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

Nanosphere, Inc.
c/o Fran White
MDC Associates, LLC.
180 Cabot Street
Beverly, MA 01915

September 4, 2015

Re: K143653
Trade/Device Name: Verigene® Respiratory Pathogens *Flex* Nucleic Acid Test (*RP Flex*)
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory Viral Panel Multiplex Nucleic Acid Assay
Regulatory Class: II
Product Code: OCC, OEM, OEP, OOU, OZE, OZZ, OOI
Dated: July 27, 2015
Received: July 28, 2015

Dear Ms. White:

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 Small Manufacturers, International and Consumer Assistance 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" (21CFR 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 Small Manufacturers, International and Consumer Assistance 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

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)*
K143653

Device Name
Verigene® Respiratory Pathogens Flex Nucleic Acid Test (RP Flex)

Indications for Use *(Describe)*
The Verigene® Respiratory Pathogens Flex Nucleic Acid Test (RP Flex) is a multiplexed qualitative test intended for the simultaneous detection and identification of multiple viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infection. The test is performed on the automated Verigene System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and microarray hybridization to detect gene sequences of the following organism types and subtypes:

Viruses	Bacteria
Adenovirus	Bordetella parapertussis/bronchiseptica
Human Metapneumovirus	Bordetella holmesii
Influenza A	Bordetella pertussis
Influenza A (Subtype H1)	
Influenza A (Subtype H3)	
Influenza B	
Parainfluenza 1	
Parainfluenza 2	
Parainfluenza 3	
Parainfluenza 4	
Respiratory Syncytial Virus A	
Respiratory Syncytial Virus B	
Rhinovirus	

Detecting and identifying specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory infection, if used in conjunction with other clinical and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or patient management decisions.

Negative results in the presence of a respiratory illness do not preclude respiratory infection and may be due to infection with pathogens that are not detected by this test or lower respiratory tract infection that is not detected by an NPS specimen. Conversely, positive results do not rule-out infection or co-infection with organisms not detected by RP Flex. The agent(s) detected may not be the definite cause of disease. The use of additional laboratory testing and clinical presentation may be necessary to establish a final diagnosis of respiratory infection.

Clinical evaluation indicates a lower sensitivity specific to RP Flex for the detection of Rhinovirus. If infection with Rhinovirus is suspected, negative samples should be confirmed using an alternative method.

Performance characteristics for Influenza A were established when Influenza A/H1 (2009 Pandemic) and A/H3 were the predominant Influenza A viruses in circulation. RP Flex may not detect novel Influenza A strains. 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 used specifically for novel virulent influenza viruses and sent to appropriate health authorities for testing. Viral culture should not be attempted in these cases unless a biosafety level (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)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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1. 510(K) Summary

510(k) Number:

K143653: Verigene® Respiratory Pathogens *Flex* Nucleic Acid Test (RP *Flex*)

Summary Preparation Date:

August 18, 2015

Submitted by:

Nanosphere, Inc.
4088 Commercial Avenue
Northbrook, IL 60062
Phone: 847-400-9000
Fax: 847-400-9199

Contact:

Fran White
MDC Associates

Proprietary Names:

For the instrument:

Verigene® System

For the assay:

Verigene® Respiratory Pathogens *Flex* Nucleic Acid Test (RP *Flex*)

Verigene® RP *Flex*

Common Names:

For the instrument:

Bench-top molecular diagnostics workstation

For the assay:

Respiratory Pathogens Nucleic Acid Test
Respiratory Pathogens *Flex* Nucleic Acid Test
Respiratory Pathogens identification and differentiation system
Respiratory assay
Respiratory test
Verigene RP *Flex*
RP *Flex*

Regulatory Information:

Regulation section:

866.3980 - Respiratory Viral Panel Multiplex Nucleic Acid Assay

Classification:

Class II

Panel:

Microbiology (83)

Product Code(s):

OCC Respiratory Virus Panel Nucleic Acid Assay System
OEM Human Metapneumovirus (hMPV) RNA Assay System
OEP Influenza A Virus Subtype Differentiation Nucleic Acid Assay
OOI Real Time Nucleic Acid Amplification System
OOU Parainfluenza Multiplex Nucleic Acid Assay
OZE Influenza A and Influenza B Multiplex Nucleic Acid Assay
OZZ Bordetella Pertussis DNA Assay System

Predicate Devices:

FilmArray Respiratory Panel (RP) System (K143080, K123620, K120267, K110764, and K103175) (BioFire Diagnostics, Inc.)

Intended Use:

The Verigene® Respiratory Pathogens *Flex* Nucleic Acid Test (RP *Flex*) is a multiplexed qualitative test intended for the simultaneous detection and identification of multiple viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infection. The test is performed on the automated Verigene System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and microarray hybridization to detect gene sequences of the following organism types and subtypes:

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Influenza A (subtype H1)	
Influenza A (subtype H3)	
Influenza B	
Parainfluenza 1	
Parainfluenza 2	
Parainfluenza 3	
Parainfluenza 4	
Respiratory Syncytial Virus A	
Respiratory Syncytial Virus B	
Rhinovirus	

Detecting and identifying specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory infection, if used in conjunction with other clinical and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or patient management decisions.

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Clinical evaluation indicates a lower sensitivity specific to RP *Flex* for the detection of Rhinovirus. If infection with Rhinovirus is suspected, negative samples should be confirmed using an alternative method.

Performance characteristics for Influenza A were established when Influenza A/H1 (2009 Pandemic) and A/H3 were the predominant Influenza A viruses in circulation. RP *Flex* may not detect novel Influenza A strains. 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 used

specifically for novel virulent influenza viruses and sent to appropriate health authorities for testing. Viral culture should not be attempted in these cases unless a biosafety level (BSL) 3+ facility is available to receive and culture specimens.

Technological Characteristics:

The Verigene Respiratory Pathogens *Flex* Nucleic Acid Test (RP *Flex*) is a molecular assay that relies on detection of specific nucleic acid targets in a microarray format. For each of the bacterial or viral nucleic acid sequences detected by RP *Flex*, unique Capture and Mediator oligonucleotides are used, with gold nanoparticle probe-based endpoint detection. The Capture oligonucleotides are covalently bound to the microarray substrate and hybridize to a specific portion of the nucleic acid targets. The Mediator oligonucleotides have a region that binds to a different portion of the same nucleic acid targets and also have a sequence that allows binding of a gold nanoparticle probe. Specific silver enhancement of the bound gold nanoparticle probes at the capture sites results in gold-silver aggregates that scatter light with high efficiency and provide accurate detection of target capture.

The RP *Flex* test is performed on the Verigene System, a "sample-to-result," fully automated, bench-top molecular diagnostics workstation. The System enables automated nucleic acid extraction from nasopharyngeal swabs (NPS) and detection of analyte-specific target nucleic acids. The Verigene System consists of two components: the Verigene Reader and the Verigene Processor *SP*.

The Reader is the Verigene System's user interface and serves as the central control unit for all aspects of test processing, automated imaging, and result generation using a touch-screen control panel and a barcode scanner. The Verigene Processor *SP* executes the test procedure, automating the steps of (1) Sample Preparation and Target Amplification – cell lysis and magnetic bead-based bacterial and viral nucleic acid isolation and amplification, and (2) Hybridization– detection and identification of analyte-specific nucleic acid in a microarray format by using gold nanoparticle probe-based technology. Once the specimen is loaded by the operator, all other fluid transfer steps are performed by an automated pipette that transfers reagents between wells of the trays and finally loads the specimen into the Test Cartridge for hybridization. Single-use disposable test consumables and a self-contained Verigene Test Cartridge are used for each sample tested with the RP *Flex* assay.

To obtain the test results after test processing is complete, the user removes the Test Cartridge from the Processor *SP*, and inserts the substrate holder into the Verigene Reader for analysis. Light scatter from the capture spots is imaged by the Verigene Reader and intensities from the microarray spots are used to make a determination regarding the presence (Detected) or absence (Not Detected) of a targeted nucleic acid sequence/analyte. This determination is made by means of software-based decision algorithm resident in the Verigene Reader.

