510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
K163636

B. Purpose for Submission:
New device

C. Measurand:
Adenovirus, Coronavirus, Human Metapneumovirus (hMPV), Human Rhinovirus/Enterovirus (HRV/HEV), Influenza A, Influenza A H1 (Flu A/H1), Influenza A H1-2009 (Flu A/H1-2009), Influenza A H3 (Flu A/H3), Influenza B (Flu B), Parainfluenza virus 1 (PIV 1), Parainfluenza virus 2 (PIV 2), Parainfluenza virus 3 (PIV 3), Parainfluenza virus 4 (PIV 4), Respiratory Syncytial Virus A (RSV A), Respiratory Syncytial Virus B (RSV B), *Chlamydia pneumoniae,* and *Mycoplasma pneumoniae* nucleic acids target sequences.

D. Type of Test:
A multiplexed nucleic acid test intended for use with the GenMark ePlex instrument for the simultaneous qualitative *in vitro* detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) collected in viral transport media and obtained from individuals suspected of respiratory tract infections.

E. Applicant:
GenMark Diagnostics, Incorporated

F. Proprietary and Established Names:
ePlex® Respiratory Pathogen (RP) Panel
Common Name: ePlex RP® Panel

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
OCC	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay	Microbiology (83)
OEM	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay	Microbiology (83)

OEP	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay	Microbiology (83)
OOU	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay	Microbiology (83)
OTG	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay	Microbiology (83)
OZE	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay	Microbiology (83)
OZX	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay	Microbiology (83)
OZY	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay	Microbiology (83)
OQW	Class II	21 CFR 866.3332 Reagents for Detection of Specific Novel Influenza A Viruses	Microbiology (83)
NSU	Class II	21 CFR 862.2570 Instrumentation for Clinical Multiplex Test Systems	Clinical Chemistry (75)

H. Intended Use:

1. Intended use:
   The ePlex® Respiratory Pathogen (RP) Panel is a multiplexed nucleic acid *in vitro* diagnostic test intended for use on the ePlex® Instrument for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals exhibiting signs and symptoms of respiratory tract infection.

   The following virus types, subtypes, and bacteria are identified using the ePlex® RP Panel: adenovirus, coronavirus, human metapneumovirus, human rhinovirus/enterovirus, influenza A, influenza A H1, influenza A H1-2009, influenza A H3, influenza B, parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3,

parainfluenza virus 4, respiratory syncytial virus (RSV) A, respiratory syncytial virus (RSV) B, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*.

The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and/or symptoms of respiratory tract infection aids in the diagnosis of respiratory infection when used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by a nasopharyngeal swab specimen. Positive results do not rule out co-infection with other organisms: the organism(s) detected by the ePlex® RP Panel may not be the definite cause of disease. Additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection.

Due to the genetic similarity between human rhinovirus and enterovirus, the ePlex® RP Panel cannot reliably differentiate them. If differentiation is required, an ePlex® RP Panel positive human rhinovirus/enterovirus result should be followed-up using an alternative method (e.g., cell culture or sequence analysis).

Performance characteristics for influenza A were established when influenza A H1-2009 and A H3 were the predominant influenza A viruses in circulation. Performance of detecting influenza A may vary if other influenza A strains are circulating or a novel influenza A virus emerges. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL-3+ facility is available to receive and culture specimens.

2. Indication for use:
   Same as Intended Use

3. Special conditions for use statement(s):
   For prescription use only

4. Special instrument requirements:
   ePlex instrument

I. Device Description:

The ePlex Respiratory Pathogen (RP) Panel is based on the principles of competitive nucleic acid hybridization using a sandwich assay format, wherein a single-stranded target binds concurrently to a sequence-specific solution-phase signal probe and a solid-

phase electrode-bound capture probe. The test employs nucleic acid extraction, target amplification via polymerase chain reaction (PCR) or reverse transcription PCR (RT-PCR), and hybridization of target DNA. In the process, the double-stranded PCR amplicons are digested with exonuclease to generate single-stranded DNA suitable for hybridization.

Nucleic acid extraction from biological specimens occurs within the cartridge via cell lysis, nucleic acid capture onto magnetic beads, and release for amplification. The nucleic acid extraction is processed through microfluidic liquid handling. Once the nucleic acid targets are captured and inhibitors are washed away, the magnetic particles are delivered to the electrowetting environment on the printed circuit board (PCB) and the targets are eluted from the particles and amplified.

During hybridization, the single-stranded target DNA binds to a complementary, single-stranded capture probe immobilized on the working gold electrode surface. Single-stranded signal probes (labeled with electrochemically active ferrocenes) bind to specific target sequence / region adjacent to the capture probe. Simultaneous hybridization of target to signal probes and capture probe is detected by alternating current voltammetry (ACV). Each working electrode on the array contains specific capture probes, and sequential analysis of each electrode allows detection of multiple analyte targets.

A summary of the ePlex RP Panel nucleic acid targets is presented in Table 1 below.

Table 1: A summary of the ePlex RP Targets

Organism	Target Gene(s)
Adenovirus	Hexon protein (L3) and Penton protein (L2)
Coronaviruses (229E, HKU1, NL63, and OC43)	Nucleoprotein (N)
Human Metapneumovirus	Nucleocapsid (N)
Influenza A	Matrix protein (M)
Influenza A H1	Hemagglutinin (HA)
Influenza A 2009 H1N1	Hemagglutinin (HA)
Influenza A/H3	Hemagglutinin (HA)
Influenza B	RNA polymerase subunit PB1
Parainfluenza 1	Hemagglutinin-neuraminidase (HN)
Parainfluenza 2	Hemagglutinin-neuraminidase (HN)
Parainfluenza 3	Hemagglutinin-neuraminidase (HN)
Parainfluenza 4	Hemagglutinin-neuraminidase (HN)
Human Rhinovirus/Enterovirus	5'- Untranslated Region (UTR)
RSV A	Nucleocapsid (N)
RSV B	Nucleocapsid (N)
Chlamydia pneumoniae	Major Outer Membrane Protein A (OmpA)
Mycoplasma pneumonia	Cytadhesin (P1)

Materials Provided:
Each ePlex Respiratory Pathogen Panel kit contains sufficient reagents to test 12 samples (EA001012):

- ePlex Respiratory Pathogen Panel Cartridge (12)
- Sample Delivery Device – RP Panel; 200 µl (12)

Materials Needed but Not Provided:
Equipment

- GenMark ePlex instrument and software
- Pipettes calibrated to deliver 200 µl
- Vortex mixer
- Printer (Optional)

Consumables

- Pipette tips, aerosol resistant, RNase/DNase-free
- Disposable, powder free gloves
- 10% bleach for decontamination of appropriate surfaces
- 70% ethanol or isopropyl alcohol

Interpretation of Results
Results interpretation of the ePlex Respiratory Pathogen Panel is performed by the ePlex instrument, and the interpretation of results on the ePlex RP Panel Detection Report for each targeted analyte is summarized in Table 2 below.

Table 2: Interpretation of Results on the ePlex RP Panel Detection Report

Target Result	Explanation	Action
Target Detected	The test was completed successfully, and the target has generated signal above its defined threshold, and the Internal Control was reported as PASS.	All results are displayed on the RP Panel Detection Report. Test is valid, report results.
Multiple Targets Detected	The test was completed successfully, and multiple targets have generated signals above its defined threshold, and the Internal Control was reported as PASS.	All results are displayed on the RP Panel Detection Report. Test is valid, report results. Detection of more than 3 pathogens may indicate contamination. Re-test of the sample is recommended to confirm results.
Not Detected	The test was completed successfully, and the target did not generate signal above its defined threshold, and the Internal Control was reported as PASS.	All results are displayed on the RP Panel Detection Report. Test is valid, report results.
Invalid	The test has not successfully completed, and results for this test are not valid. This is often due to an instrument or software error or failure of an internal control.	No results are displayed on the RP Panel Detection Report. Test is not valid, repeat test.

Influenza A Results

The ePlex RP Panel detects Influenza A and the A/H1, A/H1-2009, and A/H3 subtypes using unique assays for each. Interpretation of results for Influenza A are described in Table 3 below.

Table 3: Possible Assay Results for Influenza A and the Corresponding Interpretation

Results for Influenza A and Subtypes	**Explanation**	**Results on Report**	**Recommended Action**
Influenza A detected, at least one subtype (A/H1, A/H1-2009, or A/H3) reported as detected.	This is an expected result.	Result reported as influenza A and influenza A subtype detected.	None
Influenza A detected, all subtypes (A/H1, A/H1-2009, and A/H3) reported as not detected	Low virus titers can result in detection of influenza A matrix without a subtype. Detection of influenza A matrix without a subtype can also indicate the presence of a novel strain.	Result reported as influenza A detected (no subtype detected)	Re-test to confirm result. If the re-test provides a different result, test the sample a third time to ensure the accuracy of the result. If the re-test provides the same result, then the function of the ePlex RP Panel cartridge should be verified by testing with appropriate external positive control materials (known positive samples for influenza A H1, influenza A H3 and influenza A H1-2009), and a negative control should also be run to test for potential PCR-product contamination. If the ePlex RP Panel accurately identifies the external and negative controls, contact the appropriate public health authorities for confirmatory testing.
Influenza A detected and more than one subtype (A/H1, A/H1-2009, or A/H3) reported as detected.	Sample is co-infected with multiple influenza subtypes. Infections with multiple subtypes of influenza are possible but rare. A live intranasal multivalent influenza virus vaccine may cause	Result reported as influenza A and multiple subtypes detected.	Re-test to confirm result. If the re-test result confirms the original result, it is recommended that the sample be further investigated using a different FDA-cleared influenza A subtyping assay.

Results for Influenza A and Subtypes	Explanation	Results on Report	Recommended Action
	false positive results for influenza A, A/H1, A/H3, A/H1-2009, and/or influenza B. Contamination has occurred.		
Influenza A not detected, at least one subtype (A/H1, A/H1-2009, or A/H3) reported as detected.	Low virus titers can result in detection of influenza A subtype without the influenza A matrix. Detection of influenza A subtype without the influenza A matrix can also indicate the presence of a novel strain.	Influenza A (subtype) detected. Re-testing of this sample to confirm influenza A (subtype) is recommended. Refer to package insert for additional information.	Re-test to confirm result. If the re-test result confirms the original result, the influenza A subtype is considered positive. It is recommended that the sample be further investigated using a different FDA-cleared influenza A subtyping assay and/or sending the residual sample to local public health laboratory for further testing.

ePlex RP Panel Test Reports

There are several different reports that are available on the ePlex instrument. Results are provided in a printable format, may be viewed electronically, or may be exported for additional analysis. Reports can be customized with account specific information such as the address, logo, and institution specific footers on each report.

Detection Report

The ePlex RP Panel Detection Report includes the results for each individual sample run on the ePlex instrument. The Summary section indicates the overall test result and lists all detected analytes in that sample. The Results section includes a list of all analytes on the panel with an individual result for each. Results for each analyte are reported as Detected, Not Detected, or Invalid (displayed as a red x); results for the Internal Control are reported as PASS, FAIL, INVALID, or N/A.

External Control Report

The ePlex RP Panel External Control Report is generated for an external control that has been pre-defined in the ePlex RP Panel software. The Summary section indicates the overall result (Pass or Fail status) and lists all detected analytes for that external control. The Results section includes a list of all panel analytes with the result, expected result, and Pass/Fail status for each. Results are reported as Detected, Not Detected, or Invalid (displayed as a red x). An analyte is reported as Pass if the actual result matches the expected result (as defined for that control); an analyte is reported as Fail if the actual result does not match the expected result. If the actual results for each analyte match the

expected results (all analytes reported as Pass), the overall result for the external control is reported as Pass in the Summary section. If the actual result for any analyte does not match the expected result, the overall result for the external control is reported as Fail in the Summary section.

Summary Report
The Summary Report allows the operator to use defined searchable criteria to create customized reports, using specified analytes, dates, range of dates, sample, external control, test bay, or operator.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   FilmArray® Respiratory Panel (RP)

2. Predicate K number(s):

   K160068

3. Comparison with predicate(s):

Similarities or Differences		
Element	**ePlex Respiratory Pathogen (RP) Panel (K163636)**	**FilmArray Respiratory Panel (RP) (K160068)**
Specimen Types	NPS (in transport media)	Same
Organisms Detected	Adenovirus, Coronavirus, Human Metapneumovirus, Influenza A, Influenza A H1, Influenza A H3, Influenza A H1-2009, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Human Rhinovirus/Enterovirus, Respiratory Syncytial Virus A, , Respiratory Syncytial Virus B, *Chlamydia pneumoniae,* and *Mycoplasma pneumoniae*	Same, except that it also detects and differentiates Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, and Coronavirus OC43; it also detects *Bordetella pertussis*; it does not differentiate Respiratory Syncytial Virus A and Respiratory Syncytial Virus B
Analyte	RNA/DNA	Same
Technological Principles	Multiplex nucleic acid amplification test	Same
Instrumentation	ePlex Instrument	FilmArray 2.0 or FilmArray® Torch

Chemistry	Reagents contained within cartridge to allow sample lysis and nucleic acid extraction, RT-PCR amplification, and hybridization-based electrochemical detection reagents.	Nested multiplex RT-PCR followed by high resolution melting analysis to confirm identity of amplified product.
Reagent Storage	Room temperature	Same
Test Interpretation	Automated test interpretation and report generation. User cannot access raw data.	Same
Controls	Eight internal controls to demonstrate that sample extraction, amplification and detection processes functioned as intended.	Two controls are included in each reagent pouch to control for sample processing and both stages of PCR and melt analysis.

K. Standard/Guidance Documents Referenced (if applicable):

None

L. Test Principle:

The ePlex instrument automates all aspects of nucleic acid testing including extraction, amplification, and detection, combining electrowetting and GenMark's eSensor technology in a single-use cartridge. eSensor technology is based on the principles of competitive DNA hybridization and electrochemical detection, which is highly specific and is not based on fluorescent or optical detection.

Electrowetting, or digital microfluidics, uses electrical fields to directly manipulate discrete droplets on the surface of a hydrophobically coated printed circuit board (PCB). Sample and reagents are moved in a programmable fashion in the ePlex cartridge to complete all portions of the sample processing from nucleic acid extraction to detection.

A sample is loaded onto the ePlex cartridge and nucleic acids are extracted and purified from the specimen via magnetic solid phase extraction. For RNA targets, a reverse transcription step is performed to generate complementary DNA from the RNA, followed by PCR to amplify the targets. Exonuclease digestion creates single-stranded DNA in preparation for eSensor detection.

The target DNA is mixed with ferrocene-labeled signal probes that are complementary to the specific targets on the panel. Target DNA hybridizes to its complementary signal probe and capture probes, which are bound to gold-plated electrodes, as shown below in Figure 1.

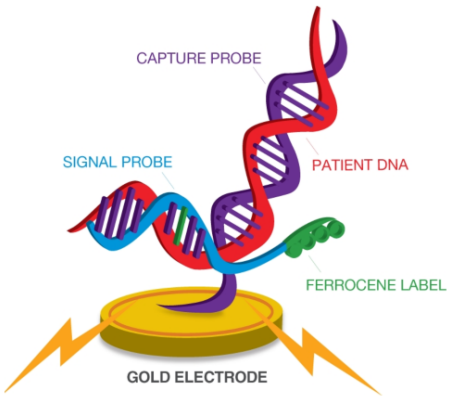

Figure 1: Hybridization complex. Target-specific capture probes are bound to the gold electrodes in the eSensor microarray on the ePlex cartridge. The amplified target DNA hybridizes to the capture probe and to a complementary ferrocene-labeled signal probe. Electrochemical analysis determines the presence or absence of targets using voltammetry.

The presence of each target is determined by voltammetry which generates specific electrical signals from the ferrocene-labeled signal.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

a. Reproducibility Study :

A multisite reproducibility study of the ePlex RP Panel was conducted at three testing sites (two external sites and one internal site) across major potential sources of variability, such as site-to-site, lot-to-lot, day-to-day, and operator-to-operator. One ePlex instrument per study site with either three or four towers was employed in this reproducibility study. Two operators performed testing at each site on six days (five nonconsecutive days) with three unique lots of ePlex RP Panel cartridges. A reproducibility study panel consisting of three panel members with six organisms that represent seven RP Panel targets (multi-spiked[1]) at three concentrations (moderate positive - 3x LoD, low positive - 1x LoD, and negative) was tested in triplicate. The six representative organisms in the reproducibility study panel included adenovirus, coronavirus OC43 (CoV-OC43), human metapneumovirus,

[1] Due to the design of ePlex RP Panel, where each of the representative targets are located in separate PCR reactions/lanes (except for CoV-OC43 and RSV A), nucleic acid amplification of each target is conducted in a reaction that is physically separated from the other targets. Therefore, pooling of multiple analyte targets in one specimen (i.e., the multi-spiking approach) most likely does not perform differently than a test sample containing a single analyte target. Analytical study results also demonstrated that the same analytical sensitivity was achieved for CoV-OC43 and RSV A regardless of whether they were tested separately/independently or together within the same sample mix.

