.7**	**167/167**	**100.0**	**97.8 - 100.0**
6.0 x 10^5/2x LoD	29/30	96.7	83.3 - 99.4			
ATCC BAA-2523	5/6					
NCTC 13442	6/6					
NRZ-00176	6/6					
NRZ-00472	6/6					
NRZ-22060	6/6					
1.1 x 10^6/3.5x LoD	15/15	100.0	79.6 - 100.0			
ATCC BAA-2523	3/3					
NCTC 13442	3/3					
NRZ-00176	3/3					
NRZ-00472	3/3					
NRZ-22060	3/3					
1.5 x 10^6/5x LoD	15/15	100.0	79.6 - 100.0			
ATCC BAA-2523	3/3					
NCTC 13442	3/3					
NRZ-00176	3/3					
NRZ-00472	3/3					
NRZ-22060	3/3					
***oxa*-58**	**59/60**	**98.3**	**91.1 - 99.7**	**180/180**	**100.0**	**97.9 - 100.0**
1.0 x 10^5/2x LoD	30/30	100.0	88.7 - 100.0			
NCTC 13305	18/18					
NRZ-00518	12/12					
1.8 x 10^5/3.5x LoD	14/15	93.3	70.2 - 98.8			
NCTC 13305	8/9					
NRZ-00518	6/6					
2.5 x 10^5/5x LoD	15/15	100.0	79.6 - 100.0			
NCTC 13305	9/9					
NRZ-00518	6/6					
vim	**60/60**	**100.0**	**94.0 - 100.0**	**144/144**	**100.0**	**97.4 - 100.0**
4.0 x 10^4/2x LoD	30/30	100.0	88.7 - 100.0			
DSM-24600	6/6					
NCTC 13437	6/6					
NCTC 13439	6/6					
NCTC 13440	6/6					

	Agreement with Expected Results					
	# Pos./ # Exp.	%	95 %CI	# Neg./ # Exp.	%	95 %CI
NRZ-00452	6/6					
7.0 x 10^4/3.5x LoD	15/15	100.0	79.6 - 100.0			
DSM-24600	3/3					
NCTC 13437	3/3					
NCTC 13439	3/3					
NCTC 13440	3/3					
NRZ-00452	3/3					
1.0 x 10^5/5x LoD	15/15	100.0	79.6 - 100.0			
DSM-24600	3/3					
NCTC 13437	3/3					
NCTC 13439	3/3					
NCTC 13440	3/3					
NRZ-00452	3/3					

For other LRT BAL panel analytes not evaluated in the contrived study, the following agreement rates with the expected negative result were observed: 120/120 (100.0%, 95% CI 96.9 - 100.0 for *Acinetobacter* spp., 200/200 (100.0%, 95% CI 98.1 - 100.0) for *E. coli*, 228/228 (100.0%, 95% CI 98.3 - 100.0) for *H. influenzae*, 232/232 (100.0%, 95% CI 98.4 - 100.0) for *mec*A, 239/239 (100.0%, 95% CI 98.4 - 100.0) for *M. catarrhalis*, 208/208 (100.0%, 95% CI 98.2 - 100.0) for *P. aeruginosa*, 179/180 (99.4 %, 95% CI 96.9 - 99.9) for *S. aureus*, 240/240 (100.0%, 95% CI 98.4 - 100.0) for *S. maltophilia*, 239/240 (99.6%, 95% CI 97.7 - 99.9) for *S. pneumoniae*, and 82/82 (100%, 95% CI 95.5 - 100.0) for *tem*.

Expected Values

Expected values were determined for the prospective study cohort with 1,016 evaluable BAL specimens collected from hospitalized patients suspected for lower respiratory tract infection at nine US sites between June 2015 and July 2016. The LRT BAL Application reported 363 specimens with at least one positive LRT BAL panel microorganism, including 113 specimens with multi-detections of two or more LRT BAL panel microorganisms.

