

## **510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY**

**A. 510(k) Number:** K122189

**B. Purpose for Submission:** The purpose of this submission is to show that the Quidel Molecular RSV + hMPV Assay is substantially equivalent to Prodesse's ProFlu™ Assay and Pro hMPV+ Assay. The ProFlu Assay detects RSV RNA while the Pro hMPV+ assay detects hMPV RNA.

**C. Measurand:** This assay detects the presence of RSV and hMPV nucleic acid using RT-PCR. Both viruses have negative single-stranded RNA genomes. The RT-PCR primers are developed to bind to conserved regions of L viral polymerase and NS2 genes for RSV and RNA polymerase gene for hMPV.

**D. Type of Test:** This is a nucleic acid based test using reverse transcription polymerase chain reaction (RT-PCR). The negative sense RNA genomes of RSV and hMPV are reverse transcribed and then amplified during the RT-PCR reaction. The presence of RSV or hMPV RNA is then detected through sequence-specific labeled probe that is cleaved during PCR amplification releasing the fluorescence reporter dye from the quencher dye.

**E. Applicant:** Quidel Corporation

**F. Proprietary and Established Names:** Quidel® Molecular RSV + hMPV Assay

**G. Regulatory Information:**

1. Regulation section: 21 CFR866.3980, Respiratory viral panel multiplex nucleic acid assay
2. Classification: Class II
3. Product code: OEM, OCC
4. Panel: Microbiology (83)

**H. Intended Use:**

1. Intended use(s):

The Quidel Molecular RSV + hMPV assay is a multiplex Real Time RT-PCR assay for the qualitative detection and identification of respiratory syncytial virus and human metapneumovirus RNA extracted from nasal and nasopharyngeal swabs specimens from patients with signs and symptoms of respiratory infection. This *in vitro* diagnostic test is intended to aid in the differential diagnosis of respiratory syncytial virus and human metapneumovirus infections in humans in conjunction with clinical and epidemiological risk factors. This test is not intended to differentiate the two subtypes of RSV or the four genetic sub-lineages of hMPV.

Negative results do not preclude RSV infection and/or hMPV infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions.

2. Indication(s) for use:

Same as intended use.

3. Special conditions for use statement(s):

Prescription only.

4. Special instrument requirements:

Cepheid SmartCycler II or ABI 7500 Fast Dx

**I. Device Description:** The Quidel Molecular RSV + hMPV Assay detects viral nucleic acids that have been extracted from a patient sample using the NucliSENS® easyMAG® automated extraction platform. An RT-PCR reaction is then performed in a single tube generating amplicons for each of the target viruses present in the sample. This reaction is performed utilizing either the Cepheid SmartCycler® II or the Applied Biosystems 7500 Fast DX. Identification of RSV and hMPV and the PRC occurs by the use of target-specific primers and fluorescent-labeled probes that hybridize to conserved regions in the genomes of RSV and hMPV and the PRC. Results are analyzed by either the Cepheid or ABI software and reported to the user.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

