510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:
K141927

B. Purpose for Submission:
To obtain a Substantial Equivalence Determination for a new 510(k) application for Lyra™ Parainfluenza Virus Assay

C. Measurand:
The Lyra™ Parainfluenza Virus Assay detects viral RNA from Parainfluenza 1, 2, and 3 viruses. The primer and probe sets amplify and detect the Parainfluenza type 1 nuclear protein gene, Parainfluenza type 2 phosphate protein gene, and Parainfluenza type 3 phosphate protein gene, using real time reverse transcriptase polymerase chain reaction.

D. Type of Test:
The Lyra™ Parainfluenza Virus Assay is a multiplex nucleic acid detection assay that uses real time polymerase chain reaction.

E. Applicant:
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F. Proprietary and Established Names:
Lyra™ Parainfluenza Virus Assay

G. Regulatory Information:
1. Regulation section:

21CFR 866.3980 - Respiratory viral panel multiplex nucleic acid assay

2. Classification:

Class II

3. Product code:

OOU

4. Panel:

Microbiology (83)

H. Intended Use:

1. Intended use(s):

The Lyra™ Parainfluenza Virus Assay is a Real-Time PCR assay for the qualitative detection and identification of human parainfluenza virus types 1, 2 and 3 viral RNA from nasal and nasopharyngeal swab specimens from symptomatic patients. It is intended for use as an aid in the differential diagnosis of parainfluenza virus types 1, 2 and 3. This test is not intended to detect Parainfluenza 4a or Parainfluenza 4b viruses.

Negative results do not preclude parainfluenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.

2. Indication(s) for use:

Same as intended use.

3. Special conditions for use statement(s):

This is a prescription only test. The assay is intended for use in hospital, reference, or state laboratory settings. The device is not intended for point-of-care use.

4. Special instrument requirements:

The assay can be performed using the Applied Biosystems® 7500 Fast Dx.

I. Device Description:

The Lyra™ Parainfluenza Virus Assay detects viral RNA of Parainfluenza 1, 2 and 3 viruses in nasal and nasopharyngeal swab specimens. First the nucleic acid is extracted from the patient sample using the NucliSENS® easyMAG® System. The extracted nucleic acid is then introduced into the master mix which contains the primers, probes, revise transcriptase, and TaqMan® chemistry and the reaction is loaded into the Applied Biosystems® (ABI) 7500 Fast Dx instrument. First, the reverse transcriptase generates a complimentary DNA strand from the viral RNA. The primer sets hybridize to the Parainfluenza type 1 nuclear protein gene, Parainfluenza type 2 phosphate protein gene, and Parainfluenza type 3 phosphate protein gene, and transcription and DNA amplification of the target sequence occur using real time reverse transcriptase polymerase chain reaction. Fluorescent-labeled probes bind to the target sequences and the dye is released from the probe containing quencher via the TaqMan® polymerase 5'-3' exonuclease activity. The un-quenched dye is detected in the ABI 7500 Fast Dx and if sufficient fluorescence is detected the result is reported as positive.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Prodesse ProParaflu™+ Assay

2. Predicate 510(k) number(s):

K091053

3. Comparison with predicate:

	Subject Device: **Lyra Parainfluenza Virus Assay**	**Predicate Device:** **Prodesse ProParaflu™+ Assay** **K091053**
Intended Use	The Lyra™ Parainfluenza Virus Assay is a Real-Time RT-PCR assay for the qualitative detection and identification of human parainfluenza virus types 1, 2 and 3 viral RNA from nasal and nasopharyngeal swab specimens from symptomatic patients. It is intended for use as an aid in the differential diagnosis of parainfluenza virus types 1, 2 and 3. This test is not intended to detect Parainfluenza 4a or Parainfluenza 4b viruses. Negative results do not preclude parainfluenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.	The ProParaflu+ Assay is a multiplex Real Time RT-PCR *in vitro* diagnostic test for the qualitative detection and discrimination of Parainfluenza I Virus, Parainfluenza 2 Virus and Parainfluenza 3 Virus (HPIV-1, HPIV-2 and HPIV-3) nucleic acids isolated and purified from nasopharyngeal (NP) swab specimens obtained from individuals exhibiting signs and symptoms of respiratory tract infections. This assay targets the conserved regions of the Hemagglutinin- Neuraminidase (HN) gene of HPIV-1, HPIV-2 and HPIV-3, respectively. The detection and discrimination of HPIV- I, HPIV-2 and HPIV-3 nucleic acids from symptomatic patients aid in the diagnosis of human respiratory tract parainfluenza infections if used in conjunction with other clinical and laboratory findings. This test is not intended to detect Parainfluenza 4a or Parainfluenza 4b Viruses. Negative test results are presumptive and should be confirmed by cell culture.

		Negative results do not preclude Parainfluenza 1, 2 or 3 virus infections and should not be used as the sole basis for treatment or other management decisions.
DNA Amplification Technology	Real time polymerase chain reaction	Same
Target Sequence Detected	Parainfluenza type 1 nuclear protein gene, Parainfluenza type 2 phosphate protein gene, Parainfluenza type 3 phosphate protein gene	Conserved regions of the Hemagglutinin-Neuraminidase (HN) gene of HPIV-1, HPIV-2 and HPIV-3
Sample Types	Nasal and Nasopharyngeal swabs	Nasopharyngeal swabs
Extraction	NucliSENS® easyMAG™ (bioMérieux)	MagNA Pure LC System (Roche), NucliSENS® easyMAG™ (bioMérieux)
Detection Techniques	Applied Biosystems 7500 Fast Dx	Cepheid SmartCycler II

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

Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay

L. Test Principle:

The assay detects viral nucleic acids that have been extracted from a patient respiratory sample. A multiplex Real-time RT-PCR reaction is carried out under optimized conditions in a single tube generating amplicons for PIV-1, PIV-2, PIV-3, and the Process Control (PRC). Identification of PIV-1, PIV-2, PIV-3, and the PRC occurs by the use of target-specific primers and fluorescent-labeled probes that hybridize to conserved regions in the genomes of PIV-1, PIV-2, PIV-3, and the PRC.