Table 52 lists expected values for microorganisms; Tables 53 and 54 list expected values for antibiotic resistance markers of the LRT BAL panel (for all specimens and stratified for each host microorganism). Note that antibiotic resistance markers are reported by LRT BAL only for specimens positive for a corresponding host microorganism.

Table 52: Expected values of LRT BAL panel microorganisms as reported for the prospective study stratified by all specimens (N = 1,016), as well as by single-detection (N = 250) or multi-detection specimens (N = 113). Expected value rates are given as percentage of 1,016 prospective study specimens.

Expected Values (N = 1,016 Prospective Specimens)							
Microorganism		**# Spec.**	**%**	**Microorganism**		**# Spec.**	**%**
Acinetobacter spp.	**all**	**21**	**2.1%**	*Moraxella catarrhalis*	**all**	**15**	**1.5%**
	single	8	0.8%		single	4	0.4%
	multi	13	1.3%		multi	11	1.1%
Chlamydia pneumoniae	**all**	**0**	**0.0%**	*Morganella morganii*	**all**	**3**	**0.3%**
	single	0	0.0%		single	0	0.0%
	multi	0	0.0%		multi	3	0.3%
Citrobacter freundii	**all**	**4**	**0.4%**	*Mycoplasma pneumoniae*	**all**	**6**	**0.6%**
	single	1	0.1%		single	4	0.4%
	multi	3	0.3%		multi	2	0.2%
Enterobacter cloacae complex	**all**	**20**	**2.0%**	*Pneumocystis jirovecii*	**all**	**22**	**2.2%**
	single	8	0.8%		single	14	1.4%
	multi	12	1.2%		multi	8	0.8%
Escherichia coli	**all**	**47**	**4.6%**	*Proteus* spp.	**all**	**10**	**1.0%**
	single	24	2.4%		single	0	0.0%
	multi	23	2.3%		multi	10	1.0%
Haemophilus influenzae	**all**	**56**	**5.5%**	*Pseudomonas aeruginosa*	**all**	**112**	**11.0%**
	single	30	3.0%		single	61	6.0%
	multi	26	2.6%		multi	51	5.0%
Klebsiella oxytoca	**all**	**14**	**1.4%**	*Serratia marcescens*	**all**	**17**	**1.7%**
	single	2	0.2%		single	5	0.5%
	multi	12	1.2%		multi	12	1.2%
Klebsiella pneumoniae	**all**	**30**	**3.0%**	*Staphylococcus aureus*	**all**	**104**	**10.2%**
	single	9	0.9%		single	60	5.9%
	multi	21	2.1%		multi	44	4.3%
Klebsiella variicola	**all**	**2**	**0.2%**	*Stenotrophomonas maltophilia*	**all**	**41**	**4.0%**
	single	0	0.0%		single	12	1.2%
	multi	2	0.2%		multi	29	2.9%
Legionella pneumophila	**all**	**1**	**0.1%**	*Streptococcus pneumoniae*	**all**	**13**	**1.3%**
	single	1	0.1%		single	7	0.7%
	multi	0	0.0%		multi	6	0.6%

Table 53: Expected values of LRT BAL panel antibiotic resistance markers as determined by the LRT BAL Application for the prospective study.

	Expected Values, All Specimens (N = 1,016)	
	# Spec.	**%**
ctx-M	9	0.9%
kpc	4	0.4%
*mec*A	47	4.6%
ndm	1	0.1%
oxa-23	3	0.3%
oxa-24	4	0.4%
oxa-48	1	0.1%
oxa-58	0	0.0%
tem	16	1.6%
vim	1	0.1%

Table 54: Expected values of LRT BAL panel antibiotic resistance markers, stratified by reported host microorganism.