ProFlu™ Assay, Pro hMPV+ Assay

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

k092500, k082688

3. Comparison with predicate:

<b>Subject Device and Comparator Device Comparison</b>			
<b>Item</b>	<b>Subject Device</b> Quidel Molecular RSV + hMPV Assay	<b>Predicate Device</b> Prodesse ProFlu+	<b>Predicate Device</b> Prodesse ProhMPV+
Intended Use	<p>The Quidel Molecular RSV + hMPV assay is a multiplex Real Time RT-PCR assay for the <i>in vitro</i> qualitative detection and identification of respiratory syncytial virus and human metapneumovirus viral RNA extracted from nasal and nasopharyngeal swabs specimens with signs and symptoms of respiratory infection. This <i>in vitro</i> diagnostic test is intended to aid in the differential diagnosis of respiratory syncytial virus and human metapneumovirus infections. This test is not intended to differentiate the four genetic sub-lineages of hMPV.</p> <p>Negative results do not preclude RSV or hMPV infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions.</p>	<p>The ProFlu™+ Assay is a multiplex Real-Time PCR (RT-PCR) <i>in vitro</i> 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. This test is intended for use to aid in the differential diagnosis of Influenza A, Influenza B and RSV viral infections in humans and is not intended to detect Influenza C.</p> <p>Negative results do not preclude influenza or RSV virus infection and should not be used as the sole basis for treatment or other management decisions. It is recommended that negative RSV results be confirmed by culture.</p> <p>Performance characteristics for Influenza A Virus were established when Influenza A/H3 and</p>	<p>The Pro hMPV+ Assay is a Real Time RT-PCR <i>in vitro</i> diagnostic test for the qualitative detection of human Metapneumovirus (hMPV) nucleic acid isolated and purified from nasopharyngeal swab (NP) specimens obtained from individuals exhibiting signs and symptoms of acute respiratory infection. This assay targets a highly conserved region of the Nucleocapsid gene of hMPV. The detection of hMPV nucleic acid from symptomatic patients aids in the diagnosis of human respiratory hMPV infection if used in conjunction with other clinical and laboratory findings. This test is not intended to differentiate the four genetic sub-lineages of hMPV.</p> <p>Negative results do not preclude hMPV infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.</p>

<b>Subject Device and Comparator Device Comparison</b>			
<b>Item</b>	<b>Subject Device</b> Quidel Molecular RSV + hMPV Assay	<b>Predicate Device</b> Prodesse ProFlu+	<b>Predicate Device</b> Prodesse ProhMPV+
		<p>A/H1 were the predominant Influenza A viruses in circulation. When other Influenza A viruses are emerging, performance characteristics may vary.</p> <p>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 department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.</p>	
Assay Target	Respiratory syncytial virus, hMPV	Influenza A virus, influenza B virus, respiratory syncytial virus	hMPV
Sample Types	Nasal swab, nasopharyngeal swab	Nasopharyngeal swab	Nasopharyngeal swab
Extraction Methods	bioMérieux easyMAG Automated Magnetic Extraction Reagents	Roche MagNA Pure LC Total Nucleic Acid Isolation Kit or the bioMérieux easyMAG	Roche MagNA Pure LC Total Nucleic Acid Isolation Kit or the bioMérieux easyMAG

<b>Subject Device and Comparator Device Comparison</b>			
<b>Item</b>	<b>Subject Device</b> Quidel Molecular RSV + hMPV Assay	<b>Predicate Device</b> Prodesse ProFlu+	<b>Predicate Device</b> Prodesse ProhMPV+
		Automated Magnetic Extraction Reagents	Automated Magnetic Extraction Reagents
Assay Methodology	PCR-based system for detecting the presence or absence of viral RNA in clinical specimens	PCR-based system for detecting the presence or absence of viral RNA in clinical specimens	PCR-based system for detecting the presence or absence of viral RNA in clinical specimens
Detection Techniques	Multiplex assay using different reporter dyes for each target	Multiplex assay using different reporter dyes for each target	Multiplex assay using different reporter dyes for each target
Viral Targets	RSV: L viral polymerase and NS2 genes hMPV: RNA polymerase gene	Influenza A: Matrix gene; Influenza B: Non- structural NS1 and NS2	Nucleocapsid
LoD	The analytical sensitivity (limit of detection or LoD) of the Quidel Molecular hMPV assay was determined using quantified (TCID <sub>50</sub> /mL) cultures of 2 RSV strains and 4 hMPV strains (A1, A2, B1, B2) serially diluted in negative nasopharyngeal matrix. Each dilution was extracted using the NucliSENS easyMAG System and tested in replicates of 20 per concentration of virus on both the Applied Biosystems® 7500 Fast Dx platform. Analytical sensitivity (LoD) is defined as the lowest concentration at which 95% of all replicates	The analytical sensitivity (limit of detection or LoD) of the ProFlu+ Assay was determined using quantified (TCID <sub>50</sub> /mL) cultures of 4 Influenza A (2 H1N1 and 2 H3N2), 2 Influenza B, 2 Respiratory Syncytial Virus Type A, and 2 Respiratory Syncytial Virus Type B strains serially diluted in nasopharyngeal clinical matrix. Each viral strain was extracted using the Roche MagNA Pure LC instrument and tested in replicates of 20 per concentration of virus. Analytical sensitivity (LoD), as defined as the lowest concentration at which 95% of all replicates tested positive,	The analytical sensitivity (limit of detection or LoD) of the Pro hMPV+ Assay was determined using quantified (TCID <sub>50</sub> /mL) cultures of 2 hMPV (subtype A2 and subtype B2) strains serially diluted in nasopharyngeal clinical matrix. Each viral strain was extracted using the Roche MagNA Pure LC instrument and tested in replicates of 20 per concentration of virus. Analytical sensitivity (LoD) as defined as the lowest concentration at which ≥ 95% of all replicates tested positive, ranged from 10 <sup>2</sup> – 10 <sup>1</sup> TCID <sub>50</sub> /mL.

