DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
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Silver Spring, MD 20993-0002

GenMark Diagnostics, Incorporated
Alan Maderazo, Ph.D., RAC
Vice President, Quality, Regulatory and Clinical Affairs
5964 La Place Court
Carlsbad, CA 92008

June 9, 2017

Re: K163636
Trade/Device Name: ePlex® Respiratory Pathogen (RP) Panel
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory Viral Panel Multiplex Nucleic Acid Assay
Regulatory Class: II
Product Code: OCC, OEM, OEP, OOU, OTG, OZE, OZX, OZY, OQW, NSU
Dated: May 3, 2017
Received: May 5, 2017

Dear Dr. Maderazo:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

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

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Tamara V. Feldblyum -S for

Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K163636

Device Name
ePlex® Respiratory Pathogen (RP) Panel

Indications for Use *(Describe)*

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.

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

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

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510(k) Summary

Summary of Safety and Effectiveness

Submitter Information

Submitter: GenMark Diagnostics, Incorporated
5964 La Place Court
Carlsbad, CA 92008

Manufacturer: GenMark Diagnostics, Incorporated
5964 La Place Court
Carlsbad, CA 92008

Establishment Registration Number: 3008632402

Contact: Alan Maderazo, Ph.D., RAC
Vice President, Quality, Regulatory and Clinical Affairs

Phone: 760-448-4308

Fax: 760-683-6961

E-mail: Al.Maderazo@genmarkdx.com

Alternate Contact: Joseph McMullen
Consultant, Regulatory Affairs

Phone: 760-410-5052

Fax: 760-683-6961

E-mail: Joseph.McMullen@genmarkdx.com

Date Prepared: December 21, 2016

Name of Device and Classification

Product Name: ePlex® Respiratory Pathogen (RP) Panel

Device Classification: 866.3980, Respiratory viral panel multiplex nucleic acid assay, Class II

Product Code(s): OCC, OEM, OOU, OEP, OTG, OQW, OZE, OZY, OZX, NSU

Predicate Device

Predicate: FilmArray Respiratory Panel; BioFire Diagnostics; K160068

Device Description

The ePlex RP Panel is an automated qualitative nucleic acid multiplex *in vitro* diagnostic test for simultaneous detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS). The test is able to detect 15 respiratory viral targets and 2 bacterial targets as summarized in the table below. This test is performed on the ePlex Instrument.

Targets Detected by the ePlex RP Panel

Target	Classification (Genome Type)
Adenovirus (A-F)	Adenovirus (DNA)
Coronavirus (229E, HKU1, NL63, OC43)	Coronavirus (RNA)
Human Metapneumovirus	Paramyxovirus (RNA)
Human Rhinovirus/ Enterovirus	Picornavirus (RNA)
Influenza A	Orthomyxovirus (RNA)
Influenza A H1	
Influenza A H1-2009	
Influenza A H3	
Influenza B	
Parainfluenza Virus 1	Paramyxovirus (RNA)
Parainfluenza Virus 2	
Parainfluenza Virus 3	
Parainfluenza Virus 4	
Respiratory Syncytial Virus A	Paramyxovirus (RNA)
Respiratory Syncytial Virus B	
Chlamydia pneumoniae	Bacterium (DNA)
Mycoplasma pneumoniae	Bacterium (DNA)

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

Intended Use/Indications for 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.

Summary of Technological Characteristics of the Device Compared to the Predicate Device

The GenMark ePlex Respiratory Pathogen (RP) Panel ("Proposed Device") and the legally marketed device, FilmArray Respiratory Panel ("Predicate Device") are described below:

Characteristic	Proposed Device	Predicate Device
Product Name	ePlex Respiratory Pathogen (RP) Panel	FilmArray Respiratory Panel
Manufacturer	GenMark Diagnostics	BioFire Diagnostics
Regulation	866.3980 Respiratory viral panel multiplex nucleic acid assay	866.3980 Respiratory viral panel multiplex nucleic acid assay
Product Code(s)	OCC, OEM, OOU, OEP, OTG, OZE, OQW, OZY, OZX, NSU	OCC, OEM, OOU, OEP, OTG, OQW, OOI, OZZ, OZY, OZX
Device Class	Class II	Class II
Organisms Detected	Influenza A Influenza A subtype H1 Influenza A subtype H3 Influenza A subtype H1-2009 Influenza B Respiratory Syncytial Virus A and B Parainfluenza Virus 1, 2, 3, 4 Human Metapneumovirus Adenovirus Human Rhinovirus/Enterovirus Coronavirus *Chlamydia pneumoniae* *Mycoplasma pneumoniae*	Influenza A Influenza A subtype H1 Influenza A subtype H3 Influenza A subtype H1-2009 Influenza B Respiratory Syncytial Virus Parainfluenza Virus 1, 2, 3, 4 Human Metapneumovirus Adenovirus Human Rhinovirus/Enterovirus Coronavirus HKU1, NL63, 229E, OC43 *Bordetella pertussis* *Chlamydia pneumoniae* *Mycoplasma pneumoniae*
Analyte	RNA/DNA	RNA/DNA
Technological Principles	Multiplex nucleic acid amplification test (NAAT)	Multiplex nucleic acid amplification test (NAAT)
Specimen Type	Nasopharyngeal swabs (NPS) in VTM	Nasopharyngeal swabs (NPS) in VTM
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.
Instrumentation	ePlex Instrument	FilmArray Instrument
Test Interpretation	Automated test interpretation and report generation. User cannot access raw data.	Automated test interpretation and report generation. User cannot access raw data.
Sample Preparation Method	Sample processing is automated in the ePlex RP cartridge.	Sample processing is automated in the FilmArray RP pouch.
Controls	Eight internal controls are used 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.

Characteristic	Proposed Device	Predicate Device
User Complexity	Moderate	Moderate
Reagent Storage	Cartridge (which contains reagents) is stored at refrigerated (2-8°C) temperature.	Reagents are stored at room temperature.

Analysis of the similarities and differences indicate that the devices are substantially equivalent in their intended uses/indications for use, and are generally the same regarding user process, ease of use and general operator protocol. Comparison of technological similarities and differences between the proposed device and the predicate do not raise new or different questions of safety and effectiveness, and therefore render the proposed device as substantially equivalent to the predicate device.

Expected Values

A prospective, multicenter clinical study was conducted to evaluate the clinical performance of the ePlex RP Panel in nasopharyngeal swab samples. 2462 nasopharyngeal swab samples were prospectively-collected at 8 collection sites in 2 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 samples were prospectively-collected and frozen; from September 2016 through October 2016, 511 samples were prospectively-collected and tested fresh (never frozen). The expected values of individual analytes based on ePlex RP Panel results in prospective samples for each phase are summarized in **Tables 1-4**.

Table 1: 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 2: 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 3: Expected Value (As Determined by ePlex RP Panel) Summary By Sample 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 4: Expected Value (As Determined by ePlex RP Panel) Summary By Sample 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)

Summary of Performance Data

Clinical performance

Comparator Method

The performance of the ePlex RP Panel was compared to an FDA-cleared multiplexed molecular respiratory pathogen panel and analytically validated PCR tests with bi-directional sequencing for confirmation of RSV subtypes.. Details of the comparator method are described in **Table 5**.