	ctx-M	*kpc*	ndm	*oxa*-23	*oxa*-24	*oxa*-48	*oxa*-58	*vim*	*tem*	*mec*A
total reported	9	4	1	3	4	1	0	1	16	47
multi host detections	8	4	1	-	-	1	-	1	-	-
Acinetobacter spp. (N = 21)	2/21 9.5%	2/21 9.5%	1/21 4.8%	3/21 14.3%	4/21 19.0%		-	-		
Citrobacter freundii (N = 4)	-	-	-			-		-		
Enterobacter cloacae complex (N = 20)	-	1/20 5.0%	-			-		-		
Escherichia coli (N = 47)	8/47 17.0%	1/47 2.1%	1/47 2.1%			1/47 2.1%		-		
Klebsiella oxytoca (N = 14)	-	-	-			-		-		
Klebsiella pneumoniae (N = 30)	4/30 13.3%	4/30 13.3%	1/30 3.3%			1/30 3.3%		-		
Klebsiella variicola (N = 2)	-	-	-			-		-		
Morganella morganii (N = 3)	1/3 33.3%	-	-			-		-		
Proteus spp. (N = 10)	2/10 20.0%	-	-			-		1/10 10.0%		
Pseudomonas aeruginosa (N = 112)	7/112 6.3%	2/112 1.8%	1/112 0.9%					1/112 0.9%		
Serratia marcescens (N = 17)	2/17 11.8%	-	-			-		-		
Haemophilus influenzae (N = 56)									16/56 28.6%	
Staphylococcus aureus (N = 104)										47/104 45.2%

Specimens reported as negative, as single detection, or as multi-detection, together with reported antibiotic resistance markers are listed in Table 55. For example, for eight specimens, LRT BAL reported a single detection of *Acinetobacter* spp. For four of these specimens (50%), an antibiotic resistance marker indicating a possible carbapenem resistance was simultaneously reported, while for four other specimens, no corresponding antibiotic resistance marker was reported. Similarly, for 60 specimens, LRT BAL reported a single detection of *S. aureus*. For 32 of these specimens (53%), a *mec*A was simultaneously reported, while for the remaining 28 specimens, *mec*A was not reported.

Table 55: Rates of negative, single detection and multi-detection specimens together with reported antibiotic resistance markers as detected by the LRT BAL Application for the prospective study (N = 1,016 specimens). Note that resistance marker *oxa*-58 was not detected in the prospective study cohort.

		Antibiotic Resistance Marker Result											
			Single Marker Detected						Multiple Markers Detected				
Microorganism Result	**# cases**	**none**	*tem*	*ctx*-M	*kpc*	*oxa*-23	*oxa*-24	*mec*A	*ctx*-M, *mec*A	*tem*, *vim*	*ctx*-M, *mec*A, *oxa*-23	*ctx*-M, *ndm*, *oxa*-48	*kpc*, *mec*A, *oxa*-24
Negative	**653 (64.3%)**	-	-	-	-	-	-	-	-	-	-	-	-
Single Organism Detected	**250 (24.6%)**												
Acinetobacter spp.	8	4	-	-	-	1	3	-	-	-	-	-	-
Citrobacter freundii	1	1	-	-	-	-	-	-	-	-	-	-	-
Enterobacter cloacae complex	8	8	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli	24	24	-	-	-	-	-	-	-	-	-	-	-
Haemophilus influenzae	30	26	4	-	-	-	-	-	-	-	-	-	-
Klebsiella oxytoca	2	2	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	9	9	-	-	-	-	-	-	-	-	-	-	-
Legionella pneumophila	1	1	-	-	-	-	-	-	-	-	-	-	-
Moraxella catarrhalis	4	4	-	-	-	-	-	-	-	-	-	-	-
Mycoplasma pneumoniae	4	4	-	-	-	-	-	-	-	-	-	-	-
Pneumocystis jirovecii	14	14	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	61	61	-	-	-	-	-	-	-	-	-	-	-
Serratia marcescens	5	5	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	60	32	-	-	-	-	-	28	-	-	-	-	-
Stenotrophomonas maltophilia	12	12	-	-	-	-	-	-	-	-	-	-	-
Streptococcus pneumoniae	7	7	-	-	-	-	-	-	-	-	-	-	-
Two Organisms Detected	**78 (7.7%)**												
Acinetobacter spp., *Citrobacter freundii*	1	1	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter spp., *Enterobacter cloacae* complex	1	1	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter spp., *Klebsiella pneumoniae*	1	1	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter spp., *Pseudomonas aeruginosa*	1	1	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter spp., *Stenotrophomonas maltophilia*	1	1	-	-	-	-	-	-	-	-	-	-	-
Citrobacter freundii, *Pseudomonas aeruginosa*	1	1	-	-	-	-	-	-	-	-	-	-	-
Enterobacter cloacae complex, *Mycoplasma pneumoniae*	1	1	-	-	-	-	-	-	-	-	-	-	-
Enterobacter cloacae complex, *Staphylococcus aureus*	2	2	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli, *Haemophilus influenzae*	1	0	1	-	-	-	-	-	-	-	-	-	-
Escherichia coli, *Klebsiella oxytoca*	1	1	-	-	-	-	-	-	-	-	-	-	-