Lyra™ Probe Targets	
Virus	**Target**
PIV-1	Nuclear Protein gene
PIV-2	Phosphate Protein gene
PIV-3	Phosphate Protein gene

Lyra™ Probe Labels	
Target	**Dye**
PIV-1	FAM
PIV-2	JOE
PIV-3	Tex Red
PRC	CY5

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The reproducibility studies were conducted over 14 days and testing was performed on 5 non-consecutive days at two clinical sites and one in-house laboratory. Each site was provided with 5 complete panels, which were stored at -70°C until used. Operators were blinded to the sample identity, which was randomized each day. Testing was conducted following the package insert instructions for use. One kit lot was used to perform the study. One strain of each parainfluenza virus was used at 3 different concentrations, 5x LoD, 2x LoD and 0.5x LoD for the Moderate Positive, Low Positive and High Negative samples respectively. The following strains were used; Parainfluenza-1 strain C35, Parainfluenza-2 strain Greer, Parainfluenza-3 strain C243.

The following table shows the summary of results for the reproducibility study. The results show that the assay had appropriate reproducibility at both the external clinical sites and the in-house laboratory. The reproducibility for this device is acceptable. The instances where the expected result differed from the actual result are within what is reasonable for C_5 and C_{95} samples.

	Site 1			Site 2			Site 3			Combined		
	Rate of Detection	Ave. Ct	%CV	Rate of Detection	Ave. Ct	%CV	Rate of Detection	Ave. Ct	%CV	Rate of Detection	Ave. Ct	%CV
PIV-1 High Negative	18/30	32.3	5.6	7/30	32.7	4.2	11/30	32.4	4.9	36/90	32.4	5
PIV-1 Low Positive	30/30	27.3	4.3	30/30	26.3	2.7	30/30	27.1	9.1	90/90	26.9	6.3
PIV-1 Moderate Positive	30/30	24.5	2.8	30/30	23.8	2.6	30/30	24.2	3.8	90/90	24.2	3.3
PIV-1 Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/29	N/A	N/A	0/89	N/A	N/A
PIV-2 High Negative	24/30	30.2	6.7	2/30	34.3	7	6/30	32.9	7.1	32/90	31	7.7
PIV-2 Low Positive	30/30	25.4	4.7	30/30	27	4.3	30/30	27.9	9.4	90/90	26.8	7.7
PIV-2 Moderate Positive	30/30	22.8	1.9	30/30	23.7	2.8	30/30	24.5	7.6	90/90	23.7	5.7
PIV-2 Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/29	N/A	N/A	0/89	N/A	N/A

PIV-3 High Negative	30/30	29.1	5.8	29/30	32.2	4.4	14/30	32	3.6	73/90	30.9	6.7
PIV-3 Low Positive	30/30	25.5	6	30/30	26.1	2.9	30/30	26.9	8.2	90/90	26.2	1.7
PIV-3 Moderate Positive	30/30	22.8	1.7	30/30	23.6	3	30/30	24.3	5.5	90/90	23.2	4.6
PIV-3 Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/29	N/A	N/A	0/89	N/A	N/A
PIV-1 Positive Control	30/30	20.6	9.1	30/30	22.6	8.7	30/30	19.3	3.4	90/90	21	11.2
PIV-2 Positive Control	30/30	17.2	1.8	30/30	19.4	4.3	30/30	20.6	2	90/90	19.1	8
PIV-3 Positive Control	30/30	16.7	7.3	30/30	19.2	8.2	30/30	18.5	2.3	90/90	18.3	11.5
Negative Control	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A

* One (1) replicate had an invalid PRC value and was removed for analysis.

b. Baseline, Threshold and Cut-off:

Baseline:
The baseline was determined using 38 parainfluenza virus negative clinical specimens. Next, known positive samples were tested and analyzed using two different baseline settings, manual and auto baseline on the ABI 7500 Fast Dx. When using the manual baseline background noise was reduced for all channels but the Ct values for all samples were changed, in some cases more than 2Cts. This resulted in a false positive for PIV-1 in a PIV-2 sample. This did not occur with the auto baseline setting. Therefore the auto baseline setting was used for all future analysis and is the chosen baseline setting in the package insert.

Signal Threshold:

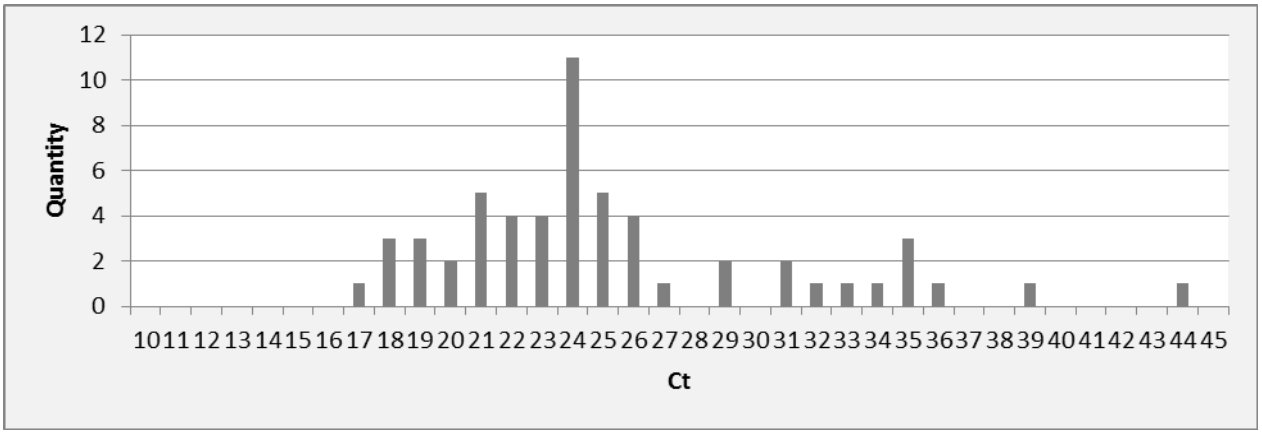
For the ABI 7500 Fast Dx Instrument, the threshold is defined as the level of fluorescence above which the ABI 7500 Fast Dx software will assign a "positive" call. The software uses the baseline and threshold values to calculate the threshold cycle (Ct).