Table 5: Comparator Methods Used to Assess ePlex RP Panel Clinical Performance

Target	Comparator Method
Adenovirus	FDA-cleared multiplexed molecular respiratory pathogen panel
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 A	FDA-cleared multiplexed molecular respiratory pathogen panel followed by a PCR test with bi-directional sequencing confirmation
Respiratory Syncytial Virus B	
Chlamydia pneumoniae	FDA-cleared multiplexed molecular respiratory pathogen panel
Mycoplasma pneumoniae	

Prospective Clinical Samples

Clinical performance was evaluated in clinical nasopharyngeal swab samples in VTM prospectively-collected at 8 clinical sites in 2 phases. From March 2013 through August 2014, 2218 samples were prospectively-collected and frozen; from September 2016 through October

2016, 514 samples were prospectively-collected and tested fresh (never frozen). A total of 2732 samples were collected across the 2 phases. Prior to the start of investigational testing, 263 samples were withdrawn (251 had sample handling deviations, 9 were tested outside of protocol timelines, 2 had insufficient volume, and 1 had incomplete documentation). Of the 2469 prospectively-collected samples eligible for testing, 2462 were evaluable. Samples with final, valid results and a valid comparator result were considered evaluable. Seven prospectively-collected samples were not evaluable because they did not have final, valid ePlex RP Panel results and were excluded from performance evaluations. Demographic information for prospectively-collected samples is described in **Table 6**. Subjects enrolled in this study were from a diverse demographic distribution and represent the intended patient population.

Table 6: Subject Demographic Data for Prospectively-Collected Samples 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)

Prospective Clinical Performance

Positive percent agreement (PPA) 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. The two-sided 95% confidence interval was also calculated.

A total of 2462 prospectively-collected samples (511 tested fresh and 1951 tested after previously frozen) were evaluated for 17 ePlex RP Panel organisms. PPA and NPA results are summarized by target in **Tables 7 and 8** below.

Table 7: Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) in the ePlex RP Panel Clinical Study (Fresh)

Organism	Prevalence	Positive % Agreement		Negative % Agreement	
		TP/TP+FN	PPA (95% CI)	TN/TN+FP	NPA (95% CI)
Adenovirus	1.6%	6/8[a]	75.0 (40.9-92.9)	499/503[a]	99.2 (98.0-99.7)
Coronavirus	1.4%	7/7	100 (64.6-100)	503/504	99.8 (98.9-100)
Human Metapneumovirus	0.0%	0/0	---	511/511	100 (99.3-100)
Human Rhinovirus/Enterovirus	35.8%	176/183[b]	96.2 (92.3-98.1)	316/328[b]	96.3 (93.7-97.9)
Influenza A	0.0%	0/0	---	511/511	100 (99.3-100)
Influenza A H1	0.0%	0/0	---	511/511	100 (99.3-100)
Influenza A H1-2009	0.0%	0/0	---	511/511	100 (99.3-100)
Influenza A H3	0.0%	0/0	---	511/511	100 (99.3-100)
Influenza B	0.2%	1/1	100 (20.7-100)	509/510	99.8 (98.9-100)
Parainfluenza Virus 1	0.2%	1/1	100 (20.7-100)	510/510	100 (99.3-100)
Parainfluenza Virus 2	2.5%	12/13	92.3 (66.7-98.6)	497/498	99.8 (98.9-100)
Parainfluenza Virus 3	1.0%	5/5	100 (56.6-100)	506/506	100 (99.2-100)
Parainfluenza Virus 4	0.6%	3/3	100 (43.9-100)	503/508[c]	99.0 (97.7-99.6)
RSV A	1.8%	8/9	88.9 (56.5-98.0)	501/501	100 (99.2-100)
RSV B	2.0%	9/10	90.0 (59.6-98.2)	500/500	100 (99.2-100)
Chlamydia pneumoniae	0.0%	0/0	---	511/511	100 (99.3-100)
Mycoplasma pneumoniae	0.6%	3/3	100 (43.9-100)	507/508[d]	99.8 (98.9-100)

[a] Adenovirus was not detected in 2 of 2 FN samples and detected in 4 of 4 FP samples using PCR/sequencing.
[b] Human rhinovirus/enterovirus was not detected in 1 of 7 FN samples and detected in 9 of 12 FP samples using PCR/sequencing.
[c] Parainfluenza virus 4 was detected in 3 of 5 FP samples using PCR/sequencing.
[d] *M. pneumoniae* was detected in the 1 FP sample using PCR/sequencing.

Table 8: Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) in the ePlex RP Panel Clinical Study (After Previously Frozen)

Organism	Prevalence	Positive % Agreement		Negative % Agreement	
		TP/TP+FN	PPA (95% CI)	TN/TN+FP	NPA (95% CI)
Adenovirus	2.7%	48/53[a]	90.6 (79.7-95.9)	1874/1898[a]	98.7 (98.1-99.1)
Coronavirus	5.6%	89/110[b]	80.9 (72.6-87.2)	1828/1841[b]	99.3 (98.8-99.6)
Human Metapneumovirus	5.8%	107/113[c]	94.7 (88.9-97.5)	1832/1838[c]	99.7 (99.3-99.9)
Human Rhinovirus/Enterovirus	17.2%	317/336[d]	94.3 (91.3-96.4)	1544/1615[d]	95.6 (94.5-96.5)
Influenza A[e]	5.7%	106/111[f]	95.5 (89.9-98.1)	1836/1840[f]	99.8 (99.4-99.9)
Influenza A H1	0.0%	0/0	---	1951/1951	100 (99.8-100)
Influenza A H1-2009	3.6%	70/71	98.6 (92.4-99.8)	1874/1880[g]	99.7 (99.3-99.9)
Influenza A H3	1.9%	34/37[h]	91.9 (78.7-97.2)	1914/1914	100 (99.8-100)
Influenza B	3.3%	58/65[i]	89.2 (79.4-94.7)	1882/1886[i]	99.8 (99.5-99.9)
Parainfluenza Virus 1	1.2%	23/24	95.8 (79.8-99.3)	1926/1927	99.9 (99.7-100)
Parainfluenza Virus 2	0.5%	9/9	100 (70.1-100)	1941/1942	99.9 (99.7-100)
Parainfluenza Virus 3	5.3%	94/104[j]	90.4 (83.2-94.7)	1842/1847[j]	99.7 (99.4-99.9)
Parainfluenza Virus 4	0.3%	5/5	100 (56.6-100)	1944/1946	99.9 (99.6-100)
RSV A	1.6%	27/31	87.1 (71.1-94.9)	1917/1918	99.9 (99.7-100)
RSV B	4.4%	81/86	94.2 (87.1-97.5)	1861/1863[k]	99.9 (99.6-100)
Chlamydia pneumoniae	0.3%	2/5[l]	40.0 (11.8-76.9)	1945/1946[l]	99.9 (99.7-100)
Mycoplasma pneumoniae	0.3%	4/5[m]	80.0 (37.6-96.4)	1945/1946	99.9 (99.7-100)

[a] Adenovirus was not detected in 1 of 5 FN samples and detected in 9 of 24 FP samples using PCR/sequencing.
[b] Coronavirus was not detected in 2 of 21 FN samples and detected in 3 of 13 FP samples using PCR/sequencing.
[c] Human Metapneumovirus was not detected in 1 of 6 FN samples and detected in 4 of 6 FP samples using PCR/sequencing.
[d] Human rhinovirus/enterovirus was not detected in 6 of 19 FN samples and detected in 33 of 71 FP samples using PCR/sequencing.
[e] Influenza A comparator results contain 71 samples with A H1-2009, 37 samples with A H3, and 3 samples with no subtype detected.
[f] Influenza A was not detected in 1 of 3 FN samples (2 samples were not tested by PCR/sequencing) and detected in 1 of 4 FP samples using PCR/sequencing.
[g] Influenza A H1-2009 was detected in 4 of 6 FP samples using PCR/sequencing.
[h] Influenza A H3 was not detected in 1 of 3 FN samples using PCR/sequencing.
[i] Influenza B was not detected in 3 of 7 FN samples and detected in 2 of 4 FP samples using PCR/sequencing.
[j] Parainfluenza virus 3 was not detected in 3 of 10 FN samples and detected in 4 of 5 FP samples using PCR/sequencing.
[k] RSV B was detected in 1 of 2 FP samples using PCR/sequencing.
[l] *C. pneumoniae* was not detected in 1 of 3 FN samples and detected in the 1 FP sample using PCR/sequencing.
[m] *M. pneumoniae* was not detected in the 1 FN sample using PCR/sequencing.