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influenza A H3, parainfluenza virus 1, and RSV A; organisms were diluted in natural clinical matrix (pooled, negative nasopharyngeal swab in VTM samples). Negative samples consisted of the same natural clinical matrix (pooled, negative nasopharyngeal swab in VTM samples) only. Each contrived sample was divided into aliquots and stored frozen (-70 °C) prior to testing. Each operator tested nine samples (i.e., three member reproducibility panel in triplicate) each day; each panel member was tested 108 times (3 replicates x 3 sites x 2 operators x 3 lots x 2 days of testing/operator/lot) for a minimum of 324 tests.

Summary results for the ePlex RP Panel reproducibility study are provided in Table 4 below.

Table 4: Reproducibility of ePlex RP Panel Results

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result			
			Site 1	Site 2	Site 3 [a]	All Sites (95% CI)
Viruses						
Adenovirus	**Moderate Positive (3× LoD)** (6.0E+00 $TCID_{50}$/mL)	**Detected**	**36/36** 100%	**36/36** 100%	**36/36** 100%	**108/108 100%** (96.6%-100%)
	Low Positive (1× LoD) (2.0E+00 $TCID_{50}$/mL)	**Detected**	**36/36** 100%	**34/36** 94.4%	**28/35** 80.0%	**98/107 91.6%** (84.8%-95.5%)
	None (no analyte)	**Not Detected**	**36/36** 100%	**36/36** 100%	**36/36** 100%	**108/108 100%** (96.6%-100%)
Coronavirus	**Moderate Positive (3× LoD)** (1.5E+03 $TCID_{50}$/mL)	**Detected**	**36/36** 100%	**36/36** 100%	**36/36** 100%	**108/108 100%** (96.6%-100%)
	Low Positive (1× LoD) (5.0E+02 $TCID_{50}$/mL)	**Detected**	**36/36** 100%	**36/36** 100%	**35/35** 100%	**107/107 100%** (96.5%-100%)
	None (no analyte)	**Not Detected**	**36/36** 100%	**36/36** 100%	**36/36** 100%	**108/108 100%** (96.6%-100%)
Human Metapneumovirus	**Moderate Positive (3× LoD)** (6.8E+02 $TCID_{50}$/mL)	**Detected**	**36/36** 100%	**36/36** 100%	**36/36** 100%	**108/108 100%** (96.6%-100%)
	Low Positive (1× LoD) (2.3E+02 $TCID_{50}$/mL)	**Detected**	**36/36** 100%	**36/36** 100%	**35/35** 100%	**107/107 100%** (96.5%-100%)

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result			
			Site 1	Site 2	Site 3 [a]	All Sites (95% CI)
	None (no analyte)	**Not Detected**	**36/36** 100%	**36/36** 100%	**36/36** 100%	**108/108 100%** (96.6%-100%)
Human Rhinovirus/ Enterovirus	**None** (no analyte)	**Not Detected**	**108/108** 100%	**108/108** 100%	**104/107** 97.2%	**320/323 99.1%** (97.3%-99.7%)
Influenza A	**Moderate Positive (3× LoD)** (1.5E+02 $TCID_{50}$/mL)	**Detected**	**36/36** 100%	**36/36** 100%	**36/36** 100%	**108/108 100%** (96.6%-100%)
	Low Positive (1× LoD) (5.0E+01 $TCID_{50}$/mL)	**Detected**	**36/36** 100%	**36/36** 100%	**35/35** 100%	**107/107 100%** (96.5%-100%)
	None (no analyte)	**Not Detected**	**36/36** 100%	**36/36** 100%	**36/36** 100%	**108/108 100%** (96.6%-100%)
Influenza A H1-2009	**None** (no analyte)	**Not Detected**	**108/108** 100%	**108/108** 100%	**107/107** 100%	**323/323 100%** (98.8%-100%)
Influenza A H1	**None** (no analyte)	**Not Detected**	**108/108** 100%	**108/108** 100%	**107/107** 100%	**323/323 100%** (98.8%-100%)
Influenza A H3	**Moderate Positive (3× LoD)** (1.5E+02 $TCID_{50}$/mL)	**Detected**	**36/36** 100%	**36/36** 100%	**36/36** 100%	**108/108 100%** (96.6%-100%)
	Low Positive (1× LoD) (5.0E+01 $TCID_{50}$/mL)	**Detected**	**36/36** 100%	**36/36** 100%	**35/35** 100%	**107/107 100%** (96.5%-100%)
	None (no analyte)	**Not Detected**	**36/36** 100%	**36/36** 100%	**36/36** 100%	**108/108 100%** (96.6%-100%)
Influenza B	**None** (no analyte)	**Not Detected**	**108/108** 100%	**108/108** 100%	**107/107** 100%	**323/323 100%** (98.8%-100%)
Parainfluenza Virus 1	**Moderate Positive (3× LoD)** (1.2E+00 $TCID_{50}$/mL)	**Detected**	**36/36** 100%	**36/36** 100%	**36/36** 100%	**108/108 100%** (96.6%-100%)
	Low Positive (1× LoD) (4.0E-01 $TCID_{50}$/mL)	**Detected**	**36/36** 100%	**36/36** 100%	**35/35** 100%	**107/107 100%** (96.5%-100%)

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result			
			Site 1	Site 2	Site 3 [a]	All Sites (95% CI)
	None (no analyte)	**Not Detected**	**36/36** 100%	**36/36** 100%	**36/36** 100%	**108/108 100%** (96.6%-100%)
Parainfluenza Virus 2	**None** (no analyte)	**Not Detected**	**108/108** 100%	**108/108** 100%	**107/107** 100%	**323/323 100%** (98.8%-100%)
Parainfluenza Virus 3	**None** (no analyte)	**Not Detected**	**108/108** 100%	**108/108** 100%	**106/107** 99.1%	**322/323 99.7%** (98.3%-99.9%)
Parainfluenza Virus 4	**None** (no analyte)	**Not Detected**	**108/108** 100%	**108/108** 100%	**107/107** 100%	**323/323 100%** (98.8%-100%)
Respiratory Syncytial Virus A	**Moderate Positive (3× LoD)** (4.5E+00 $TCID_{50}$/mL)	**Detected**	**36/36** 100%	**36/36** 100%	**36/36** 100%	**108/108 100%** (96.6%-100%)
	Low Positive (1× LoD) (1.5E+00 $TCID_{50}$/mL)	**Detected**	**36/36** 100%	**36/36** 100%	**35/35** 100%	**107/107 100%** (96.5%-100%)
	None (no analyte)	**Not Detected**	**36/36** 100%	**36/36** 100%	**36/36** 100%	**108/108 100%** (96.6%-100%)
Respiratory Syncytial Virus B	**None** (no analyte)	**Not Detected**	**108/108** 100%	**108/108** 100%	**107/107** 100%	**323/323 100%** (98.8%-100%)
Bacteria						
Chlamydia pneumoniae	**None** (no analyte)	**Not Detected**	**108/108** 100%	**108/108** 100%	**107/107** 100%	**323/323 100%** (98.8%-100%)
Mycoplasma pneumoniae	**None** (no analyte)	**Not Detected**	**108/108** 100%	**107/108** 99.1%	**106/107** 99.1%	**321/323 99.4%** (97.8%-99.8%)

[a] 1/324 reproducibility panel samples had initial and re-tested invalid results (tested at Site 3) and was excluded from this analysis.

Over the course of the reproducibility study, a total of three different ePlex instruments and 66 different ePlex bays (one 4-tower ePlex instrument each at Site 1 and Site 2, and one 3-tower ePlex instrument at Site 3, each tower has six bays) were used by six different operators at three different sites. All bays were utilized at least once during the conduct of this study.

In total, 334 runs were attempted with one pre-flight check failure. Of the 333 runs that initiated, all runs completed and valid results were obtained from 323 out of the 333 runs that were initiated (323/333, 97.0%).

Of the 324 reproducibility panel samples initially tested, 315 (97.2%; 95% CI: 94.8% - 98.5%) had valid results and nine (2.8%; 95% CI: 1.5% - 5.2%) had invalid results. Initial validity rates were similar across the testing sites: 96.3% at Site 1, 98.1% at Site 2, 97.2% at Site 3. Initially invalid samples were re-tested and eight had valid results. The final validity rate was 99.7% (95% CI: 98.3%-99.9%).

b. Linearity/assay reportable range:

Not applicable, qualitative assay

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

Assay Controls

Internal Controls

Each ePlex RP Panel cartridge includes internal controls that monitor performance of each step of the testing process. A DNA control verifies extraction, amplification and detection of DNA targets, and RNA controls verify amplification and detection of RNA targets. Each amplification reaction on the cartridge has at least one internal control and in each reaction either the internal control or a target must generate signal above the defined threshold for a valid test result. Internal control results are interpreted by the ePlex software and displayed on ePlex RP Panel Reports as Internal Control with a result of PASS, FAIL, N/A, or INVALID. Table 5 below includes detailed interpretation of Internal Control results.

Table 5: Internal Control Results

Internal Control Result	Explanation	Action
PASS	The internal control or a target from each amplification reaction has generated signal above the threshold. The test was completed and internal controls were successful, indicating valid results were generated.	All results are displayed on the RP Panel Detection Report. Test is valid, report results.
FAIL	Neither the internal control nor any target in at least one amplification reaction generates signal above the threshold. The test was completed but at least one internal control was not detected, indicating that results are not valid.	No results are displayed on the RP Panel Detection Report. Test is not valid, repeat the test using a new cartridge.

Internal Control Result	Explanation	Action
N/A	The internal control in every amplification reaction does not generate signal above the threshold, but a target in every amplification reaction does generate signal above the threshold. The test was completed and internal controls were not successful, however detection of signal above the threshold for a target in every amplification reaction indicates valid results were generated.	All results are displayed on the RP Panel Detection Report. Test is valid, report results.
INVALID	An error has occurred during processing that prevents analysis of signal data. The test has not successfully completed and results for this test are not valid. This is often due to an instrument or software error.	No results are displayed on the RP Panel Detection Report. Test is not valid, repeat the test using a new cartridge.

External Controls

External controls are not provided with the ePlex RP Panel. However, during the prospective clinical evaluation of the ePlex RP Panel, each testing site was provided with positive and negative external control samples. There were four external positive controls (A, B, C, D) and one external negative control. The positive controls together represent all the targets on the ePlex RP Panel. On each day of testing with the ePlex RP Panel, each site tested at least one positive and one negative control. Sites rotated through the four positive controls throughout the study.

Table 6: External Controls Utilized in the Clinical Evaluations

External Controls	Expected Calls
Positive Control A	Adenovirus, Coronavirus, Influenza A H3, PIV 4, RSV A, and *C. pneumoniae*
Positive Control B	Adenovirus, Coronavirus, Human Metapneumovirus, Influenza A H1-2009, PIV 2, and RSV B
Positive Control C	Adenovirus, Coronavirus, Influenza A H1, PIV 1, and PIV
Positive Control D	Coronavirus, Human Rhinovirus/Enterovirus, Influenza B, and *M. pneumoniae*
Negative Control	Negative (Not Detected)

Over 104 days of investigational testing across five testing sites, there were 209 external controls tested with valid results: 27 Positive Control A, 24 Positive Control B, 27 Positive Control C, 25 Positive Control D, and 106 Negative Control. Of these, 203/209 had the expected results. The remaining six controls had the expected results except as follows:

- two Negative Controls each had one false positive (FP) result: one for Adenovirus, one for Coronavirus

- one Negative Control had four FP results: Coronavirus, Human Rhinovirus/Enterovirus, Influenza B, and *Mycoplasma pneumoniae*. It is likely the site inadvertently tested a Positive Control D sample and not a Negative Control since these four organisms are the expected results for Positive Control D
- two Positive Controls (A and B) each had a FP result for Human Rhinovirus/Enterovirus
- one Positive Control A had a false negative (FN) result for PIV 4.

The sponsor is also recommending the following in the product package insert: *"Positive and negative external controls should be tested with each new lot of reagents or monthly, whichever occurs first. Viral transport medium can be used as the negative control. Previously characterized positive samples or viral transport medium spiked with well characterized organisms can be used as the external positive control. External controls should be run in accordance with laboratory protocols and accrediting organizations, as applicable."*

Specimen Stability

The ePlex RP Panel package insert claims that clinical specimens can be stored at room temperature (15–30ºC) for up to 12 hours or refrigerated at 4°C for up to 10 days after collection in transport media. Specimens can also be stored at -20°C or -80°C for six months with up to two freeze/thaw cycles.

These clinical specimen stability claims are supported by analytical study which investigated the effect of different specimen storage conditions on specimen integrity (as determined by performance on the ePlex RP Panel).

A representative panel of six analytes (Table 7) was spiked into a pooled nasopharyngeal (NP) sample to approximately 1x LoD concentrations. It is noted that the six-analyte test sample generates seven results for the ePlex RP Panel.

Table 7: Representative Analytes Contained in Pooled NP Sample for Analytical Studies

Analyte Strain	Strain	Expected Positive Result on the ePlex RP Panel
Influenza A H3	H3N2 Brisbane/10/07	Flu A and Flu A H3
RSV A	2006 Isolate	RSV-A
Parainfluenza Virus 1	Clinical Isolate	PIV-1
Human Metapneumovirus	B2 Peru1-2002	hMPV
Coronavirus	OC43	Coronavirus
Adenovirus B	Type 7	Adenovirus

Baseline condition (T0) was established with 20 replicates. Each subsequent time point/test condition was assessed with 10 replicates unless otherwise noted. The overall study design (identifying all of the test conditions assessed in the study) is summarized in Table 8 below.

Table 8: Study Design – Specimen Storage and Testing Time points

Storage Condition	Time Points											
Ambient Storage Conditions												
15°C – 30°C	T0	4h	8h	12h	24h							
Refrigerated Storage Conditions												
2°C - 8°C	T0	1d	3d	5d	7d	10d	14d					
Frozen Storage Conditions												
-20°C & -80°C	T0	1w	2w	1m	3m	6m	12m	18m	24m	30m	36m	42m

Note: Shaded boxes indicate future time points that have not yet been tested.

The specimen stability study acceptance criteria were the following:

- Performance at each time point will be considered acceptable if the positivity rate for each analyte (either from the first 10 replicates or from the final 20 replicates in case of additional testing) is equivalent to the positivity rate for the corresponding analyte at time point T0 (baseline time point). "Equivalent" is defined as positivity rates that differ by 5% or less.
- If a time point did not meet the positivity acceptance criteria, the next time point was evaluated. If the subsequent time point met the acceptance criteria, the study was continued. If two consecutive time points failed, the specimen stability was determined to be the last time point when the acceptance criteria were met.

The specimen stability assessment data are summarized in Table 9 to Table 13 below.

Table 9: Ambient Storage Conditions (Positivity and Mean Signal)

Target	T0		4 Hrs		8 Hrs		12 Hrs		24 Hrs	
	% Pos	Mean nA	% Pos	Mean nA	% Pos	Mean nA	% Pos	Mean nA	% Pos	Mean nA
Flu A H3	100	279.2	100	318.3	100	306.1	100	309.0	100	158.9
Flu A	100	852.2	100	872.2	100	659.9	100	651.2	100	576.0
RSV-A	100	619.0	100	669.6	100	567.6	100	604.4	95	500.5
hMPV	100	421.9	100	448.6	100	373.5	100	404.1	85	259.8
PIV-1	100	619.5	100	645.3	100	629.6	100	661.8	95	388.2
Adv B	100	376.1	100	405.9	100	299.4	100	361.6	95	296.7
CoV	100	776.0	100	820.7	100	745.8	100	790.6	100	776.8

Table10: Refrigerated Storage Conditions (Positivity)

Target	% Positivity						
	T0	1 Day	3 Days	5 Days	7 Days	10 days	14 days
Flu A H3	100	100	100	100	100	100	100
Flu A	100	100	100	100	100	100	100
RSV-A	100	100	100	100	100	100	100
hMPV	100	100	100	100	100	100	95
PIV-1	100	100	100	100	100	100	100
Adv B	100	100	100	100	100	100	90
CoV	100	100	100	100	100	100	100

Table 11: Refrigerated Storage Conditions (Mean Signal)

Target	Mean Signal (nA)						
	T0	1 Day	3 Days	5 Days	7 Days	10 days	14 days
Flu A H3	330.6	159.6	270.5	269.1	322.0	351.0	174.6
Flu A	725.4	691.8	719.3	586.1	844.0	762.7	546.7
RSV-A	589.3	522.0	589.9	577.8	600.2	588.5	462.8
hMPV	404.0	361.4	347.9	328.8	391.7	352.4	245.3
PIV-1	475.6	613.1	453.8	576.9	413.7	558.9	411.2
Adv B	328.7	277.5	319.1	248.2	341.4	280.2	187.1
CoV	767.1	669.9	752.2	696.8	815.8	802.1	531.0

Table 12: Frozen Storage Conditions (Positivity)

Target	T0	1 Week		2 Weeks		1 Month		3 Months		6 Months	
		-20°C	-80°C	-20°C	-80°C	-20°C	-80°C	-20°C	-80°C	-20°C	-80°C
Flu A H3	100	100	100	100	100	100	100	100	100	100	100
Flu A	100	100	100	100	100	100	100	100	100	100	100
RSV-A	100	100	95*	100	100	100	100	100	100	100	100
hMPV	100	100	100	100	100	100	100	100	100	100	100
PIV-1	100	100	100	100	100	100	100	100	100	100	100
Adv B	100	100	100	100	100	100	100	100	100	100	100
CoV	100	100	100	100	100	100	100	100	100	100	95

* When the first 10 replicates were tested at the one week time point, RSV-A was missed in 1/10 replicates. Ten additional replicates were tested and the combined percent positivity for RSV-A was 95%.