On the ABI 7500 Fast Dx Platform, PIV-1 and PIV-3 targets required no threshold changes from the default threshold of 1.00E+05 baseline-corrected normalized reporter units (ΔRn). However, the initial PIV-2 target threshold of 1.00E+05 ΔRn resulted in a loss of one positive specimen previously determined positive by culture with DFA confirmation. After re-analyzing the PIV-2 target using a lower threshold (8.00E+04 ΔRn), this specimen had a detectable Ct value. Therefore, the data indicated that lowering the threshold for this target would allow for maximum sensitivity to detect late positive specimens, but did not decrease specificity (no false positives were detected with the lower threshold). The threshold for

PIV-3 (1.60E+05 ΔRn) was raised to compensate for excess background fluorescence noise resulting from the auto baseline setting in this channel. These threshold levels are the levels used for all future analysis and are the chosen threshold settings in the package insert.

Cut-Off:

The cut-off was determined using the threshold for PIV-1 (1.00E+05), PIV-2 (8.00E+04) and PIV-3 (1.60E+05). Auto baseline was used for all targets. These conditions follow the package insert directions. Most Ct values were recorded between 18-27 cycles; however, at least 15 percent of samples were recorded after 30 cycles. The figure below shows the Ct Distribution for all three PIV targets from the ABI 7500 Fast Dx. The highest Ct value was detected at 44 cycles. The Ct range on this platform was from 17 cycles to 44 cycles, indicating that the cutoff should be at 45 cycles. Forty five cycles is the cut-off indicated in the package insert.



c. Stability:

Kit Stability:

Three kit lots were used to determine the real-time stability of the kit reagents when being stored at 4°C. PIV-1, PIV-2 and PIV-3 samples were tested at 2x LoD for each time point. Testing was conducted on days 0, 5, 7, 10, 15, 21, 38, 56, 76 and 90 (3 months), testing was then conducted monthly from months 4-11 and will continue through month 25. The following strains were used; Parainfluenza-1 strain C35, Parainfluenza-2 strain Greer, Parainfluenza-3 strain C243. Testing was conducted according to the directions in the package insert. Testing showed there was no change in Ct value greater than 3 Ct, which would indicate a significant change in detection and loss of stability, during the first 11 months of storage. Stability of the kit will be listed as 12 months in the package insert, and will be modified as more real time stability data is available.

Rehydrated Master Mix Stability:

The stability of rehydrated master mix, when stored at -20°C, 4°C, and at room temperature, was determined in this study. Testing was conducted on the ABI 7500 Dx according to the package instructions. Parainfluenza-1 strain C35, Parainfluenza-2 strain Greer, Parainfluenza-3 strain C243 were tested at 2x LoD. Testing for the 4°C and room

temperature conditions was performed at 1, 2, 4, 8, 9, 24, 28, 48, 52, 72 and 76 hours. Testing for the -20°C condition was performed at 24, 28, 48, 52, 72 and 76 hours. Viral RNA was extracted only once (a minimum of 24 extractions), the RNA was then pooled, aliquoted and stored at -70°C until used. Each strain was tested in triplicate, with three separate vials of rehydrated master mix, for every time and temperature condition. At the end of each incubation period an aliquot of viral RNA was added to the rehydrated Master Mix and loaded into the ABI 7500 Dx. The time point selected for stability claims in the package insert is the time point one point earlier than where all replicates were not detected. The table below shows the stability of the rehydrated Master Mix.

Storage Temperature	**Stability Time Limit**
Room Temperature	**2 hours**
2-8°C	**4 hours**
-20°C	**52 hours**

Extracted Specimen Stability:

The stability of extracted samples was tested at Room Temperature, 4°C, -20°C, and -70°C at various time points. Testing was conducted on the ABI 7500 Dx according to the package instructions. Parainfluenza-1 strain C35, Parainfluenza-2 strain Greer, and Parainfluenza-3 strain C243 were prepared at 3X LoD in negative nasal matrix and used in this study. Sufficient volume was prepared to extract RNA for testing all time points. Samples stored at -20°C and -70°C were also tested for stability after 1, 2, and 3 freeze/thaw cycles. The table below shows the different time points tested for each temperature. Testing showed that there was no change in Ct value greater than 3 Ct, which would indicate a significant change in detection and loss of stability, at any of the time points tested. The package insert will list the stability of extracted RNA as stable if stored at Room Temperature, 4°C, -20°C and -70°C for up to 30 days.

Temperature	Hours post extraction	Days post extraction
Room Temperature	4, 8 and 9	1, 2, 3, 7, 14, 21, 28, 30, 31
4°C	4, 8 and 9	1, 2, 3, 7, 14, 21, 28, 30, 31
-20°C	N/A	1, 2, 3, 7, 14, 21, 28, 30, 31
-70°C	N/A	1, 2, 3, 7, 14, 21, 28, 30, 31

d. Expected values (controls, calibrators, or methods):

No external controls are provided with this assay.