		Antibiotic Resistance Marker Result											
			Single Marker Detected						Multiple Markers Detected				
Microorganism Result	**# cases**	**none**	*tem*	*ctx*-M	*kpc*	*oxa*-23	*oxa*-24	*mec*A	*ctx*-M, *mec*A	*tem*, *vim*	*ctx*-M, *mec*A, *oxa*-23	*ctx*-M, *ndm*, *oxa*-48	*kpc*, *mec*A, *oxa*-24
Escherichia coli, *Klebsiella pneumoniae*	1	1	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli, *Pseudomonas aeruginosa*	1	0	-	1	-	-	-	-	-	-	-	-	-
Escherichia coli, *Serratia marcescens*	2	1	-	1	-	-	-	-	-	-	-	-	-
Escherichia coli, *Staphylococcus aureus*	2	1	-	-	-	-	-	-	1	-	-	-	-
Haemophilus influenzae, *Klebsiella oxytoca*	1	1	-	-	-	-	-	-	-	-	-	-	-
Haemophilus influenzae, *Moraxella catarrhalis*	4	3	1	-	-	-	-	-	-	-	-	-	-
Haemophilus influenzae, *Mycoplasma pneumoniae*	1	0	1	-	-	-	-	-	-	-	-	-	-
Haemophilus influenzae, *Pseudomonas aeruginosa*	3	1	2	-	-	-	-	-	-	-	-	-	-
Haemophilus influenzae, *Staphylococcus aureus*	4	2	2	-	-	-	-	-	-	-	-	-	-
Haemophilus influenzae, *Streptococcus pneumoniae*	3	1	2	-	-	-	-	-	-	-	-	-	-
Klebsiella oxytoca, *Klebsiella pneumoniae*	1	1	-	-	-	-	-	-	-	-	-	-	-
Klebsiella oxytoca, *Proteus* spp.	1	1	-	-	-	-	-	-	-	-	-	-	-
Klebsiella oxytoca, *Serratia marcescens*	1	1	-	-	-	-	-	-	-	-	-	-	-
Klebsiella oxytoca, *Staphylococcus aureus*	1	1	-	-	-	-	-	-	-	-	-	-	-
Klebsiella oxytoca, *Stenotrophomonas maltophilia*	1	1	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae, *Pseudomonas aeruginosa*	2	1	-	1	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae, *Staphylococcus aureus*	3	2	-	-	-	-	-	1	-	-	-	-	-
Moraxella catarrhalis, *Pneumocystis jirovecii*	1	1	-	-	-	-	-	-	-	-	-	-	-
Moraxella catarrhalis, *Streptococcus pneumoniae*	1	1	-	-	-	-	-	-	-	-	-	-	-
Pneumocystis jirovecii, *Pseudomonas aeruginosa*	1	1	-	-	-	-	-	-	-	-	-	-	-
Pneumocystis jirovecii, *Staphylococcus aureus*	1	1	-	-	-	-	-	-	-	-	-	-	-
Pneumocystis jirovecii, *Stenotrophomonas maltophilia*	2	2	-	-	-	-	-	-	-	-	-	-	-
Proteus spp., *Pseudomonas aeruginosa*	1	1	-	-	-	-	-	-	-	-	-	-	-
Proteus spp., *Staphylococcus aureus*	1	0	-	-	-	-	-	1	-	-	-	-	-
Pseudomonas aeruginosa, *Serratia marcescens*	1	1	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa, *Staphylococcus aureus*	14	4	-	-	-	-	-	10	-	-	-	-	-
Pseudomonas aeruginosa, *Stenotrophomonas maltophilia*	6	6	-	-	-	-	-	-	-	-	-	-	-
Serratia marcescens, *Staphylococcus aureus*	1	0	-	-	-	-	-	1	-	-	-	-	-
Staphylococcus aureus, *Stenotrophomonas maltophilia*	5	3	-	-	-	-	-	2	-	-	-	-	-
Three Organisms Detected	**19 (1.9%)**												
Acinetobacter spp., *Klebsiella pneumoniae*, *Staphylococcus aureus*	1	0	-	-	-	-	-	-	-	-	-	-	1