Table 13: Frozen Storage Conditions (Mean Signal)

Target	T 0	1 Week		2 Weeks		1 Month		3 Months		6 Months	
		-20°C	-80°C	-20°C	-80°C	-20°C	-80°C	-20°C	-80°C	-20°C	-80°C
Flu A H3	330.6	260.7	281.3	282.6	209.6	249.5	301.9	286.2	274.0	308.4	349.0
Flu A	725.4	901.0	665.5	595.7	626.0	621.6	723.5	574.4	640.2	714.7	708.1
RSV-A	589.3	587.7	518.8	497.5	516.0	525.9	561.7	458.9	515.7	523.2	567.2
hMPV	404.0	395.2	384.1	345.2	369.0	298.8	318.0	251.7	254.0	267.2	298.0
PIV-1	475.6	422.0	465.6	577.1	567.7	464.4	555.1	422.8	470.1	484.7	478.9
Adv B	328.7	349.6	324.1	303.2	296.2	272.5	273.1	234.4	226.0	237.7	265.5
CoV	767.1	802.9	987.1	666.5	291.6	621.1	677.2	476.3	515.1	473.2	487.2

The data from the specimen stability studies supported the following specimen stability claims:

- storage under ambient conditions (15–30ºC) for up to 12 hours in VTM

- storage under refrigerated conditions (4ºC) for up to 10 days in VTM
- storage under frozen conditions (-20°C and -80°C) for up to six months

Fresh vs. Frozen Study

An analytical study was conducted to demonstrate clinical specimen stability after subjecting samples to one or two freeze-thaw cycles. A representative panel of six analytes (Table 7 above) was spiked into pooled negative NPS in VTM to approximately 1x LoD concentrations and tested fresh after one and after two freeze-thaw cycles at 10 replicates each.

The specimen freeze-thaw study results are summarized in Table 14 below.

Table 14: Freeze-Thaw Study (Positivity and Mean Signal)

Target	T0		T1		T2	
	% Pos	Mean nA	% Pos	Mean nA	% Pos	Mean nA
Flu A H3	100	229.6	100	355.3	100	379.1
Flu A	100	790.6	100	720.7	100	714.0
RSV-A	100	622.7	100	588.6	100	593.9
hMPV	100	424.6	100	389.5	100	410.1
PIV-1	100	585.6	100	385.4	100	355.1
Adv B	100	365.2	100	351.9	100	361.4
CoV	100	816.4	100	703.4	100	685.1

The data from the specimen freeze-thaw study supported the following specimen stability claim:

- specimens in VTM are stable after two freeze-thaw cycles

In-Cartridge Sample Stability Study

An analytical study was carried out to demonstrate that the cartridge can be stored at room temperature (15–30ºC) for two hours after the sample has been loaded prior to running the cartridge in an ePlex bay.

A representative panel of six analytes (Table 7 above) was spiked into pooled negative NPS as matrix. Each target mix was tested at a final concentration of approximately 1x LoD. Forty (40) cartridges were loaded following package insert

instructions. Twenty (20) of these cartridges were run within 10 minutes of loading; the other 20 cartridges were kept for two hours at room temperature and then run following package insert instructions.

The acceptance criteria for this analytical study were the following:

- The sample is stable at room temperature after it has been loaded into the cartridge for up to 120 minutes prior to running the cartridge. Stability is defined as equal positivity rates for each target in each of the tested conditions that will be compared to each other. Equivalent positivity rate is defined as positivity rates that differ by 5% or less.

The in-cartridge sample stability study results are summarized in Table 15 and Table 16 below.

Table 15: In-Cartridge Sample Stability Study Comparison of Positivity Rate at 10 and 120 Minutes

Target	% Positive	
	Sample loaded after 10 min	Sample loaded after 120 min
Flu A H3	100% (20/20)	95% (19/20)
Flu A	100% (20/20)	100% (20/20)
RSV-A	100% (20/20)	100% (20/20)
hMPV	100% (20/20)	95% (19/20)
PIV-1	100% (20/20)	100% (20/20)
Adv B	100% (20/20)	100% (20/20)
CoV	100% (20/20)	95% (19/20)

Table 16: In-Cartridge Sample Stability Study Comparison of Mean and Mean minus 2 SD at 10 and 120 Minutes

Target	Cutoff (nA)	Sample loaded after 10 min		Sample loaded after 120 min	
		mean signal (nA)	mean signal minus 2SD (nA)	mean signal (nA)	mean signal minus 2SD (nA)
Flu A H3	5	314.8	181.2	225.0	10.1
Flu A	20	712.9	420.9	688.6	568.9
RSV-A	10	588.5	535.6	580.1	438.7
hMPV	5	413.0	313.7	353.8	167.7
PIV-1	10	542.5	333.3	561.0	369.1
Adv B	10	339.6	254.9	382.7	235.3
CoV	10	774.8	558.8	605.5	270.8

The study results demonstrated that the cartridge can be stored at room temperature (15–30ºC) for two hours after the sample has been loaded prior to running the cartridge in an ePlex bay.

Simulated vs. Natural NPS in VTM Specimen Study

An analytical study was conducted to verify sample matrix equivalency between Remel M5 VTM (simulated matrix) and pooled negative nasopharyngeal swabs (NPS) collected in commercially available VTM (natural matrix) for the ePlex RP Panel for targets that are tested at 10x LoD and above.

A representative panel of six analytes (Table 7 above) was spiked either into Remel M5 VTM or pooled negative NPS at a final concentration of approximately 10x LoD. A negative control was run in duplicate for VTM and the NPS pool. Twenty (20) cartridge runs of spiked NPS matrix and 20 cartridge runs of spiked VTM were performed, as were two negative controls with each set of 20 runs (i.e., 44 cartridge runs were performed).

The acceptance criteria for this analytical study were the following:

- The assay will be determined to not be impacted by sample matrix if the positivity rate for each target is equivalent to the positivity rate for the corresponding target in the simulated matrix control. Equivalent is defined as positivity rates that differ by 5% or less.

The sample matrix equivalence study results are summarized in Table 17 below.

Table 17: Sample Matrix Equivalence Study Positivity rate and mean signals for all targets in VTM and NPS

Target	Remel M5 VTM				NPS in VTM				Cut off in nA	% difference in positivity rate
	% positive	Mean (nA)	SD (nA)	Mean nA-	% positive	Mean (nA)	SD (nA)	Mean nA-2SD		
Flu A H3	100% (20/20)	308.9	125.5	57.9	100% (20/20)	317.8	97.8	122.2	5	0%
Flu A	100% (20/20)	724.5	93.7	537.1	100% (20/20)	763.0	112.6	537.8	20	0%
RSV-A	100% (20/20)	631.3	98.6	434.1	100% (20/20)	623.7	79.0	465.7	10	0%
hMPV	100% (20/20)	466.2	77.6	311	100% (20/20)	474.1	71.7	330.8	5	0%
PIV-1	100% (20/20)	484.5	133.5	217.5	100% (20/20)	502.9	138.3	226.3	10	0%
Adv B	100% (20/20)	371.0	47.2	276.6	100% (20/20)	354.3	52.4	249.5	10	0%
CoV	100% (20/20)	790.3	90.2	609.9	100% (20/20)	736.2	106.7	522.8	10	0%

The results from the sample matrix equivalency study demonstrated the equivalency between Remel M5 VTM and natural nasopharyngeal swabs (NPS) collected in Remel M5 VTM for targets at approximately 10x LoD.

d. Detection limit:

The limit of detection (LoD), or analytical sensitivity was identified and verified for each viral and bacterial target on the ePlex RP Panel using quantified reference strains/isolates. Serial dilutions were prepared in a natural clinical matrix (pooled, negative nasopharyngeal swab in VTM samples) with one or more organisms per series, and at least 20 replicates per target were tested in the verification. The limit of detection was defined as the lowest concentration at which each target is detected at least 95% of the time. The confirmed LoD for each ePlex RP Panel organism is shown in Table 18 below.

Table 18: LoD Results Summary

Target	Strain	LoD Concentration
Adenovirus	Type 1 (C)	1.0E+03 $TCID_{50}$/mL
	Type 4 (E)	2.0E+00 $TCID_{50}$/mL
	Type 7 (B)	2.0E+00 $TCID_{50}$/mL
Coronavirus 229E	229E	1.0E+00 $TCID_{50}$/mL
Coronavirus HKU1	HKU1[a]	5.0E+04 copies/mL
Coronavirus NL63	NL63	7.5E+00 $TCID_{50}$/mL
Coronavirus OC43	OC43	5.0E+02 $TCID_{50}$/mL
Human Metapneumovirus	A1 IA3-2002	2.0E-01 $TCID_{50}$/mL
	A2 IA14-2003	2.0E+03 $TCID_{50}$/mL
	B1 Peru2-2002	2.0E+02 $TCID_{50}$/mL
	B2 Peru1-2002	2.3E+02 $TCID_{50}$/mL
Human Rhinovirus/Enterovirus	Enterovirus Type 68 (2007)	1.0E+00 $TCID_{50}$/mL
	Rhinovirus 1A	1.5E+00 $TCID_{50}$/mL
	Rhinovirus B14	1.0E+00 $TCID_{50}$/mL
	Rhinovirus C [a]	1.0E+05 copies/mL
Influenza A	H1N1 Brisbane/59/07	3.0E-01 $TCID_{50}$/mL
Influenza A H1	H1N1 Brisbane/59/07	3.0E-01 $TCID_{50}$/mL
Influenza A H1-2009	NY/01/2009	1.0E-01 $TCID_{50}$/mL
Influenza A H3	A/Perth/16/2009	1.0E+01 $TCID_{50}$/mL
	A/Texas/50/2012	1.0E+00 $TCID_{50}$/mL
	A/Victoria/361/2011	5.0E-01 $TCID_{50}$/mL
	H3N2 Brisbane/10/07	5.0E+01$TCID_{50}$/mL
Influenza B	Florida/02/06	1.0E-01 $TCID_{50}$/mL
Influenza B (Victoria Lineage)	B/Brisbane/60/2008	1.0E+00 $TCID_{50}$/mL
	B/Montana/5/2012	1.0E+00 $TCID_{50}$/mL
	B/Nevada/03/2011	1.0E+00 $TCID_{50}$/mL
Influenza B (Yamagata Lineage)	B/Massachusetts/02/2012	1.0E+02 $TCID_{50}$/mL
	B/Texas/06/2011	1.0E-01 $TCID_{50}$/mL
	B/Wisconsin/01/2010	1.0E+00 $TCID_{50}$/mL
Parainfluenza Virus 1	Clinical Isolate	4.0E-01 $TCID_{50}$/mL
Parainfluenza Virus 2	Clinical Isolate	5.0E+01 $TCID_{50}$/mL
Parainfluenza Virus 3	Clinical Isolate	5.0E+00 $TCID_{50}$/mL
Parainfluenza Virus 4	4a	3.0E+01 $TCID_{50}$/mL
Respiratory Syncytial Virus A	2006 Isolate	1.5E+00 $TCID_{50}$/mL
Respiratory Syncytial Virus B	CH93(18)-18	2.0E-01 $TCID_{50}$/mL
Chlamydia pneumoniae	AR-39	3.0E+02 $TCID_{50}$/mL
Mycoplasma pneumoniae	FH strain of Eaton Agent [NCTC 10119]	3.0E+02 CCU/mL

[a] Clinical samples confirmed positive for coronavirus HKU1 and human rhinovirus C by bi-directional sequencing and quantified by real-time RT-PCR were used for determination of LoD.

e. Analytical Reactivity:

A panel of 101 strains/isolates representing the genetic, temporal, and geographic diversity of each target on the ePlex RP Panel was evaluated to demonstrate analytical reactivity. Each strain/isolate was tested in triplicate at 3x LoD in natural clinical matrix (pooled, negative nasopharyngeal swab in VTM samples); if the organism was not detected at this concentration, testing of higher concentrations was performed. Additional *in silico* analysis was also performed on a subset of ePlex RP Panel organisms.

Results of analytical reactivity study, including *in silico* analysis results, are shown in Tables 19 to Table 29.

Table 19: Analytical Reactivity (Inclusivity) Results for Adenovirus

Adenovirus Species	Serotype	Concentration	Multiple of LoD Detected
A	Type 31	3.0E+03 $TCID_{50}$/mL	3x
B	Type 3	6.0E+00 $TCID_{50}$/mL	3x
	Type 11	6.0E+00 $TCID_{50}$/mL	3x
	De Wit Type 14	6.0E+00 $TCID_{50}$/mL	3x
	Ch.79 Type 16	2.0E+02 $TCID_{50}$/mL	100x [a]
	Type 21	6.0E+00 $TCID_{50}$/mL	3x
	Compton Type 34	6.0E+00 $TCID_{50}$/mL	3x
	Holden Type 35	6.0E+00 $TCID_{50}$/mL	3x
	Wan Type 50	2.0E+01 $TCID_{50}$/mL	10x [b]
C	Type 2	3.0E+03 $TCID_{50}$/mL	3x
	Type 5	3.0E+03 $TCID_{50}$/mL	3x
	Type 6	3.0E+03 $TCID_{50}$/mL	3x
D	Type 26	3.0E+03 $TCID_{50}$/mL	3x
	Type 37	3.0E+03 $TCID_{50}$/mL	3x
F	Type 40 Dugan	3.0E+03 $TCID_{50}$/mL	3x
	Type 41/ Strain Tak	3.0E+03 $TCID_{50}$/mL	3x

[a] *In silico* analysis revealed good homology to primers and probes. Lower sensitivity is likely the result of incorrect estimation of genetic material present in the culture of this or the reference strain ($TCID_{50}$ value is based only on infectious virus particles).

[b] *In silico* analysis revealed that lower sensitivity may be a result of mismatches in the assay primers and/or probes.

Table 20: Analytical Reactivity (Inclusivity) Results for Human Metapneumovirus

Metapneumovirus Subtype	Strain	Concentration	Multiple of LoD Detected
Human metapneumovirus	Peru6-2003 G, B2	6.8E+02 $TCID_{50}$/mL	3x

Table 21: Analytical Reactivity (Inclusivity) Results for Human Rhinovirus/Enterovirus

Rhinovirus/Enterovirus	Strain	Concentration	Multiple of LoD Detected
Human Rhinovirus	Type A2	4.5E+00 $TCID_{50}$/mL	3x
	Type A7	1.5E+01 $TCID_{50}$/mL	10x [a]
	Type A16	4.5E+00 $TCID_{50}$/mL	3x
	Type A18	1.5E+02 $TCID_{50}$/mL	100x [a]
	Type A34	4.5E+00 $TCID_{50}$/mL	3x
	Type A57	4.5E+00 $TCID_{50}$/mL	3x
	Type A77	4.5E+00 $TCID_{50}$/mL	3x
	277G	4.5E+00 $TCID_{50}$/mL	3x
	Type B3	1.5E+01 $TCID_{50}$/mL	10x [a]
	Type B17	1.5E+01 $TCID_{50}$/mL	10x [a]
	Type B42	4.5E+00 $TCID_{50}$/mL	3x
	Type B83	4.5E+00 $TCID_{50}$/mL	3x
	Type B84	4.5E+00 $TCID_{50}$/mL	3x
	FO2-2547	4.5E+00 $TCID_{50}$/mL	3x
Enterovirus	Type 71	3.0E+00 $TCID_{50}$/mL	3x
Coxsackievirus	A9	3.0E+00 $TCID_{50}$/mL	3x
	A10	3.0E+00 $TCID_{50}$/mL	3x
	A21	3.0E+00 $TCID_{50}$/mL	3x
	A24	3.0E+00 $TCID_{50}$/mL	3x
	B2	1.0E+02 $TCID_{50}$/mL	100x [a]
	B3	3.0E+00 $TCID_{50}$/mL	3x
	B4	3.0E+00 $TCID_{50}$/mL	3x
	B5	1.0E+01 $TCID_{50}$/mL	10x [a]
Echovirus	9	3.0E+00 $TCID_{50}$/mL	3x
	E6	1.0E+01$TCID_{50}$/mL	10x [b]
	25	1.0E+01$TCID_{50}$/mL	10x [a]
	30	3.0E+00 $TCID_{50}$/mL	3x
Poliovirus	1	1.0E+02 $TCID_{50}$/mL	100x [a]

[a] *In silico* analysis revealed that lower sensitivity may be a result of mismatches in the assay primers and/or probes.
[b] *In silico* analysis revealed good homology to primers and probes. Lower sensitivity is likely the result of incorrect estimation of genetic material present in the culture of this or the reference strain ($TCID_{50}$ value is based only on infectious virus particles).