There is an internal control called the 'process control' (PRC). It is the MS2 bacteriophage (Zeptometrix) which is a single stranded RNA virus that can be extracted, amplified, and detected by primers and probes included in the master mix. Detection of the PRC indicates that assay conditions allowed proper sample extraction, amplification, and detection.

e. Limit of Detection:

The limit of detection (LOD) (defined as the lowest $TCID_{50}$/mL that is detected ≥95% of the time) of the Lyra Parainfluenza Virus Assay was determined on the ABI 7500 Fast Dx platform using three separate validation lots of lyophilized master mix and limiting dilutions of viral stocks. Parainfluenza-1 strain C35, Parainfluenza-2 strain Greer, and Parainfluenza-3 strain C243 were used in this study. Twenty replicates for each dilution of each parainfluenza virus type were prepared and tested. Viral stocks were diluted in negative nasal matrix and then extracted and stored for up to one month at -70°C. Testing was then conducted according to the package insert. Data showing the results for each parainfluenza virus strain using each validation lot was provided at one concentration above and below the determined LoD. The table below shows the LoD determined for each parainfluenza virus type.

Virus	LoD on ABI 7500 Fast Dx	Average Ct Value at LoD
Parainfluenza-1, strain C-35	2.50 $TCID_{50}$/mL	38.0
Parainfluenza-2, strain Greer	250 $TCID_{50}$/mL	37.0
Parainfluenza-3, strain C243	80 $TCID_{50}$/mL	38.1

f. Analytical Inclusivity:

In silico analysis to demonstrate inclusivity for all three parainfluenza virus types was performed. No laboratory studies were performed because there are a very limited number of characterized strains of any human parainfluenza virus type. *In silico* analysis was performed comparing the sequence of the chosen target region for each parainfluenza virus type with the NCBI database for human parainfluenza virus sequences. Three hundred and eight human parainfluenza virus type 1, 458 human parainfluenza virus type 2, and 509 parainfluenza virus type 3 full-genome sequences were analyzed. Primers and probes were designed against consensus sequences found for each virus type and have 100% homology to all sequences analyzed but do not have homology with other parainfluenza virus types. Therefore, *in silico* analysis and primer and probe design demonstrates that the assay should detect all human parainfluenza viruses of types 1, 2, and 3.

g. Analytical Specificity/Cross-Reactivity:

Cross Reactivity:

This study was designed to determine potential cross-reactivity of the assay with non-parainfluenza respiratory viruses, yeast, and bacteria. Organisms were sourced from DHI, Zeptometrix, or ATCC. Bacteria were tested at 1.00E+6 cfu/ml or higher and viruses were tested at 1.00E+05 TCID50.mL or higher, stock concentrations permitting. Stocks were diluted in negative nasal matrix and the samples were extracted and amplified according to the package insert instructions. All samples were tested in triplicate starting from the extraction step. The results were analyzed for any cross-reactivity for all three parainfluenza virus types and are listed below. No Cross-reactivity was observed in this study.

Organism	Vendor	Final Concentration TCID50/mL or CFU/mL	Result (Avg. Ct)
Adenovirus type 1	DHI	1.00E+05	Negative
Coronavirus 229E	DHI	1.00E+05	Negative
Coronavirus NL63	Zeptometrix	7.05E+04	Negative
Coronavirus OC43	DHI	1.00E+05	Negative
Coxsackievirus B4:ODH-42385	DHI	1.00E+05	Negative
Coxsackievirus B5:ODH-594484	DHI	1.00E+05	Negative
Cytomegalovirus	DHI	1.00E+05	Negative
Echovirus 6	DHI	7.60E+08	Negative
Echovirus 7	DHI	1.00E+05	Negative
Echovirus 9	DHI	1.00E+05	Negative
Echovirus 11	DHI	1.00E+05	Negative
Enterovirus 70	DHI	1.00E+05	Negative
Enterovirus 71	DHI	1.00E+05	Negative
Epstein Barr Virus	Zeptometrix	9.27E+07 Copies/mL	Negative
HSV Type 1 MacIntyre Strain	Zeptometrix	5.89E+06	Negative
HSV Type 2 G strain	DHI	1.00E+05	Negative
Human Metapneumovirus (A1)	DHI	1.00E+05	Negative
Human Rhinovirus 45	DHI	2.94E+04	Negative
Human Rhinovirus 52	DHI	2.63E+04	Negative
Influenza A/Mexico/4108/2009	DHI	1.00E+05	Negative
Influenza A/Port Chalmers	DHI	1.00E+05	Negative
Influenza B/Florida/04/2006	DHI	1.00E+05	Negative
Measles	Zeptometrix	1.95E+06	Negative
Mumps Virus	Zeptometrix	2.75E+08	Negative
RSV A (Long)	DHI	1.00E+05	Negative
RSV B Strain (Wash/18537/62)	DHI	1.00E+05	Negative
Varicella Zoster Virus	DHI	1.00E+05	Negative
Bordetella pertussis	DHI	1.00E+06	Negative
Bordetella bronchiseptica	DHI	1.00E+06	Negative
Chlamydophilia pneumonia	ATC	1.00E+06 copies/mL	Negative
Chlamydophila trachomatis	DHI	1.00E+06	Negative
Legionella pneumophila	DHI	1.00E+06	Negative
Mycobacterium intracellulare	DHI	1.00E+06	Negative
Mycobacterium tuberculosis	DHI	1.00E+06	Negative
Mycobacterium avium	DHI	1.00E+06	Negative
Mycoplasma pneumoniae	Zeptometrix	3.16E+06 Color Changing Unit/mL	Negative
Haemophilus influenzae	DHI	1.00E+06	Negative
Pseudomonas aeruginosa	DHI	1.00E+06	Negative

Proteus vulgaris	DHI	1.00E+06	Negative
Proteus mirabilis	DHI	1.00E+06	Negative
Neisseria gonorrhoeae	DHI	1.00E+06	Negative
Neisseria meningitidis	DHI	1.00E+06	Negative
Neisseria mucosa	DHI	1.00E+06	Negative
Klebsiella pneumoniae	DHI	1.00E+06	Negative
Escherichia coli	DHI	1.00E+06	Negative
Moraxella catarrhalis	DHI	1.00E+06	Negative
Corynebacterium diptheriae	DHI	1.00E+06	Negative
Lactobacillus plantarum	DHI	1.00E+06	Negative
Streptococcus pneumoniae	DHI	1.00E+06	Negative
Streptococcus pyogenes	DHI	1.00E+06	Negative
Streptococcus salivarius	DHI	1.00E+06	Negative
Staphylococcus epidermidis	DHI	1.00E+06	Negative
Staphylococcus aereus	DHI	1.00E+06	Negative
Candida albicans	DHI	1.00E+06	Negative
Parainfluenza Type 4A	Zeptometrix	1.04E+05	Negative
Parainfluenza Type 1	DHI	1.00E+05	18.0 for PIV1
Parainfluenza Type 2	DHI	1.00E+05	13.0 for PIV2
Parainfluenza Type 3	DHI	1.00E+05	15.2 for PIV3
Negative Matrix Control	DHI	N/A	Negative