Performance Data - Analytical Testing

Analytical Sensitivity / Limit of Detection (LoD)

Limit of Detection (LoD) of the Verigene RP *Flex* test was determined for twenty-eight (28) strains of respiratory pathogens, representing all sixteen (16) Verigene RP *Flex* reportable target analytes. The LoD was defined as the concentration at which the test produces a positive result greater than or equal to 95% of the time. Serial dilutions of the strains were tested and the initial tentative LoD confirmed with 20 replicates. To ensure the accuracy of the LoD determination, if the initial detection rate was 100%, an additional 20 replicates were performed at the next lower concentration until ≤95% was achieved. The confirmed LoDs for the twenty-eight (28) strains tested and the corresponding LoDs for the RP *Flex* test reportable targets are shown in the table below.

Table 1: Limit of Detection (LoD)

Viral Species and Bacterial Genus	Viral Strains and Bacterial Species	LoD
Adenovirus	C (AdV-1)	1.2×10^{-1} $TCID_{50}$/mL
	B (AdV-3)	1.1×10^{0} $TCID_{50}$/mL
	E (AdV-4)	4.1×10^{-2} $TCID_{50}$/mL
Human Metapneumovirus	Metapneumovirus 9 (A1)	3.0×10^{1} $TCID_{50}$/mL
	Metapneumovirus 27 (A2)	1.1×10^{0} $TCID_{50}$/mL
	Metapneumovirus 3 (B1)	1.0×10^{1} $TCID_{50}$/mL
	Metapneumovirus 8 (B2)	3.3×10^{0} $TCID_{50}$/mL
Influenza A	Brisbane/59/2007 (H1N1)	3.0×10^{1} $TCID_{50}$/mL
		1.0×10^{1} $TCID_{50}$/mL
	California/04/2009pdm09 (H1N1)	3.0×10^{1} $TCID_{50}$/mL
		1.0×10^{1} $TCID_{50}$/mL
	Port Chalmers/1/73 (H3N2)	3.3×10^{0} $TCID_{50}$/mL
		3.3×10^{0} $TCID_{50}$/mL
	Victoria/361/2011 (H3N2)	3.7×10^{-1} $TCID_{50}$/mL
		1.2×10^{-1} $TCID_{50}$/mL
	Wisconsin/67/05 (H3N2)	3.3×10^{0} $TCID_{50}$/mL
		3.3×10^{0} $TCID_{50}$/mL
Influenza B	Brisbane/60/2008	1.2×10^{-1} $TCID_{50}$/mL
	Florida/02/2006	3.0×10^{1} $TCID_{50}$/mL
	Massachusetts/02/2012	1.2×10^{-1} $TCID_{50}$/mL
Parainfluenza	Parainfluenza 1	9.0×10^{1} $TCID_{50}$/mL
	Parainfluenza 2	1.0×10^{1} $TCID_{50}$/mL
	Parainfluenza 3	3.3×10^{0} $TCID_{50}$/mL
	Parainfluenza 4a	2.7×10^{2} $TCID_{50}$/mL
Rhinovirus	A (Rhinovirus 39)	1.0×10^{1} $TCID_{50}$/mL
	B (Rhinovirus 14)	9.0×10^{1} $TCID_{50}$/mL
	C (Rhinovirus C41)	2.4×10^{3} PFU/mL
Respiratory Syncytial Virus	RSV A (A2)	3.3×10^{0} $TCID_{50}$/mL
	RSV B (Wash/18537/62)	3.7×10^{-1} $TCID_{50}$/mL
Bordetella	*parapertussis*	2.4×10^{3} CFU/mL
	bronchiseptica	2.4×10^{3} CFU/mL
	holmesii	2.4×10^{3} CFU/mL
	pertussis	8.1×10^{2} CFU/mL

Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) of the RP *Flex* test was demonstrated with a comprehensive panel of one-hundred and eight (108) strains representing temporal, evolutionary, and geographic diversity for each of the RP *Flex* panel organisms. Together with the twenty-eight (28) strains evaluated as part of the Limit of Detection Study, a total of one-hundred and thirty-six (136) strains were evaluated for analytical inclusivity to RP *Flex* through empirical testing.

The organisms in the inclusivity panel were prepared in Simulated NPS. Thirteen (13) strains of Influenza A (subtypes H2N2, H2N3, H5N1, H5N3, H7N2, H7N7, H7N9, H9N2 & H10N7) were prepared and tested at a BSL 3 laboratory. Each sample was tested with the RP *Flex* in triplicate at an initial concentration 3-fold higher than the LoD determined for each analyte. In cases where the expected targets were not detected in one or more replicates, concentrations at a 3-fold higher level were evaluated.

RP *Flex* demonstrated analytical reactivity to all one-hundred and eight (108) strains tested, with some strains requiring higher titers for detection. The individual strains and concentrations at which positive test results were obtained for all three (3) replicates are presented by target organism in the tables below.

Adenovirus Inclusivity Results

Table 2: Adenovirus Inclusivity Results

Adenovirus Species	Serotype	Strain #	Source	Concentration ($TCID_{50}$/mL)	Multiples of LoD
A	31	0810073CF	Zeptometrix	1.1×10^{0}	1x
B1	7	VR-7	ATCC	3.3×10^{0}	3x
	21	VR-1099	ATCC	3.3×10^{0}	3x
B2	11	VR-12	ATCC	3.3×10^{0}	3x
	14	0810108CF	Zeptometrix	3.3×10^{0}	3x
	34	VR-716	ATCC	3.3×10^{0}	3x
	35	VR-718	ATCC	1.0×10^{1}	9x
C	2	111010	TriCore	3.3×10^{0}	3x
	5*	0810020CF	Zeptometrix	8.1×10^{2}	729x
	6*	0810111CF	Zeptometrix	2.7×10^{2}	243x
D	26	0810117CF	Zeptometrix	1.1×10^{0}	1x
	37	0810119CF	Zeptometrix	1.1×10^{0}	1x
F	40	0810084CF	Zeptometrix	1.1×10^{0}	1x
	41	0810085CF	Zeptometrix	1.1×10^{0}	1x

* Based on *in silico* analysis, the oligonucleotide identities of all the tested Adenovirus C subtypes have very similar ranges. Based on the investigation of viral stocks titers using a quantitative TaqMan real-time PCR developed at Nanosphere that is specific for all Adenovirus species (note: the primers for the TaqMan assay are not the same primers used in the RP *Flex*), it appears that the amplifiable genome equivalents available in these two adenovirus viral stocks are significantly reduced comparing to that of the other adenovirus stocks tested in the study.

Influenza A Inclusivity Results

Table 3: Influenza A Inclusivity Results

Influenza A Subtype	Strain	Source	Influenza A		A/H1 or A/H3	
			Concentration ($TCID_{50}$/mL)	Multiple of LoD	Concentration ($TCID_{50}$/mL)	Multiples of LoD
H1N1	A/California/07/2009pdm09	IRR	9.0×10^{1}	3x	9.0×10^{1}	9x
	A/New Caledonia/20/99	Zeptometrix	9.0×10^{1}	3x	9.0×10^{1}	9x
	A/New Jersey/8/76	TriCore	2.7×10^{2}	9x	3.0×10^{1}	3x
	A/NWS/33	TriCore	3.0×10^{1}	1x	3.0×10^{1}	3x
	A/PR/8/ 34	Charles River Labs	3.0×10^{1}	1x	3.0×10^{1}	3x
	A1/Denver/1/57	TriCore	3.0×10^{1}	1x	3.0×10^{1}	3x
	A1/FM/1/47	TriCore	3.0×10^{1}	1x	3.0×10^{1}	3x
	A/ Solomon Islands/3/2006	Zeptometrix	3.0×10^{1}	1x	3.0×10^{1}	3x
	A/Hawaii/15/2001	IRR	2.7×10^{2}	9x	2.7×10^{2}	27x
H3N2	A/ Aichi/ 68	Charles River Labs	1.0×10^{1}	<1x	1.0×10^{1}	3x
	A/ Hong Kong/ 8/ 68	Charles River Labs	3.0×10^{1}	1x	1.0×10^{1}	3x
	A/ Victoria/ 3/ 75*	Charles River Labs	2.4×10^{3}	81x	2.4×10^{3}	729x
	A/Ohio/02/2012	IRR	2.7×10^{2}	9x	2.7×10^{2}	81x

