510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
K083088

B. Purpose for Submission:
New device

C. Measurand:
Influenza virus and Respiratory Syncytial Virus (RSV) nucleic acids target sequences. Influenza A, Influenza B, and RSV are detected.

D. Type of Test:
Multiplex nucleic acid assay for qualitative detection and identification of Influenza A Virus, Influenza B Virus, and Respiratory Syncytial Virus (RSV) target sequences in nasopharyngeal swab specimens including nucleic acid isolation, multiplex RT-PCR amplification, capture of the amplicons hybridized to gold-labeled probes on a microarray-based chip, and detection of the signal enhanced with elemental silver using the Verigene® System

E. Applicant:
Nanosphere, Inc.

F. Proprietary and Established Names:
Verigene® Respiratory Virus Nucleic Acid Test
Common or Usual Name: VRNAT

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
OCC and NSU	Class II	21 CFR 866.3980 Respiratory viral panel multiplex nucleic acid assay	Microbiology (83)

H. Intended Use:

1. Intended use(s):

The Verigene® Respiratory Virus Nucleic Acid Test is a qualitative multiplex *in vitro* diagnostic test for the detection and identification of Influenza A Virus, Influenza B Virus, and Respiratory Syncytial Virus (RSV) nucleic acids purified from nasopharyngeal swab specimens obtained from patients symptomatic for viral upper respiratory infection. The test is intended to be used on the Verigene® System as an aid in the differential diagnosis of Influenza A, Influenza B, and RSV infections. The test is not intended to detect influenza C virus.

Negative results do not preclude influenza virus or RSV infection and should not be used as the sole basis for treatment or other management decisions. It is recommended that negative test results be confirmed by culture.

Performance characteristics for Influenza A Virus were established when Influenza A/H3 and A/H1 were the predominant Influenza A viruses in circulation. When other Influenza A viruses are emerging, performance characteristics may vary.

If infections 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 department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

2. Indication(s) for use:
   Same as Intended Use

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

4. Special instrument requirements:
   Verigene® System consisting of the Verigene Processor (software version 0.7) and the Verigene Reader (software version 1.5)

I. **Device Description:**
The Verigene® Respiratory Virus Nucleic Acid Test (VRNAT) is a set of reagents and supplies designed to be utilized in RT-PCR and hybridization assay using the Verigene® instrument system for detection and identification of human influenza A, influenza B, and RSV viral RNA. The VRNAT primers target matrix (M) gene of influenza A, non structural (NS) and M genes of influenza B, and L gene and F gene of RSV. The amplicons are hybridized to gold nanoparticle probes through a mediator oligonucleotide and target-specific capture oligonucleotides on a microarray-based chip in a disposable test cartridge. The signal enhanced with elemental silver at the test site is detected using the Verigene Processor.

The VRNAT also includes control materials:

Internal Positive Control
The internal positive control (PC) refers to a set of 'capture' oligonucleotides on the test microarray that directly hybridize to a specific gold nanoparticle probe present in the Reagent Pack of each Test Cartridge. The PC spots are further amplified during the silver amplification step.

Internal Negative Control
The internal negative control (NC) refers to a set of oligonucleotides on the test microarray that have no significant homology to Influenza A, Influenza B, and RSV sequences available in public

databases.

Inhibition Control
Inhibition Control (PC1) or positive amplification control is diagnostic of potential inhibition effects during the target amplification step. It is a synthetic DNA construct designed to act as an artificial target within the VRNAT. It is included in the Primer Mix along with PC1-specific forward and reverse primer. The PC1 target binds specifically to a capture spot on the test chip and the Verigene Reader analyzes the PC1 signal.

Process Control
The process control (PC2) is an intact MS2 bacteriophage and therefore controls for the nucleic acid extraction step and the target amplification or the RT-PCR step. In the VRNAT, isolated MS2 bacteriophage RNA is amplified in the RT-PCR step by virus-specific primers. The amplicon is then mixed with the target buffer containing MS2-specific mediators, it binds to the MS2-specific capture spot and to the probe via the mediator oligonucleotide, and is detected in the Verigene® system.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Prodesse Multiplex RT-PCR ProFlu™ Plus Assay