Subject Device and Comparator Device Comparison			
Item	Subject Device Quidel Molecular RSV + hMPV Assay	Predicate Device Prodesse ProFlu+	Predicate Device Prodesse ProhMPV+
	tested positive, ranged from 10 <sup>-1</sup> to 10 <sup>1</sup> TCID <sub>50</sub> /mL.	ranged from 10 <sup>2</sup> to 10 <sup>-1</sup> TCID <sub>50</sub> /mL.	

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

N/A

**L. Test Principle:**

This is a nucleic acid based test using reverse transcription polymerase chain reaction (RT-PCR). Viral infection is detected through the use of PCR to amplify and detect viral RNA. The negative sense RNA genomes of RSV and hMPV are reverse-transcribed and then amplified during the RT-PCR reaction. The presence of RSV or hMPV RNA is then detected through sequence-specific labeled probe that is cleaved during PCR amplification releasing the fluorescence reporter dye from the quencher dye.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

*a. Precision/Reproducibility:*

Reproducibility studies were performed on both thermal cycler instruments, the Cepheid SmartCycler II and ABI 7500 Fast Dx. The studies were conducted at three separate testing sites where two users tested each virus sample in triplicate over 5 days. The hMPV-A2 or RSV A viruses were tested at High Negative (0.05xLoD), Low Positive, close to the detection limit (2x LoD) and Medium Positive (5x LoD) concentrations. The viruses were diluted in a negative nasal matrix. Negative and positive controls were included in the study. The positive control was each virus diluted in UTM and the negative controls were UTM only (Negative control) and negative nasal matrix (RSV and hMPV negative). To conduct each test, the contrived samples were extracted using the bioMérieux easyMAG system and tested on either the Cepheid SmartCycler II or the ABI 7500 Fast Dx.

Reproducibility Results – Cepheid SmartCycler II										
Panel Member ID	Site 1			Site 2			Site 3			Total Positive Results
	Positive Results	AVE Ct*	%CV	Positive Results	AVE Ct*	%C V	Positive Results	AVE Ct*	%CV	
RSV High Negative 0.05x LoD	8/30	44.3	9.3	6/30	47.4	5.4	1/30	48.7	N/A	15/90

Reproducibility Results – Cepheid SmartCycler II										
Panel Member ID	Site 1			Site 2			Site 3			Total Positive Results
	Positive Results	AVE Ct*	%CV	Positive Results	AVE Ct*	%C V	Positive Results	AVE Ct*	%CV	
RSV Low Positive 2x LoD	30/30	31.2	7.3	30/30	31.4	4.1	30/30	30.9	1.5	90/90
RSV Med Positive 5x LoD	30/30	29.6	5.7	30/30	29.7	3.1	30/30	29.5	2.0	90/90
RSV Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90
RSV Negative Control	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90
RSV Positive Control	30/30	30.0	1.8	30/30	31.0	3.3	30/30	30.6	1.2	90/90
hMPV High Negative 0.15x LoD	12/30	34.3	8.3	6/30	41.8	6.3	8/30	34.9	6.4	26/90
hMPV Low Positive 2x LoD	30/30	31.1	6.7	30/30	32.2	6.2	30/30	31.1	3.3	90/90
hMPV Med Positive 5x LoD	30/30	29.3	2.0	30/30	29.9	3.2	30/30	29.8	3.4	90/90
hMPV Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90
hMPV Negative Control	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90
hMPV Positive Control	30/30	30.3	1.4	30/30	30.9	1.9	30/30	30.8	2.5	90/90