Influenza A Subtype	Strain	Source	Influenza A		A/H1 or A/H3	
			Concentration ($TCID_{50}$/mL)	Multiple of LoD	Concentration ($TCID_{50}$/mL)	Multiples of LoD
H3N2v	A/Indiana/08/2011	IRR	1.0×10^{1}	<1x	1.0×10^{1}	3x
	A/Minnesota/11/2010**	IRR	2.4×10^{3}	81x	9.0×10^{1}	27x
	A/Indiana/10/2011	IRR	1.0×10^{1}	<1x	3.0×10^{1}	9x
H2N2	Japan/305/1957	MRI	9.0×10^{1}	3x	-	-
H2N3	Mallard/Albert79/03	MRI	9.0×10^{1}	3x	-	-
H5N1	A/Duck/Hunan/795/02	MRI	9.0×10^{1}	3x	-	-
	A/Chicken/Korea/IS/2006	MRI	9.0×10^{1}	3x	-	-
	A/Scaly-breasted Munia/ HongKong/2006	MRI	9.0×10^{1}	3x	-	-
H5N3	A/Duck/Singapore/645/1997	MRI	8.1×10^{2}	27x	-	-
H7N2	A/New York/107/2003	MRI	9.0×10^{1}	3x	-	-
H7N7	A/Netherlands/219/2003	MRI	2.7×10^{2}	9x	-	-
	Equine-1/Prague/1956	MRI	9.0×10^{1}	3x	-	-
H7N9	Anhui/01/2013	MRI	9.0×10^{1}	3x	-	-
H9N2	Hong Kong/1073/99	MRI	9.0×10^{1}	3x	-	-
	Chicken/Hong Kong/G9/97	MRI	9.0×10^{1}	3x	-	-
H10N7	Chick/Germany/n/1949	MRI	9.0×10^{1}	3x	-	-

* Based on *in silico* analysis, the oligonucleotide identities of all the tested Influenza A/H3N2 strains have very similar ranges. Based on the investigation of viral stocks titers using a quantitative TaqMan real-time PCR developed at Nanosphere that is specific for Influenza A/H3 strains (note: the primers for the TaqMan assay are not the same primers used in the RP *Flex*), it appears that the amplifiable genome equivalents available in this Influenza A/H3N2 viral stock are significantly reduced comparing to that of the other Influenza A/H3N2 stocks tested in the study.

** Based on *in silico* analysis, the oligonucleotide identities to this strain have slightly lower ranges than the other two H3N2v strains tested.

Influenza B Inclusivity Results

Table 4: Influenza B Inclusivity Results

Type	Strain	Source	Concentration ($TCID_{50}$/mL)	Multiples of LoD
Influenza B	B/ Allen/45	TriCore	9.0×10^{1}	3x
	B/Florida/07/2004	TriCore	9.0×10^{1}	3x
	B/GL/1739/54	TriCore	9.0×10^{1}	3x
	B/Hong Kong/5/72	ATCC	9.0×10^{1}	3x
	B/Malaysia/2506/2004	TriCore	9.0×10^{1}	3x
	B/Maryland/1/59	TriCore	9.0×10^{1}	3x
	B/Taiwan/2/62	TriCore	9.0×10^{1}	3x
	B/Wisconsin/01/2010	IRR	9.0×10^{1}	3x
	B/ Lee/40	Charles River Lab	9.0×10^{1}	3x
	B/Florida/04/2006	Zeptometrix	9.0×10^{1}	3x

Human Metapneumovirus Inclusivity Results

Table 5: Human Metapneumovirus Inclusivity Results

Subtype	Strain	Source	Concentration ($TCID_{50}$/mL)	Multiples of LoD
hMPV A1	16	Zeptometrix 0810161CF	9.0×10^{1}	3x
hMPV A2	20	Zeptometrix 0810163CF	9.0×10^{1}	3x
hMPV B1	5	Zeptometrix 0810158CF	9.0×10^{1}	3x
hMPV B2	4	Zeptometrix 0810157CF	9.0×10^{1}	3x
	18	Zeptometrix 0810162CF	9.0×10^{1}	3x

Parainfluenza 1-4 Inclusivity Results

Table 6: Parainfluenza 1-4 Inclusivity Results

Type		Source/Strain	Concentration ($TCID_{50}$/mL)	Multiples of LoD
Parainfluenza 1		Zeptometrix 0810014CF	2.7×10^{2}	3x
Parainfluenza 2		Zeptometrix 0810015CF	3.0×10^{1}	3x
Parainfluenza 3		ATCC VR-93*	2.7×10^{2}	81x
		BEI NR-3233	3.0×10^{1}	9x
		TriCore (ATCC VR-1782)	9.0×10^{1}	27x
Parainfluenza 4	a	Zeptometrix 0810060CF	8.1×10^{2}	3x
	b	VR-1377	8.1×10^{2}	3x
		Zeptometrix 0810060BCF	8.1×10^{2}	3x

* For Parainfluenza 3, the extracted eluate from the three strains tested in the inclusivity study were each evaluated with PCR/bi-directional sequencing, and the sequence information were used to assess the homology to the RP *Flex* oligos. Based on the *in silico* analysis, the three strains have the identical homology to the RP *Flex* oligos, indicating that the apparent difference in sensitivity was not due to sequence diversity in the gene targeted by the RP *Flex*. The apparent variation in the sensitivity of the RP *Flex* test for these strains is likely attributable to inconsistencies in the quantification of the viral stocks.

RSV Inclusivity Results

Table 7: RSV Inclusivity Results

Subtype	Source/Strain	Concentration ($TCID_{50}$/mL)	Multiples of LoD
Respiratory Syncytial Virus A	ATCC VR-26	1.0×10^{1}	3x
	Zeptometrix 0810040ACF	1.0×10^{1}	3x
Respiratory Syncytial Virus B	Zeptometrix 0810040CF	1.1×10^{0}	3x
	ATCC VR-1400	1.1×10^{0}	3x
	ATCC VR-955	3.3×10^{0}	9x

Rhinovirus A and B Inclusivity Results

Table 8: Rhinovirus A and B Inclusivity Results

Rhinovirus Species	Strain	Source	Concentration ($TCID_{50}$/mL)	Multiples of LoD
Rhinovirus A	1	Zeptometrix 0810012CFN	2.7×10^2	3x
	2	ATCC VR-482	2.7×10^2	3x
	7	ATCC VR-1601	2.7×10^2	3x
	16	ATCC VR-283	2.7×10^2	3x
	34	ATCC VR-507	2.7×10^2	3x
	57	ATCC VR-1600	2.7×10^2	3x
	77	ATCC VR-1187	2.7×10^2	3x
	85	ATCC VR-1195	2.7×10^2	3x
Rhinovirus B	3	ATCC VR-483	2.7×10^2	3x
	17	ATCC VR-1663	2.7×10^2	3x
	27	ATCC VR-1137	2.7×10^2	3x
	42	ATCC VR-338	2.7×10^2	3x
	83	ATCC VR-1193	2.7×10^2	3x

Rhinovirus C Inclusivity Results

Table 9: Rhinovirus C Inclusivity Results

Rhinovirus Species	Strain	Source	Concentration (PFU/mL)*	Multiples of LoD
Rhinovirus C	C2	UW-Madison	7.3×10^3	3x
	C15	UW-Madison	7.3×10^3	3x

* As there is no susceptible cell line to grow Rhinovirus C, the strains were cloned into a plasmid vector and transfected into WisL cells (primary human lung fibroblasts). All were sequenced to confirm identity. The titers were established by qPCR using serial dilutions of Rhinovirus 16 as a surrogate to provide actual PFU/mL values for the standard curve. Therefore, it has been assumed that Rhinovirus 16 has similar virulence rates to Rhinovirus C.