2. Predicate K number(s):
   K073029

1) Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
	Verigene® Respiratory Virus Nucleic Acid Test	Prodesse ProFlu+ Assay
Intended use	The Verigene® Respiratory Virus Nucleic Acid Test is a qualitative multiplex *in vitro* diagnostic test for the detection and identification of Influenza A Virus, Influenza B Virus, and Respiratory Syncytial Virus (RSV) nucleic acids purified from nasopharyngeal swab specimens obtained from patients symptomatic for viral upper respiratory infection.	The ProFlu+ Assay is a multiplex Real Time RT-PCR *in vitro* diagnostic test for the rapid and qualitative detection and discrimination of Influenza A Virus, Influenza B Virus, and Respiratory Syncytial Virus (RSV) nucleic acids isolated and purified from nasopharyngeal (NP) swab specimens obtained from symptomatic patients.
Targets	Influenza A Influenza B RSV	Influenza A Influenza B RSV
Specimen Types	Nasopharyngeal swabs	Nasopharyngeal swabs

Sample Preparation	Automated extraction of nucleic acids	Automated extraction of nucleic acids
Amplification Method	Multiplex RT-PCR; M-MLV Reverse Transcriptase	Multiplex RT-PCR; M-MLV Reverse Transcriptase
Results	Positive or negative qualitative results	Positive or negative qualitative results

Differences		
Item	**Device**	**Predicate**
	Verigene® Respiratory Virus Nucleic Acid Test	Prodesse ProFlu+ Assay
Test Principle	Gold nanoparticle probe-based chemistry	TaqMan chemistry
Amplification Enzyme	Tfi polymerase	Taq polymerase
Test Format	Microarray: multiplex capability allows for greater coverage of viral strain variants	Real-time PCR format: limited multiplex capability
Contamination Control	Heat-labile Uracil DNA Glycosylase (UDG) to limit amplicon contamination	None
Detection Method	Single-image sensor; nanoparticles illuminated using a fixed-wavelength light source	Fluorescence-based detection
Quality control	Two internal procedural controls: Process Control –and Inhibition Control	Single internal procedural control

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

- Guidance on In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path - *http://www.fda.gov/cdrh/oivd/guidance/1594.pdf*.
- Draft Guidance for Industry and FDA Staff: Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses -*http://www.fda.gov/cdrh/oivd/guidance/1638.pdf*

L. Test Principle:

The Verigene® Respiratory Virus Nucleic Acid Test (VRNAT) includes a set of target-specific oligonucleotides immobilized on the Test Substrate (microarray); target-specific mediator oligonucleotides, and Universal Gold Nanoparticle Probe for use in multiplex RT-PCR amplification and hybridization assay on the Verigene® System instruments.

Summary of Steps in the VRNAT Assay:

Nucleic acids are isolated from clinical specimens on an automated NucliSENS easyMAG System; the targeted regions of viral RNA are transcribed into complimentary DNA and

amplified in a multiplex polymerase chain reaction (PCR). The amplicons are mixed with a sample buffer containing target-specific mediator oligonucleotides and applied to the Test Substrate on a microarray chip in a disposable test cartridge. The cartridge is inserted into the Verigene Processor. The mediator oligonucleotides are chimeras with one part complementary to the target and another part complementary to the Universal Gold Nanoparticle Probe. The Probe mix is added to form the capture-target-mediator-Gold Nanoparticle Probe complex. The signal is amplified by deposition of elemental silver at the test sites to generate nanoscopic gold-silver aggregates which are illuminated and the light scatter is measured. The test results are analyzed in the Verigene Reader.

Interpretation of Results:

The Verigene System software automatically determines the specimen results for influenza A virus, influenza B virus, and RSV. The test is designed to provide a "Detected" or "Not Detected" decision for each of the three viruses evaluated in each test sample. A valid 'call' is made only when both inhibition control and process control are 'Detected' during analysis of each test signifying that the extraction and target amplification processes performed correctly. Failure of the system, cartridge or sample to produce a test result of "Detected" or "Not Detected" for one or more of the targeted viral agents can produce a "No call" result. The descriptions of possible "No call" results with the recommended recourse to be taken can be found in the table below:

Result Failure	Cause	Action
No Call – POS CTRL	Detection criteria failure of the internal controls which guide decisions regarding the validity of the test process.	1) Repeat the VRNAT involving the steps (i) sample preparation, (ii) RT-PCR, and (iii) Verigene Test. 2) If the repeat test provides "Detected" or "Not Detected" results for the three targets, the results are accepted. 3) If the repeat test provides the same "No Call – Pos Ctrl" decision, the sample is inadequate for testing on the VRNAT. A fresh specimen should be collected and tested.
No Call – IND ZONE	An inability of the test to make a decisive call because signal intensities for a specific agent fall into an indeterminate zone close to the signal cut-off limits.	1) Repeat the VRNAT using leftover amplified DNA sample. 2) If the repeat test also provides a "No call – IND ZONE" result, repeat the VRNAT steps (i) sample preparation, (ii) RT-PCR, and (iii) Verigene Test. 3) If this repeat test provides the same "No Call" decision using the 2nd amplified DNA sample, the sample is inadequate for testing on the VRNAT. A fresh specimen should be collected and tested.
No Call – VARIATION	An inability to obtain the test result because of high variability in the target-specific signals.	Repeat the Verigene test with amplified DNA. Results are accepted if the repeat test provides "Detected" or "Not Detected" results for the three targets.
No Call – No GRID **No Call – BKGD**	Verigene System or the Test Cartridge failure.	Repeat the Verigene test with amplified DNA. Results are accepted if the repeat test provides "Detected" or "Not Detected" results for the three targets.