Microbial Interference:

Parainfluenza virus types 1, 2 and 3 were spiked with clinically relevant concentrations of viruses, bacteria or yeast in order to evaluate possible interference from organisms not targeted by the assay. Parainfluenza virus types were used at a 2X LoD concentration; all dilutions were made in negative nasal matrix. Clinically relevant levels of viruses and bacteria were used when stock concentrations permitted. Samples were extracted and amplified according to the package insert instructions. All samples were tested in triplicate starting from the extraction step. The results were analyzed for any interference with any of the three parainfluenza virus types. The final concentration tested and results are listed in the table below. No interference was observed in the study.

Organism Name	**Concentration Tested (TCID50/ml or CFU/mL)**	**PIV-1 Avg. Ct**	**PIV-2 Avg. Ct**	**PIV-3 Avg. Ct**	**Interference (Yes/No)**
Adenovirus 1	1.51E+05	33.9	34.5	34.6	No
Coronavirus 229E	2.46E+06	33.6	33.7	33.8	No
Coronavirus NL63	1.41E+04	35.0	35.5	34.6	No
Coronavirus OC43	2.42E+06	33.6	34.4	34.0	No
Coxsackievirus B4	2.00E+06	34.3	34.7	33.9	No

Coxsackievirus B5/10/2006	3.62E+05	33.8	34.5	34.4	No
Cytomegalovirus	2.14E+05	34.1	33.9	33.8	No
Echovirus 6	1.52E+08	34.5	34.0	34.6	No
Echovirus 7	4.58E+05	34.3	32.9	34.3	No
Echovirus 9	2.17E+06	34.0	33.9	34.1	No
Echovirus 11	2.17E+05	34.1	34.7	34.1	No
Enterovirus 70	2.41E+05	34.4	34.4	33.9	No
Enterovirus 71	2.03E+05	34.1	34.3	34.2	No
Epstein Barr Virus	9.27E+07 copies/mL	35.8	34.9	35.3	No
HSV Type 1 MacIntyre Strain	5.89E+06	34.0	33.5	33.8	No
HSV Type 2 Strain G	1.96E+05	33.8	34.7	33.9	No
Human Metapneumovirus (A1)	3.66E+05	34.8	34.6	34.5	No
Human Rhinovirus 45	1.47E+04	34.2	33.8	33.7	No
Human Rhinovirus 52	1.31E+04	34.7	34.0	33.8	No
Influenza A/Mexico/4108/2009	4.08E+05	34.5	35.3	34.7	No
Influenza A/Port Chalmers	3.55E+07	34.8	34.6	34.0	No
Influenza B/Florida/04/2006	1.54E+05	34.2	34.5	33.8	No
Measles/7/2000	1.95E+06	34.4	33.3	34.0	No
Mumps Virus	2.75E+08	34.4	34.6	34.9	No
RSV A (Long)	4.36E+04	34.5	34.5	34.5	No
RSV B (Wash/18537/62)	3.43E+05	33.9	34.2	34.1	No
Varicella Zoster Virus	1.11E+04	34.0	34.6	33.6	No
Bordetella pertussis	9.08E+07	33.7	35.4	34.2	No
Bordetella bronchiseptica	5.40E+07	33.3	33.8	34.0	No
Chlamydophila pneumoniae	22 ng/mL (DNA)	33.6	33.8	34.1	No
Chlamydia trachomatis	2.10E+06	33.7	34.0	34.2	No
Legionella pneumophila	1.42E+08	34.8	34.1	34.6	No
Mycobacterium intracellualre	1.53E+08	33.9	33.9	35.6	No
Mycobacterium tuberculosis	9.30E+06	34.7	34.4	33.5	No
Mycobacterium avium	3.18E+08	33.5	34.0	34.9	No

Mycoplasma pneumoniae	3.16E+06 color changing units	34.3	34.0	34.3	No
Haemophilus influenzae	4.00E+07	34.6	34.7	33.4	No
Pseudomonas aeruginosa	1.32E+08	33.8	33.3	34.1	No
Proteus vulgaris	6.53E+07	33.7	33.3	34.0	No
Proteus mirabilis	1.19E+08	34.3	34.1	34.5	No
Neisseria gonorrhoeae	1.40E+08	34.4	34.2	35.0	No
Neisseria meningitidis	1.29E+07	33.9	34.6	34.4	No
Neisseria mucosa	1.61E+08	34.0	34.0	33.7	No
Klebsiella pneumoniae	9.75E+07	34.0	34.6	34.0	No
Escherichia coli	1.13E+08	34.8	34.7	34.7	No
Moraxella catarrhalis	1.26E+08	34.6	34.5	34.3	No
Corynebacterium diptheriae	3.44E+07	34.2	34.4	34.2	No
Lactobacillus plantarum	3.18E+07	34.1	34.6	34.1	No
Streptococcus pneumoniae	1.43E+07	35.4	35.6	35.0	No
Streptococcus pyogenes	6.38E+07	34.6	34.8	34.2	No
Streptococcus salivarius	5.40E+07	35.1	34.2	34.4	No
Staphylococcus epidermidis	9.23E+07	34.6	34.6	34.0	No
Staphylococcus aureus	6.08E+07	35.1	36.1	34.4	No
Candida albicans	9.70E+07	34.9	34.2	34.2	No
Parainfluenza Type 4A	4.17E+04	34.6	34.8	34.2	No
Parainfluenza Type 1		**35.0**	Neg	Neg	N/A
Parainfluenza Type 2		Neg	**34.2**	Neg	N/A
Parainfluenza Type 3		Neg	Neg	**34.7**	N/A
Negative Nasal Matrix		Neg	Neg	Neg	N/A

Competitive Interference:

The purpose of this study is to determine whether competitive interference exists between analytes when more than one parainfluenza type is present in the same reaction. All samples were tested in triplicate starting from the extraction step. The results were analyzed for any competitive interference with any of the three parainfluenza virus types. The final concentration tested and results are listed in the table below.