Retrospective Clinical Samples

To supplement the number of positives for targets that were not sufficiently represented in the prospective collection, additional nasopharyngeal swab in VTM samples were retrospectively collected from 6 sites. A total of 535 nasopharyngeal swab samples 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 samples were withdrawn due to noncompliance with the study protocol, and 52 samples were withdrawn because the organisms present had sufficient representation in other samples. In addition, the composition and integrity of the retrospective samples were 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 samples were withdrawn because the original SOC testing positive results for the intended organisms were not confirmed when tested with the comparator method. Of the remaining 446 retrospectively-collected samples eligible for testing, all 446 were evaluable. Demographic information for retrospectively-collected samples is described in **Table 9**. Subjects enrolled in this study were from a diverse demographic distribution and represent the intended patient population.

Table 9: Subject Demographic Data for Retrospectively-Collected Samples 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 Performance

A total of 446 retrospectively-collected samples were evaluated for 17 ePlex RP Panel organisms. The following specimens with the original positive SOC results for the unintended organisms that were not confirmed by the comparator method were excluded from the performance calculation for the respective organism: 1 coronavirus positive specimen, 3 human rhinovirus/enterovirus positive specimens, 1 influenza A positive specimen, 1 influenza A H3 positive specimen, 1 parainfluenza virus positive specimen. In addition, 5 unintended RSV positive specimens by the comparator method were not confirmed by PCR/sequencing with regard to determining RSV subtypes and therefore were excluded from the performance calculations for RSV A and RSV B. PPA and NPA results are summarized by target in **Table 10** below.

Table 10: Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of the ePlex RP Panel With Comparator Methods (Retrospective Collection)

Organism	**Positive % Agreement**		**Negative % Agreement**	
	TP/TP+FN	**PPA (95% CI)**	**TN/TN+FP**	**NPA (95% CI)**
Adenovirus	55/56[a]	98.2 (90.6-99.7)	386/390[a]	99.0 (97.4-99.6)
Coronavirus	121/138[b]	87.7 (81.2-92.2)	307/307	100 (98.8-100)
Human Metapneumovirus	5/7	71.4 (35.9-91.8)	439/439	100 (99.1-100)
Human Rhinovirus/Enterovirus	37/41	90.2 (77.5-96.1)	384/402	95.5 (93.0-97.1)
Influenza A[c]	75/82[d]	91.5 (83.4-95.8)	363/363	100 (99.0-100)
Influenza A H1	0/0	---	446/446	100 (99.1-100)
Influenza A H1-2009	27/31[e]	87.1 (71.1-94.9)	415/415	100 (99.1-100)
Influenza A H3	45/51[f]	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	43/48[g]	89.6 (77.8-95.5)	396/397	99.7 (98.6-100)

Organism	Positive % Agreement		Negative % Agreement	
	TP/TP+FN	PPA (95% CI)	TN/TN+FP	NPA (95% CI)
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	25/27	92.6 (76.6-97.9)	414/414	100 (99.1-100)
RSV B	21/22	95.5 (78.2-99.2)	419/419	100 (99.1-100)
Chlamydia pneumoniae	1/1	100 (20.7-100)	445/445	100 (99.1-100)
Mycoplasma pneumoniae	7/7	100 (64.6-100)	439/439	100 (99.1-100)

[a] Adenovirus was not detected in the 1 FN sample and detected in 2 of 4 FP samples using PCR/sequencing.
[b] Coronavirus was not detected in 2 of 16 FN samples using PCR/sequencing (1 sample was not tested by PCR/sequencing).
[c] Influenza A comparator results contain 31 samples with A H1-2009 and 51 samples with A H3 detected.
[d] Influenza A was not detected in 3 of 7 FN samples using PCR/sequencing.
[e] Influenza A H1-2009 was not detected in 2 of 4 FN samples using PCR/sequencing.
[f] Influenza A H3 was not detected in 1 of 6 FN samples using PCR/sequencing.
[g] Parainfluenza virus 1 was not detected in 2 of 5 FN samples using PCR/sequencing.

Contrived Sample Performance

There were 327 contrived samples created and tested to supplement the low prevalence targets on the RP Panel; 104 contained one or more low prevalence organisms and 223 were negative for the contrived organisms. All 327 contrived samples were tested with the ePlex RP Panel and 326 were evaluable. PPA and NPA results are summarized for these low prevalence organisms in **Table 11** below.

Table 11: Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of the ePlex RP Panel With Comparator Method (Contrived Samples)

Organism	Positive % Agreement		Negative % Agreement	
	TP/TP+FN	PPA (95% CI)	TN/TN+FP	NPA (95% CI)
Chlamydia pneumoniae	52/52	100 (93.1-100)	274/274	100 (98.6-100)
Influenza A H1	51/51	100 (93.0-100)	275/275	100 (98.6-100)

Co-Detections in Prospective Clinical Samples

The ePlex RP Panel identified a total of 135 prospective samples with multiple organisms detected, or 5.5% of all prospectively-collected samples. Of these, 118 (4.8%) had two organisms, 14 (0.6%) had three organisms, and 3 (0.1%) had four organisms detected. Of the 135 co-detected samples, 58 included 1 or more organisms that had not been detected by the comparator method(s). Results are summarized in **Tables 12 and 13**.

Table 12: Distinct Co-Detection Combinations Detected by the ePlex RP Panel in the Prospective Clinical Samples

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 AH3			1 (0.04%)	0	
ADV	Flu B	HRV/EV	RSV B	1 (0.04%)	1	ADV (1), Flu B (1)
ADV	FluA09H1			1 (0.04%)	1	ADV (1), FluA09H1 (1)
ADV	FluA09H1	HRV/EV		1 (0.04%)	0	
ADV	FluA09H1	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	FluA09H1			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)
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)

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			
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(s).

[b] 64/64 discrepant organisms were investigated using PCR/sequencing; the discrepant organism was detected in 20/64 cases:
-In 8/18 samples, adenovirus was detected by PCR/sequencing.
-In 1/4 samples, coronavirus was detected by PCR/sequencing.
-In 7/25 samples, human rhinovirus/enterovirus was detected by PCR/sequencing.
-In 1/1 sample, influenza A H1-2009 was detected by PCR/sequencing.
-In 1/1 sample, parainfluenza virus 3 was detected by PCR/sequencing.
-In 2/6 samples, parainfluenza virus 4 was detected by PCR/sequencing.

Table 13: Additional Co-Detection Combinations Detected by the Comparator Method in the Prospective Clinical Samples

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	FluA09H1		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 AH3	HRV/EV	PIV 3	1 (0.04%)	1	Flu AH3 (1), PIV 3 (1)
Flu AH3	PIV 3		1 (0.04%)	1	PIV 3 (1)
FluA09H1	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)

[a] A discrepant organism is defined as one that was detected by the comparator method(s) 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, *Chlamydia 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.