M. Performance Characteristics (if/when applicable):

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

   a. Precision/Reproducibility:

   The Verigene® Respiratory Virus Nucleic Acid Test reproducibility study was performed at each of three sites. Eight unique samples were created by diluting known concentrations of viral particles with Viral Transport Medium. The following strains representing Influenza A, Influenza B, RSV A, and RSV B were used to prepare the samples: Influenza A/Wisconsin/67/2005; Influenza B/ Florida/04/2006; RSV A Strain Long; RSV B Strain B-1 Wild Type (B WV/14617/85). Each strain was represented at 3 distinct concentrations: high negative, low positive, and moderate positive.

Unique Samples	Viral Strains and Levels
1	Influenza A - High Negative; Influenza B - High Negative
2	RSV A - High Negative; RSV B - High Negative
3	Influenza A - Low Positive
4	Influenza B - Low Positive
5	RSV A - Low Positive
6	RSV B - Low Positive
7	Influenza A - Moderate Positive; RSV A - Moderate Positive
8	Influenza B - Moderate Positive; RSV B - Moderate Positive

At Site 1, the reproducibility study was part of a larger precision study (see below) which used the same set of 8 samples and was performed over 12 non-consecutive days. On each test day, two operators performed in duplicate the VRNAT, involving sample isolation, target amplification, and the Verigene Test. In the reproducibility study performed at sites 2 and 3, the samples were analyzed on the Verigene System in triplicate daily by 2 operators on each of five non-consecutive days after undergoing sample isolation and target amplification.

In the 'Reproducibility and Precision' studies, the moderate positive samples served as positive controls since they were expected to provide positive decisions 100% of the time. The same samples also served as negative controls; 'Not Detected' decisions were expected at Influenza B and RSV B in the moderate positive samples containing Influenza A and RSV A and conversely 'Not Detected' decisions were expected at Influenza A and RSV A in the moderate positive samples containing Influenza B and RSV B. The positive and negative controls provided expected decisions during the studies.

Reproducibility Study Summary for the Verigene® Respiratory Virus Nucleic Acid Test.

Panel Member	Site 1			Site 2			Site 3			Total		
	Agree with expected results	Mean Ratio	Ratio CV, %	Agree with exp. results	Mean Ratio	Ratio CV, %	Agree with exp. results	Mean Ratio	Ratio CV, %	Agree with exp. results	% Agree with exp. result	95% Score CI
Flu A High Neg (0.001 TCID50/mL)	46/48	n/a	n/a	14/15	n/a	n/a	15/15	n/a	n/a	75/78	96.20%	89.3%-98.7%
Flu A Low Pos (10 TCID 50/mL)	48/48	0.9994	0.11%	14/15	0.9972	0.09%	15/15	0.9981	0.07%	77/78	98.70%	93.1%-99.8%
Flu A Mod Pos (50 TCID50/mL)	48/48	0.9986	0.17%	15/15	0.997	0.17%	15/15	0.9977	0.13%	78/78	100%	95.3%-100%
Flu B High Neg (0.03 TCID50/mL)	48/48	n/a	n/a	15/15	n/a	n/a	15/15	n/a	n/a	78/78	100%	95.3%-100%
FluB Low Pos (60 TCID50/mL)	47/48	0.9995	0.10%	15/15	0.9985	0.02%	15/15	0.9978	0.31%	77/78	98.70%	93.1%-99.8%
Flu B Mod Posi (600 TCID50/mL)	48/48	0.9992	0.10%	15/15	0.9986	0.02%	15/15	0.999	0.07%	78/78	100%	95.3%-100%
RSV A High Neg (0.01 TCID50/mL)	48/48	n/a	n/a	13/15	n/a	n/a	15/15	n/a	n/a	76/78	97.40%	91.1%-99.3%
RSV A Low Pos (50 TCID50/mL)	47/48	0.9988	0.19%	15/15	0.9968	0.35%	15/15	0.9985	0.10%	77/78	98.70%	93.1%-99.8%
RSV A Mod Pos (250 TCID50/mL)	48/48	0.9948	1.44%	15/15	0.9882	1.46%	15/15	0.9885	1.88%	78/78	100%	95.3%-100%
RSV B High Neg (0.005 TCID50/mL)	48/48	n/a	n/a	14/15	n/a	n/a	14/15	n/a	n/a	74/78	94.90%	87.5%-98.0%
RSV B Low Pos (10 TCID50/mL)	48/48	0.999	0.18%	15/15	0.9985	0.02%	15/15	0.9987	0.03%	78/78	100%	95.3%-100%
RSV B Mod Pos (100 TCID50/mL)	48/48	0.9989	0.32%	15/15	0.9984	0.05%	15/15	0.9992	0.05%	78/78	100%	95.3%-100%