Bordetella Species Inclusivity Results

Table 10: *Bordetella* Species Inclusivity

***Bordetella* Species**	**Source**	**RP Flex Target**	**Concentration (CFU/mL)**	**Multiples of LoD**
B. pertussis	ATCC 51445	*B. pertussis*	2.4×10^3	3x
	ATCC 10380		2.4×10^3	3x
	ATCC 9340		2.4×10^3	3x
	ATCC BAA-589		2.4×10^3	3x
	ATCC BAA-1335		2.4×10^3	3x
	ATCC 53894		2.4×10^3	3x
	ATCC 9306		2.4×10^3	3x
	ATCC 8467		7.3×10^3	9x
B. parapertussis	ATCC 15237	*Bordetella Parapertussis/ bronchiseptica*	7.3×10^3	3x
	ATCC 9305		7.3×10^3	3x
	ATCC BAA-587		7.3×10^3	3x
	ATCC 15989		7.3×10^3	3x
	Zeptometrix 0801461		2.2×10^4	9x
B. bronchiseptica	ATCC 4617	*Bordetella Parapertussis/ bronchiseptica*	7.3×10^3	3x
	ATCC 7773		7.3×10^3	3x
	ATCC 785		7.3×10^3	3x
	ATCC 14064		7.3×10^3	3x
	ATCC 10580		7.3×10^3	3x
	ATCC 19395		7.3×10^3	3x
B. holmesii	Zeptometrix 0801464	*B. holmesii*	2.2×10^4	9x
	ATCC 700053		2.4×10^3	1x
	ATCC 700052		2.4×10^3	1x

Analytical Specificity (Exclusivity)

One hundred and seven (107) organisms (tables below), consisting of forty-six (46) bacterial/fungal strains (tested at 1×10^6 CFU/mL), twenty-six (26) viruses, twenty-two in-panel tested in the LoD study, and thirteen (13) additional influenza A virus strains with other hemagglutinin (HA) types were tested with RP *Flex* to determine analytical specificity (exclusivity).

The viral and bacterial/fungal samples were contrived in Simulated NPS at high concentrations (1×10^5 $TCID_{50}$/mL for viral targets and at 1×10^6 CFU/mL for bacterial and fungal targets, except for Mumps virus which was tested at the highest available concentration of 1.60×10^4 $TCID_{50}$/mL). Four (4) organisms which were not available as titered stocks were evaluated using genomic DNA at 1×10^6 copies/mL. All samples were tested in triplicate with the RP *Flex*.

Bacterial and Fungal Organisms Tested for RP *Flex* Analytical Specificity

Table 11: Bacterial and Fungal Organisms Tested for Analytical Specificity

Genus	Species	Strain Number
Acinetobacter	*baumannii*	ATCC 19606
Bordetella	*avium*	ATCC 35086
Bordetella	*hinzii*	ATCC 51784
Bordetella	*petrii*	ATCC BAA-461
Bordetella	*trematum*	ATCC 700309
Candida	*albicans*	ATCC 18804
Candida	*glabrata*	ATCC 38326
Chlamydophila	*pneumoniae*	ATCC VR-1360
Chlamydia	*trachomatis* Serovar D	ATCC VR-885
Corynebacterium	*pseudodiphtheriticum*	ATCC 10700
Corynebacterium	*diphtheriae*	ATCC 14779
Corynebacterium	*striatum*	ATCC BAA-1293
Escherichia	*coli*	ATCC 25922
Haemophilus	*influenzae*	ATCC 49144
Haemophilus	*parainfluenzae*	ATCC 9796
Klebsiella	*pneumoniae* subsp. *pneumoniae*	ATCC 13883
Lactobacillus	*acidophilus*	Zeptometrix 0801540
Lactobacillus	*plantarum*	ATCC BAA-793
Legionella	*pneumophilia*	ATCC 33152
Legionella	*longbechiae*	ATCC 33462
Legionella	*micdadei*	ATCC 33204
Listeria	*innocua*	ATCC 51742
Listeria	*monocytogenes* serotype 4b	ATCC 19115
Moraxella (Branhamella)	*catarrhalis*	ATCC 43617
Mycobacterium	*tuberculosis*	ATCC BAA-2237D-2[a]
Mycoplasma	*genitalium*	ATCC 49123[a]
Mycoplasma	*hominis*	ATCC 27545-TTR
Mycoplasma	*pneumoniae*	ATCC 15531-TTR
Neisseria	*elongata* subsp. *elongata*	ATCC 25295
Neisseria	*gonorrhoeae*	ATCC 31426
Neisseria	*meningitidis*	ATCC 53415D-5[a]
Neisseria	*lactamica*	ATCC 23970
Neisseria	*mucosa*	ATCC 49233
Neisseria	*sicca*	ATCC 29256
Pneumocystis	*jirovecii*	Erasme-Belgium-Clinical Sample*
Proteus	*vulgaris*	ATCC 6380
Pseudomonas	*aeruginosa*	ATCC 27853
Serratia	*marcescens*	ATCC 29021
Staphylococcus	*aureus* subsp. *aureus*	ATCC 12600
Staphylococcus	*epidermidis*	ATCC 12228
Staphylococcus	*haemolyticus*	ATCC 29970
Streptococcus	*agalactiae*	ATCC 12386
Streptococcus	*pneumoniae*	ATCC 6303
Streptococcus	*pyogenes*	ATCC 14289
Streptococcus	*salivarius*	ATCC 13419
Ureaplasma	*urealyticum*	ATCC 27618[a]

[a] Genomic DNA tested at 1×10^6 copies/mL

Viral Organisms Tested for RP *Flex* Analytical Specificity

Table 12: Viral Organisms Tested for Analytical Specificity

Virus Name	Type	Source/Strain Number
Bocavirus	-	Clinical Sample
Coronavirus	229E	Zeptometrix 0810229CF
Coronavirus	NL63	Zeptometrix 0810228CF
Coronavirus	OC43	Zeptometrix 0810024CF
Coronavirus	HKU1	LIJ-Clinical Sample
Cytomegalovirus	-	ATCC VR-977
Enterovirus A	Type 71	Zeptometrix 0810047CF
Enterovirus A	Coxsackievirus A2	ATCC VR-1550
Enterovirus A	Coxsackievirus A10	Zeptometrix 0810106CF
Enterovirus B	Coxsackievirus A9	Zeptometrix 0810017CF
Enterovirus B	Coxsackievirus B4	ATCC VR-184
Enterovirus B	Coxsackievirus B5	ATCC VR-185
Enterovirus B	Echovirus 6	Zeptometrix 0810076CF
Enterovirus B	Echovirus 9	Zeptometrix 0810077CF
Enterovirus B	Echovirus 11	Zeptometrix 0810023CF
Enterovirus B	Echovirus 30	Zeptometrix 0810078CF
Enterovirus C	Coxsackievirus A21	Zeptometrix 0810235CF
Enterovirus C	Coxsackievirus A24*	ATCC VR-1662
Enterovirus C	Poliovirus 2 (attenuated)*	ATCC VR-301
Enterovirus C	Poliovirus 3 (attenuated)*	ATCC VR-193
Enterovirus D	Type 68*	ATCC VR-561
Epstein Barr Virus	-	Zeptometrix 0810008CF
Herpes Simplex virus	Type 1	Zeptometrix 0810005CF
Measles	-	ATCC VR-24
Mumps virus	-	ATCC VR-106
Varicella-Zoster virus	-	Zeptometrix 0810026CF

In-Panel RP *Flex* Organisms (Viruses and Bacteria) and Additional Influenza A Virus Strains with Other Hemagglutinin (HA) Types Tested for Analytical Specificity