Table 22: Analytical Reactivity (Inclusivity) Results for Influenza A

Note: Due to different assays for influenza A matrix and influenza A subtypes on the ePlex RP Panel, if different LoDs are observed for inclusivity for a Flu A matrix vs. a subtype, the differences are noted in the Multiple of LoD Detected column.

Influenza A Subtype	Strain	Concentration	Multiple of LoD Detected
Influenza A H1	A/FM/1/47	3.0E+00 $TCID_{50}$/mL	10x (Influenza A matrix) [a] 10000x H1 subtype [b]
	A/New Caledonia/20/1999	9.0E-01 $TCID_{50}$/mL	3x
	A/New Jersey/8/76	9.0E-01 $TCID_{50}$/mL	3x (Influenza A matrix) H1 subtype not detected [c]
	A/NWS/33	3.0E+00 $TCID_{50}$/mL	10x (Influenza A matrix) [a] H1 subtype not detected [d]
	A/PR/8/34	9.0E-01 $TCID_{50}$/mL	3x (Influenza A matrix) H1 subtype not detected [e]
	A/Solomon Islands/3/2006	9.0E-01 $TCID_{50}$/mL	3x
	A/Taiwan/42/06	9.0E+00 $TCID_{50}$/mL	30x [f]
Influenza A H3	A/Hong Kong/8/68	1.5E+02 $TCID_{50}$/mL	3x
	A/Port Chalmers/1/73		
	A/Nanchang/933/95		
	A/Victoria/3/75		
	A/Wisconsin/67/05		
Influenza A 2009 H1N1	A/California/7/2009	1.0E+00 $TCID_{50}$/mL	10x [g]
	A/Mexico/4108/09	3.0E-01 $TCID_{50}$/mL	3x
	A/NY/02/2009	1.0E+00 $TCID_{50}$/mL	10x [h]
	A/Swine NY/03/2009	3.0E-01 $TCID_{50}$/mL	3x
	A/Swine/Iowa/15/30	3.0E-01 $TCID_{50}$/mL	3x (Influenza A matrix) 100,000x (H1-2009 subtype) [i]
	A/Virginia/ATCC1/2009	1.0E+00 $TCID_{50}$/mL	10x [j]
	A/Virginia/ATCC2/2009	1.0E+01 $TCID_{50}$/mL	100x [j]
	A/Virginia/ATCC3/2009	1.0E+02 $TCID_{50}$/mL	1,000x [j]

[a] *In silico* analysis revealed good homology to primers and probes. Lower sensitivity is likely the result of incorrect estimation of genetic material present in the culture of this or the reference strain ($TCID_{50}$ value is based only on infectious virus particles).

[b] *In silico* analysis revealed that lower sensitivity may be a result of mismatches in the assay primers and/or probes.

[c] H1-2009 subtype was detected in this seasonal influenza A H1 strain at 30x LoD.

[d] *In silico* analysis revealed little homology between this non-contemporary strain sequence and the H1 signal probe/capture probe sequences.

[e] *In silico* analysis revealed little homology between this non-contemporary influenza strain sequence and the H1 primer sequences.

[f] For Influenza A matrix, *in silico* analysis revealed good homology to primers and probes. Lower sensitivity is likely the result of incorrect estimation of genetic material present in the culture of this or the reference strain ($TCID_{50}$ value is based only on infectious virus particles). For H1 subtype, *in silico* analysis revealed that lower sensitivity may be a result of mismatches in the assay primers and/or probes.

[g] For Influenza A matrix, *in silico* analysis revealed that lower sensitivity may be a result of mismatches in the assay primers and/or probes. For H1 subtype, *in silico* analysis revealed good homology to primers and probes. Lower sensitivity is likely the result of incorrect estimation of genetic material present in the culture of this or the reference strain ($TCID_{50}$ value is based only on infectious virus particles).

[h] For Influenza A matrix, *in silico* analysis revealed good homology to primers and probes. Lower sensitivity is likely the result of incorrect estimation of genetic material present in the culture of this or the reference strain

($TCID_{50}$ value is based only on infectious virus particles). For H1-2009 subtype, *in silico* analysis revealed that lower sensitivity may be a result of mismatches in the assay primers and/or probes.

[i] *In silico* analysis revealed little homology between the strain sequence and the H1 or H1-2009 primer, signal probe and capture probe sequences.

[j] No sequence data was available to investigate lower sensitivity of the influenza A 2009 H1N1 A/Virginia/ATCC1/2009, A/Virginia/ATCC2/2009 and A/Virginia/ATTC3/2009 strains.

Table 23: Analytical Reactivity (Inclusivity) Results for Influenza A Strains Titered with Methods Different From the Reference Strain

Influenza A Subtype	Strain	Concentration Deteced
Influenza A H1	A/Denver/1/57	1.6E+02 $CEID_{50}$/mL (Influenza A matrix) 1.6E+08 $CEID_{50}$/mL (H1 subtype)
	A/Mal/302/54	1.6E+02 $CEID_{50}$/mL (Influenza A matrix) 1.6E+05 $CEID_{50}$/mL (H1 subtype)
Influenza A H3	A/Aichi/2/68 H3N2	1.6E+03 $CEID_{50}$/mL
	Alice (vaccine) A/England/42/72	5.0E+00 EID_{50}/mL (Influenza A matrix) 5.00E+01 EID_{50}/mL (H3 subtype)
	MRC-2 Recombinant Strain	8.9E+02 $CEID_{50}$/mL (Influenza A matrix) 8.9E+03 $CEID_{50}$/mL (H3 subtype)
Influenza A H1N1	A/Washington/24/2012 (A/H1 pdm09)	3.2E+03 EID_{50}/mL (Influenza A matrix) 3.2E+02 EID_{50}/mL (H1-2009 subtype)
Influenza A H1N2	Kilbourne F63: A/NWS/34 (HA) x A/Rockefeller Institute/5/57 (NA), Reassortant NWS-F- Matrix	8.89 x 10^1 $CEID_{50}$/mL (Influenza A matrix) No subtype detected [a]
Influenza A H5N8	A/Gyrfalcon/Washington/41088-6/2014 BPL	1.6E+03 EID_{50}/mL (Influenza A matrix) No subtype detected [b]
Influenza A H5N2	A/Northern Pintail/Washington/40964/2014 BPL	2.5E+03 EID_{50}/mL (Influenza A matrix) No subtype detected [b]
Influenza A H7N9	A/ANHUI/1/2013	7.9E+03 EID_{50}/mL (Influenza A matrix) No subtype detected [c]
Influenza A H3N2v	A/Indiana/21/2012	2.5E+04 EID_{50}/mL (Influenza A matrix and H3 subtype)

[a] *In silico* analysis revealed little homology between this non-contemporary strain sequence and the H1 Signal Probe/Capture Probe sequences.

[b] Detection of the H5 Subtype not expected

[c] Detection of the H7 Subtype not expected

NOTE: $CEID_{50}$/mL= Chick Embryo Infectious Dose; EID_{50}/mL= Egg Infectious Dose

For human, avian, and swine influenza A strains that were not available for wet testing on the ePlex RP Panel, *in silico* analysis was performed. Bioinformatics analysis was used to predict a result based on the number and location of mismatches in the primers, capture probes, and signal probes found in the ePlex RP Panel relative to an alignment of GenBank sequences. The *in silico* analysis results are summarized in Table 24 below.

Table 24: Predicted (*in silico*) Reactivity (Inclusivity) Results for Influenza A

Influenza A Subtype	Host	Strain	GenBank ID	Predicted ePlex Result
H2N2	Human	A/Albany/20/1957(H2N2)	CY022014	Influenza A
		Kilbourne F38: A/Korea/426/68 (HA, NA) x A/Puerto Rico/8/34	CY037296	Influenza A
	Avian	A/chicken/New York/13828-3/1995(H2N2)	CY014822	Influenza A
		A/Japan/305/1957(H2N2)	CY014977	Influenza A
		A/Korea/426/1968(H2N2)	CY031596	Influenza A
H4N6	Avian	A/Blue-winged teal/Minnesota/Sg-00043/2007(H4N6)	CY063978	Influenza A
H5N1		A/Peregrine falcon/Aomori/7/2011	AB629716	Influenza A
		A/Chicken/West Bengal/239022/2010	CY061305	Influenza A
		A/Chicken/West Bengal/193936/2009	GU272009	Influenza A
		A/Chicken/Hunan/1/2009	HM172150	Influenza A
		A/Chicken/Hunan/8/2008	GU182162	Influenza A
		A/Chicken/West Bengal/106181/2008	GU083632	Influenza A
		A/Chicken/Primorsky/85/2008	FJ654298	Influenza A
		A/Chicken/West Bengal/82613/2008	GU083648	Influenza A
		A/Duck/France/080036/2008	CY046185	Influenza A
		A/Duck/Vietnam/G12/2008	AB593450	Influenza A
		A/Chicken/Thailand/PC-340/2008	EU620664	Influenza A
		A/Great egret/Hong Kong/807/2008	CY036240	Influenza A
		A/Rook/Rostov-on-Don/26/2007(H5N1)	EU814504	Influenza A
		A/Turkey/VA/505477-18/2007(H5N1)	GU186510	Influenza A
		A/Chicken/Bangladesh/1151-10/2010(H5N1)	HQ156766	Influenza A
	Human	A/Bangladesh/3233/2011	CY088772	Influenza A
		A/Cambodia/R0405050/2007(H5N1)	HQ200572	Influenza A
		A/Cambodia/S1211394/2008	HQ200597	Influenza A
		A/Hong Kong/486/97(H5N1)	AF255368	Influenza A
	Swine	A/Swine/East Java/UT6010/2007(H5N1)	HM440124	Influenza A
H5N2	Avian	A/Duck/Pennsylvania/10218/1984(H5N2)	AB286120	Influenza A
		A/American black duck/Illinois/08OS2688/2008	CY079453	Influenza A
		A/American green-winged teal/California/HKWF609/2007	CY033447	Influenza A
		A/Canada goose/New York/475813-2/2007	GQ923358	Influenza A
		A/Blue-winged teal/Saskatchewan/22542/2007	CY047705	Influenza A
		A/Chicken/Taiwan/A703-1/2008	AB507267	Influenza A
		A/Duck/France/080032/2008	CY046177	Influenza A
		A/Duck/New York/481172/2007	GQ117202	Influenza A
		A/Gadwall/Altai/1202/2007	CY049759	Influenza A
		A/Mallard/Louisiana/476670-4/2007	GQ923390	Influenza A
		A/Waterfowl/Colorado/476466-2/2007	GQ923374	Influenza A
H5N3	Avian	A/Duck/Singapore/F119/3/1997(H5N3)	GU052803	Influenza A
H6N1		A/Duck/PA/486/1969(H6N1)	EU743287	Influenza A

Influenza A Subtype	Host	Strain	GenBank ID	Predicted ePlex Result
H6N2		A/Mallard/Czech Republic/15902-17K/2009(H6N2)	HQ244433	Influenza A
H7N2	Avian	A/Chicken/Hebei/1/2002	AY724263	Influenza A
		A/Chicken/PA/149092-1/02	AY241609	Influenza A
		A/Chicken/NJ/294508-12/2004	EU743254	Influenza A
		A/Chicken/New York/23165-6/2005	CY031077	Influenza A
		A/Muscovy duck/New York/23165-13/2005	CY033226	Influenza A
		A/Muscovy duck/New York/87493-3/2005	CY034791	Influenza A
		A/Mallard/Netherlands/29/2006	CY043833	Influenza A
		A/Northern shoveler/California/JN1447/2007	CY076873	Influenza A
	Human	A/New York/107/2003(H7N2)	EU587373	Influenza A
H7N3		A/Canada/rv504/2004(H7N3)	CY015007	Influenza A
H7N7	Avian	A/American green-winged teal/Mississippi/09OS046/2009	CY079309	Influenza A
		A/Chicken/Germany/R28/03	AJ619676	Influenza A
		A/Chicken/Netherlands/1/03	AY340091	Influenza A
		A/Mallard/California/HKWF1971/2007	CY033383	Influenza A
		A/Mallard/Korea/GH171/2007	FJ959087	Influenza A
		A/Mute swan/Hungary/5973/2007	GQ240816	Influenza A
		A/Northern shoveler/Mississippi/ 09OS643/2009	CY079413	Influenza A
	Human	A/Netherlands/219/03(H7N7)	AY340089	Influenza A
H7N9	Human	A/Shanghai/1/2013(H7N9)	EPI439493	Influenza A
	Avian	A/Northern shoveler/Mississippi/11OS145/2011(H7N9)	CY133650	Influenza A
		A/Ruddy turnstone/Delaware Bay/220/1995(H7N9)	CY127254	Influenza A
		A/Turkey/Minnesota/1/1988(H7N9)	CY014787	Influenza A
		A/Blue-winged teal/Ohio/566/2006(H7N9)	CY024819	Influenza A
H9N2	Human	A/Hong Kong/1073/99(H9N2)	AJ278647	Influenza A
	Avian	A/Turkey/Wisconsin/1/1966(H9N2)	CY014664	Influenza A
H10N7		A/chicken/Germany/N/1949(H10N7)	GQ176135	Influenza A
H11N9		A/Duck/Memphis/546/1974(H11N9)	GQ257441	Influenza A
H1N1	Swine	A/Swine/Wisconsin/1/1971(H1N1)	CY022414	Influenza A
	Human	A/California/UR06-0393/2007(H1N1)	CY026540	Influenza A H1
			CY026539	
H1N2		A/New York/297/2003(H1N2)	CY002664	Influenza A H1
			CY002665	
H1N1 (2009)		A/Aalborg/INS133/2009(H1N1)	CY063606	Influenza A H1-2009
			CY063607	
		A/South Carolina/02/2010(H1N1)	KC781370	Influenza A H1-2009
			KC781372	
H1N2	Swine	A/Swine/Hong Kong/NS857/2001(H1N2)	GQ229350	Influenza A
		A/Swine/Sweden/1021/2009(H1N2)	GQ495135	Influenza A

Influenza A Subtype	Host	Strain	GenBank ID	Predicted ePlex Result
H3N1	Avian	A/Blue-winged teal/ALB/452/1983(H3N1)	CY004635	Influenza A
H3N2v	Human	A/Iowa/07/2011(H3N2)	JQ070760	Influenza A H3
			JQ290177	
		A/Iowa/08/2011(H3N2)	JQ070768	Influenza A H3
			JQ290167	
		A/Iowa/09/2011(H3N2)	JQ070776	Influenza A H3
			JQ290183	
		A/Indiana/08/2011(H3N2)	JQ070800	Influenza A H3
			JQ070795	
		A/Maine/06/2011(H3N2)	JN866181	Influenza A H3
			JN866186	
		A/Maine/07/2011(H3N2)	JN992746	Influenza A
		A/Pennsylvania/09/2011(H3N2)	JN655534	Influenza A
		A/Pennsylvania/11/2011(H3N2)	JN655540	Influenza A
		A/Pennsylvania/10/2011(H3N2)	JN655550	Influenza A
		A/West Virginia/06/2011(H3N2)	JQ290159	Influenza A H3
			JQ290164	
		A/West Virginia/07/2011(H3N2)	JQ348839	Influenza A
		A/Indiana/10/2011(H3N2)	KJ942592	Influenza A H3
			JQ070787	
		A/Boston/38/2008(H3N2)	CY044580	Influenza A H3
			CY044581	
	Swine	A/swine/NY/A01104005/2011(H3N2v)	JN940422	Influenza A H3
		A/Maine/06/2011(H3N2)	JN866181	Influenza A H3
			JN866186	Influenza A H3
		A/Indiana/08/2011(H3N2)	JN655558	Influenza A H3
			JN638733	
	Avian	A/American black duck/North Carolina/675-075/2004(H3N2)	GU051135	Influenza A
			GU051136	Influenza A
H3N5		A/Mallard/Netherlands/2/1999(H3N5)	CY060261	Influenza A
			CY060264	Influenza A
H3N6		A/American black duck/New Brunswick/25182/2007(H3N6)	CY047696	Influenza A
			CY047697	Influenza A
H3N7		A/Northern shoveler/California/HKWF1367/2007(H3N7)	CY033372	Influenza A
			CY033375	Influenza A
H3N8		A/American black duck/Washington/699/1978(H3N8)	GU052300	Influenza A H3
			GU052299	

Table 25: Analytical Reactivity (Inclusivity) Results for Influenza B

Influenza B Subtype	Strain	Concentration	Multiple of LoD Detected
Influenza B (Yamagata Lineage)	B/Lee/40	3.0E-01 $TCID_{50}$/mL	3x
	B/Allen/45	1.0E+00 $TCID_{50}$/mL	10x [a]
	B/Maryland/1/59	1.0E+01 $TCID_{50}$/mL	100x [a]
	B/Taiwan/2/62	1.0E+01 $TCID_{50}$/mL	100x [a]
Influenza B (Victoria Lineage)	B/Hong Kong/5/72	1.0E+01 $TCID_{50}$/mL	100x [b]
	B/Malaysia/2506/04	3.0E-01 $TCID_{50}$/mL	3x
Influenza B (Lineage unknown)	B/GL/1739/54	3.0E-01 $TCID_{50}$/mL	3x

[a] No sequence data available. Lower sensitivity may be a result of mismatches in the assay primers and/or probes. In addition, the reduced sensitivity may be the result of incorrect estimation of genetic material present in the culture of this or the reference strain ($TCID_{50}$ value is based only on infectious virus particles).
[b] *In silico* analysis revealed that lower sensitivity may be a result of mismatches in the assay primers and/or probes.