b. *Linearity/assay reportable range:*

Not applicable, qualitative assay

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

Assay Controls
The Verigene System quality control regimen rests on the foundation of a system design that reduces the probability for errors and includes a unit-use disposable Test Cartridge containing all the reagents needed to run a single test:

- A series of automated on-line quality measurements that monitor instrument functionality, software performance, fluidics, test conditions, reagent integrity, and procedural steps in each assay each time a test is performed.
- A series of automated on-line procedural checks guide the user each time a test is performed.
- Several layers of controls built into the VRNAT ensure that failures at any step within the VRNAT are identified in the end-point image analysis of the Test Cartridge.

Control	Description	Function
Inhibition Control	Double-stranded DNA target present in the primer mix. Amplified with every RT-PCR reaction.	Controls for PCR inhibition due to sample- or process-related inhibitors or due to reagent failures.
Process Control	MS2 bacteriophage with an intact viral RNA genome. Added to each test sample including external positive and negative controls.	Controls for sample isolation step (or nucleic acid extraction step) and the RT-PCR step.
External Positive Control	Any of the three viral particles (Influenza A, Influenza B, or RSV) to which Process Control (MS2 bacteriophage) is added prior to sample isolation.	Serve as external controls for the extraction, target amplification, and detection steps. Used to verify reagent performance
External Negative Control	Viral Transport Media to which the Process Control (MS2 bacteriophage) is added prior to sample isolation.	Controls for reagent and/or environmental contamination.

External Controls
Regardless of the choice of quality control materials, all external quality control requirements and testing should be performed in conformance with local, state, and federal regulations or accreditation organizations as applicable and should follow the user's laboratory's standard quality control procedures. External Controls can be prepared by using influenza A, influenza B, and RSV viral particles diluted in negative clinical matrix or Universal Transport Media. It is recommended that the user refer to CLSI document C24-A2, Statistical Quality Control for Quantitative Measurements: Principles and Definitions: [Approved Guideline-Second Edition] or other published guidelines for general quality

control recommendations. For further guidance on appropriate quality control practices, refer to 42CFR 493.1202(c).

d. Detection limit:

The analytical sensitivity was demonstrated by determining the Limit of Detection or (LoD) of the VRNAT using strains with established titers for the following viruses: Influenza A, Influenza B, RSV A, and RSV B. Each virus stock was serially diluted into a sample matrix (Universal Transport Media, Copan). The dilution series was taken through the VRNAT process and tested in triplicate at each dilution level for each virus. The limit of detection defined as the lowest concentration at which ≥95% of the replicates tested positive, was assessed based on the performance of the samples within each dilution series as judged by the Verigene calls. This LoD was confirmed by testing an additional 20 replicates for each strain in order to demonstrate that the virus was detected ≥95% of the time.

Limit of Detection Summary

Limits of Detection	Concentration
Influenza A strains	
Influenza A/Wisconsin/67/05 (H3N2)	2 $TCID_{50}$/mL
Influenza A/New Caledonia/20/99 (H1N1)	50 $TCID_{50}$/mL
Influenza A/Port Chalmers/1/73 (H3N2)	50 $TCID_{50}$/mL
Influenza B strains	
Influenza B/Florida/04/2006	60 $TCID_{50}$/mL
Influenza B/Lee/40	0.01 EID_{50}/mL
Influenza B/Hong Kong/5/72	0.05 EID_{50}/mL
RSV A strains	
RSV A Strain A2	10 $TCID_{50}$/mL
RSV A Strain Long	10 $TCID_{50}$/mL
RSV B strains	
RSV B Strain B-1 Wild Type (B WV/14617/85)	2 $TCID_{50}$/mL
RSV B Strain Wash/18537/62	0.5 $TCID_{50}$/mL
RSV B Strain 9320	0.05 $TCID_{50}$/mL

e. Analytical specificity:

Analytical specificity of Verigene® Respiratory Virus Nucleic Acid Test was evaluated with respect to 1) reactivity (inclusivity) with a number of geographically diverse influenza virus and RSV strains and 2) potential cross-reactivity with pathogens associated with respiratory tract infections.