Clinical Study ePlex Instrument Performance

A total of 3281 samples (including prospective, retrospective, and contrived samples) were initially tested in the clinical evaluations and 3127/3281 = 95.3% (95% CI: 94.5%-96.0%) generated valid results on the first attempt. After re-test, 8 samples had invalid results; final validity rate was 3273/3281 = 99.8% (95% CI: 99.5%-99.9%).

Selected Analytical Studies

Limit of Detection

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 study. 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 14**.

Table 14: LoD Results Summary

Target	Strain	LoD Concentration
Adenovirus	Type 1 (C)	1×10^3 $TCID_{50}$/mL
	Type 4 (E)	2×10^0 $TCID_{50}$/mL
	Type 7 (B)	2×10^0 $TCID_{50}$/mL
Coronavirus 229E	229E	1×10^0 $TCID_{50}$/mL
Coronavirus HKU1	HKU1[a]	5×10^4 copies/mL
Coronavirus NL63	NL63	7.5×10^0 $TCID_{50}$/mL
Coronavirus OC43	OC43	5×10^2 $TCID_{50}$/mL
Human Metapneumovirus	A1 IA3-2002	2×10^{-1} $TCID_{50}$/mL
	A2 IA14-2003	2×10^3 $TCID_{50}$/mL
	B1 Peru2-2002	2×10^2 $TCID_{50}$/mL
	B2 Peru1-2002	2.25×10^2 $TCID_{50}$/mL
Human Rhinovirus/Enterovirus	Enterovirus Type 68 (2007)	1×10^0 $TCID_{50}$/mL
	Rhinovirus 1A	1.5×10^0 $TCID_{50}$/mL
	Rhinovirus B14	1×10^0 $TCID_{50}$/mL
	Rhinovirus C [a]	1×10^5 copies/mL
Influenza A	H1N1 Brisbane/59/07	3×10^{-1} $TCID_{50}$/mL
Influenza A H1	H1N1 Brisbane/59/07	3×10^{-1} $TCID_{50}$/mL
Influenza A H1-2009	NY/01/2009	1×10^{-1} $TCID_{50}$/mL

Target	Strain	LoD Concentration
Influenza A H3	A/Perth/16/2009	1×10^{1} $TCID_{50}$/mL
	A/Texas/50/2012	1×10^{0} $TCID_{50}$/mL
	A/Victoria/361/2011	5×10^{-1} $TCID_{50}$/mL
	H3N2 Brisbane/10/07	5×10^{1} $TCID_{50}$/mL
Influenza B	Florida/02/06	1×10^{-1} $TCID_{50}$/mL
Influenza B (Victoria Lineage)	B/Brisbane/60/2008	1×10^{0} $TCID_{50}$/mL
	B/Montana/5/2012	1×10^{0} $TCID_{50}$/mL
	B/Nevada/03/2011	1×10^{0} $TCID_{50}$/mL
Influenza B (Yamagata Lineage)	B/Massachusetts/02/2012	1×10^{2} $TCID_{50}$/mL
	B/Texas/06/2011	1×10^{-1} $TCID_{50}$/mL
	B/Wisconsin/01/2010	1×10^{0} $TCID_{50}$/mL
Parainfluenza Virus 1	Clinical Isolate	4×10^{-1} $TCID_{50}$/mL
Parainfluenza Virus 2	Clinical Isolate	5×10^{1} $TCID_{50}$/mL
Parainfluenza Virus 3	Clinical Isolate	5×10^{0} $TCID_{50}$/mL
Parainfluenza Virus 4	4a	3×10^{1} $TCID_{50}$/mL
Respiratory Syncytial Virus A	2006 Isolate	1.5×10^{0} $TCID_{50}$/mL
Respiratory Syncytial Virus B	CH93(18)-18	2×10^{-1} $TCID_{50}$/mL
Chlamydia pneumoniae	AR-39	3×10^{2} $TCID_{50}$/mL
Mycoplasma pneumoniae	FH strain of Eaton Agent [NCTC 10119]	3×10^{2} 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.

Analytical Reactivity (Inclusivity)

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.

All of the 101 strains/isolates tested for inclusivity were detected by the ePlex RP Panel. Results of analytical reactivity are shown in **Tables 15-25**.

Table 15: Analytical Reactivity (Inclusivity) Results for Adenovirus

Adenovirus Species	Serotype	Concentration	Multiple of LoD Detected
A	Type 31	3×10^{3} $TCID_{50}$/mL	3x
B	Type 3	6×10^{0} $TCID_{50}$/mL	3x
	Type 11	6×10^{0} $TCID_{50}$/mL	3x
	De Wit Type 14	6×10^{0} $TCID_{50}$/mL	3x

Adenovirus Species	Serotype	Concentration	Multiple of LoD Detected
	Ch.79 Type 16	2 x 10^2 $TCID_{50}$/mL	100x[a]
	Type 21	6 x 10^0 $TCID_{50}$/mL	3x
	Compton Type 34	6 x 10^0 $TCID_{50}$/mL	3x
	Holden Type 35	6 x 10^0 $TCID_{50}$/mL	3x
	Wan Type 50	2 x 10^1 $TCID_{50}$/mL	10x[b]
C	Type 2	3 x 10^3 $TCID_{50}$/mL	3x
	Type 5	3 x 10^3 $TCID_{50}$/mL	3x
	Type 6	3 x 10^3 $TCID_{50}$/mL	3x
D	Type 26	3 x 10^3 $TCID_{50}$/mL	3x
	Type 37	3 x 10^3 $TCID_{50}$/mL	3x
F	Type 40 Dugan	3 x 10^3 $TCID_{50}$/mL	3x
	Type 41/ Strain Tak	3 x 10^3 $TCID_{50}$/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 16: Analytical Reactivity (Inclusivity) Results for Human Metapneumovirus

Metapneumovirus Subtype	Strain	Concentration	Multiple of LoD Detected
Human metapneumovirus	Peru6-2003 G, B2	6.75 x 10^2 $TCID_{50}$/mL	3x

Table 17: Analytical Reactivity (Inclusivity) Results for Human Rhinovirus/Enterovirus

Rhinovirus/Enterovirus	Strain	Concentration	Multiple of LoD Detected
Human Rhinovirus	Type A2	4.5 x 10^0 $TCID_{50}$/mL	3x
	Type A7	1.5 x 10^1 $TCID_{50}$/mL	10x[a]
	Type A16	4.5 x 10^0 $TCID_{50}$/mL	3x
	Type A18	1.5 x 10^2 $TCID_{50}$/mL	100x[a]
	Type A34	4.5 x 10^0 $TCID_{50}$/mL	3x
	Type A57	4.5 x 10^0 $TCID_{50}$/mL	3x
	Type A77	4.5 x 10^0 $TCID_{50}$/mL	3x
	277G	4.5 x 10^0 $TCID_{50}$/mL	3x
	Type B3	1.5 x 10^1 $TCID_{50}$/mL	10x[a]
	Type B17	1.5 x 10^1 $TCID_{50}$/mL	10x[a]
	Type B42	4.5 x 10^0 $TCID_{50}$/mL	3x
	Type B83	4.5 x 10^0 $TCID_{50}$/mL	3x
	Type B84	4.5 x 10^0 $TCID_{50}$/mL	3x
	FO2-2547	4.5 x 10^0 $TCID_{50}$/mL	3x
Enterovirus	Type 71	3 x 10^0 $TCID_{50}$/mL	3x
Coxsackievirus	A9	3 x 10^0 $TCID_{50}$/mL	3x
	A10	3 x 10^0 $TCID_{50}$/mL	3x
	A21	3 x 10^0 $TCID_{50}$/mL	3x
	A24	3 x 10^0 $TCID_{50}$/mL	3x