Table 26: Analytical Reactivity (Inclusivity) Results for Parainfluenza Virus

Parainfluenza Subtype	Strain	Concentration	Multiple of LoD Detected
Parainfluenza Virus 1	C35	1.2E+00 $TCID_{50}$/mL	3x
Parainfluenza Virus 2	Greer	1.5E+02 $TCID_{50}$/mL	3x
Parainfluenza Virus 3	C-243	5.0E+01 $TCID_{50}$/mL	10x [a]
Parainfluenza Virus 4	4b	9.0E+01 $TCID_{50}$/mL	3x

[a] *In silico* analysis revealed that lower sensitivity may be a result of mismatches in the assay primers and/or probes.

Table 27: Analytical Reactivity (Inclusivity) Results for Respiratory Syncytial Virus

RSV Subtype	Strain	Concentration	Multiple of LoD Detected
Respiratory Syncytial Virus A	A2	4.5E+00 $TCID_{50}$/mL	3x
	Long	4.5E+00 $TCID_{50}$/mL	3x
Respiratory Syncytial Virus B	9320	6.0E-01 $TCID_{50}$/mL	3x
	Wash/18537/62	6.0E-01 $TCID_{50}$/mL	3x
	WV/14617/85	6.0E-01 $TCID_{50}$/mL	3x

Table 28: Analytical Reactivity (Inclusivity) Results for *Chlamydia pneumoniae*

	Strain	Concentration	Multiple of LoD Detected
Chlamydia pneumoniae	CWL-029	9.0E+02 CFU/mL	3x
	TWAR strain 2043	9.0E+02 CFU/mL	3x

Table 29: Analytical Reactivity (Inclusivity) Results for *Mycoplasma pneumoniae*

	Strain	Concentration	Multiple of LoD Detected
Mycoplasma pneumoniae	[Bru]	9 x 10^2 CCU/mL	3x
	M129-B170	9 x 10^2 CCU/mL	3x
	M129-B7	9 x 10^2 CCU/mL	3x
	[M52]	9 x 10^2 CCU/mL	3x
	[Mac]	9 x 10^2 CCU/mL	3x
	Mutant 22	3 x 10^4 CCU/mL	100x [a]
	PI 1428	3 x 10^4 CCU/mL	100x [b]

[a] No sequence data available. Lower sensitivity may be a result of mismatches in the assay primers and/or probes. In addition, the reduced sensitivity may be the result of incorrect estimation of genetic material present in the culture of this or the reference strain (CCU/ml value is based only on live bacteria).
[b] *In silico* analysis revealed good homology to primers and probes. The reduced sensitivity is likely the result of incorrect estimation of genetic material present in the culture of this or the reference strain (CCU/ml value is based only on live bacteria).

All of the 101 strains/isolates wet tested for inclusivity were detected by the ePlex RP Panel.

f. Analytical Specificity/Cross-reactivity Evaluation:

<u>On-Panel Organisms Testing</u>

Potential cross-reactivity of each viral and bacterial target on the ePlex RP Panel was evaluated at high concentrations (i.e., 1.0E+05 $TCID_{50}$/mL or >1.0E+05 EID_{50}/mL for viruses; 1.0E+06 CFU/mL or CCU/mL for bacterial isolates, or 1.0E+06 copies/mL for *in vitro* transcripts) of quantified strains/isolates diluted in VTM. *In vitro* transcripts for coronavirus HKU1 was diluted in PBS. Table 30 below summarizes the results of the on-panel viral and bacterial strains/isolates tested.

Table 30: Cross-reactivity Study with ePlex RP Panel Target Organisms

Target	Strain	Concentration	Cross-Reactivity Results
Adenovirus A	Type 31	1.0E+05 $TCID_{50}$/mL	Not observed
Adenovirus B	Type 7A	1.0E+05 $TCID_{50}$/mL	Not observed
Adenovirus C	Type 1	1.0E+05 $TCID_{50}$/mL	Not observed
Adenovirus D	Type 9	1.0E+05 $TCID_{50}$/mL	Not observed
Adenovirus E	Type 4	1.0E+05 $TCID_{50}$/mL	Not observed
Adenovirus F	Type 41	1.0E+05 $TCID_{50}$/mL	Not observed
Coronavirus	229E	1.0E+05 $TCID_{50}$/mL	Not observed
Coronavirus	HKU1 *in vitro* transcript	1.0E+06 copies/mL	Not observed
Coronavirus	NL63	1.0E+05 $TCID_{50}$/mL	Not observed
Coronavirus	OC43	1.0E+05 $TCID_{50}$/mL	Not observed
Enterovirus	Type 68 2007 isolate	1.0E+05 $TCID_{50}$/mL	Not observed

Target	Strain	Concentration	Cross-Reactivity Results
Human metapneumovirus	B1	1.0E+05 $TCID_{50}$/mL	Not observed
Human rhinovirus	1A	1.0E+05 $TCID_{50}$/mL	Not observed
Influenza A	A/Brisbane/59/07	1.0E+05 $TCID_{50}$/mL	Not observed
Influenza A H1	A/Brisbane/59/07	1.0E+05 $TCID_{50}$/mL	Not observed
Influenza A H1-2009	A/NY/01/2009	1.0E+05 $TCID_{50}$/mL	Not observed
Influenza A H3	A/Brisbane/10/07	1.0E+05 $TCID_{50}$/mL	Not observed
Influenza A H3N2v [a]	A/Indiana/21/2012	2.5E+05 EID_{50}/mL	Not observed
Influenza A H5N2 [b]	A/Northern Pintail Washington/40964/14BPL	2.5E+05 EID_{50}/mL	Not observed
Influenza A H5N8 [c]	A/Gyrfalcon/Washington /410886/2014 BPL	1.6E+05 EID_{50}/mL	Not observed
Influenza A H7N9 [d]	A/ANHUI/1/2013	7.9E+05 EID_{50}/mL	Not observed
Influenza B	B/Florida/02/06	1.0E+05 $TCID_{50}$/mL	Not observed
Parainfluenza Virus 1	C35	1.0E+05 $TCID_{50}$/mL	Not observed
Parainfluenza Virus 2	Type 2	1.0E+05 $TCID_{50}$/mL	Not observed
Parainfluenza Virus 3	Type 3	1.0E+05 $TCID_{50}$/mL	Not observed
Parainfluenza Virus 4	Type 4a	1.0E+05 $TCID_{50}$/mL	Not observed
RSV A	2006 Isolate	1.0E+05 $TCID_{50}$/mL	Not observed
RSV B	CH93(18)-18	1.0E+05 $TCID_{50}$/mL	Not observed
Chlamydia pneumoniae	AR-39	1.0E+06 CFU/mL	Not observed
Mycoplasma pneumoniae	FH strain of Eaton Agent [NCTC 10119]	1.0E+06 CCU/mL	Not observed

[a] Influenza A H3N2v detected as Influenza A, Influenza A H3
[b] Influenza A H5N2 detected as Influenza A
[c] Influenza A H5N8 detected as Influenza A
[d] Influenza A H7N9 detected as Influenza A

No cross-reactivity was observed between any of the on-panel viruses or bacteria.

Off-Panel Organisms Testing

Potential cross-reactivity of viruses, bacteria, and fungi that are not targets on the ePlex RP Panel was evaluated at high concentrations (i.e., 1.0E+05 $TCID_{50}$/mL or copies/mL for viruses, 1.0E+06 CFU/mL for bacterial and yeast isolates, or 1.0E+06 copies/mL for plasmid DNA or genomic RNA) by diluting quantified strains/isolates in VTM. Plasmid for Bocavirus and genomic RNA for MERS-CoV were diluted in PBS. Table 31 below summarizes the results of the strains/isolates tested.

Table 31: Cross-reactivity with Organisms Not Targeted by the ePlex RP Panel (Exclusivity)

Target	Strain	Concentration	Cross-Reactivity Results
Acinetobacter baumanii	ATCC 19606	1.0E+06 CFU/mL	Not observed
Bordetella pertussis	18323 [NCTC 10739]	1.0E+06 CFU/mL	Not observed
Bordetella parapertussis	ATCC 15311	1.0E+06 CFU/mL	Not observed
Burkholderia cepacia	ATCC 25416	1.0E+06 CFU/mL	Not observed
Candida albicans	ATCC 10231	1.0E+06 CFU/mL	Not observed
Candida glabrata	ATCC 15126	1.0E+06 CFU/mL	Not observed
MERS-CoV	EMC/2012 [a]	1.0E+05 copies/mL	Not observed
Corynebacterium diphtheriae	ATCC 13812	1.0E+06 CFU/mL	Not observed
Cytomegalovirus	AD 169	1.0E+05 $TCID_{50}$/mL	Not observed
Epstein Barr Virus	Strain B95-8	1.0E+05 $TCID_{50}$/mL	Not observed
Escherichia coli	ATCC 10279	1.0E+06 CFU/mL	Not observed
Haemophilus influenzae	ATCC 43065	1.0E+06 CFU/mL	Not observed
Herpes Simplex Virus	Isolate 2	1.0E+05 $TCID_{50}$/mL	Not observed
Human bocavirus	Bocavirus plasmid [b]	1.0E+06 copies/mL	Not observed
Klebsiella pneumoniae	ATCC 51504	1.0E+06 CFU/mL	Not observed
Lactobacillus acidophilus	ATCC 314	1.0E+06 CFU/mL	Not observed
Lactobacillus plantarum	ATCC 8014	1.0E+06 CFU/mL	Not observed
Legionella pneumophila	Philadelphia-1	1.0E+06 CFU/mL	Not observed
Measles	N/A	1.0E+05 $TCID_{50}$/mL	Not observed
Moraxella catarrhalis	ATCC 23246	1.0E+06 CFU/mL	Not observed
Mumps	Isolate 2	1.0E+05 $TCID_{50}$/mL	Not observed
Mycobacterium tuberculosis	ATCC 25177	1.0E+06 CFU/mL	Not observed
Neisseria meningiditis	ATCC 13077	1.0E+06 CFU/mL	Not observed
Neisseria sicca	ATCC 29193	1.0E+06 CFU/mL	Not observed
Porphyromonas gingivalis	ATCC 33277	1.0E+06 CFU/mL	Not observed
Proteus vulgaris	ATCC 33420	1.0E+06 CFU/mL	Not observed
Pseudomonas aeruginosa	ATCC 15442	1.0E+06 CFU/mL	Not observed
Serratia marcescens	ATCC 13880	1.0E+06 CFU/mL	Not observed
Staphylococcus aureus (MRSA)	NRS384	1.0E+06 CFU/mL	Not observed
Staphylococcus aureus (MSSA)	ATCC 25923	1.0E+06 CFU/mL	Not observed
Staphylococcus epidermidis (MRSE)	ATCC 35983	1.0E+06 CFU/mL	Not observed
Staphylococcus epidermidis (MSSE)	ATCC 49134	1.0E+06 CFU/mL	Not observed
Staphylococcus haemolyticus	ATCC 29970	1.0E+06 CFU/mL	Not observed
Streptococcus agalactiae	ATCC 12401	1.0E+06 CFU/mL	Not observed
Streptococcus dysgalactiae	ATCC 35666	1.0E+06 CFU/mL	Not observed
Streptococcus mitis	ATCC 15914	1.0E+06 CFU/mL	Not observed
Streptococcus pneumoniae	ATCC 49619	1.0E+06 CFU/mL	Not observed
Streptococcus pyogenes	ATCC 12384	1.0E+06 CFU/mL	Not observed

Target	Strain	Concentration	Cross-Reactivity Results
Streptococcus salivarius	ATCC 13419	1.0E+06 CFU/mL	Not observed
Varicella Zoster Virus	82	8.9E+03 $TCID_{50}$/mL	Not observed

[a] Extracted genomic RNA
[b] Plasmid does not contain full length viral genome.

No cross-reactivity was observed between any of the off-panel viruses, bacteria or fungi with the ePlex RP Panel targets.

g. Assay cut-off:

Analytical studies were conducted to establish the signal boundaries for all targets and controls of the ePlex RP Panel.

Positive and negative NPS samples were obtained from internal and external sources. Supplemental samples were contrived for analytes that were under-represented in the existing sample set. Contrived samples included viral and bacterial cultures prepared in negative NPS. Contrived positive samples were formulated using cultured analytes that were diluted into negative NPS matrix. For each target, the signals for positive and negative tests were analyzed. An appropriate boundary was established wherein specificity and sensitivity were maximized. The analysis was verified by ROC analysis. The final boundary set points are listed in Table 32 below.

Table 32: ePlex RP Panel Boundary Set Points

Target Name	Boundary (nA)
Adenovirus B	10
Adenovirus C	10
Adenovirus E	15
Adenovirus C (Penton)	14
Adenovirus (Pan)	10
Coronavirus 229E	10
Coronavirus HKU1	10
Coronavirus NL63	10
Coronavirus OC43	10
Human Metapneumovirus	5
Human Rhinovirus A/C	15
Human Rhinovirus B	15
Human Rhinovirus 4a5	15
Enterovirus	15
Influenza A	20
Influenza A H1	10

Influenza A H1-2009	10
Influenza A H3	5
Influenza B	10
Parainfluenza Virus 1	10
Parainfluenza Virus 2	10
Parainfluenza Virus 3	10
Parainfluenza Virus 4	5
Respiratory Syncytial Virus A	10
Respiratory Syncytial Virus B	20/30 [a]
Chlamydia pneumoniae	10
Mycoplasma pneumoniae	5
Control Name	**Boundary (nA)**
Control 1 (IC4 – hMPV)	20
Control 2 (IC2 – PIV2)	5
Control 4 (IC1 – H3)	20
Control 6 (IC2 – Flu A)	5
Control 7 (IC2 – 229E)	10
Control 8 (IC1 – PIV3)	20
Control 3 (Pombe – Bact)	20
Control 5 (Pombe – Adv)	20

[a] Due to elevated background signal on RSV B in the presence of RSV A virus, there is a conditional threshold for RSV B for when an RSV A signal is detected. In the absence of RSV A signal above 10 nA, the boundary set point for RSV B is 20 nA; if an RSV A signal is detected at greater than 10 nA on a cartridge, the boundary set point for RSV B is set to 30 nA.

h. Interfering Substances:

Substances commonly found in respiratory samples, substances that could be introduced during specimen collection, or medications commonly used to treat congestion, allergies, or asthma symptoms that could potentially interfere with the ePlex RP Panel were individually evaluated in an analytical study. To simulate clinical samples, quantified representative viral and bacterial strains were diluted to 1x LoD in a natural clinical matrix (pooled, negative nasopharyngeal swab specimens) and tested in triplicate for negative and positive interference. Natural clinical matrix (pooled, negative nasopharyngeal swab samples) with no organisms added was used as a control.

All substances and organisms tested for interference were shown to be compatible with the ePlex RP Panel. No potentially interfering substances or microorganisms were found to inhibit the ePlex RP Panel at the concentrations tested in Table 33 below.