		Antibiotic Resistance Marker Result											
			Single Marker Detected						Multiple Markers Detected				
Microorganism Result	**# cases**	**none**	*tem*	*ctx*-M	*kpc*	*oxa*-23	*oxa*-24	*mec*A	*ctx*-M, *mec*A	*tem*, *vim*	*ctx*-M, *mec*A, *oxa*-23	*ctx*-M, *ndm*, *oxa*-48	*kpc*, *mec*A, *oxa*-24
Acinetobacter spp., *Klebsiella pneumoniae, Stenotrophomonas maltophilia*	1	1	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter spp., *Pseudomonas aeruginosa, Stenotrophomonas maltophilia*	1	0	-	-	-	1	-	-	-	-	-	-	-
Enterobacter cloacae complex, *Haemophilus influenzae, Moraxella catarrhalis*	1	1	-	-	-	-	-	-	-	-	-	-	-
Enterobacter cloacae complex, *Klebsiella oxytoca, Stenotrophomonas maltophilia*	1	1	-	-	-	-	-	-	-	-	-	-	-
Enterobacter cloacae complex, *Klebsiella pneumoniae, Moraxella catarrhalis*	1	1	-	-	-	-	-	-	-	-	-	-	-
Enterobacter cloacae complex, *Klebsiella pneumoniae, Pseudomonas aeruginosa*	1	0	-	-	1	-	-	-	-	-	-	-	-
Escherichia coli, Haemophilus influenzae, Staphylococcus aureus	1	1	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli, Morganella morganii, Pseudomonas aeruginosa	1	1	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli, Pneumocystis jirovecii, Pseudomonas aeruginosa	1	0	-	1	-	-	-	-	-	-	-	-	-
Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens	1	1	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus	1	1	-	-	-	-	-	-	-	-	-	-	-
Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae	1	1	-	-	-	-	-	-	-	-	-	-	-
Klebsiella oxytoca, Pseudomonas aeruginosa, Staphylococcus aureus	1	1	-	-	-	-	-	-	-	-	-	-	-
Klebsiella oxytoca, Pseudomonas aeruginosa, Stenotrophomonas maltophilia	1	1	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae, Pseudomonas aeruginosa, Stenotrophomonas maltophilia	1	0	-	-	1	-	-	-	-	-	-	-	-
Proteus spp., *Pseudomonas aeruginosa, Staphylococcus aureus*	1	0	-	-	-	-	-	1	-	-	-	-	-
Pseudomonas aeruginosa, Serratia marcescens, Stenotrophomonas maltophilia	2	2	-	-	-	-	-	-	-	-	-	-	-
Four Organisms Detected	**7 (0.7%)**												
Acinetobacter spp., *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*	1	0	-	-	-	-	-	-	-	-	-	1	-
Acinetobacter spp., *Escherichia coli, Klebsiella pneumoniae, Stenotrophomonas maltophilia*	1	0	-	-	1	-	-	-	-	-	-	-	-
Acinetobacter spp., *Escherichia coli,*	1	1	-	-	-	-	-	-	-	-	-	-	-