Rhinovirus/Enterovirus	Strain	Concentration	Multiple of LoD Detected
	B2	1 x 10^2 $TCID_{50}$/mL	100x[a]
	B3	3 x 10^0 $TCID_{50}$/mL	3x
	B4	3 x 10^0 $TCID_{50}$/mL	3x
	B5	1 x 10^1 $TCID_{50}$/mL	10x[a]
Echovirus	9	3 x 10^0 $TCID_{50}$/mL	3x
	E6	1 x 10^1 $TCID_{50}$/mL	10x[b]
	25	1 x 10^1 $TCID_{50}$/mL	10x[a]
	30	3 x 10^0 $TCID_{50}$/mL	3x
Poliovirus	1	1 x 10^2 $TCID_{50}$/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 18: 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 x 10^0 $TCID_{50}$/mL	10x (Influenza A matrix)[a] 10000x H1 subtype[b]
	A/New Caledonia/20/1999	9 x 10^{-1} $TCID_{50}$/mL	3x
	A/New Jersey/8/76	9 x 10^{-1} $TCID_{50}$/mL	3x H1 subtype not detected[c]
	A/NWS/33	3 x 10^0 $TCID_{50}$/mL	10x (Influenza A matrix)[a] H1 subtype not detected[d]
	A/PR/8/34	9 x 10^{-1} $TCID_{50}$/mL	3x (Influenza A matrix) H1 subtype not detected[e]
	A/Solomon Islands/3/2006	9 x 10^{-1} $TCID_{50}$/mL	3x
	A/Taiwan/42/06	9 x 10^0 $TCID_{50}$/mL	30x[f]
Influenza A H3	A/Hong Kong/8/68	1.5 x 10^2 $TCID_{50}$/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 x 10^0 $TCID_{50}$/mL	10x[g]
	A/Mexico/4108/09	3 x 10^{-1} $TCID_{50}$/mL	3x
	A/NY/02/2009	1 x 10^0 $TCID_{50}$/mL	10x[h]
	A/Swine NY/03/2009	3 x 10^{-1} $TCID_{50}$/mL	3x
	A/Swine/Iowa/15/30	3 x 10^{-1} $TCID_{50}$/mL	3x (Influenza A matrix) 100,000x (H1-2009 subtype)[i]
	A/Virginia/ATCC1/2009	1 x 10^0 $TCID_{50}$/mL	10x[j]
	A/Virginia/ATCC2/2009	1 x 10^1 $TCID_{50}$/mL	100x[j]
	A/Virginia/ATCC3/2009	1 x 10^2 $TCID_{50}$/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 19: Analytical Reactivity (Inclusivity) Results for Influenza A Strains Titered with Methods Different From the Reference Strain

Influenza A Subtype	Strain	Concentration Detected
Influenza A H1	A/Denver/1/57	1.6×10^2 $CEID_{50}$/mL (Influenza A matrix) 1.6×10^8 $CEID_{50}$/mL (H1 subtype)
	A/Mal/302/54	1.58×10^2 $CEID_{50}$/mL (Influenza A matrix) 1.58×10^5 $CEID_{50}$/mL (H1 subtype)
Influenza A H3	A/Aichi/2/68 H3N2	1.58×10^3 $CEID_{50}$/mL
	Alice (vaccine) A/England/42/72	5×10^0 EID_{50}/mL (Influenza A matrix) 5×10^1 EID_{50}/mL (H3 subtype)
	MRC-2 Recombinant Strain	8.89×10^2 $CEID_{50}$/mL (Influenza A matrix) 8.89×10^3 $CEID_{50}$/mL (H3 subtype)
Influenza A H1N1	A/Washington/24/2012 (A/H1 pdm09)	3.16×10^3 EID_{50}/mL (Influenza A matrix) 3.16×10^2 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	8.89×10^1 $CEID_{50}$/mL (Influenza A matrix) No subtype detected[a]
Influenza A H5N8	A/Gyrfalcon/Washington/41088-6/2014 BPL	1.58×10^3 EID_{50}/mL (Influenza A matrix) No subtype detected[b]
Influenza A H5N2	A/Northern Pintail/Washington/40964/2014 BPL	2.51×10^3 EID_{50}/mL (Influenza A matrix) No subtype detected[b]
Influenza A H7N9	A/ANHUI/1/2013	7.94×10^3 EID_{50}/mL (Influenza A matrix) No subtype detected[c]
Influenza A H3N2v	A/Indiana/21/2012	2.51×10^4 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

Supplemental Analytical Reactivity (Inclusivity) for Influenza A

For human, avian, and swine influenza strains not available for 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.

Table 20: 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

Influenza A Subtype	Host	Strain	GenBank ID	Predicted ePlex Result
		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
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

Influenza A Subtype	Host	Strain	GenBank ID	Predicted ePlex Result
			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
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	CY047696	Influenza A

Influenza A Subtype	Host	Strain	GenBank ID	Predicted ePlex Result
		Brunswick/25182/2007(H3N6)	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 21: Analytical Reactivity (Inclusivity) Results for Influenza B

Influenza B Subtype	Strain	Concentration	Multiple of LoD Detected
Influenza B (Yamagata Lineage)	B/Lee/40	3×10^{-1} $TCID_{50}$/mL	3x
	B/Allen/45	1×10^{0} $TCID_{50}$/mL	10x[a]
	B/Maryland/1/59	1×10^{1} $TCID_{50}$/mL	100x[a]
	B/Taiwan/2/62	1×10^{1} $TCID_{50}$/mL	100x[a]
Influenza B (Victoria Lineage)	B/Hong Kong/5/72	1×10^{1} $TCID_{50}$/mL	100x[b]
	B/Malaysia/2506/04	3×10^{-1} $TCID_{50}$/mL	3x
Influenza B (Lineage unknown)	B/GL/1739/54	3×10^{-1} $TCID_{50}$/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 22: Analytical Reactivity (Inclusivity) Results for Parainfluenza Virus

Parainfluenza Subtype	Strain	Concentration	Multiple of LoD Detected
Parainfluenza Virus 1	C35	1.2×10^{0} $TCID_{50}$/mL	3x
Parainfluenza Virus 2	Greer	1.5×10^{2} $TCID_{50}$/mL	3x
Parainfluenza Virus 3	C-243	5×10^{1} $TCID_{50}$/mL	10x[a]
Parainfluenza Virus 4	4b	9×10^{1} $TCID_{50}$/mL	3x

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

Table 23: Analytical Reactivity (Inclusivity) Results for Respiratory Syncytial Virus

RSV Subtype	Strain	Concentration	Multiple of LoD Detected
Respiratory Syncytial Virus A	A2	4.5×10^{0} $TCID_{50}$/mL	3x
	Long	4.5×10^{0} $TCID_{50}$/mL	3x
Respiratory Syncytial Virus B	9320	6×10^{-1} $TCID_{50}$/mL	3x
	Wash/18537/62	6×10^{-1} $TCID_{50}$/mL	3x
	WV/14617/85	6×10^{-1} $TCID_{50}$/mL	3x

Table 24: Analytical Reactivity (Inclusivity) Results for *Chlamydia pneumoniae*

	Strain	Concentration	Multiple of LoD Detected
Chlamydia pneumoniae	CWL-029	9×10^{2} CFU/mL	3x
	TWAR strain 2043	9×10^{2} CFU/mL	3x

Table 25: Analytical Reactivity (Inclusivity) Results for *Mycoplasma pneumoniae*

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

Analytical Specificity (Cross-Reactivity and Exclusivity)

Cross-reactivity of each viral and bacterial target on the ePlex RP Panel was evaluated at high concentrations (1×10^5 $TCID_{50}$/mL or $>1 \times 10^5$ EID_{50}/mL for viruses, 1×10^6 CFU/mL or CCU/mL for bacterial isolates, or 1×10^6 copies/mL for *in vitro* transcripts) of quantified strains/isolates diluted in viral transport media. *In vitro* transcript for coronavirus HKU1 was diluted in PBS. **Table 26** summarizes the results of the on-panel viral and bacterial strains/isolates tested. No cross-reactivity was observed between any of the on-panel viruses or bacteria.