1) Reactivity/Inclusivity
The analytical reactivity was evaluated against strains of Influenza A, Influenza B, RSV A, and RSV B that are representative of temporal and geographical diversity. Known concentrations of the viral strains grown in culture were diluted in sample matrix (Universal Transport Media, Copan) to achieve the titers listed in the table below. A total of 14 strains for Influenza A, 10 strains for influenza B, and five (5) RSV strains were tested in triplicate at 100 $TCID_{50}$/mL. All viral cultures were detected; the VRNAT analytical reactivity demonstrated 100% concordance with expected results for all primer and probe sets included in the device.

Analytical Reactivity	Concentration	Influenza A	Influenza B	RSV
Influenza A strains				
Influenza A/Canada/578/04 (H3N2)	100 $TCID_{50}$/mL	+	-	-
Influenza A/New Caledonia/20/99 (H1N1)	100 $TCID_{50}$/mL	+	-	-
Influenza A/Hong Kong/29/2006 (H1N1)	100 $TCID_{50}$/mL	+	-	-
Influenza A/Wisconsin/67/05 (H3N2)	100 $TCID_{50}$/mL	+	-	-
Influenza A/PR/8/34 (H1N1)	100 $TCID_{50}$/mL	+	-	-
Influenza A/Aichi/68 (H3N2)	100 $TCID_{50}$/mL	+	-	-
Influenza A/WS/33 (H1N1)	100 $TCID_{50}$/mL	+	-	-
Influenza A/Port Chalmers/1/73 (H3N2)	100 $TCID_{50}$/mL	+	-	-
Influenza A/ Brisbane/59/ 2007 (H1N1)	100 $TCID_{50}$/mL	+	-	-
Influenza A/Brisbane/10/2007 (H3N2)	100 $TCID_{50}$/mL	+	-	-
Influenza A1/Denver/1/57 (H1N1)	100 $TCID_{50}$/mL	+	-	-
Influenza A1/FM/1/47 (H1N1)	100 $TCID_{50}$/mL	+	-	-
Influenza A/New Jersey/8/76 (H1N1)	100 $TCID_{50}$/mL	+	-	-
Influenza A/NWS/33 (H1N1)	100 $TCID_{50}$/mL	+	-	-
Influenza B strains				
Influenza B/Yamanashi/166/98	100 $TCID_{50}$/mL	-	+	-
Influenza B/Panama/45/90	100 $TCID_{50}$/mL	-	+	-
Influenza B/Florida/02/2006	100 $TCID_{50}$/mL	-	+	-
Influenza B/Florida/04/2006	100 $TCID_{50}$/mL	-	+	-
Influenza B/ Lee/40	100 $TCID_{50}$/mL	-	+	-
Influenza B/Egypt/2040/04	100 $TCID_{50}$/mL	-	+	-
Influenza B/GL/1739/54	100 $TCID_{50}$/mL	-	+	-
Influenza B/Hong Kong/5/72	100 $TCID_{50}$/mL	-	+	-
Influenza B/Maryland/1/59	100 $TCID_{50}$/mL	-	+	-
Influenza B/Taiwan/2/62	100 $TCID_{50}$/mL	-	+	-
RSV A strains				
RSV A Strain A2	100 $TCID_{50}$/mL	-	-	+
RSV A Strain Long	100 $TCID_{50}$/mL	-	-	+
RSV B strains				
RSV B Strain B-1 Wild Type (B WV/14617/85)	100 $TCID_{50}$/mL	-	-	+
RSV B Strain Wash/18537/62	100 $TCID_{50}$/mL	-	-	+
RSV B Strain 9320	100 $TCID_{50}$/mL	-	-	+

Cross-Reactivity Evaluation

Analytical specificity (cross-reactivity) studies were performed to assess potential cross-reactivity of the VRNAT with common respiratory pathogens and other microorganisms commonly present in specimens collected from the nasopharynx. A total of 38 organisms of interest were identified as respiratory pathogens with which the majority of the population may be infected. These included 15 viruses and 23 bacterial strains which were propagated and the titers were determined. To assess cross-reactivity, each organism was diluted in a sample matrix (Universal Transport Medium, Copan) to the concentration listed in the table below and taken through all the steps of the VRNAT protocol.