		Antibiotic Resistance Marker Result											
			Single Marker Detected						Multiple Markers Detected				
Microorganism Result	**# cases**	**none**	*tem*	*ctx*-M	*kpc*	*oxa*-23	*oxa*-24	*mec*A	*ctx*-M, *mec*A	*tem*, *vim*	*ctx*-M, *mec*A, *oxa*-23	*ctx*-M, *ndm*, *oxa*-48	*kpc*, *mec*A, *oxa*-24
Proteus spp., *Pseudomonas aeruginosa*													
Enterobacter cloacae complex, *Escherichia coli*, *Klebsiella oxytoca*, *Stenotrophomonas maltophilia*	1	1	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli, *Haemophilus influenzae*, *Pneumocystis jirovecii*, *Staphylococcus aureus*	1	1	-	-	-	-	-	-	-	-	-	-	-
Haemophilus influenzae, *Proteus* spp., *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*	1	0	-	-	-	-	-	-	-	1	-	-	-
Pseudomonas aeruginosa, *Serratia marcescens*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*	1	1	-	-	-	-	-	-	-	-	-	-	-
Five Organisms Detected	**7 (0.7%)**												
Acinetobacter spp., *Enterobacter cloacae* complex, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*	1	1	-	-	-	-	-	-	-	-	-	-	-
Citrobacter freundii, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcescens*	1	1	-	-	-	-	-	-	-	-	-	-	-
Enterobacter cloacae complex, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella variicola*, *Proteus* spp.	1	1	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Stenotrophomonas maltophilia*	1	0	-	1	-	-	-	-	-	-	-	-	-
Escherichia coli, *Morganella morganii*, *Proteus* spp., *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*	1	0	-	1	-	-	-	-	-	-	-	-	-
Haemophilus influenzae, *Klebsiella pneumoniae*, *Klebsiella variicola*, *Proteus* spp., *Staphylococcus aureus*	1	1	-	-	-	-	-	-	-	-	-	-	-
Haemophilus influenzae, *Klebsiella pneumoniae*, *Moraxella catarrhalis*, *Pneumocystis jirovecii*, *Serratia marcescens*	1	0	1	-	-	-	-	-	-	-	-	-	-
Six Organisms Detected	**2 (0.2%)**												
Acinetobacter spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*	1	0	-	-	-	-	-	-	-	-	1	-	-
Enterobacter cloacae complex,	1	0	1	-	-	-	-	-	-	-	-	-	-

		Antibiotic Resistance Marker Result											
		none	Single Marker Detected						Multiple Markers Detected				
Microorganism Result	**# cases**		*tem*	*ctx*-M	*kpc*	*oxa*-23	*oxa*-24	*mec*A	*ctx*-M, *mec*A	*tem*, *vim*	*ctx*-M, *mec*A, *oxa*-23	*ctx*-M, *ndm*, *oxa*-48	*kpc*, *mec*A, *oxa*-24
Haemophilus influenzae, Moraxella catarrhalis, Morganella morganii, Staphylococcus aureus, Stenotrophomonas maltophilia													
Total	**1,016**	**938**	**15**	**6**	**3**	**2**	**3**	**44**	**1**	**1**	**1**	**1**	**1**

807.92 (b)(3): Conclusions from Nonclinical and Clinical Data

The conclusions drawn from the analytical and clinical data demonstrate that the device is safe and effective for its intended use.