	Analyte Concentration TCID50/mL				
Test Analyte (at 2xLoD)	**PIV1**	**PIV2**	**PIV3**	**Replicates of Test Analyte Detected**	**Competitive Interference (Yes/No)**
PIV1	5.00E+00	-	-	3/3	No
	5.00E+00	2.50E+02	-	3/3	No
	5.00E+00	1.25E+03	-	3/3	No
	5.00E+00	1.25E+04	-	3/3	No
	5.00E+00	1.25E+05	-	3/3	No
	5.00E+00	1.25E+06	-	3/3	No
	5.00E+00	1.25E+07	-	0/3	Yes
	5.00E+00	-	8.00E+01	3/3	No
	5.00E+00	-	4.00E+02	3/3	No
	5.00E+00	-	4.00E+03	3/3	No
	5.00E+00	-	4.00E+04	3/3	No
	5.00E+00	-	4.00E+05	3/3	No
	5.00E+00	-	4.00E+06	0/3	Yes
PIV2		2.50E+02	-	3/3	No
	5.00E+00	2.50E+02	-	3/3	No
	2.50E+01	2.50E+02	-	3/3	No
	2.50E+02	2.50E+02	-	3/3	No
	2.50E+03	2.50E+02	-	3/3	No
	2.50E+04	2.50E+02	-	3/3	No
	-	2.50E+02	8.00E+01	3/3	No
	-	2.50E+02	4.00E+02	3/3	No
	-	2.50E+02	4.00E+03	3/3	No
	-	2.50E+02	4.00E+04	3/3	No
	-	2.50E+02	4.00E+05	3/3	No
	-	2.50E+02	4.00E+06	0/3	Yes
PIV3	-	-	8.00E+01	3/3	No
	5.00E+00	-	8.00E+01	3/3	No
	2.50E+01	-	8.00E+01	3/3	No
	2.50E+02	-	8.00E+01	3/3	No
	2.50E+03	-	8.00E+01	3/3	No
	2.50E+04	-	8.00E+01	3/3	No
	-	2.50E+02	8.00E+01	3/3	No
	-	1.25E+03	8.00E+01	3/3	No
	-	1.25E+04	8.00E+01	3/3	No
	-	1.25E+05	8.00E+01	3/3	No
	-	1.25E+06	8.00E+01	3/3	No
	-	1.25E+07	8.00E+01	0/3	Yes

The results indicate that competitive interference exists for PIV-1 when combined with PIV-2 at a concentration 1.25 x10^7 $TCID_{50}$ or greater and at 4.0 x10^6 $TCID_{50}$ or greater when combined with PIV-3. When evaluating PIV-2, it was determined that interference existed when combined with PIV-3 at a concentration 4.0 x10^6 $TCID_{50}$ or greater. No interference was seen when PIV-2 was combined with PIV-1. For PIV-3, there was competitive interference when combined with PIV-2 at a concentration of 1.25 x10^7 $TCID_{50}$ or greater. No interference was seen when PIV-3 was combined with PIV-1.

For all virus combinations, detectable interference was seen at concentrations equal to or greater than 4.0 x10^6 $TCID_{50}$. These titers are high and not likely to be found in clinical samples; it is therefore highly unlikely that competitive interference will be seen in the clinical settings. A limitation indicating competitive interference at high concentrations will be included in the package insert.

h. Interfering Substances:

A study was conducted to demonstrate that the Lyra Parainfluenza Virus Assay specifically detects parainfluenza virus types 1, 2, and 3 in the presence of clinically relevant concentrations of potentially interfering substances. To prepare samples for testing, parainfluenza virus at 4x LoD in negative nasal matrix was diluted 1:1 with each test substance. The final concentration after dilution for each substance is listed in the table below and the final concentration of parainfluenza virus was 2x LoD. Each mixed sample was then extracted and tested according to the package insert.

Ct values for all testing were reported and there was no significant change from the control. No interference was detected in the presence of any of the substances tested.

Name	Active Ingredients	Concentration to be Tested	Testing Level Rationale
Mucin (Bovine Submaxillary Gland, type I-S)	Purified mucin protein	60µg/mL	1000x maximum level present in serum
Blood (human), EDTA anticoagulated	N/A	2% (vol/vol)	
Neo-Synephrine	Phenylephrine HCl	15% (vol/vol)	10% of total recommended dose
Afrin Nasal Spray	Oxymetazoline Hydrochloride	15% (vol/vol)	10% of total recommended dose

Zicam Homeopathic Non-Drowsy Allergy Relief No Drip Liquid Nasal Gel	Luffa Operculata, Galphimia Glauca, Histaminum Hydrochloricum, Sulphur	5% (vol/vol)	10% of total recommended dose
Saline Nasal Spray:	Sodium chloride with preservatives	15% (vol/vol) of dose	10% of total recommended dose
OTC Throat Lozenges: LNegens Wild Cherry	Menthol	25% (mass/vol)	Same menthol level as other products
Zanamivir	Zanamivir	3.3-5mg/mL (target 4mg/mL)	10% of total spray dose
Tobramycin	Tobramycin	4.0µg/mL	10% of total recommended dose
Mupirocin	Mupirocin	6.6-10mg/mL (target 8mg/mL)	10% of total recommended dose in Mupirocin ointment
Oseltamivir phosphate (Tamiflu)	Oseltamivir phosphate	7.5-25mg/mL (target 16mg/mL)	10% of total recommended dose

i. Carry-Over/Cross-Contamination:

Simulated parainfluenza high positive samples were analyzed in series alternating with negative samples (checkerboard pattern) in order to determine whether carry-over/cross-contamination occurs with the use of the Lyra Parainfluenza Virus Assay. The high positive samples in this study were at concentration of 1.0×10^5 $TCID_{50}$/mL in negative nasal matrix. Parainfluenza-1, Parainfluenza-2, and Parainfluenza-3 analytes were combined into one sample for this study. Samples were processed and tested according to the package insert. Testing was performed on 5 separate days where each 96-well plate contained 48 high positive samples alternating with 48 negative samples (negative nasal matrix only). No cross-contamination was observed during the study. All negative samples tested had negative results and all high positive samples had all three parainfluenza viruses detected.

2. Comparison studies:

   a. Method comparison with predicate device:
   N/A

b. Matrix comparison:

This study was designed to verify that the assay does not have variable performance when samples are transported in various viral transport media. Parainfluenza-1 strain C35, Parainfluenza-2 strain Greer, Parainfluenza-3 strain C243 were used in this study. Viruses were diluted in the transport media to 2x LoD and testing was performed using six replicates per virus/media combination.

Transport Media Name	Vendor	PIV-1 Avg Ct	PIV-2 Avg Ct	PIV-3 Avg Ct
UTM	Copan	34.8	33.6	34.4
M4	Remel	35.2	34.0	33.5
M4-RT	Remel	35.2	33.8	34.8
M5	Remel	34.5	34.1	33.9
M6	Remel	33.8	34.0	34.3

Varying the transport media did not affect the performance of this assay, with no observed changes in the Ct value. The data demonstrates that all transport media included in the study are suitable for use with the Lyra Parainfluenza Virus assay.

c. Fresh vs. Frozen sample comparison:

Parainfluenza virus stocks were diluted to 2X and 5X LoD using negative nasal matrix. The five panels created contained 2X LoD (combined PIV-1, PIV-2 and PIV-3), 5X LoD (combined PIV-1, PIV-2 and PIV-3) and negative samples. Each panel consisted of 30 aliquots of 5X LoD, 30 aliquots of 2X LoD and 10 aliquots of negative nasal matrix. Panel 1 was extracted on the day of preparation. Panels 2 and 3 were stored at 4°C and then extracted on day 7 and 8, respectively. Panels 4 and 5 were stored at -20°C and then extracted on day 7 and 8, respectively. A single replicate of each panel member was extracted and tested according to the instructions in the package insert.

		5X LoD			2X LoD			
Panel #	Conditions	PIV1	PIV2	PIV3	PIV1	PIV2	PIV3	Neg.
1	Day 0	33.4	32.4	32.6	36.2	35.0	35.0	NEG
2	Day 7, 4°C	34.4	33.0	32.7	37.6	35.5	34.9	NEG
3	Day 8, 4°C	34.0	32.7	32.2	37.1	35.4	34.5	NEG
4	Day 7, -20°C	33.5	32.5	32.4	36.1	35.3	34.7	NEG
5	Day 8, -20°C	33.5	32.6	32.6	36.6	35.6	35.3	NEG

The average Ct values for all samples at 5x LoD and 2x LoD are substantially equivalent

among all conditions. The data presented demonstrates that the performance of this test with frozen samples is equal to the performance with fresh samples.

3. Clinical studies:

The evaluation of the Lyra™ Parainfluenza Virus Assay occurred in two separate studies: a prospective multi-center study using 1241 fresh specimens from the upper respiratory tract; and a retrospective study using 105 frozen specimens from the upper respiratory tract. In both studies the bioMérieux NucliSENS® easyMag® was used at all sites for the extraction of nucleic acids from the clinical specimens. The Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument was used with the Lyra™ Parainfluenza Virus Assay for the amplification and detection of the target nucleic acids.

The prospective specimens were also tested with direct specimen fluorescent antibody (DSFA) and cell culture with DFA (CCFA). Specimens for DSFA and CCFA were shipped to central location daily with cold packs, and were cultured within 72- hours of collection. The DSFA and CCFA of the specimens were performed at a central location, DHI reference laboratory (Diagnostic Hybrids, Athens, Ohio).

The study utilized fresh and frozen specimens from the 2013 and 2014 respiratory seasons. Clinical samples were collected at three geographically diverse locations, all clinical laboratories

Prospective Study:

A prospective study was conducted with 1241 fresh specimens from male and female patients with age range of ≤2 to 102 years. The table below shows the age and gender distribution of the patients in this study.

Gender	**Female**	**Male**
Total	651	590
Age		
< 2 years	111	158
2 to 5 years	130	126
6 to 21 years	150	129
22 to 59 years	160	108
≥ 60 years	100	69

Similar performance was observed at the 3 clinical sites participating in the study during the 2013 and 2014 respiratory seasons. Therefore, performance results from all sites were pooled and the summary data for PIV-1, PIV-2 and PIV-3 are presented in the tables below.

PIV-1			
Lyra™ Parainfluenza Virus Assay	**Comparator: DSFA and Culture with DFA**		
	Positive	Negative	Total
Positive	10	3*	13
Negative	0	1228	1228
Total	10	1231	1241
			95% CI
Sensitivity	10/10	100%	72.2% to 100%
Specificity	1228/1231	99.8%	99.3 % to 99.9%

* Two (2) of the three (3) samples positive by Lyra but negative by the comparator method were positive when tested by an additional RT-PCR assay.