Table 26: Cross-reactivity with ePlex RP Panel Target Organisms

Target	Strain	Concentration	Cross-Reactivity Results
Adenovirus A	Type 31	1×10^5 $TCID_{50}$/mL	Not observed
Adenovirus B	Type 7A	1×10^5 $TCID_{50}$/mL	Not observed
Adenovirus C	Type 1	1×10^5 $TCID_{50}$/mL	Not observed
Adenovirus D	Type 9	1×10^5 $TCID_{50}$/mL	Not observed
Adenovirus E	Type 4	1×10^5 $TCID_{50}$/mL	Not observed
Adenovirus F	Type 41	1×10^5 $TCID_{50}$/mL	Not observed
Coronavirus	229E	1×10^5 $TCID_{50}$/mL	Not observed
Coronavirus	HKU1 *in vitro* transcript	1×10^6 copies/mL	Not observed
Coronavirus	NL63	1×10^5 $TCID_{50}$/mL	Not observed
Coronavirus	OC43	1×10^5 $TCID_{50}$/mL	Not observed
Enterovirus	Type 68 2007 isolate	1×10^5 $TCID_{50}$/mL	Not observed
Human metapneumovirus	B1	1×10^5 $TCID_{50}$/mL	Not observed
Human rhinovirus	1A	1×10^5 $TCID_{50}$/mL	Not observed

Target	Strain	Concentration	Cross-Reactivity Results
Influenza A	A/Brisbane/59/07	1 x 10^5 $TCID_{50}$/mL	Not observed
Influenza A H1	A/Brisbane/59/07	1 x 10^5 $TCID_{50}$/mL	Not observed
Influenza A H1-2009	A/NY/01/2009	1 x 10^5 $TCID_{50}$/mL	Not observed
Influenza A H3	A/Brisbane/10/07	1 x 10^5 $TCID_{50}$/mL	Not observed
Influenza A H3N2v[a]	A/Indiana/21/2012	2.51 x 10^5 EID_{50}/mL	Not observed
Influenza A H5N2[b]	A/Northern Pintail Washington/40964/14BPL	2.51 x 10^5 EID_{50}/mL	Not observed
Influenza A H5N8[c]	A/Gyrfalcon/Washington /410886/2014 BPL	1.58 x 10^5 EID_{50}/mL	Not observed
Influenza A H7N9[d]	A/ANHUI/1/2013	7.94 x 10^5 EID_{50}/mL	Not observed
Influenza B	B/Florida/02/06	1 x 10^5 $TCID_{50}$/mL	Not observed
Parainfluenza Virus 1	C35	1 x 10^5 $TCID_{50}$/mL	Not observed
Parainfluenza Virus 2	Type 2	1 x 10^5 $TCID_{50}$/mL	Not observed
Parainfluenza Virus 3	Type 3	1 x 10^5 $TCID_{50}$/mL	Not observed
Parainfluenza Virus 4	Type 4a	1 x 10^5 $TCID_{50}$/mL	Not observed
RSV A	2006 Isolate	1 x 10^5 $TCID_{50}$/mL	Not observed
RSV B	CH93(18)-18	1 x 10^5 $TCID_{50}$/mL	Not observed
Chlamydia pneumoniae	AR-39	1 x 10^6 CFU/mL	Not observed
Mycoplasma pneumoniae	FH strain of Eaton Agent [NCTC 10119]	1 x 10^6 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

Cross-reactivity of viruses, bacteria, and fungi that are not targets on the ePlex RP Panel was evaluated at high concentrations (1 x 10^5 $TCID_{50}$/mL or copies/mL for viruses, 1 x 10^6 CFU/mL for bacterial and yeast isolates, or 1 x 10^6 copies/mL for plasmid DNA or genomic RNA) by diluting quantified strains/isolates in viral transport media. Plasmid for bocavirus and genomic RNA for MERS coronavirus (MERS-CoV) were diluted in PBS. **Table 27** summarizes the results of the strains tested. No cross-reactivity was observed between any of the off-panel viruses, bacteria or fungi with the ePlex RP Panel targets.

Table 27: Cross-reactivity with Organisms Not Detected by the ePlex RP Panel (Exclusivity)

Target	Strain	Concentration	Cross-Reactivity Results
Acinetobacter baumanii	ATCC® 19606	1 x 10^6 CFU/mL	Not observed
Bordetella pertussis	18323 [NCTC 10739]	1 x 10^6 CFU/mL	Not observed
Bordetella parapertussis	ATCC 15311	1 x 10^6 CFU/mL	Not observed
Burkholderia cepacia	ATCC 25416	1 x 10^6 CFU/mL	Not observed
Candida albicans	ATCC 10231	1 x 10^6 CFU/mL	Not observed

Target	Strain	Concentration	Cross-Reactivity Results
Candida glabrata	ATCC 15126	1 x 10^6 CFU/mL	Not observed
MERS Coronavirus (MERS-CoV)	EMC/2012[a]	1 x 10^5 copies/mL	Not observed
Corynebacterium diphtheriae	ATCC 13812	1 x 10^6 CFU/mL	Not observed
Cytomegalovirus	AD 169	1 x 10^5 $TCID_{50}$/mL	Not observed
Epstein Barr Virus	Strain B95-8	1 x 10^5 $TCID_{50}$/mL	Not observed
Escherichia coli	ATCC 10279	1 x 10^6 CFU/mL	Not observed
Haemophilus influenzae	ATCC 43065	1 x 10^6 CFU/mL	Not observed
Herpes Simplex Virus	Isolate 2	1 x 10^5 $TCID_{50}$/mL	Not observed
Human bocavirus	Bocavirus plasmid[b]	1 x 10^6 copies/mL	Not observed
Klebsiella pneumoniae	ATCC 51504	1 x 10^6 CFU/mL	Not observed
Lactobacillus acidophilus	ATCC 314	1 x 10^6 CFU/mL	Not observed
Lactobacillus plantarum	ATCC 8014	1 x 10^6 CFU/mL	Not observed
Legionella pneumophila	Philadelphia-1	1 x 10^6 CFU/mL	Not observed
Measles	N/A	1 x 10^5 $TCID_{50}$/mL	Not observed
Moraxella catarrhalis	ATCC 23246	1 x 10^6 CFU/mL	Not observed
Mumps	Isolate 2	1 x 10^5 $TCID_{50}$/mL	Not observed
Mycobacterium tuberculosis	ATCC 25177	1 x 10^6 CFU/mL	Not observed
Neisseria meningiditis	ATCC 13077	1 x 10^6 CFU/mL	Not observed
Neisseria sicca	ATCC 29193	1 x 10^6 CFU/mL	Not observed
Porphyromonas gingivalis	ATCC 33277	1 x 10^6 CFU/mL	Not observed
Proteus vulgaris	ATCC 33420	1 x 10^6 CFU/mL	Not observed
Pseudomonas aeruginosa	ATCC 15442	1 x 10^6 CFU/mL	Not observed
Serratia marcescens	ATCC 13880	1 x 10^6 CFU/mL	Not observed
Staphylococcus aureus (MRSA)	NRS384	1 x 10^6 CFU/mL	Not observed
Staphylococcus aureus (MSSA)	ATCC 25923	1 x 10^6 CFU/mL	Not observed
Staphylococcus epidermidis (MRSE)	ATCC 35983	1 x 10^6 CFU/mL	Not observed
Staphylococcus epidermidis (MSSE)	ATCC 49134	1 x 10^6 CFU/mL	Not observed
Staphylococcus haemolyticus	ATCC 29970	1 x 10^6 CFU/mL	Not observed
Streptococcus agalactiae	ATCC 12401	1 x 10^6 CFU/mL	Not observed
Streptococcus dysgalactiae	ATCC 35666	1 x 10^6 CFU/mL	Not observed
Streptococcus mitis	ATCC 15914	1 x 10^6 CFU/mL	Not observed
Streptococcus pneumoniae	ATCC 49619	1 x 10^6 CFU/mL	Not observed
Streptococcus pyogenes	ATCC 12384	1 x 10^6 CFU/mL	Not observed
Streptococcus salivarius	ATCC 13419	1 x 10^6 CFU/mL	Not observed
Varicella Zoster Virus	82	8.9 x 10^3 $TCID_{50}$/mL	Not observed