Cross-reactivity Test Results with Common Human Respiratory Pathogens

			Influenza A	Influenza B	RSV
Viruses	**Strain**	**pfu/mL**	-	-	-
Human Adenovirus Type 1	Adenoid 71	3.1×10^5	-	-	-
Human Adenovirus Type 7	Gomen	3.1×10^5	-	-	-
Human coronavirus (OC43)	OC43	1.1×10^5	-	-	-
Human coronavirus (229E)	229E	1.1×10^5	-	-	-
Cytomegalovirus	68-1	1×10^5	-	-	-
Enterovirus, H. coxsackievirus B4	J.V.B.	1×10^5	-	-	-
Epstein Barr Virus	B95-8	6.2×10^6	-	-	-
Human Parainfluenza Type 1	C35	6.2×10^6	-	-	-
Human Parainfluenza Type 2	Greer	2×10^5	-	-	-
Human Parainfluenza Type 3	C243	1.1×10^7	-	-	-
Measles	Edmonston	1.1×10^5	-	-	-
Mumps virus	Enders	1.1×10^6	-	-	-
Human Parainfluenza Type 4a	M-25	1.1×10^5	-	-	-
Human Rhinovirus Type 1A	2060	1.1×10^5	-	-	-
Human Metapneumovirus	B-1	5.6×10^4	-	-	-
			Influenza A	Influenza B	RSV
Bacteria		**cfu/mL**			
Acinetobacter baumannii		3×10^6	-	-	-
Bordetella bronchiseptica		3×10^5	-	-	-
Chlamydia pneumoniae	CM-1	1.6×10^6	-	-	-
Corynebacterium pseudodiphtheriticum		8.2×10^6	-	-	-
Escherichia coli		1.5×10^6	-	-	-
Haemophilus influenzae		1×10^7	-	-	-
Klebsiella pneumoniae		5×10^5	-	-	-
Lactobacillus acidophilus		2.1×10^7	-	-	-
Legionella pneumophila		2.1×10^6	-	-	-
Listeria innocua		1.2×10^7	-	-	-
Moraxella catarrhalis		1×10^6	-	-	-
Neisseria gonorrhoeae		5×10^6	-	-	-
Neisseria meningitidis		1×10^5	-	-	-
Proteus vulgaris		6×10^8	-	-	-
Pseudomonas aeruginosa		6.9×10^6	-	-	-

Staphylococcus aureus	$6.1x10^6$	-	-	-
Staphylococcus epidermidis	$7.3x10^6$	-	-	-
Streptococcus pneumoniae	$9x10^6$	-	-	-
Streptococcus agalactiae	$2x10^6$	-	-	-
Streptococcus pyogenes	$2.5x10^6$	-	-	-
Streptococcus salivarius	$8.3x10^6$	-	-	-
Mycoplasma pneumoniae	$3x10^6$	-	-	-
*Mycobacterium tuberculosis, attenuated**	$2.6x10^6$	-	-	-

None of the organisms tested interfered with the internal controls. For all the organisms tested in the VRNAT for cross-reactivity 'Not Detected' calls were made by the Verigene Reader for each of the three viruses in the VRNAT, Influenza A, Influenza B, and RSV. No cross-reactivity was observed with the common respiratory pathogens and organisms infecting the majority of the population.

f. Assay cut-off:

The Verigene Respiratory Virus Nucleic Acid Test (VRNAT) uses a microarray-based platform in which the viruses and the inhibition control (PC1) and process control (PC2) are represented by recognition elements on the Test Substrate. The recognition elements are virus-specific oligonucleotides that bind to the amplified viral targets, which in turn bind to gold nanoparticle probes via additional recognition elements. A gold nanoparticle probe-specific signal enhancement reaction deposits silver at the virus-specific spots. The scatter from the spots is detected by the Verigene Reader and registered as signal intensity. In addition to the above recognition elements, the Test Substrate has spots specific to positive control (PC) and negative control (NC). Three conditions were identified that together served as a single set of clinical cutoff criteria.

Condition 1: Noise Threshold

Condition 2: Normalized 'Ratio to Negative Control' (Ratio-to-NC) – intensity at the virus-specific recognition element normalized against the intensity values at the negative control elements.

Condition 3: Normalized 'Ratio to Positive Control' (Ratio-to-PC) – intensity at the virus-specific recognition element normalized against the intensity values at the positive control elements.