Table 13: In-Panel Organisms Tested for Analytical Specificity

Bacteria/Virus Name	Type	Source/Strain Number
Adenovirus A	Type 31	Zeptometrix ×810073CF
Adenovirus D	Type 26	Zeptometrix 0810117CF
Adenovirus D	Type 37	Zeptometrix 0810119CF
Adenovirus F	Type 40	Zeptometrix 0810084CF
Adenovirus F	Type 41	Zeptometrix 0810085CF
Adenovirus E	Type 4	Zeptometrix 0810070CF
Bordetella holmesii	-	ATCC 51541
Bordetella pertussis	-	ATCC 9797
Influenza A /Brisbane/59/2007	H1N1	TriCore
Influenza A /Wisconsin/67/05	H3N2	Zeptometrix N/A
Influenza A/California/04/2009pdm09	H1N1 - pandemic	TriCore
Influenza A/Victoria/361/2011	H3N2	Zeptometrix 0810240CF
Influenza A	H2N2[a]	Japan/305/1957
Influenza A	H5N1[a]	A/Duck/Hunan/795/02

Bacteria/Virus Name	Type	Source/Strain Number
Influenza A	H5N1[a]	A/Chicken/Korea/IS/2006
Influenza A	H5N1[a]	Scaly-breasted Munia/HongKong/2006
Influenza A	H7N2[a]	New York/107/2003
Influenza A	H7N7[a]	Netherlands/219/2003
Influenza A	H7N9[a]	Anhui/01/2013
Influenza A	H9N2[a]	Hong Kong/1073/99
Influenza A	H2N3[a]	Mallard/Albert79/03
Influenza A	H5N3[a]	Duck/Singapore/645/1997
Influenza A	H7N7[a]	Equine-1/Prague/1956
Influenza A	H9N2[a]	Chicken/Hong Kong/G9/97
Influenza A	H10N7[a]	Chick/Germany/n/1949
Influenza B /Florida/02/2006	-	TriCore
Metapneumovirus 9	Type A1	TriCore
Metapneumovirus 8	Type B2	TriCore
Parainfluenza 1	-	TriCore VR-94
Parainfluenza 2	-	TriCore VR-92
Parainfluenza 3	-	Zeptometrix 0810016CF
Parainfluenza 4a	-	TriCore VR-1378
Respiratory Syncytial Virus	Type A2	TriCore VR-1540
Respiratory Syncytial Virus	Type B	TriCore VR-1580
Rhinovirus 14	Type B	TriCore

[a] Prepared and tested at a BSL 3 laboratory.

All of the organisms tested yielded the expected "Not Detected" results at the concentrations tested with the exception of the enteroviruses marked with an asterisk and *Pneumocystis jirovecii* (from a clinical sample) marked with an asterisk, which gave "Rhinovirus detected" results in some of the replicates.

Based on *in silico* analyses, a number of Enterovirus strains have a relatively high homology to RP *Flex* Rhinovirus oligos, with some percent identities to Rhinovirus RP *Flex* oligos of 84%. As a result, some cross- reactivity at high titer was expected.

In silico analysis also determined that *Pneumocystis jirovecii* sequences have a maximum Oligo Identity to RP *Flex* targets of 67% and therefore *Pneumocystis jirovecii* was not predicted to be cross-reactive to RP *Flex* Rhinovirus probes. Extracted nucleic acids from all Rhinovirus positive tests of the *Pneumocystis jirovecii* positive clinical sample were evaluated with an analytically validated PCR/BDS Rhinovirus assay. PCR/BDS test results confirmed the presence of Rhinovirus in all samples, indicating that the *Pneumocystis jirovecii* positive clinical sample also contains Rhinovirus nucleic acids.

Interference (Exogenous and Endogenous Substances)

Microbial Interference

Three (3) representative target organisms detected by RP *Flex*, Adenovirus 3 (B), Influenza A (H1N1), and *Bordetella pertussis* were evaluated at 3x their respective LoD for potential interference in the presence of seven (7) potentially interfering microorganisms not detected by RP *Flex*: *Staphylococcus aureus, Neisseria meningitidis, Corynebacterium diphtheria, Haemophilus influenza, Streptococcus pneumoniae, Mycoplasma pneumoniae,* and cytomegalovirus. These seven (7) microorganisms represent the most prevalent microorganisms known to be present in the human upper respiratory tract and therefore the most likely to be encountered in NPS specimens. These normal flora organisms were tested at a concentration of 1×10^6 CFU/mL with the exception of *Mycoplasma pneumoniae,* which was tested at 1×10^6 CCU/mL, and *Neisseria meningitidis,* which was tested at 1×10^6 genomic copies/mL and cytomegalovirus, which was tested at 1×10^5 PFU/mL. No interference was observed with the RP *Flex* test for any of these samples tested.

Exogenous and Endogenous Substances

A comprehensive interfering substances study was performed to assess the potential effects of endogenous and exogenous substances that can commonly be found in clinical upper respiratory specimens. Three (3) representative target organisms detected by RP *Flex*, Adenovirus 3 (B), Influenza A (H1N1), and *Bordetella pertussis* were evaluated at 3x their respective LoD for potential interference in the presence of thirty-six (36) potentially interfering exogenous substances (table below). Two (2) endogenous substances were also included, human blood and human DNA. None of the substances at the concentrations tested showed any inhibitory effect on the detection of target respiratory pathogens using the RP *Flex* test.

Table 14: Interfering Substances

Interfering Substances Tested	
Wal-Four® Nasal Spray	*Staphylococcus aureus*
Anefrin Nasal Spray	*Neisseria meningitidis*
Saline Nasal Spray	*Corynebacterium diphtheriae*
Similasan Sinus Relief	*Haemophilus influenzae*
Anbesol® (Anesthetic)	*Streptococcus pneumoniae*
Qvar® (Nasal corticosteroid)	*Mycoplasma pneumoniae*
Dexacort® (Nasal corticosteroid)	Cytomegalovirus
AeroBid® (Nasal corticosteroid)	Mucin, bovine submaxillary Type I-S
Triamcinolone (Nasal corticosteroid)	Mucin, porcine stomach Type II
Pulmicort® (Nasal corticosteroid)	Mucin, porcine stomach Type III
Elocon® (Nasal corticosteroid)	BD Universal Viral Transport Media

Interfering Substances Tested	
Flonase® (Nasal corticosteroid)	Remel M4®
Veramyst® (Fluticasone furoate)	Remel M4-RT®
Tobramycin (systemic antibiotic)	Remel M5®
Relenza™ (Anti-viral)	Remel M6™
Tamiflu® (Anti-viral)	BD Liquid Amies
Sulfur (Boiron®)	Remel Regan Lowe Semi-Solid Transport Media
Galphimia Glauca (Boiron®)	Copan ClassiqSwabs (Aluminum, rayon tipped, sterile)
Histaminum Hydrochloricum	Copan FloqSwabs (Nylon® ,regular, sterile)
Mupirocin (antibiotic)	Ethyl Alcohol, Absolute 200 Proof
Menthol	Acetonitrile
Human Blood	FluMist® Influenza Vaccine Live, Intranasal
Human DNA	

Competitive Interference

In order to assess potential competitive inhibition for RP *Flex*, binary combinations of all test panel organisms representing all possible dual infections, were evaluated. Contrived samples were prepared in negative simulated NPS matrix, with one panel organism present at a Low Positive titer (3x LoD) and a second organism present at a High Positive titer (1×10^5 $TCID_{50}$/mL for viruses, 1×10^6 CFU/mL for bacteria). The performance of Verigene RP *Flex* was evaluated with each of the one-hundred and eighty-two (182) unique sample combinations tested in replicates of three (3). No evidence of competitive inhibition was observed at the titers tested.

Carryover and Cross-Contamination

The potential for carryover and cross-contamination on the Verigene system was assessed by alternately testing three (3) high positive respiratory pathogen samples; Adenovirus 3 (B), Influenza A (H1N1) (both at 1×10^5 $TCID_{50}$/mL), and *Bordetella pertussis* (at 1×10^6 CFU/mL), followed by testing a negative NPS sample. The high-titer sample was alternated with the negative sample five (5) times on six (6) unique Processor *SPs*. No carryover or cross-contamination was observed.

Specimen Stability

Fourteen (14) viral and bacterial strains in pooled Negative Clinical NPS were evaluated at Low Positive (2x LoD) and Moderate Positive (5x LoD) concentrations. Samples were stored at various temperature conditions and tested at defined timepoints in triplicate. The results of this stability study support the stability claim for RP *Flex* testing of clinical NPS specimens preserved in UTM at the following storage conditions: 4 hours at 20-25°C, 72 hours at 2-8°C, and 30 days at ≤-70°C.