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

Reproducibility

A multisite reproducibility study of the ePlex RP Panel was performed to evaluate agreement with expected results across major sources of variability, such as site-to-site, lot-to-lot, day-to-day, and operator-to-operator. Testing occurred at 3 sites (2 external, 1 internal) on one ePlex instrument per site with either 3 or 4 towers. Two operators performed testing at each site on 6 days (5 nonconsecutive days) with 3 unique lots of RP Panel cartridges. A reproducibility panel consisting of 3 panel members with 6 organisms (representing 7 RP Panel targets) at 3 concentrations (moderate positive- 3x LoD, low positive- 1x LoD, and negative) was tested in triplicate. The 6 organisms tested included adenovirus, coronavirus, human metapneumovirus, influenza A H3, parainfluenza virus 1, and RSV A*;* organisms were diluted in natural clinical matrix (pooled, negative nasopharyngeal swab samples). Negative samples consisted of natural clinical matrix only. Each simulated sample was divided into aliquots and stored frozen (-70 °C) prior to testing. Each operator tested 9 samples (3 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 maximum of 324 tests. After completion of initial and repeat testing for invalid results, 1 low positive sample tested at Site 3 had an invalid result and was excluded from these analyses.

Percent agreement (95% CI) with expected results was 100% for all 7 targets for the moderate positive and negative panel, and 100% for 6 of 7 low positive panel targets (coronavirus, human metapneumovirus, influenza A, influenza A H3, parainfluenza 1, and RSV A); percent agreement was 91.6% for adenovirus. Summary results for the 7 ePlex RP Panel targets that correspond to the 6 organisms in the reproducibility panel are provided in **Tables 28-34**. Summary results for the 10 ePlex RP Panel targets that did not have organisms included in the reproducibility panel are provided in **Table 35**.

Table 28: Percent Agreement for Adenovirus

Adenovirus Concentration	Site	Agreement with Expected Results		
		Agreed / N	%	95% CI
Moderate Positive 3x LoD 6 x 10^0 $TCID_{50}$/mL	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	36/36	100	(90.4-100)
	All	108/108	100	(96.6-100)
Low Positive 1x LoD 2 x 10^0 $TCID_{50}$/mL	1	36/36	100	(90.4-100)
	2	34/36	94.4	(81.9-98.5)
	3	28/35	80.0	(64.1-90.0)
	All	98/107	91.6	(84.8-95.5)
Negative	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	36/36	100	(90.4-100)
	All	108/108	100	(96.6-100)

CI=Confidence Interval

Table 29: Percent Agreement for Coronavirus

Coronavirus Concentration	Site	Agreement with Expected Results		
		Agreed / N	%	95% CI
Moderate Positive 3x LoD 1.5 x 10^3 $TCID_{50}$/mL	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	36/36	100	(90.4-100)
	All	108/108	100	(96.6-100)
Low Positive 1x LoD 5 x 10^2 $TCID_{50}$/mL	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	35/35	100	(90.1-100)
	All	107/107	100	(96.5-100)
Negative	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	36/36	100	(90.4-100)
	All	108/108	100	(96.6-100)

Table 30: Percent Agreement for Human Metapneumovirus (hMPV)

hMPV Concentration	Site	Agreement with Expected Results		
		Agreed / N	%	95% CI
Moderate Positive 3x LoD 6.75×10^2 $TCID_{50}$/mL	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	36/36	100	(90.4-100)
	All	108/108	100	(96.6-100)
Low Positive 1x LoD 2.25×10^2 $TCID_{50}$/mL	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	35/35	100	(90.1-100)
	All	107/107	100	(96.5-100)
Negative	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	36/36	100	(90.4-100)
	All	108/108	100	(96.6-100)

Table 31: Percent Agreement for Influenza A

Influenza A Concentration	Site	Agreement with Expected Results		
		Agreed / N	%	95% CI
Moderate Positive 3x LoD 1.5×10^2 $TCID_{50}$/mL	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	36/36	100	(90.4-100)
	All	108/108	100	(96.6-100)
Low Positive 1x LoD 5×10^1 $TCID_{50}$/mL	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	35/35	100	(90.1-100)
	All	107/107	100	(96.5-100)
Negative	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	36/36	100	(90.4-100)
	All	108/108	100	(96.6-100)

Table 32: Percent Agreement for Influenza A H3

Influenza A H3 Concentration	Site	Agreement with Expected Results		
		Agreed / N	%	95% CI
Moderate Positive 3x LoD 1.5×10^{2} $TCID_{50}$/mL	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	36/36	100	(90.4-100)
	All	108/108	100	(96.6-100)
Low Positive 1x LoD 5×10^{1} $TCID_{50}$/mL	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	35/35	100	(90.1-100)
	All	107/107	100	(96.5-100)
Negative	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	36/36	100	(90.4-100)
	All	108/108	100	(96.6-100)

Table 33: Percent Agreement for Parainfluenza Virus (PIV) 1

PIV 1 Concentration	Site	Agreement with Expected Results		
		Agreed / N	%	95% CI
Moderate Positive 3x LoD 1.2×10^{0} $TCID_{50}$/mL	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	36/36	100	(90.4-100)
	All	108/108	100	(96.6-100)
Low Positive 1x LoD 4×10^{-1} $TCID_{50}$/mL	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	35/35	100	(90.1-100)
	All	107/107	100	(96.5-100)
Negative	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	36/36	100	(90.4-100)
	All	108/108	100	(96.6-100)

Table 34: Percent Agreement for Respiratory Syncytial Virus (RSV) A

RSV A Concentration	Site	Agreement with Expected Results		
		Agreed / N	%	95% CI
Moderate Positive 3x LoD 4.5 x 10^0 $TCID_{50}$/mL	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	36/36	100	(90.4-100)
	All	108/108	100	(96.6-100)
Low Positive 1x LoD 1.5 x 10^0 $TCID_{50}$/mL	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	35/35	100	(90.1-100)
	All	107/107	100	(96.5-100)
Negative	1	36/36	100	(90.4-100)
	2	36/36	100	(90.4-100)
	3	36/36	100	(90.4-100)
	All	108/108	100	(96.6-100)