The Noise Threshold was determined empirically to be equal to Negative Control + 2 SD. The cut-offs for the normalized ratios, 'Ratio-to-NC' and the 'Ratio-to-PC', were determined by using ROC curves. For a positive 'Detected' decision the following criteria apply:

Condition 1: Signal intensity is above the noise threshold

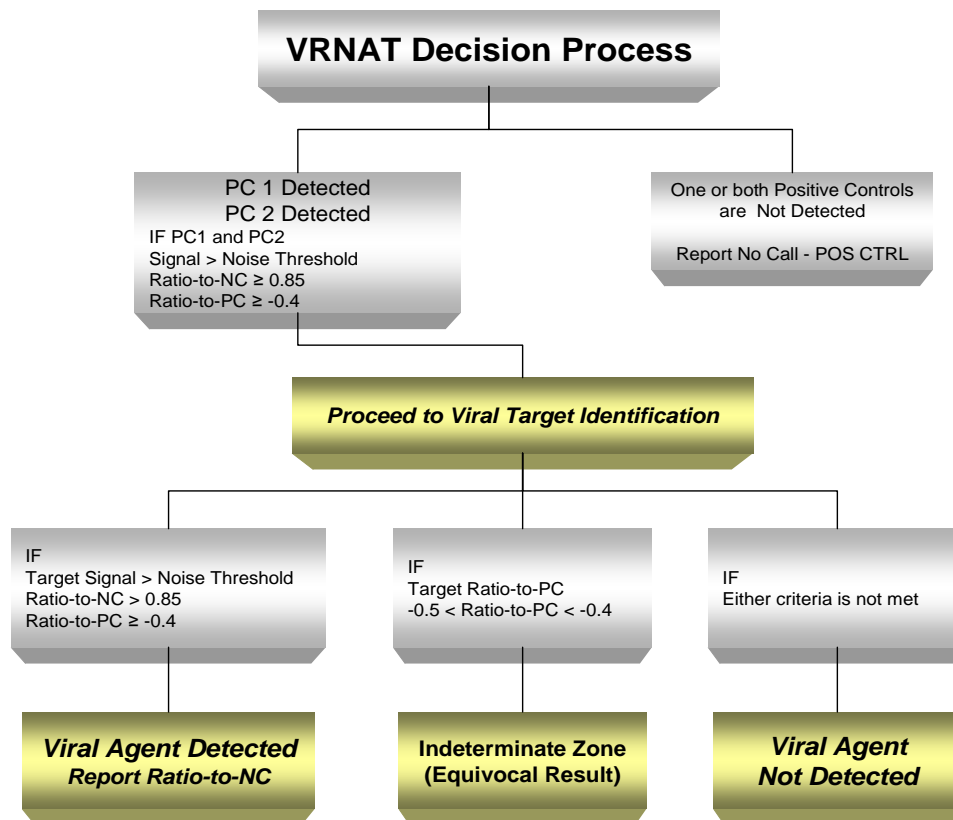Condition 2: Ratio-to-NC is above 0.85

Condition 3: Ratio-to-PC is above -0.4

If any one of these criteria is not met, a negative 'Not Detected' decision is provided. If the Ratio-to-PC for any one of the three viruses falls between -0.4 and -0.5, an equivocal (i.e., No Call – IND ZONE) decision is provided. Criteria set for each of the three conditions are

required to be met for a 'Detected' call.

VRNAT Decision Process

For a result, the decision tree examines the presence of both PC1 and PC2 initially (see Schematic). Both PC1 and PC2 signal intensities have to meet the detection criteria before the analysis is allowed to proceed toward establishing the presence or absence (i.e., "Detected" or "Not Detected") of the individual viruses.

VRNAT Decision Process

PC 1 Detected
PC 2 Detected
IF PC1 and PC2
Signal > Noise Threshold
Ratio-to-NC ≥ 0.85
Ratio-to-PC ≥ -0.4

One or both Positive Controls are Not Detected

Report No Call - POS CTRL

Proceed to Viral Target Identification

IF
Target Signal > Noise Threshold
Ratio-to-NC > 0.85
Ratio-to-PC ≥ -0.4

IF
Target Ratio-to-PC
-0.5 < Ratio-to-PC < -0.4

IF
Either criteria is not met

Viral Agent Detected
Report Ratio-to-NC

Indeterminate Zone
(Equivocal Result)

Viral Agent
Not Detected

2. Comparison studies:

    a. *Method comparison with predicate device:*

    Not applicable, performance of the assay was evaluated in comparison to the gold standard/reference method, viral culture followed by DFA and/or viral culture followed by sequencing

    b. *Matrix comparison:*

    Not applicable

3. Clinical studies:

Performance characteristics of the Verigene® Respiratory Virus Nucleic Acid Test were established during a prospective study during the 2007-2008 respiratory virus season. A total of 720 nasopharyngeal swab specimens were prospectively collected for routine influenza or RSV testing by DFA/culture methods. The residual specimens were frozen and later tested at three clinical sites, 240 at each site, using the Verigene® Respiratory Virus Nucleic Acid Test. The patient population was distributed by age as presented in the table below:

Patient Population by Age	Number of Subjects
0 – 1	120
1 – 5	229
5 – 20	129
20 – 65	204
>65	38
Total	720

The VRNAT performance was compared to a culture-based reference method followed by direct fluorescent antibody (DFA) identification of all culture positive samples. Samples which demonstrated discordant results between the VRNAT and the reference method were further analyzed by using bi-directional sequencing at an independent reference laboratory.