Table 33: List of Substances/Organisms and Concentrations for Testing in the Interfering Substances Study

Potentially Interfering Substance	Active Ingredient	Testing Concentration
Control Sample Matrix [a]	Becton Dickinson UVT	N/A
Transport Medium [a]	Copan eSwab (Liquid Amies media)	N/A
Viral Transport Medium [a]	MicroTest M4	N/A
	MicroTest M4-RT	N/A
	MicroTest M5	N/A
	MicroTest M6	N/A
Flocked Swabs	Copan Minitip in UVT	N/A
	Copan Regular Tip in UVT	N/A
Blood (human)	Blood	2% v/v
	Human gDNA	50 ng/rxn
Throat lozenges, oral anesthetic and analgesic	Benzocaine, menthol	26% w/v
Mucin	Purified mucin protein	1% w/v
Nasal sprays or drops	Phenylephrine HCl (Neo-Synephrine®)	1.5% v/v
	Oxymetazoline HCl (Afrin®)	1% v/v
	Sodium chloride	0.8% w/v
Antibacterial, systemic	Tobramycin [b]	1% w/v
Antibiotic, nasal ointment	Mupirocin	2% w/v
Nasal corticosteroids	Beclomethasone	1.5% w/v
	Dexamethasone	1.5% w/v
	Flunisolide	1.5% w/v
	Budesonide (Rhinocort®)	0.9% v/v
	Triamcinolone (Nasacort®)	1.5% v/v
	Fluticasone (Flonase®)	1.5% v/v
ZICAM® Allergy Relief Nasal Gel	Luffa opperculata	1% v/v
	Sulfur	
	Galphimia glauca	
	Histaminum hydrochloricum	
Anti-viral drugs	Zanamivir	550 ng/mL
	Oseltamivir	142 ng/mL
Virus	Cytomegalovirus	1.0E+05 $TCID_{50}$/mL
Bacteria	*Streptococcus pneumoniae*	1.0E+06 CFU/mL
	Bordetella parapertussis	
	Haemophilus influenza	
	Staphylococcus aureus	
	Neisseria meningitides	
	Corynebacterium diptheriae	

[a] Testing of media was done by adding a negative NPS collected in the specified media and diluting in the natural clinical matrix.
[b] At concentrations greater than 1% weight/volume in the sample, tobramycin was found to inhibit assay performance.

Note: Nasal influenza vaccines (e.g., FluMist) were not evaluated in this study, but are predicted to be reactive with the ePlex RP Panel Influenza A (including subtype) and Influenza B assays. Therefore, contamination of specimens with vaccine or recent administration of the vaccine prior to NPS specimen collection could lead to accurate detection by the ePlex RP Panel of the viruses contained in the vaccine, but would not represent infection by those agents.

i. Co-Detected Organisms Study:

Detection of more than one clinically relevant viral and/or bacterial organism in a sample was evaluated in an analytical study with the ePlex RP Panel, using a natural clinical matrix (pooled, negative nasopharyngeal swab samples) spiked with two ePlex RP Panel targeted organisms: one organism at a low concentration (i.e., 1-3x LoD) and the second organism at a high concentration (i.e., 1.0E+05 $TCID_{50}$/mL).

Study results are summarized in Table 34 below.

Table 34: Detection of Co-Infections

ePlex RP Panel Result Organism 1	Organism 1 (High Titer)	ePlex RP Panel Result Organism 2	Organism 2 (Low Titer)	Organism 2 Multiple of LoD
Influenza A H3	1.0E+05 $TCID_{50}$/mL	Adenovirus B	2.0E+00 $TCID_{50}$/mL	1x
Adenovirus	1.0E+05 $TCID_{50}$/mL	Influenza A H3	5.0E+01 $TCID_{50}$/mL	1x
Influenza A H3	1.0E+05 $TCID_{50}$/mL	RSV A	1.5E+00 $TCID_{50}$/mL	1x
RSV A	1.0E+05 $TCID_{50}$/mL	Influenza A H3	5.0E+01 $TCID_{50}$/mL	1x
Influenza A H1-2009	1.0E+05 $TCID_{50}$/mL	RSV B	6.0E-01 $TCID_{50}$/mL	3x
RSV B	1.0E+05 $TCID_{50}$/mL	Influenza A H1-2009	1.0E-01$TCID_{50}$/mL	1x
Influenza A H1-2009	1.0E+05 $TCID_{50}$/mL	Rhinovirus	1.5E+00 $TCID_{50}$/mL	1x
Rhinovirus	1.0E+05 $TCID_{50}$/mL	Influenza A H1-2009	3.0E-01 $TCID_{50}$/mL	3x
Influenza A H1-2009	1.0E+05 $TCID_{50}$/mL	Parainfluenza Virus 3	5.0E+00 $TCID_{50}$/mL	1x
Parainfluenza Virus 3	1.0E+05 $TCID_{50}$/mL	Influenza A H1-2009	1×10^{-1} $TCID_{50}$/mL	1x
Rhinovirus	1.0E+05 $TCID_{50}$/mL	RSV A	1.5E+00 $TCID_{50}$/mL	1x
RSV A	1.0E+05 $TCID_{50}$/mL	Rhinovirus	1.5E+00 $TCID_{50}$/mL	1x
Coronavirus	1.0E+05 $TCID_{50}$/mL	RSV A	1.5E+00 $TCID_{50}$/mL	1x
RSV A	1.0E+05 $TCID_{50}$/mL	Coronavirus	7.5E+00 $TCID_{50}$/mL	1x

Human Metapneumovirus	1.0E+05 $TCID_{50}$/mL	Adenovirus	2.0E+00 $TCID_{50}$/mL	1x
Adenovirus	1.0E+05 $TCID_{50}$/mL	Human Metapneumovirus	2.3E+02 $TCID_{50}$/mL	1x
Adenovirus	1.0E+05 $TCID_{50}$/mL	RSV A	1.5E+00 $TCID_{50}$/mL	1x
RSV A	1.0E+05 $TCID_{50}$/mL	Adenovirus	2.0E+00 $TCID_{50}$/mL	1x

The study results demonstrated the ability of the ePlex RP Panel to detect two clinically relevant co-infecting organisms in a sample at both high and low concentrations.

j. Carry-Over Contamination:

The carryover/cross-contamination rate of the ePlex RP Panel and ePlex instrument was evaluated using a checkerboard approach by running high positive and negative samples interspersed in all bays of a four-tower ePlex instrument (i.e., 24 bays total) over five separate runs on five separate days. Quantified parainfluenza virus 3 was prepared in VTM at a high concentration (1.0E+05 $TCID_{50}$/mL) to simulate a clinically relevant high positive and was tested as a representative target organism. VTM was used to represent negative samples. On each round of testing, 24 ePlex RP Panel cartridges were evaluated. 100% of parainfluenza 3-positive samples generated a result of Detected and 100% of parainfluenza 3-negative samples generated a parainfluenza 3 result of No Target Detected, indicating no carryover or cross-contamination was observed between bays or within bays with the ePlex RP Panel when testing samples consecutively or in adjacent bays with an ePlex instrument .

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable. Refer to the Clinical Studies Section of this document.

b. Matrix comparison:

Not applicable

3. Clinical studies:

Prospective Clinical Study

The clinical performance of the ePlex RP Panel was established during multi-center clinical studies conducted at five distinct U.S. test sites. Each test site was

representative of the intended use setting (clinical laboratories) and testing was performed by trained clinical laboratory personnel.

Clinical performance of the ePlex RP Panel was evaluated testing clinical nasopharyngeal swab (NPS) samples prospectively-collected in VTM at eight geographically distinct U.S. clinical sites in two phases. From March 2013 through August 2014 (Phase 1), NPS in VTM samples were prospectively-collected from all comers meeting the study eligibility criteria at four specimen collection sites and immediately frozen (N=2218 specimens) for later testing as prospective archived/frozen (Category II) specimens. From September 2016 through October 2016 (Phase 2), NPS in VTM samples were prospectively-collected from all comers meeting the study eligibility criteria and tested fresh at five specimen collection and testing sites (N=514 specimens) as prospective fresh (Category I) specimens. Category II specimens were distributed to sites beginning in September 2016. Study sites also began testing Category I specimens at this time. At each site, Category II specimens were thawed and tested according to the study procedures as time permitted over the remaining duration of the clinical study.

A total of 2732 prospective specimens (Category I and II) were collected across the two phases. Prior to the start of investigational testing the ePlex RP Panel, 263 specimens were withdrawn (251 specimens had sample handling deviations, nine specimens were outside of the protocol allowed timelines, two specimens had insufficient volume, and one specimen had incomplete documentation) from the study. Of the 2469 remaining prospective specimens eligible for testing, 2462 (511 Category I and 1951 Category II specimens) were evaluable. Samples with final, valid results and a valid comparator result were considered evaluable. Seven (7) prospective specimens were not evaluable due to the lack of final, valid ePlex RP Panel results and were excluded from performance evaluations.

Demographic information for the 2462 evaluable prospective specimens is described in Table 35 below.

Table 35: Subject Demographic Data for All Evaluable Prospective Specimens by Collection Site (N=2462)

	All Sites N=2462 n (%)	Site 1 N=165 n (%)	Site 2 N=248 n (%)	Site 3 N=350 n (%)	Site 4 N=892 n (%)	Site 5 N=345 n (%)	Site 6 N=101 n (%)	Site 7 N=161 n (%)	Site 8 N=200 n (%)
Sex									
Male	1247 (50.6)	96 (58.2)	118 (47.6)	186 (53.1)	450 (50.4)	188 (54.5)	43 (42.6)	84 (52.2)	82 (41.0)
Female	1215 (49.4)	69 (41.8)	130 (52.4)	164 (46.9)	442 (49.6)	157 (45.5)	58 (57.4)	77 (47.8)	118 (59.0)
Age (years)									
0–1	388 (15.8)	17 (10.3)	21 (8.5)	74 (21.1)	164 (18.4)	45 (13.0)	28 (27.7)	3 (1.9)	36 (18.0)
> 1–5	325 (13.2)	12 (7.3)	22 (8.9)	62 (17.7)	64 (7.2)	100 (29.0)	39 (38.6)	16 (9.9)	10 (5.0)
> 5–21	321 (13.0)	15 (9.1)	6 (2.4)	38 (10.9)	82 (9.2)	116 (33.6)	34 (33.7)	18 (11.2)	12 (6.0)
> 21–65	926 (37.6)	87 (52.7)	131 (52.8)	98 (28.0)	385 (43.2)	55 (15.9)	0 (0.0)	92 (57.1)	78 (39.0)
> 65	502 (20.4)	34 (20.6)	68 (27.4)	78 (22.3)	197 (22.1)	29 (8.4)	0 (0.0)	32 (19.9)	64 (32.0)

A total of five test sites participated in the prospective clinical evaluation of ePlex RP Panel. One test site had a 4-tower ePlex instrument (24 bays) and the remaining four test sites had a 3-tower ePlex instrument (18 bays). A total of 13 ePlex RP Panel cartridge lots were used in the prospective clinical evaluation.

The performance of the ePlex RP Panel was evaluated by comparing the ePlex RP Panel results with those from an FDA-cleared multiplexed respiratory pathogen panel (i.e., the main comparator) and an analytically validated PCR test with bi-directional sequencing for confirmation of RSV subtypes.

Prospective Clinical Study Performance

Positive percent agreement (PPA) for each panel organism was calculated by dividing the number of true positive (TP) results by the sum of TP and false negative (FN) results, while negative percent agreement (NPA) was calculated by dividing the number of true negative (TN) results by the sum of TN and false positive (FP) results. A TP result was one where the "detected" ePlex RP Panel result matched the "detected" comparator method result, while a TN result was one where a "negative" ePlex RP Panel result matched a "negative" comparator method result. A FN result was one where the "negative" ePlex RP Panel result did not match the "detected" comparator method result, while a FP result was one where the "detected" ePlex RP Panel result did not match the "negative" comparator method result. The two-sided 95% confidence interval was also calculated.

Samples for which FP and/or FN results (i.e., discrepant results) were obtained when comparing the ePlex RP Panel results to the comparator method results were further investigated. The discrepancy investigation was mainly conducted by performing analytically validated PCR/Sequencing assays.

The ePlex RP Panel prospective performance data in positive percent and negative percent agreements against the comparator methods (all sites combined) are presented in the following Table 36 by analyte:

Table 36: ePlex RP Panel Prospective Clinical Performance Summary

Organism		Positive Percent Agreement			Negative Percent Agreement		
		TP/ (TP + FN)	%	95%CI	TN/ (TN + FP)	%	95%CI
Viruses							
Adenovirus [a]	Fresh	6/8	75.0	40.9-92.9	499/503	99.2	98.0-99.7
	Frozen	48/53	90.6	79.7-95.9	1874/1898	98.7	98.1-99.1
	Overall	***54/61***	***88.5***	***78.2-94.3***	***2373/2401***	***98.8***	***98.3-99.2***
Coronavirus [b]	Fresh	7/7	100	64.6-100	503/504	99.8	98.9-100
	Frozen	89/110	80.9	72.6-87.2	1828/1841	99.3	98.8-99.6
	Overall	***96/117***	***82.1***	***74.1-88.0***	***2331/2345***	***99.4***	***99.0-99.6***
hMPV [c]	Fresh	0/0	NA	NA	511/511	100	99.3-100
	Frozen	107/113	94.7	88.9-97.5	1832/1838	99.7	99.3-99.9
	Overall	***107/113***	***94.7***	***88.9-97.5***	***2343/2349***	***99.7***	***99.4-99.9***
HRV/EV [d]	Fresh	176/183	96.2	92.3-98.1	316/328	96.3	93.7-97.9
	Frozen	317/336	94.3	91.3-96.4	1544/1615	95.6	94.5-96.5
	Overall	***493/519***	***95.0***	***92.8-96.6***	***1860/1943***	***95.7***	***94.7-96.5***
FluA [e]	Fresh	0/0	NA	NA	511/511	100	99.3-100
	Frozen	106/111	95.5	89.8-98.1	1836/1840	99.8	99.4-99.9
	Overall	***106/111***	***95.5***	***89.8-98.1***	***2347/2351***	***99.8***	***99.6-99.9***
FluA H1	Fresh	0/0	NA	NA	511/511	100	99.3-100
	Frozen	0/0	NA	NA	1951/1951	100	99.8-100
	Overall	***0/0***	***NA***	***N/A***	***2462/2462***	***100***	***99.8-100***
FluA H1-2009 [f]	Fresh	0/0	NA	NA	511/511	100	99.3-100
	Frozen	70/71	98.6	92.4-99.8	1874/1880	99.7	99.3-99.9
	Overall	***70/71***	***98.6***	***92.4-99.8***	***2385/2391***	***99.7***	***99.5-99.9***
FluA H3 [g]	Fresh	0/0	NA	NA	511/511	100	99.3-100
	Frozen	34/37	91.9	78.7-91.2	1914/1914	100	99.8-100
	Overall	***34/37***	***91.9***	***78.7-91.2***	***2425/2425***	***100***	***99.8-100***
FluB [h]	Fresh	1/1	100	20.7-100	509/510	99.8	98.9-100
	Frozen	58/65	89.2	79.4-94.7	1882/1886	99.8	99.5-99.9
	Overall	***59/66***	***89.4***	***79.7-94.8***	***2391/2396***	***99.8***	***99.5-99.9***
PIV1	Fresh	1/1	100	20.7-100	510/510	100	99.3-100
	Frozen	23/24	95.8	79.8-99.3	1926/1927	99.9	99.7-100
	Overall	***24/25***	***96.0***	***80.5-99.3***	***2436/2437***	***100***	***99.8-100***
PIV2	Fresh	12/13	92.3	66.7-98.6	497/498	99.8	98.9-100
	Frozen	9/9	100	70.1-100	1941/1942	99.9	99.7-100
	Overall	***21/22***	***95.5***	***78.2-99.2***	***2438/2440***	***99.9***	***99.7-100***
PIV3 [i]	Fresh	5/5	100	56.6-100	506/506	100	99.2-100
	Frozen	94/104	90.4	83.2-94.7	1842/1847	99.7	99.4-99.9
	Overall	***99/109***	***90.8***	***83.9-94.9***	***2348/2353***	***99.8***	***99.5-99.9***
PIV4 [j]	Fresh	3/3	100	43.9-100	503/508	99.0	97.7-99.6
	Frozen	5/5	100	56.6-100	1944/1946	99.9	99.6-100
	Overall	***8/8***	***100***	***67.6-100***	***2447/2454***	***99.7***	***99.4-99.9***
RSV A	Fresh	8/9	88.9	56.5-98.0	501/501	100	99.2-100

Organism		Positive Percent Agreement			Negative Percent Agreement		
		TP/ (TP + FN)	%	95%CI	TN/ (TN + FP)	%	95%CI
	Frozen	27/31	87.1	71.1-94.9	1917/1918	99.9	99.7-100
	Overall	***35/40***	***87.5***	***73.9-94.5***	***2418/2419***	***100***	***99.8-100***
RSV B [k]	Fresh	9/10	90.0	59.6-98.2	500/500	100	99.2-100
	Frozen	81/86	94.2	87.1-97.5	1861/1863	99.9	99.6-100
	Overall	***90/96***	***93.8***	***87.0-97.1***	***2361/2363***	***99.9***	***99.7-100***
Bacteria							
C. pneumoniae [l]	Fresh	0/0	NA	NA	511/511	100	99.3-100
	Frozen	2/5	40.0	11.8-76.9	1945/1946	99.9	99.7-100
	Overall	***2/5***	***40.0***	***11.8-76.9***	***2456/2457***	***100***	***99.8-100***
M. pneumoniae [m]	Fresh	3/3	100	43.9-100	507/508	99.8	98.9-100
	Frozen	4/5	80.0	37.6-96.4	1945/1946	99.9	99.7-100
	Overall	***7/8***	***87.5***	***52.9-97.8***	***2452/2454***	***99.9***	***99.7-100***

[a] Adenovirus was not detected in 3/7 FN specimens using PCR/Sequencing. Adenovirus was detected in 13/28 FP specimens using PCR/Sequencing.