PIV-2			
Lyra™ Parainfluenza Virus Assay	**Comparator: DSFA and Culture with DFA**		
	Positive	Negative	Total
Positive	5	0	5
Negative	0	1236	1241
Total	5	1236	1246
			95% CI
Sensitivity	5/5	100%	56.6% to 100%
Specificity	1236/1236	100%	99.7% to 100%

PIV-3			
Lyra™ Parainfluenza Virus Assay	**Comparator: DSFA and Culture with DFA**		
	Positive	Negative	Total
Positive	17	5*	22
Negative	0	1219	1219
Total	17	1224	1241
			95% CI
Sensitivity	17/17	100%	81.6% to 100%
Specificity	1219/1224	99.6%	99.0% to 99.8%

* Five (5) of the five (5) positive by Lyra but negative by the comparator method, were positive when tested by an additional RT-PCR assay.

Retrospective Study:

A Retrospective study was also conducted at site 1 because of the low prevalence of parainfluenza virus seen at all clinical sites during the study period. One hundred five frozen specimens from the upper respiratory tract were tested concurrently with the Lyra™

Parainfluenza Virus Assay and the Prodesse ProParaFlu+ assay (K091053).

PIV-1			
Lyra™ Parainfluenza Virus Assay	**Comparator: Prodesse ProParaFlu+ assay**		
	Positive	Negative	Total
Positive	24	1*	25
Negative	0	80	80
Total	24	81	105
			95% CI
Positive Percent Agreement	24/24	100%	86.2% to 100%
Negative Percent Agreement	80/81	98.8%	93.3% to 99.8%

* One (1) sample positive by Lyra but negative by the comparator method, was positive when tested by an additional RT-PCR assay.

PIV-2			
Lyra™ Parainfluenza Virus Assay	**Comparator: Prodesse ProParaFlu+ assay**		
	Positive	Negative	Total
Positive	22	5*	27
Negative	0	78	78
Total	22	83	105
			95% CI
Positive Percent Agreement	22/22	100%	85.1% to 100%
Negative Percent Agreement	78/83	94.0%	86.7% to 97.4%

* Five (5) of five (5) positive samples were positive by Lyra but negative by the comparator method, were positive when tested by an additional RT-PCR assay.

PIV-3			
Lyra™ Parainfluenza Virus Assay	**Comparator: Prodesse ProParaFlu+ assay**		
	Positive	Negative	Total
Positive	24	0	24
Negative	0	81	81
Total	24	81	105
			95% CI
Positive Percent Agreement	24/24	100%	86.2% to 100%
Negative Percent Agreement	81/81	100%	95.5% to 100%

When performed on the Applied Biosystems® 7500 Fast Dx the Lyra™ Parainfluenza Virus Assay yielded good sensitivity and specificity when compared to direct specimen fluorescent antibody (DSFA) and cell culture with DFA (CCFA).

When performed on the Applied Biosystems® 7500 Fast Dx the Lyra™ Parainfluenza

Virus Assay yielded good Positive Percent Agreement and Negative Percent Agreement when compared to the Prodesse ProParaFlu+ assay.

Based on the performance data presented from the clinical study this device performs as well as the predicate device, Prodesse ProParaFlu+ (K0910533).

4. Clinical cut-off**:**

Please refer to the "Baseline, Threshold, Cut-off" sections of this document.

5. Expected values/Reference range:

The prevalence (positives as determined by the reference method) of PIV-1, PIV-2 and PIV-3 during the study (2013 and 2014 respiratory seasons) is presented in the table below. The highest prevalence of PIV1 was observed among adults 60 years or older (2.4%) and of PIV-3 was observed among children younger than 2 years of age (3.7%).

Combined Study – Prevalence (N=1241)									
	PIV-1			**PIV-2**			**PIV-3**		
Age	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
< 2 years	269	1	0.40%	269	2	0.70%	269	10	3.70%
2 to 5 years	256	2	0.80%	256	2	0.80%	256	8	3.10%
6 to 21 years	279	1	0.40%	279	0	N/A	279	2	0.70%
22 to 59 years	268	5	1.90%	268	1	0.40%	268	2	0.80%
≥ 60 years	169	4	2.40%	169	0	N/A	169	0	N/A

N. Instrument Name:

Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument with the SDS Software version 1.4

O. System Descriptions:

The Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with the SDS Software version 1.4 is a real-time nucleic acid amplification and detection system that measures fluorescence and converts the signal to comparative quantitative readouts using fluorescent detection of dual-labeled hydrolysis probes. The 7500 Fast Dx is to be used only by technologists trained in laboratory techniques, procedures and on use of the analyzer.

1. Modes of Operation:

Instructions for programming the Lyra Direct Strep Assay, using the Version 1.4 software, are included in the package insert. The computer system is locked and controlled by ABI.

No additional software can be loaded by the end-user.

2. Software:

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

Yes ___X_____ or No _____

Level of Concern:
Moderate

Software Description:

The Sequence Detection Software (SDS) version 1.4 for the 7500 Fast Dx Instrument is used for instrument control, data collection and data analysis. The software can measure cycle-by-cycle real-time signals from the sample. The software provides a variety of tools to help the user analyze the data extracted from the samples. The software also provides lamp-life monitoring and other instrument maintenance information. The software runs as an application on a Windows operating system.

3. Specimen Identification:

Specimen identification is described in the "Test Principle" and "Baseline, Threshold, Cut-off" sections of this document.

4. Specimen Sampling and Handling:

All specimen sampling and handling is manual.

5. Calibration:
N/A

6. Quality Control:

An internal control 'process control' (PRC) is included in the device. It is the MS2 bacteriophage (Zeptometrix) which is a single stranded RNA virus that can be extracted, amplified and detected by primers and probes included in the master mix. Detection of the PRC indicates that assay conditions allowed proper sample extraction, amplification and detection.

No external controls are provided with this assay, but they are available from Quidel. External controls for PIV-1, PIV-2 and PIV-3 from the Lyra Parainfluenza Control Set #M1XX are recommended to serve as external positive controls. Laboratories may use previously characterized specimens that are negative for PIV-1, PIV-2 and PIV-3 as negative controls.

P. Proposed Labeling:

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

Q. Conclusion:

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