Precision

The Precision Study involved the testing of a representative test panel daily by two (2) operators for twelve (12) non-consecutive days for a total of forty-eight (48) tests per panel sample. The Precision Study used three (3) lots of each of the consumables (cartridges, extraction trays and amplification trays). All precision testing was performed at a single laboratory site with one (1) Verigene reader and twelve (12) Verigene Processor *SP*s. The test panel, representing all the RP *Flex* analytes except for *B. parapertussis and B. bronchiseptica,* consisted of two (2) negative samples (one negative simulated NPS matrix and one *Staphylococcus aureus* spiked in negative simulated NPS matrix), as well as seven (7) positive mixed samples at two (2) different concentrations for a total of sixteen (16) unique samples. Samples were prepared by spiking previously characterized and quantified organism stocks into simulated NPS matrix at Moderate Positive (5x LoD) and Low Positive (2x LoD) concentrations.

The results of the precision study are summarized below, which provides the percent agreement between the expected results and the obtained results for each sample tested.

Table 15: Precision Study

Verigene RP *Flex* Target	Positive Percent Agreement (95% CI)		Negative Percent Agreement* (95% CI)
	Low	Moderate	
Parainfluenza 1	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	100% 671/671 (99.4-100)
Parainfluenza 2	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	100% 671/671 (99.4-100)
Parainfluenza 3	100% 48/48 (92.6-100)	95.8 46/48 (86.0-98.8)	100% 671/671 (99.4-100)
Parainfluenza 4	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	99.9% 670/671 (99.2-100)
RSV A	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	100% 671/671 (99.4-100)
RSV B	93.8% 45/48 (83.2-97.9)	100% 48/48 (92.6-100)	100% 671/671 (99.4-100)
Influenza A	100% 96/96 (96.2-100)	100% 96/96 (96.2-100)	100% 575/575 (99.3-100)
Influenza A/H1	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	100% 671/671 (92.4-100)
Influenza A/H3	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	100% 671/671 (92.4-100)
Influenza B	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	100% 671/671 (92.4-100)

Verigene RP *Flex* Target	Positive Percent Agreement (95% CI)		Negative Percent Agreement* (95% CI)
	Low	Moderate	
Rhinovirus	97.9% 47/48 (89.1-99.6)	100% 48/48 (92.6-100)	99.9% 670/671 (99.2-100)
hMPV	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	100% 671/671 (92.4-100)
Adenovirus	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	99.7% 669/671 (98.9-99.9)
B. pertussis	100% 48/48 (92.6-100)	97.9% 46/47 (88.9-99.6)	100% 672/672 (99.4-100)
B. holmesii	100% 47/48 (89.1-99.6)	100% 47/47 (92.4-100)	100% 672/672 (99.4-100)

* Negative Percent Agreement (NPA) was determined with all samples that did not contain the target analyte.

Reproducibility

The Reproducibility Study involved the testing of a representative test panel daily by two (2) operators for five (5) non-consecutive days at three (3) sites for a total of ninety (90) tests per sample. The test panel, representing all the RP *Flex* analytes except for *B. parapertussis and B. bronchiseptica,* consisted of two (2) negative samples (one negative simulated NPS matrix and one *Staphylococcus aureus* spiked in negative simulated NPS matrix), as well as seven (7) positive mixed samples at two (2) different concentrations for a total of sixteen (16) unique samples. Samples were prepared by spiking previously characterized and quantified organism stocks into simulated NPS matrix at Moderate Positive (5x LoD) and Low Positive (2x LoD) concentrations.

The results of the reproducibility study are summarized below, which provides the percent agreement between the expected results and the obtained results for each sample tested.

Table 16: Reproducibility Study

Verigene RP *Flex* Target	Positive Percent Agreement (95% CI)		Negative Percent Agreement* (95% CI)
	Low	Moderate	
Parainfluenza 1	100% 90/90 (96.2-100)	100% 90/90 (96.2-100)	100% 1258/1258 (99.7-100)
Parainfluenza 2	100% 89/89 (95.9-100)	100% 90/90 (96.2-100)	99.8% 1256/1259 (99.3-99.9)
Parainfluenza 3	100% 90/90 (96.2-100)	100% 90/90 (96.2-100)	100% 1258/1258 (99.7-100)
Parainfluenza 4	100% 90/90 (96.2-100)	100% 89/89 (95.9-100)	100% 1259/1259 (99.7-100)
RSV A	98.9% 89/90 (94.0-99.8)	97.8% 88/90 (92.3-99.4)	100% 1258/1258 (99.7-100)
RSV B	100% 90/90 (96.2-100)	100% 90/90 (96.2-100)	99.9% 1257/1258 (99.6-100)
Influenza A	99.4 179/179 (97.9-100)	100% 180/180 (97.9-100)	100% 1079/1079 (99.6-100)
Influenza A/H1	100% 90/90 (96.2-100)	100% 90/90 (96.2-100)	99.8% 1256/1258 (99.4-100)
Influenza A/H3	98.9% 88/89 (93.9-99.8)	100% 90/90 (96.2-100)	99.6% 1254/1259 (99.1-99.8)
Influenza B	100% 90/90 (96.2-100)	100% 90/90 (96.2-100)	99.8% 1255/1258 (99.3-99.9)
Rhinovirus	100% 90/90 (96.2-100)	100% 90/90 (96.2-100)	99.9% 1257/1258 (99.6-100)
hMPV	100% 90/90 (96.2-100)	100% 89/89 (95.9-100)	99.9% 1258/1259 (99.6-100)
Adenovirus	100% 90/90 (96.2-100)	100% 90/90 (96.2-100)	99.8% 1255/1258 (99.3 -99.9)
Bordetella para/bronch	100% 90/90 (96.2-100)	100% 90/90 (96.2-100)	99.8% 1256/1258 (99.4-100)
B. pertussis	96.7% 87/90 (90.7-98.9)	100% 90/90 (96.2-100)	99.9% 1257/1258 (99.6-100)
B. holmesii	100% 90/90 (96.2-100)	100% 90/90 (96.2-100)	99.9% 1257/1258 (99.6-100)

* Negative Percent Agreement (NPA) was determined with all samples that did not contain the target analyte.

Performance Data – Clinical Testing

The clinical performance characteristics of the RP *Flex* test were determined by comparing viral and bacterial test results to an FDA-cleared molecular respiratory panel and/or PCR amplification followed by confirmatory bi-directional sequencing in a multi-site prospective investigation study at six (6) external clinical study sites. Subjects included individuals whose routine care called for respiratory pathogen testing.

A total of 3299 specimens were enrolled, of which 18 specimens were excluded from the study due to protocol violations, and 15 specimens which yielded a final "No Call" result were excluded from the performance analyses. Therefore, a total of 3266 specimens were included in the performance analyses. Enrolled specimens included 1082 prospectively-collected fresh specimens (of which 1069 were included in the performance analyses), 1330 prospectively-collected frozen specimens (of which 1317 were included in the performance analyses), 526 retrospectively-collected frozen specimens (of which 520 were included in the performance analyses), and 361 contrived frozen specimens (of which 360 were included in the performance analyses). The following table provides a summary of demographic information for the 2412 prospectively-collected specimens in the enrolled dataset.

Table 17: Prospective Clinical Studies

Age Range	***Prospective Fresh***		***Prospective Frozen***		***Combined***	
	No. of Specimens	***Percentage***	***No. of Specimens***	***Percentage***	***No. of Specimens***	***Percentage***
0-1	151	14.0%	165	12.4%	316	13.1%
>1-5	176	16.3%	382	28.7%	558	23.1%
>5-12	73	6.7%	98	7.4%	171	7.1%
>12-21	74	6.8%	67	5.0%	141	5.8%
>21-65	426	39.4%	275	20.7%	701	29.1%
>65	163	15.1%	155	11.7%	318	13.2%
Not Provided	19	1.8%	188	14.1%	207	8.6%
Total	1082	100%	1330	100%	2412	100%

The comparator methods were a composite of an FDA-cleared molecular respiratory panel and analytically validated PCR with bi-directional sequencing. The tables below contain the clinical performance data generated during the RP *Flex* test clinical studies, stratified by specimen type, which, as previously described, were categorized as fresh and frozen prospectively collected specimens, frozen selected specimens and frozen contrived specimens.