A total of 21 samples (2.9%) generated a "No Call" result; ten (10) of the samples (1.4%) gave a 'No Call – Indeterminate Zone' result indicating an equivocal call. Another eleven (11) samples (1.5%) gave a 'No Call – Pos Ctrl' call indicating an inability to detect an internal control. All the samples with an initial 'No Call' result were re-tested and resolved successfully by following the recommendations in the 'Results Interpretation' section (see above).

a and b. Clinical Sensitivity and Specificity:

Influenza A Results

All Sites Influenza A		DFA/Viral Culture			
		Positive	Negative	Total	
Verigene System	Positive	122	59 [a]	181	Sensitivity 99.2% (95%CI=95.5% - 99.9%)
	Negative	1 [b]	538	539	Specificity 90.1% (95%CI=87.5% - 92.3%)
	Total	123	597	720	

a. 58 samples were positive by sequencing. 1 sample was negative by sequencing.
b. 1 sample was negative by sequencing

Influenza B Results

All Sites Influenza B		DFA/Viral Culture			
		Positive	Negative	Total	
Verigene System	Positive	30	10 [a]	40	Sensitivity 96.8% (95%CI=83.5% - 99.4%)
	Negative	1 [b]	679	680	Specificity 98.5% (95%CI=97.3% - 99.2%)
	Total	31	689	720	

    a.   4 samples were positive by sequencing. 6 samples were negative by sequencing
    b.   1 sample was negative by sequencing

RSV Results

All Sites RSV		DFA/Viral Culture			
		Positive	Negative	Total	
Verigene System	Positive	44 [a]	57 [b]	101	Sensitivity 89.8% (95%CI=78.2% - 95.6%)
	Negative	5 [c]	614	619	Specificity 91.5% (95%CI=89.2% - 93.4%)
	Total	49	671	720	

    a.   All 44 samples were positive by sequencing.
    b.   46 samples were positive by sequencing. 11 samples were negative by sequencing.
    c.   4 samples were positive by sequencing. 1 sample was negative by sequencing.

Dual Infections

Of the 720 samples included in the clinical study, 12 samples were positive for 2 infections by the VRNAT, translating to 1.7% of the samples tested. Comparable RT-PCR—based real-time detection methods have reported a significantly lower percentage for dual infections (~0.1%). Two inter-related factors may contribute to the higher percentage of the dual infections observed in the VRNAT. Competitive inhibition is often observed in dual infections, especially when one infective agent is at a much lower initial concentration resulting thereby in amplicons below the detection limits of fluorescence-based methods.

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

During the 2005-2006 flu season, 12% of 148,636 samples tested for influenza were positive for either Influenza A or Influenza B [based on data from laboratories in the US which collaborated with World Health Organization (WHO) and National Respiratory and Enteric Virus Surveillance System (NREVSS)]. During the 2006-2007 and 2007-2008 flu seasons, the prevalence was 13% of 179,268 samples tested and 18% of 225,329 samples tested, respectively.

According to data reported to the National Respiratory and Enteric Virus Surveillance System (NREVSS), the prevalence of RSV was 16% in the 120,503 samples tested for RSV during the 2005-2006 season and 17% of the 126,617 samples tested during the 2006-2007 season.

In the Verigene Respiratory Virus Nucleic Acid Test multi-site study, which analyzed 720 samples collected during the 2007 – 2008 flu season, the prevalence of Influenza A observed by culture and DFA was 17.1%, of Influenza B was 4.3%, and of RSV 6.8%. By VRNAT, of the set of 720 samples, 25.0% were positive for Influenza A, 4.0% were positive for Influenza B, and 14.0% were positive for RSV. While no dual infections were detected by culture and DFA, the VRNAT detected 12 dual infections or 1.7% (see "Dual Infections" in the Performance Characteristics section). Because the incidence of a triple infection of Influenza A, Influenza B, and RSV is low, it is recommended that the samples undergo repeat testing if nucleic acids from all three analytes are detected in a single sample.

Virus Infection	Identified by DFA/Culture (% samples tested)	Identified by VRNAT (% samples tested)
Influenza A	17.1	25.0
Influenza B	4.3	5.6
RSV	6.8	14.0
Dual infections	None	1.7

N. Proposed Labeling:

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

O. Conclusion:

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