[b] Twenty (20) FN prospective-frozen specimens were repeat tested with the comparator method and 12 had Coronavirus detected. Of these 12 specimens, 11 were repeat tested with the ePlex RP Panel and 3 had Coronavirus detected. Coronavirus was not detected in 2/21 FN specimens using PCR/Sequencing. Coronavirus was detected in 3/13 FP specimens using PCR/sequencing. One (1) FP specimen was not tested by PCR/Sequencing.

[c] Human Metapneumovirus was not detected in 1/6 FN specimens using PCR/Sequencing. Human Metapneumovirus was detected in 4/6 FP specimens using PCR/sequencing.

[d] Human rhinovirus/enterovirus was not detected in 7/26 FN specimens using PCR/Sequencing. Human rhinovirus/enterovirus was detected in 42/83 FP samples using PCR/sequencing.

[e] Influenza A comparator results contain 71 specimens with A H1-2009, 37 specimens with A H3, and 3 specimens with no subtype detected. Influenza A was not detected in 1/3 FN samples using PCR/Sequencing. Two (2) FN specimens were not tested by PCR/Sequencing. Influenza A was detected in 1/4 FP specimens using PCR/Sequencing.

[f] Influenza A H1-2009 was detected in 4/6 FP specimens using PCR/Sequencing.

[g] Influenza A H3 was not detected in 1/3 FN specimens using PCR/Sequencing.

[h] Influenza B was not detected in 3/7 FN specimens using PCR/Sequencing. Influenza B was detected in 2/4 FP specimens using PCR/Sequencing. One (1) FP specimen was not tested by PCR/Sequencing

[i] Parainfluenza virus 3 was not detected in 3/10 FN specimens using PCR/Sequencing. Parainfluenza virus 3 was detected in 4/5 FP specimens using PCR/Sequencing.

[j] Parainfluenza virus 4 was detected in 3/5 FP specimens using PCR/Sequencing. 2 FP specimens were not tested by PCR/Sequencing.

[k] RSV B was detected in 1/2 FP specimens using PCR/Sequencing.

[l] *C. pneumoniae* was not detected in 1/3 FN specimens using PCR/Sequencing. *C. pneumoniae* was detected in the 1 FP specimen using PCR/Sequencing.

[m] *M. pneumoniae* was not detected in the 1 FN specimen using PCR/Sequencing. *M. pneumoniae* was detected in the 1 FP specimen using PCR/Sequencing. One (1) FP specimen was not tested by PCR/Sequencing.

Prospective Clinical Study Mixed Infection Analysis

The ePlex RP Panel identified a total of 135 prospective specimens with multiple organisms detected, or 5.5% (135/2462) of all prospective specimens. Of these, 118 (118/2462, 4.8%) had two organisms, 14 (14/2462, 0.6%) had three organisms, and 3 (3/2462, 0.1%) had four organisms detected. Of the 135 specimens with multiple

organisms detected, 58 included one or more organisms that had not been detected by the comparator method (i.e., false positive results).

All distinct co-infection combinations as detected by the ePlex RP Panel during the prospective clinical evaluation are presented in Table 37 below.

Table 37: Distinct Co-Detection Combinations Detected by the ePlex RP Panel in the Prospective Clinical Specimens

Distinct Co-Detection Combinations Detected by the ePlex RP Panel				Total Number Of Co-detections (% of samples)	Number of Discrepant Co-detections	Discrepant Organism(s) [a]
Organism 1	Organism 2	Organism 3	Organism 4			
ADV	CoV			2 (0.08%)	0	
ADV	CoV	HRV/EV		2 (0.08%)	1	ADV (1)
ADV	Flu A (unk)	Flu B	HRV/EV	1 (0.04%)	1	ADV (1), Flu A (unk) (1), Flu B (1), HRV/EV (1)
ADV	Flu A H3			1 (0.04%)	0	
ADV	Flu B	HRV/EV	RSV B	1 (0.04%)	1	ADV (1), Flu B (1)
ADV	FluA H1-2009			1 (0.04%)	1	ADV (1), FluA H1-2009 (1)
ADV	FluA H1-2009	HRV/EV		1 (0.04%)	0	
ADV	FluA H1-2009	PIV 3		1 (0.04%)	1	PIV 3 (1)
ADV	HMPV			3 (0.12%)	2	ADV (2)
ADV	HMPV	HRV/EV	RSV A	1 (0.04%)	1	RSV A (1)
ADV	HRV/EV			18 (0.73%)	7	ADV (6), HRV/EV (1)
ADV	HRV/EV	Mpneum		1 (0.04%)	0	
ADV	HRV/EV	PIV 1		1 (0.04%)	1	PIV 1 (1)
ADV	HRV/EV	PIV 4		1 (0.04%)	1	ADV (1), PIV 4 (1)
ADV	HRV/EV	RSV B		1 (0.04%)	0	
ADV	PIV 2			2 (0.08%)	1	ADV (1)
ADV	PIV 3			2 (0.08%)	1	ADV (1)
ADV	PIV 4			1 (0.04%)	1	ADV (1)
ADV	RSV B			2 (0.08%)	2	ADV (2)
CPneum	HRV/EV			1 (0.04%)	0	
CoV	FluA H1-2009			1 (0.04%)	0	
CoV	HMPV			4 (0.16%)	0	
CoV	HMPV	HRV/EV		2 (0.08%)	0	
CoV	HRV/EV			12 (0.49%)	4	CoV (1), HRV/EV (4)
CoV	HRV/EV	RSV B		1 (0.04%)	1	CoV (1)
CoV	PIV 1			1 (0.04%)	0	
CoV	RSV A			3 (0.12%)	0	
CoV	RSV B			3 (0.12%)	2	CoV (2)
Flu A (unk)	HRV/EV			1 (0.04%)	1	Flu A (unk) (1)
Flu AH3	HRV/EV			2 (0.08%)	1	HRV/EV (1)
Flu AH3	RSV B			1 (0.04%)	0	
Flu B	HRV/EV			4 (0.16%)	2	HRV/EV (2)

Distinct Co-Detection Combinations Detected by the ePlex RP Panel				Total Number Of Co-detections (% of samples)	Number of Discrepant Co-detections	Discrepant Organism(s) [a]
Organism 1	**Organism 2**	**Organism 3**	**Organism 4**			
Flu B	HRV/EV	RSV B		1 (0.04%)	0	
Flu B	PIV 3			1 (0.04%)	0	
FluA09H1	HMPV	HRV/EV		1 (0.04%)	1	HRV/EV (1)
FluA09H1	HRV/EV			2 (0.08%)	1	HRV/EV (1)
HMPV	HRV/EV			5 (0.20%)	1	HRV/EV (1)
HMPV	HRV/EV	RSV B		1 (0.04%)	1	HRV/EV (1)
HMPV	PIV 3			1 (0.04%)	0	
HRV/EV	PIV 1			3 (0.12%)	0	
HRV/EV	PIV 2			7 (0.28%)	3	HRV/EV (1), PIV 2 (2)
HRV/EV	PIV 3			11 (0.45%)	5	HRV/EV (5)
HRV/EV	PIV 4			4 (0.16%)	4	PIV 4 (4)
HRV/EV	RSV A			5 (0.20%)	0	
HRV/EV	RSV B			11 (0.45%)	6	HRV/EV (6)
PIV 1	PIV 4			1 (0.04%)	1	PIV 4 (1)
PIV 3	RSV B			1 (0.04%)	0	
RSV A	RSV B			1 (0.04%)	1	RSV B (1)
Total Number of Co-Detections				135 (5.5%)	57	64/290 [b]
Total Number with 2 Organisms Detected				118 (4.8%)	47	49/236
Total Number with 3 Organisms Detected				14 (0.6%)	7	8/42
Total Number with 4 Organisms Detected				3 (0.1%)	3	7/12

Note: ADV= adenovirus, CoV= coronavirus, HMPV= human metapneumovirus, HRV/EV= human rhinovirus/enterovirus, Flu= Influenza, (unk)= unknown subtype, PIV= parainfluenza, RSV= respiratory syncytial virus, Cpneum= *C. pneumoniae*, Mpneum= *M. pneumoniae*

[a] A discrepant organism is defined as one that was detected by the ePlex RP Panel but not by the comparator method.

[b] 64 discrepant organisms were investigated using PCR/Sequencing; the discrepant organism was detected in 20/64 cases:

-In 8/18 specimens, adenovirus was detected by PCR/Sequencing.
-In 1/4 specimens, coronavirus was detected by PCR/Sequencing.
-In 7/25 specimens, human rhinovirus/enterovirus was detected by PCR/Sequencing.
-In 1/1 specimen, influenza A H1-2009 was detected by PCR/Sequencing.
-In 1/1 specimen, parainfluenza virus 3 was detected by PCR/Sequencing.
-In 2/6 specimens, parainfluenza virus 4 was detected by PCR/Sequencing.

Additional distinct co-infection combinations detected by the comparator method, but not detected by the ePlex RP Panel in the prospective clinical evaluation are presented in Table 38 below.

Table 38: Additional Distinct Co-infection Combinations Detected by the Comparator Method, but not detected by the ePlex RP Panel in the Prospective Clinical Trial

Distinct Co-Detection Combinations Detected by the Comparator Method			Total Number Of Co-detections (% of samples)	Number of Discrepant Co-detections	Discrepant Organism(s) [a,b]
Organism 1	Organism 2	Organism 3			
ADV	CoV		1 (0.04%)	1	ADV (1), CoV (1)
ADV	HRV/EV		4 (0.16%)	4	ADV (4)
ADV	HRV/EV	PIV 3	1 (0.04%)	1	HRV/EV (1), PIV 3 (1)
ADV	HRV/EV	RSV A	1 (0.04%)	1	ADV (1)
CPneum	HRV/EV		1 (0.04%)	1	CPneum (1)
CPneum	PIV 3		1 (0.04%)	1	CPneum (1)
CoV	FluA H1-2009		2 (0.08%)	2	CoV (2)
CoV	HMPV		1 (0.04%)	1	CoV (1)
CoV	HRV/EV		6 (0.24%)	6	CoV (4), HRV/EV (2)
CoV	PIV 3		1 (0.04%)	1	CoV (1)
CoV	RSV B		3 (0.12%)	3	CoV (2), RSV B (1)
Flu A H3	HRV/EV	PIV 3	1 (0.04%)	1	Flu A H3 (1), PIV 3 (1)
Flu A H3	PIV 3		1 (0.04%)	1	PIV 3 (1)
FluA H1-2009	HMPV	HRV/EV	1 (0.04%)	1	HMPV (1), HRV/EV (1)
HMPV	HRV/EV		1 (0.04%)	1	HRV/EV (1)
HRV/EV	PIV 1		1 (0.04%)	1	HRV/EV (1)
HRV/EV	PIV 3		2 (0.08%)	2	HRV/EV (2)
HRV/EV	PIV 3	RSV B	1 (0.04%)	1	PIV 3 (1)
HRV/EV	RSV A		2 (0.08%)	2	RSV A (2)

Note: ADV= adenovirus, CoV= coronavirus, HMPV= human metapneumovirus, HRV/EV= human rhinovirus/enterovirus, Flu= Influenza, PIV= parainfluenza, RSV= respiratory syncytial virus, Cpneum= *C. pneumoniae*, Mpneum= *M. pneumoniae*

[a] A discrepant organism is defined as one that was detected by the comparator method but not by the ePlex RP Panel.

[b] 36/36 discrepant organisms were investigated using PCR/Sequencing; the discrepant organism was not detected in 10/36 cases:

-In 2/6 samples, adenovirus was not detected by PCR/Sequencing.
-In 1/2 samples, *Chlamydophila pneumoniae* was not detected by PCR/Sequencing.
-In 1/11 samples, coronavirus was not detected by PCR/Sequencing.
-In 5/8 samples, human rhinovirus/enterovirus was not detected by PCR/Sequencing.
-In 1/1 sample, influenza A H3 was not detected by PCR/Sequencing.

Retrospective Clinical Study

Some of the organisms on the ePlex RP Panel were of low prevalence and were not encountered in sufficiently large numbers during the prospective evaluation to adequately demonstrate system performance. To supplement the number of positives for targets that were not sufficiently represented in the prospective collection, additional nasopharyngeal swab in VTM specimens were retrospectively collected from six clinical sites.

A total of 535 nasopharyngeal swab in VTM specimens that had previously tested positive for one or more of the target organisms during standard-of-care (SOC) testing were collected and stored frozen. Prior to the start of investigational testing, 11 specimens were withdrawn due to incompliance to the study protocol, and 52 specimens were withdrawn because the organisms present had sufficient representation in other samples. In addition, the composition and integrity of the pre-selected specimens were also confirmed with the same comparator method employed in the prospective clinical study (i.e., an FDA-cleared multiplexed respiratory pathogen panel). As the result of this confirmation testing using the comparator method, 26 additional specimens were excluded because the original SOC testing positive results for the intended organisms were not confirmed when tested with the comparator method.

The remaining 446 retrospective specimens were eligible for testing in this study, all 446 specimens were evaluable. The retrospective specimens were distributed approximately equally across the same five testing sites that participated in the prospective evaluation of the ePlex RP Panel. Prospective and retrospective samples were intermixed and testing sites were blinded to the identity of the samples.

Demographic information for the 446 eligible retrospective specimens is described in Table 39 below.

Table 39: Subject Demographic Data for Retrospective Specimens by Collection Site (N=446)

	All Sites N=446 n (%)	**Site 1 N=1 n (%)**	**Site 2 N=1 n (%)**	Site 3 N=129 n (%)	**Site 4 N=18 n (%)**	**Site 5 N=131 n (%)**	**Site 6 N=166 n (%)**
Sex							
Male	232 (52.0)	0 (0.0)	1 (100)	76 (58.9)	11 (61.1)	68 (51.9)	76 (45.8)
Female	214 (48.0)	1 (100)	0 (0.0)	53 (41.1)	7 (38.9)	63 (48.1)	90 (54.2)
Age (years)							
0 – 1	122 (27.4)	0 (0.0)	0 (0.0)	24 (18.6)	5 (27.8)	56 (42.7)	37 (22.3)
> 1 – 5	107 (24.0)	0 (0.0)	1 (100)	51 (39.5)	3 (16.7)	16 (12.2)	36 (21.7)
> 5 – 21	59 (13.2)	0 (0.0)	0 (0.0)	9 (7.0)	2 (11.1)	19 (14.5)	29 (17.5)
> 21 – 65	99 (22.2)	1 (100)	0 (0.0)	11 (8.5)	8 (44.4)	31 (23.7)	48 (28.9)
> 65	59 (13.2)	0 (0.0)	0 (0.0)	34 (26.4)	0 (0.0)	9 (6.9)	16 (9.6)

Retrospective Clinical Study Performance

The ePlex RP Panel retrospective performance data in positive percent and negative percent agreements against the comparator methods are presented in the following Table 39 by analyte:

Table 39: ePlex RP Panel Retrospective Clinical Study Performance Summary

Organism	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Viruses						
Adenovirus [a]	55/56	98.2	90.6-99.7	386/390	99.0	97.4-99.6
Coronavirus [b]	121/138	87.7	81.2-92.2	307/307	100	98.8-100
hMPV	5/7	71.4	35.9-91.8	439/439	100	99.1-100
HRV/EV [c]	37/41	90.2	77.5-96.1	384/402	95.5	93.0-97.1
Influenza A [d]	75/82	91.5	83.4-95.8	363/363	100	99.0-100
Influenza A H1	0/0	0	NA	446/446	100	99.1-100
Influenza A H1-2009 [e]	27/31	87.1	71.1-94.9	415/415	100	99.1-100
Influenza A H3 [f]	45/51	88.2	76.6-94.5	394/394	100	99.0-100
Influenza B	1/1	100	20.7-100	445/445	100	99.1-100
Parainfluenza Virus 1 [g]	43/48	89.6	77.8-95.5	396/397	99.7	98.6-100
Parainfluenza Virus 2	46/51	90.2	79.0-95.7	395/395	100	99.0-100
Parainfluenza Virus 3	2/2	100	34.2-100	444/444	100	99.1-100
Parainfluenza Virus 4	18/20	90.0	69.9-97.2	426/426	100	99.1-100
RSV A [h]	25/27	92.6	76.6-97.9	414/414	100	99.1-100
RSV B [h]	21/22	95.5	78.2-99.2	419/419	100	99.1-100
Bacteria						
Chlamydia pneumoniae	1/1	100	20.7-100	445/445	100	99.1-100
Mycoplasma pneumoniae [g]	7/7	100	64.6-100	439/439	100	99.1-100

[a] Adenovirus was not detected in the 1 FN specimen using PCR/Sequencing. Adenovirus was detected in 2/4 FP specimens using PCR/Sequencing.

[b] One (1) unintended coronavirus positive specimen by the source laboratory was not confirmed by the comparator method and therefore was excluded from the performance calculation for coronavirus. Coronavirus was not detected in 2/16 FN specimens using PCR/Sequencing. One (1) FN specimen was not tested by PCR/Sequencing.