Table 18: Results Stratified by Target Analyte – Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza B, Respiratory Syncytial Virus (RSV)A, Respiratory Syncytial Virus (RSV)B

	Specimen Type		*n*=	*% Agreement (95% CI)*	
				Positive	***Negative***
Influenza A	Prospectively Collected	Fresh	1049	100% 12/12 (75.7 - 100)	99.4% 1030/1037 (98.6 – 99.7)
		Frozen	1144	97.9% 46/47 (88.9 – 99.6)	99.4% 1091/1097 (98.8 – 99.7)
		All	2193	98.3% 58/59 (91.0 – 99.7)	99.4% 2121/2134 (99.0 – 99.6)
	Selected		513	99.2% 122/123 (95.5 – 99.9)	99.5% 387/390 (97.8 – 99.7)
	Contrived		360	-	100% 360/360 (98.9 – 100)
Influenza A subtype H1	Prospectively Collected	Fresh	1048	-	99.8% 1046/1048 (99.3 – 99.9)
		Frozen	1144	97.8% 45/46 (88.7 – 99.6)	99.6% 1092/1096 (99.1 – 99.9)
		All	2190	97.8% 45/46 (88.7 – 99.6)	99.7% 2138/2144 (99.4 – 99.9)
	Selected		512	97.6% 40/41 (87.4 – 99.6)	99.6% 469/471 (98.5 – 99.9)
	Contrived		360	-	100% 360/360 (98.9 – 100)
Influenza A subtype H3	Prospectively Collected	Fresh	1048	100% 12/12 (75.7 – 100)	99.6% 1032/1036 (99.0 – 99.8)
		Frozen	1142	100% 1/1 (20.6 – 100)	100% 1141/1141 (99.7 – 100)
		All	2190	100% 13/13 (77.2 – 100)	99.8% 2173/2177 (99.5 – 99.9)
	Selected		512	100% 82/82 (95.5 – 100)	99.5% 428/430 (98.3 – 99.9)
	Contrived		360	-	100% 360/360 (98.9 – 100)
Influenza B	Prospectively Collected	Fresh	1052	98.0% 49/50 (89.5 – 99.6)	99.3% 995/1002 (98.6 – 99.7)
		Frozen	1145	-	99.9% 1144/1145 (99.5 – 100)
		All	2197	98.0% 49/50 (89.5 – 99.6)	99.6% 2139/2147 (99.3 – 99.8)
	Selected		516	100% 26/26 (87.1 – 100)	99.6% 488/490 (98.5 – 99.9)
	Contrived		360	-	100% 360/360 (98.9 – 100)
RSV A	Prospectively Collected	Fresh	1049	100% 11/11 (74.1 – 100)	99.8% 1036/1038 (99.3 – 99.9)
		Frozen	1121	100% 6/6 (61.0 – 100)	99.9% 1114/1115 (99.5 – 100)
		All	2170	100% 17/17 (81.6 – 100)	99.9% 2150/2153 (99.6 – 99.9)
	Selected		498	94.8% 55/58 (85.9 – 98.2)	99.3% 437/440 (98.0 – 99.8)
	Contrived		360	-	100% 360/360 (98.9 – 100)
RSV B	Prospectively Collected	Fresh	1049	100% 8/8 (67.6 – 100)	99.6% 1037/1041 (96.7 – 98.6)
		Frozen	1121	100% 165/165 (97.7 – 100)	97.9% 936/956 (96.8 – 98.6)
		All	2170	100% 173/173 (97.8 - 100)	98.8% 1973/1997 (98.2 – 99.2)
	Selected		498	100% 23/23 (85.7 – 100)	98.5% 468/475 (97.0 – 99.3)
	Contrived		360	-	99.7% 359/360 (98.4 – 99.9)

Table 19: Results Stratified by Target Analyte –Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4

	Specimen Type		*n*=	*% Agreement (95% CI)*	
				Positive	***Negative***
Parainfluenza 1	Prospectively Collected	Fresh	1052	-	100% 1052/1052 (99.6 – 100)
		Frozen	1145	90.0% 27/30 (74.4 – 96.5)	99.8% 1113/1115 (99.3 – 99.9)
		All	2197	90.0% 27/30 (74.4 – 96.5)	99.9% 2165/2167 (99.7 – 100)
	Selected		516	100% 50/50 (92.9 – 100)	100% 466/466 (99.2 – 100)
	Contrived		360	-	100% 360/360 (98.9 – 100)
Parainfluenza 2	Prospectively Collected	Fresh	1052	100% 11/11 (74.1 – 100)	99.7% 1038/1041 (99.2 – 99.9)
		Frozen	1145	50.0% 1/2 (9.5 – 90.5)	100% 1143/1143 (99.7 – 100)
		All	2197	92.3% 12/13 (66.7 – 98.6)	99.9% 2181/2184 (99.6 – 99.9)
	Selected		516	100% 28/28 (87.9 – 100)	99.8% 487/488 (98.8 – 100)
	Contrived		360	-	99.7% 359/360 (98.4 – 99.9)
Parainfluenza 3	Prospectively Collected	Fresh	1052	83.3% 10/12 (55.2 – 95.3)	99.7% 1037/1040 (99.2 – 99.9)
		Frozen	1145	80.0% 4/5 (37.5 – 96.4)	100% 1140/1140 (99.7 – 100)
		All	2197	82.4% 14/17 (59.0 – 93.8)	99.9% 2177/2180 (99.6 – 99.9)
	Selected		516	100% 31/31 (89.0 – 100)	100% 485/485 (99.2 – 100)
	Contrived		360	-	100% 360/360 (98.9 – 100)
Parainfluenza 4	Prospectively Collected	Fresh	1052	100% 3/3 (43.8 – 100)	100% 1049/1049 (99.6 – 100)
		Frozen	1145	76.2% 16/21 (54.9 – 89.4)	99.6% 1120/1124 (99.1 – 99.9)
		All	2197	79.2% 19/24 (59.3 – 90.8)	99.8% 2169/2173 (99.5 – 99.9)
	Selected		516	100% 41/41 (91.4 – 100)	99.6% 473/475 (98.5 – 99.9)
	Contrived		360	-	100% 360/360 (98.9 – 100)

Table 20: Results Stratified by Target Analyte –Adenovirus, human Metapneumovirus (hMPV), Rhinovirus

	Specimen Type		*n*=	*% Agreement (95% CI)*	
				Positive	***Negative***
Adenovirus	Prospectively Collected	Fresh	1052	91.7% 22/24 (74.1 – 97.7)	98.2% 1009/1028 (97.1 – 98.8)
		Frozen	1145	81.8% 27/33 (65.6 – 91.4)	96.4% 1072/1112 (95.1 – 97.3)
		All	2197	86.0% 49/57 (74.7 – 92.7)	97.2% 2081/2140 (96.5 – 97.9)
	Selected		516	97.4% 38/39 (86.8 – 99.5)	98.3% 469/477 (96.7 – 99.1)
	Contrived		360	-	99.4% 358/360 (98.0 – 99.8)
Rhinovirus	Prospectively Collected	Fresh	1000	85.9% 214/249 (81.1 – 89.7)	95.7% 719/751 (94.1 – 97.0)
		Frozen	1122	77.8% 193/248 (72.2 – 82.5)	98.3% 859/874[o] (97.2 – 99.0)
		All	2122	81.9% 407/497 (78.3 – 85.0)	97.1% 1578/1625 (96.2 – 97.8)
	Selected		509	80.0% 28/35 (64.1 – 90.0)	98.3% 466/474 (96.7 – 99.1)
	Contrived		360	-	99.7% 359/360 (98.4 – 99.9)