Table 35: Negative Percent Agreement for Targets With Organisms Not Included in the Reproducibility Panel

Target	Site	Agreement with Expected Negative Results		
		Agreed / N	%	95% CI
Human Rhinovirus/Enterovirus	1	108/108	100	(96.6-100)
	2	108/108	100	(96.6-100)
	3	104/107	97.2	(92.1-99.0)
	All	320/323	99.1	(97.3-99.7)
Influenza A H1	1	108/108	100	(96.6-100)
	2	108/108	100	(96.6-100)
	3	107/107	100	(96.5-100)
	All	323/323	100	(98.8-100)
Influenza A H1-2009	1	108/108	100	(96.6-100)
	2	108/108	100	(96.6-100)
	3	107/107	100	(96.5-100)
	All	323/323	100	(98.8-100)
Influenza B	1	108/108	100	(96.6-100)
	2	108/108	100	(96.6-100)
	3	107/107	100	(96.5-100)
	All	323/323	100	(98.8-100)
Parainfluenza Virus 2	1	108/108	100	(96.6-100)
	2	108/108	100	(96.6-100)
	3	107/107	100	(96.5-100)
	All	323/323	100	(98.8-100)

Target	Site	Agreement with Expected Negative Results		
		Agreed / N	%	95% CI
Parainfluenza Virus 3	1	108/108	100	(96.6-100)
	2	108/108	100	(96.6-100)
	3	106/107	99.1	(94.9-99.8)
	All	322/323	99.7	(98.3-99.9)
Parainfluenza Virus 4	1	108/108	100	(96.6-100)
	2	108/108	100	(96.6-100)
	3	107/107	100	(96.5-100)
	All	323/323	100	(98.8-100)
Respiratory Syncytial Virus B	1	108/108	100	(96.6-100)
	2	108/108	100	(96.6-100)
	3	107/107	100	(96.5-100)
	All	323/323	100	(98.8-100)
Chlamydia pneumoniae	1	108/108	100	(96.6-100)
	2	108/108	100	(96.6-100)
	3	107/107	100	(96.5-100)
	All	323/323	100	(98.8-100)
Mycoplasma pneumoniae	1	108/108	100	(96.6-100)
	2	107/108	99.1	(94.9-99.8)
	3	106/107	99.1	(94.9-99.8)
	All	321/323	99.4	(97.8-99.8)

Samples with Co-Detected Organisms

Detection of more than one clinically relevant viral and/or bacterial organism in a sample was evaluated with the ePlex RP Panel using a natural clinical matrix (pooled, negative nasopharyngeal swab samples) spiked with two RP Panel organisms: one organism at a low concentration (1-3x LoD) and the second organism at a high concentration (1×10^5 $TCID_{50}$/mL). **Table 36** contains the results of co-detection testing which demonstrated the ability of the ePlex RP Panel to detect 2 organisms in a sample at both high and low concentrations as indicated in the table.

Table 36: Detection of Co-detections

Organism 1	High Titer	Organism 2	Low Titer	Multiple of LoD
Influenza A H3	1×10^5 $TCID_{50}$/mL	Adenovirus B	2×10^0 $TCID_{50}$/mL	1x
Adenovirus	1×10^5 $TCID_{50}$/mL	Influenza A H3	5×10^1 $TCID_{50}$/mL	1x
Influenza A H3	1×10^5 $TCID_{50}$/mL	RSV A	1.5×10^0 $TCID_{50}$/mL	1x
RSV A	1×10^5 $TCID_{50}$/mL	Influenza A H3	5×10^1 $TCID_{50}$/mL	1x
Influenza A H1-2009	1×10^5 $TCID_{50}$/mL	RSV B	6×10^{-1} $TCID_{50}$/mL	3x
RSV B	1×10^5 $TCID_{50}$/mL	Influenza A H1-2009	1×10^{-1} $TCID_{50}$/mL	1x
Influenza A H1-2009	1×10^5 $TCID_{50}$/mL	Rhinovirus	1.5×10^0 $TCID_{50}$/mL	1x
Rhinovirus	1×10^5 $TCID_{50}$/mL	Influenza A H1-2009	3×10^{-1} $TCID_{50}$/mL	3x
Influenza A H1-2009	1×10^5 $TCID_{50}$/mL	Parainfluenza Virus 3	5×10^0 $TCID_{50}$/mL	1x
Parainfluenza Virus 3	1×10^5 $TCID_{50}$/mL	Influenza A H1-2009	1×10^{-1} $TCID_{50}$/mL	1x
Rhinovirus	1×10^5 $TCID_{50}$/mL	RSV A	1.5×10^0 $TCID_{50}$/mL	1x
RSV A	1×10^5 $TCID_{50}$/mL	Rhinovirus	1.5×10^0 $TCID_{50}$/mL	1x
Coronavirus	1×10^5 $TCID_{50}$/mL	RSV A	1.5×10^0 $TCID_{50}$/mL	1x
RSV A	1×10^5 $TCID_{50}$/mL	Coronavirus	7.5×10^0 $TCID_{50}$/mL	1x
Human Metapneumovirus	1×10^5 $TCID_{50}$/mL	Adenovirus	2×10^0 $TCID_{50}$/mL	1x
Adenovirus	1×10^5 $TCID_{50}$/mL	Human Metapneumovirus	2.25×10^2 $TCID_{50}$/mL	1x
Adenovirus	1×10^5 $TCID_{50}$/mL	RSV A	1.5×10^0 $TCID_{50}$/mL	1x
RSV A	1×10^5 $TCID_{50}$/mL	Adenovirus	2×10^0 $TCID_{50}$/mL	1x

Sample Matrix Equivalency

All analytical studies that utilized viral and bacterial cultures close to LoD were performed by spiking the viral and bacterial cultures into a pool of natural negative NPS in VTM as sample matrix. For analytical studies that used viral and bacterial cultures at a concentration which was at least 10x LoD or higher, the viral and bacterial cultures were spiked into MicroTest™ M5® transport media from Remel instead of negative pooled NPS for ease of use. A sample matrix equivalency study was performed to demonstrate equivalency of natural clinical matrix (pooled, negative nasopharyngeal swab in VTM samples) with viral transport media for targets spiked at a concentration of approximately 10x LoD. Quantified, representative viral and bacterial strains were diluted in a natural clinical matrix (pooled, negative nasopharyngeal swab in VTM samples) and in viral transport media. All samples were tested in duplicate. There was no difference observed in detection of targets in natural clinical matrix vs. viral transport media.

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. 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 were found to inhibit the ePlex RP Panel at the concentrations tested in **Table 37**.

Table 37: List of Substances for Testing

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 x 10^5 $TCID_{50}$/mL
Bacteria	*Streptococcus pneumoniae*	1 x 10^6 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.

Carryover and Cross-contamination

The carryover/cross-contamination rate of the ePlex RP Panel and ePlex instrument was tested in a checkerboard approach by running high positive and negative samples interspersed in all bays of a four-tower ePlex instrument (24 bays total) over 5 separate runs on 5 separate days. Quantified parainfluenza virus 3 was prepared in viral transport media at a high concentration (1 x 10^5 $TCID_{50}$/mL, 20,000x LoD) to simulate a clinically relevant high positive and was tested as a representative target organism. Transport media 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 consecutively or in adjacent bays.