[c] Three (3) unintended HRV/EV positive specimens by the source laboratory were not confirmed by the comparator method and therefore were excluded from the performance calculation for HRV/EV.

[d] One (1) unintended influenza A positive specimen by the source laboratory was not confirmed by the comparator method and therefore was excluded from the performance calculation for influenza A. Influenza A was not detected in 3/7 FN specimens using PCR/Sequencing. Influenza A comparator results contain 31 specimens with A H1-2009 and 51 specimens with A H3 detected.

[e] Influenza A H1-2009 was not detected in 2/4 FN specimens using PCR/Sequencing.

[f] One (1) unintended influenza A H3 positive specimen by the source laboratory was not confirmed by the comparator method and therefore was excluded from the performance calculation for influenza A H3. Influenza A H3 was not detected in 1/6 FN specimens using PCR/Sequencing.

[g] One (1) unintended PIV 1 positive specimen by the source laboratory was not confirmed by the comparator method and therefore was excluded from the performance calculation for PIV 1. PIV 1 was not detected in 2/5 FN specimens using PCR/Sequencing.

[h] Five (5) unintended RSV positive specimens by the comparator method were not confirmed by PCR/Sequencing with regard to determining RSV subtypes and therefore was excluded from the performance calculations for RSV A and RSV B.

Mock Clinical Study

Influenza A H1 and *Chlamydia pneumoniae* are of such rarity that both prospective and retrospective testing efforts were insufficient to demonstrate system performance for these two organisms. To supplement the prospective and retrospective data, an evaluation of contrived specimens was performed at the same five testing sites that participated in the prospective and retrospective evaluations of the ePlex RP Panel. Contrived clinical specimens were prepared using individual unique negative residual clinical NPS in VTM specimens. For each target, at least 50 individual specimens were prepared with half of the samples spiked at approximately 2 -10x LoD and the remaining samples spiked with target spanning the expected clinical range. Contrived samples were tested along with other clinical samples and/or contrived samples that were negative for the corresponding target.

There were 327 contrived specimens created and tested to supplement low prevalence targets on the RP Panel; 104 specimens contained one or more low prevalence organisms (i.e., Influenza A H1 and *Chlamydia pneumoniae*) and 223 specimens were negative for these organisms. All 327 contrived specimens were tested with the ePlex RP Panel and 326 were evaluable. The one unevaluable specimen did not have a final, valid ePlex RP Panel result after repeat testing.

Mock clinical study results are summarized for these two low prevalence organisms in Table 40 below.

Table 40: ePlex RP Panel Performance Testing Contrived Specimens

Organism	Positive Percent Agreement (PPA)				Negative Percent Agreement (NPA)		
	× LoD	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Influenza A H1	10	25/25	100	86.7-100	275/275	100	98.6-100
	50	8/8	100	67.6-100			
	100	9/9	100	70.1-100			
	1000	9/9	100	70.1-100			
	Combined	**51/51**	**100**	**93.0-100**			
Chlamydia pneumoniae	2	25/25	100	86.7-100	274/274	100	98.6-100
	5	9/9	100	70.1-100			
	10	9/9	100	70.1-100			
	100	9/9	100	70.1-100			
	Combined	**52/52**	**100**	**93.1-100**			

Clinical Study ePlex Instrument Performance

There were 3242 specimens eligible for ePlex RP Panel testing (i.e., 2469 prospective specimens, 446 retrospective specimens, and 327 contrived specimens) in the clinical evaluations of the ePlex RP Panel. Influenza A with no subtype was

detected in 11 specimens. These 11 specimens underwent an additional ePlex RP Panel test per the product instructions for use to identify the subtype. In addition, 28 specimens that had been withdrawn were inadvertently sent for investigational testing. In total, there were 3281 specimens initially tested using the ePlex RP Panel.

Of the 3281 specimens initially tested, 3127 (95.3%, 95% CI: 94.5% - 96.0%) had valid results and 154 (4.7%, 95% CI: 4.0% -5.5%) had invalid results. Initial validity rates were similar across the five testing sites. The 154 initially invalid specimens were re-tested per the product instructions for use at the same or a different testing site and 146 had valid results. The final validity rate was 99.8% (3273/3281) (95% CI: 99.5%-99.9%). Final validity rates were also similar across the testing sites.

Of the 162 invalid results (154 initial invalid and 8 re-test invalid), 161 were due to an assay algorithm failure and one was due to operator error. For the one invalid due to an operator error, the cartridge ejected due to a pre-flight failure but the operator did not notice until the following morning. The cartridge was re-inserted and testing completed; however, the sample was in the cartridge for longer than indicated in the product instructions for use so the initial result was invalidated by the operator and the specimen was re- tested.

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

A prospective, multicenter clinical study was conducted to evaluate the clinical performance of the ePlex RP Panel in nasopharyngeal swab in VTM specimens. 2462 prospective specimens were collected at eight collection sites in two phases from patients of all ages and genders presenting with signs and/or symptoms of respiratory infection. In the first phase from March 2013 through August 2014, 1951 prospective specimens were collected and frozen; from September 2016 through October 2016, 511 prospective specimens were collected and tested fresh (never frozen). The expected values of individual analytes based on ePlex RP Panel results in prospective specimens for each phase are summarized in Table 41 to Table 44 below.

Table 41: Expected Value (As Determined by ePlex RP Panel) Summary by Age Group in the Prospective Clinical Evaluation (Phase 1: March 2013 – August 2014)

Organism	All Ages (N=1951) n (%)	Age 0-1 (N=315) n (%)	Age >1-5 (N=250) n (%)	Age >5-21 (N=246) n (%)	Age >21-65 (N=745) n (%)	Age >65 (N=395) n (%)
Adenovirus	72 (3.7)	31 (9.8)	24 (9.6)	7 (2.8)	7 (0.9)	3 (0.8)
Coronavirus	102 (5.2)	19 (6.0)	18 (7.2)	16 (6.5)	32 (4.3)	17 (4.3)
Human Metapneumovirus	113 (5.8)	22 (7.0)	28 (11.2)	6 (2.4)	31 (4.2)	26 (6.6)
Human Rhinovirus/Enterovirus	388 (19.9)	113 (35.9)	94 (37.6)	58 (23.6)	87 (11.7)	36 (9.1)
Influenza A	110 (5.6)	6 (1.9)	18 (7.2)	20 (8.1)	49 (6.6)	17 (4.3)
Influenza A H1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Influenza A H1-2009	76 (3.9)	4 (1.3)	13 (5.2)	14 (5.7)	37 (5.0)	8 (2.0)
Influenza A H3	34 (1.7)	1 (0.3)	5 (2.0)	6 (2.4)	12 (1.6)	10 (2.5)
Influenza B	62 (3.2)	4 (1.3)	9 (3.6)	10 (4.1)	24 (3.2)	15 (3.8)
Parainfluenza Virus 1	24 (1.2)	4 (1.3)	12 (4.8)	4 (1.6)	3 (0.4)	1 (0.3)
Parainfluenza Virus 2	10 (0.5)	4 (1.3)	4 (1.6)	0 (0.0)	2 (0.3)	0 (0.0)
Parainfluenza Virus 3	99 (5.1)	31 (9.8)	20 (8.0)	3 (1.2)	27 (3.6)	18 (4.6)
Parainfluenza Virus 4	7 (0.4)	3 (1.0)	2 (0.8)	1 (0.4)	1 (0.1)	0 (0.0)
RSV A	28 (1.4)	13 (4.1)	6 (2.4)	3 (1.2)	2 (0.3)	4 (1.0)
RSV B	83 (4.3)	33 (10.5)	19 (7.6)	6 (2.4)	15 (2.0)	10 (2.5)
Chlamydia pneumoniae	3 (0.2)	0 (0.0)	0 (0.0)	1 (0.4)	1 (0.1)	1 (0.3)
Mycoplasma pneumoniae	5 (0.3)	1 (0.3)	1 (0.4)	2 (0.8)	1 (0.1)	0 (0.0)

Table 42: Expected Value (As Determined by ePlex RP Panel) Summary by Age Group in the Prospective Clinical Evaluation (Phase 2: September 2016 – October 2016)

Organism	All Ages (N=511) n (%)	Age 0-1 (N=73) n (%)	Age >1-5 (N=75) n (%)	Age >5-21 (N=75) n (%)	Age >21-65 (N=181) n (%)	Age >65 (N=107) n (%)
Adenovirus	10 (2.0)	3 (4.1)	4 (5.3)	1 (1.3)	1 (0.6)	1 (0.9)
Coronavirus	8 (1.6)	2 (2.7)	0 (0.0)	1 (1.3)	4 (2.2)	1 (0.9)
Human Metapneumovirus	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Human Rhinovirus/Enterovirus	188 (36.8)	37 (50.7)	40 (53.3)	33 (44.0)	58 (32.0)	20 (18.7)
Influenza A	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Influenza A H1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Influenza A H1-2009	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Influenza A H3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Influenza B	2 (0.4)	0 (0.0)	0 (0.0)	1 (1.3)	1 (0.6)	0 (0.0)
Parainfluenza Virus 1	1 (0.2)	0 (0.0)	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)
Parainfluenza Virus 2	13 (2.5)	3 (4.1)	4 (5.3)	3 (4.0)	2 (1.1)	1 (0.9)
Parainfluenza Virus 3	5 (1.0)	2 (2.7)	1 (1.3)	1 (1.3)	1 (0.6)	0 (0.0)
Parainfluenza Virus 4	8 (1.6)	1 (1.4)	4 (5.3)	2 (2.7)	1 (0.6)	0 (0.0)
RSV A	8 (1.6)	5 (6.8)	3 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)
RSV B	9 (1.8)	3 (4.1)	4 (5.3)	0 (0.0)	2 (1.1)	0 (0.0)
Chlamydia pneumoniae	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mycoplasma pneumoniae	4 (0.8)	0 (0.0)	1 (1.3)	2 (2.7)	1 (0.6)	0 (0.0)

Table 43: Expected Value (As Determined by ePlex RP Panel) Summary by Specimen Collection Site in the Prospective Clinical Evaluation (Phase 1: March 2013 – August 2014)

Organism	All Sites (N=1951) n (%)	Site 1 (N=165) n (%)	Site 2 (N=248) n (%)	Site 3 (N=350) n (%)	Site 4 (N=892) n (%)	Site 5 (N=296) n (%)
Adenovirus	72 (3.7)	4 (2.4)	8 (3.2)	28 (8.0)	23 (2.6)	9 (3.0)
Coronavirus	102 (5.2)	8 (4.8)	11 (4.4)	32 (9.1)	29 (3.3)	22 (7.4)
Human Metapneumovirus	113 (5.8)	10 (6.1)	23 (9.3)	27 (7.7)	30 (3.4)	23 (7.8)
Human Rhinovirus/Enterovirus	388 (19.9)	27 (16.4)	33 (13.3)	61 (17.4)	185 (20.7)	82 (27.7)
Influenza A	110 (5.6)	5 (3.0)	21 (8.5)	48 (13.7)	19 (2.1)	17 (5.7)
Influenza A H1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Influenza A H1-2009	76 (3.9)	3 (1.8)	22 (8.9)	31 (8.9)	5 (0.6)	15 (5.1)
Influenza A H3	34 (1.7)	2 (1.2)	0 (0.0)	18 (5.1)	12 (1.3)	2 (0.7)
Influenza B	62 (3.2)	9 (5.5)	9 (3.6)	9 (2.6)	19 (2.1)	16 (5.4)
Parainfluenza Virus 1	24 (1.2)	0 (0.0)	0 (0.0)	5 (1.4)	2 (0.2)	17 (5.7)
Parainfluenza Virus 2	10 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	10 (1.1)	0 (0.0)
Parainfluenza Virus 3	99 (5.1)	13 (7.9)	3 (1.2)	28 (8.0)	41 (4.6)	14 (4.7)
Parainfluenza Virus 4	7 (0.4)	0 (0.0)	0 (0.0)	1 (0.3)	4 (0.4)	2 (0.7)
RSV A	28 (1.4)	4 (2.4)	6 (2.4)	7 (2.0)	4 (0.4)	7 (2.4)
RSV B	83 (4.3)	6 (3.6)	15 (6.0)	24 (6.9)	15 (1.7)	23 (7.8)
Chlamydia pneumoniae	3 (0.2)	0 (0.0)	0 (0.0)	1 (0.3)	2 (0.2)	0 (0.0)
Mycoplasma pneumoniae	5 (0.3)	1 (0.6)	0 (0.0)	3 (0.9)	0 (0.0)	1 (0.3)

Table 44: Expected Value (As Determined by ePlex RP Panel) Summary by Specimen Collection Site in the Prospective Clinical Evaluation (Phase 2: September 2016 – October 2016)

Organism	All Sites (N=511) n (%)	Site 5 (N=49) n (%)	Site 6 (N=101) n (%)	Site 7 (N=161) n (%)	Site 8 (N=200) n (%)
Adenovirus	10 (2.0)	2 (4.1)	3 (3.0)	3 (1.9)	2 (1.0)
Coronavirus	8 (1.6)	0 (0.0)	2 (2.0)	4 (2.5)	2 (1.0)
Human Metapneumovirus	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Human Rhinovirus/Enterovirus	188 (36.8)	24 (49.0)	49 (48.5)	62 (38.5)	53 (26.5)
Influenza A	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Influenza A H1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Influenza A H1-2009	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Influenza A H3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Influenza B	2 (0.4)	1 (2.0)	0 (0.0)	0 (0.0)	1 (0.5)
Parainfluenza Virus 1	1 (0.2)	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)
Parainfluenza Virus 2	13 (2.5)	2 (4.1)	4 (4.0)	3 (1.9)	4 (2.0)
Parainfluenza Virus 3	5 (1.0)	2 (4.1)	2 (2.0)	0 (0.0)	1 (0.5)
Parainfluenza Virus 4	8 (1.6)	1 (2.0)	1 (1.0)	4 (2.5)	2 (1.0)
RSV A	8 (1.6)	0 (0.0)	8 (7.9)	0 (0.0)	0 (0.0)
RSV B	9 (1.8)	1 (2.0)	4 (4.0)	0 (0.0)	4 (2.0)
Chlamydia pneumoniae	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mycoplasma pneumoniae	4 (0.8)	0 (0.0)	3 (3.0)	0 (0.0)	1 (0.5)

N. Instrument Name:

ePlex Instrument

O. System Descriptions:

1. Modes of Operation:

   After samples have been loaded in the ePlex RP Panel cartridge, the remaining processing steps are executed under control of the ePlex instrument.

2. Software:

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

   Yes ____X____ or No _____

3. Specimen Identification:

   Specimen identification can be entered manually or via barcode.

4. Specimen Sampling and Handling:

   The ePlex RP Panel is intended for use with nasopharyngeal swab (NPS) collected in VTM specimens. The operator removes an ePlex RP Panel cartridge pouch and one Sample Delivery Device from Kit packaging. The operator first writes the accession ID or place a barcode label with accession ID on the ePlex RP Panel cartridge and the Sample Delivery Device, and then pipette 200 µl of specimen into the Sample Delivery Device using a calibrated pipette, close the Sample Delivery Device, and vortex it for 10 seconds. The operator subsequently removes the white cover from the tip of the Sample Delivery Device, invert the Sample Delivery Device and dispense the entire volume (~350 µL) by squeezing the vial and dispensing the drops into the sample loading port of the ePlex RP Panel cartridge. The operator then close the sample loading port by sliding the cap over the port and firmly pushing down on the cap to securely seal the sample delivery port, scan the ePlex RP Panel cartridge using the barcode reader provided with the ePlex instrument, and insert the cartridge into any available bay on the ePlex instrument, indicated by a flashing, white LED light. The test then begins automatically when the cartridge has been inserted into the bay and the pre-run check (cartridge initialization) is completed, indicated by a blue LED light.

5. Calibration:

   Not applicable

6. Quality Control:

Internal Controls
Each ePlex RP Panel cartridge includes internal controls that monitor performance of each step of the testing process. A DNA control verifies extraction, amplification and detection of DNA targets, and RNA controls verify amplification and detection of RNA targets. Each amplification reaction on the cartridge has at least one internal control and in each reaction either the internal control or a target must generate signal above the defined threshold for a valid test result. Internal control results are interpreted by the ePlex software and displayed on ePlex RP Panel Reports as Internal Control with a result of PASS, FAIL, N/A or INVALID.

External Controls
External controls are not provided with the ePlex RP Panel. However, the sponsor is making the following recommendations regarding running external controls in the product package insert:

"Positive and negative external controls should be tested with each new lot of reagents or monthly, whichever occurs first. Viral transport medium can be used as the negative control. Previously characterized positive samples or viral transport medium spiked with well characterized organisms can be used as the external positive control. External controls should be run in accordance with laboratory protocols and accrediting organizations, as applicable."

P. Other Supportive Instrument Performance Characteristics Data Not Covered In the "Performance Characteristics" Section above:

Not applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.