	Specimen Type		*n*=	*% Agreement (95% CI)*	
				Positive	***Negative***
hMPV	Prospectively Collected	Fresh	1052	100% 10/10 (72.2 – 100)	99.5% 1037/1042[h] (98.9 – 99.8)
		Frozen	1145	100% 36/36 (90.4 - 100)	99.9% 1108/1109[i] (99.5 – 100)
		All	2197	100% 46/46 (92.3 - 100)	99.7% 2145/2151 (99.4 – 99.9)
	Selected		516	92.6% 25/27[g] (76.6 – 97.9)	99.8% 488/489[j] (98.8 – 100)
	Contrived		360	-	99.4% 358/360 (98.0 – 99.8)

Table 21: Results Stratified by Target Analyte –*Bordetella parapertussis/bronchiseptica, Bordetella pertussis, Bordetella holmesii*

	Specimen Type		*n*=	*% Agreement (95% CI)*	
				Positive	***Negative***
Bordetella parapertussis/bronchiseptica	Prospectively Collected	Fresh	1041	100% 2/2 (34.2 – 100)	100% 1039/1039 (99.6 – 100)
		Frozen	1255	-	99.9% 1254/1255 (99.5 – 100)
		All	2296	100% 2/2 (34.2 – 100)	99.9% 2290/2291 (99.8 – 100)
	Selected		463	71.4% 5/7 (35.9 – 91.8)	99.8% 455/456 (98.8 – 100)
	Contrived		360	100% 104/104 (96.4 – 100)	100% 256/256 (98.5 – 100)
Bordetella pertussis	Prospectively Collected	Fresh	1052	100% 1/1 (20.6 – 100)	99.9% 1050/1051 (99.5 – 100)
		Frozen	1145	100% 7/7 (64.6 - 100)	99.9% 1137/1138 (99.5 – 100)
		All	2197	100% 8/8 (67.6 – 100)	99.9% 2187/2189 (99.7 – 100)
	Selected		516	96.6% 28/29 (82.8 – 99.4)	100% 487/487 (99.2 – 100)
	Contrived		360	-	100% 360/360 (98.9 – 100)
Bordetella holmesii	Prospectively Collected	Fresh	1043	100% 1/1 (20.6 – 100)	100% 1042/1042 (99.6 – 100)
		Frozen	1263	-	100% 1263/1263 (99.7 – 100)
		All	2306	100% 1/1 (20.6 – 100)	100% 2305/2305 (99.8 – 100)
	Selected		490	50% 1/2 (9.4 – 90.1)	100% 488/488 (99.2 – 100)
	Contrived		360	100% 56/56 (93.6 – 100)	100% 304/304 (98.6 – 100)

Substantial Equivalence

The Verigene Respiratory Pathogens *Flex* Nucleic Acid test (RP *Flex*) has been shown to be substantially equivalent to the BioFire FilmArray Respiratory Panel (RP) System. The RP *Flex* test has similar intended use and indications, technological characteristics, and performance characteristics. Performance data demonstrate that the RP *Flex* test performs comparably to the predicate device. Thus, the RP *Flex* test is substantially equivalent to the predicate device.

Table 22: Substantial Equivalence

Similarities		
Element	**New Device:** Verigene Respiratory Pathogens *Flex* Nucleic Acid Test (RP *Flex*) K143653	**Predicate:** FilmArray® Respiratory Panel (RP) K143080/K123620/K120267/K110764/K103175
Intended Use	The Verigene® Respiratory Pathogens *Flex* Nucleic Acid Test (RP *Flex*) is a multiplexed qualitative test intended for the simultaneous detection and identification of multiple viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infection. The test is performed on the automated Verigene System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and microarray hybridization to detect gene sequences of the following organism types and subtypes: Adenovirus, Human Metapneumovirus, Influenza A, Influenza A (subtype H1), Influenza A (subtype H3), Influenza B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Rhinovirus, *Bordetella parapertussis/bronchiseptica*, *Bordetella holmesii*, and *Bordetella pertussis*. Detecting and identifying specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory infection, if used in conjunction with other clinical and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or patient management decisions. Negative results in the presence of a respiratory illness do not preclude respiratory infection and may be due to infection with pathogens that are not detected by this test or lower respiratory tract infection that is not detected by an NPS specimen. Conversely, positive results do not rule-out infection or co-	The FilmArray® Respiratory Panel (RP) is a multiplexed nucleic acid test intended for use with the FilmArray Instrument for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections. The following organism types and subtypes are identified using the FilmArray RP: Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Human Metapneumovirus, Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype H1-2009, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Human Rhinovirus/Enterovirus, Respiratory Syncytial Virus, *Bordetella pertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of a respiratory infection aids in the diagnosis of respiratory infection if 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 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 is not detected by a nasopharyngeal swab specimen. Positive results do not rule out co-infection with other organisms: the agent(s) detected by the Film Array RP may not be the definite cause of

Similarities		
Element	**New Device:** Verigene Respiratory Pathogens *Flex* Nucleic Acid Test (RP *Flex*) K143653	**Predicate:** FilmArray® Respiratory Panel (RP) K143080/K123620/K120267/K110764/K103175
	infection with organisms not detected by RP *Flex*. The agent(s) detected may not be the definite cause of disease. The use of additional laboratory testing and clinical presentation may be necessary to establish a final diagnosis of respiratory infection. Clinical evaluation indicates a lower sensitivity specific to RP *Flex* for the detection of rhinovirus. If infection with Rhinovirus is suspected, negative samples should be confirmed using an alternative method. Performance characteristics for Influenza A were established when Influenza A/H1 (2009 Pandemic) and A/H3 were the predominant Influenza A viruses in circulation. RP *Flex* may not detect novel Influenza A strains. 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 used specifically for novel virulent influenza viruses and sent to appropriate health authorities for testing. Viral culture should not be attempted in these cases unless a biosafety level (BSL) 3+ facility is available to receive and culture specimens.	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 small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *Bordetella pertussis*, Coronavirus 229E, Coronavirus OC43, Influenza A H1, Influenza A H3, Influenza A H1-2009, Influenza B, *Mycoplasma pneumoniae*, Parainfluenza Virus 1, Parainfluenza. Due to the genetic similarity between Human Rhinovirus and Enterovirus, the FilmArray RP cannot reliably differentiate them. A positive FilmArray RP Rhinovirus/Enterovirus result should be followed-up using an alternate method (e.g., cell culture or sequence analysis). The FilmArray RP assay for Coronavirus OC43 may cross-react with some isolates of Coronavirus HKU1. A dual positive result may be due to cross-reactivity or may indicate a co-infection. Performance characteristics for Influenza A were established when Influenza A H1-2009, A H1, 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.
Specimen Type	Nasopharyngeal swabs (NPS)	Nasopharyngeal swabs (NPS)
Nucleic Acid Amplification	Multiplexed RT-PCR	Multiplexed RT-PCR
Organisms/NA	Adenovirus, Human Metapneumovirus,	Adenovirus, Coronavirus 229E, Coronavirus

Similarities		
Element	**New Device:** Verigene Respiratory Pathogens *Flex* Nucleic Acid Test (RP *Flex*) K143653	**Predicate:** FilmArray® Respiratory Panel (RP) K143080/K123620/K120267/K110764/K103175
Targets Detected	Influenza A, Influenza A (subtype H1), Influenza A (subtype H3), Influenza B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B., Rhinovirus, *Bordetella parapertussis/bronchiseptica*, *Bordetella holmesii*, *Bordetella pertussis*	HKU1, Coronavirus NL63, Coronavirus OC43, Human Metapneumovirus, Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype H1-2009, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Human Rhinovirus/Enterovirus, Respiratory Syncytial Virus, *Bordetella pertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*.

Differences		
Element	**New Device:** Verigene Respiratory Pathogens *Flex* Nucleic Acid test (RP *Flex*) K143653	**Predicate:** FilmArray® Respiratory Panel (RP) K123620/K120267/K110764/K103175
Time to Result	About 2 hours	About 1 hour
Detection Method	Multiplexed RT-PCR followed by a gold nanoparticle probe-based detection of microbial-specific DNA in a microarray format	Nested multiplex RT-PCR followed by high resolution melting analysis to confirm identity of amplified product
Optical Detection	Light Scattering	Fluorescence