Reproducibility Results – Applied Biosystems 7500 Fast Dx										
Panel Member ID	Site 1			Site 2			Site 3			Total Positive Results
	Positive Results	AVE Ct*	%CV	Positive Results	AVE Ct*	%CV	Positive Results	AVE Ct*	%CV	
RSV High Negative 0.15x LoD	14/30	30.7	8.3	3/30	33.8	2.4	9/30	32.3	4.0	26/90
RSV Low Positive	30/30	23.7	7.4	30/30	23.8	3.1	30/30	23.4	4.5	90/90

Reproducibility Results – Applied Biosystems 7500 Fast Dx										
Panel	Site 1			Site 2			Site 3			Total
2x LoD										
RSV Med Positive 5x LoD	30/30	20.5	4.0	30/30	21.8	2.2	29/29	21.1	2.2	89/89
RSV Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90
RSV Negative Control	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90
RSV Positive Control	30/30	19.7	6.5	30/30	20.0	8.5	30/30	19.6	1.9	90/90
hMPV High Negative 0.15x LoD	14/30	24.9	19.5	6/30	29.1	10.2	10/30	25.1	16.9	30/90
hMPV Low Positive 2x LoD	30/30	21.0	8.7	30/30	21.5	4.1	30/30	21.6	5.8	90/90
hMPV Med Positive 5x LoD	30/30	18.9	3.3	30/30	19.6	3.0	29/29	19.2	2.6	89/89
hMPV Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90
hMPV Negative Control	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90
hMPV Positive Control	30/30	19.7	3.9	30/30	20.0	1.7	30/30	19.9	1.4	90/90

- Average Ct based on the average of only positive results. Ct values of 0 were left out of this calculation.

The data reported shows 100% detection of hMPV and RSV at Low and Medium Positive levels demonstrating good reproducibility of the test. Detection was lower than 50% for the High Negative samples which is acceptable and typical for nucleic acid tests.

*b. Linearity/assay reportable range:*  
N/A

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

A Fresh vs. Frozen study was performed using the same contrived specimens in negative clinical

matrix as was used in the Reproducibility Study. Briefly, hMPV-A2 and RSV were diluted into negative clinical matrix at 5x LoD, 2x LoD and 0.15x LoD. The viral panel was tested before freezing and then after frozen storage at -70°C for 22-56 days; each sample was tested in triplicate. No intermediate time points were tested for the stability studies. Additionally, 99 clinical samples were tested within 72 hours of collection and once again after 7 days at -70°C.

		Applied Biosystems 7500 Fast Dx Data							
		RSV				hMPV			
		5x LoD	2x LoD	0.15x LoD	Negative	5x LoD	2x LoD	0.15x LoD	Negative
Fresh Testing	Average	20.9	22.7	29.6	N/A	18.4	19.2	25.0	N/A
	STDEV	0.5	0.6	2.0	N/A	0.5	0.3	2.8	N/A
	%CV	2.6%	2.8%	6.8%	N/A	2.6%	1.6%	11.1%	N/A
Post-Freeze	Average	21.7	23.0	32.4	N/A	19.5	21.0	27.7	N/A
	STDEV	0.3	0.4	1.9	N/A	0.2	0.7	3.4	N/A
	%CV	1.3%	1.7%	5.9%	N/A	1.1%	3.1%	12.4%	N/A

Sample storage at -70°C for up to 56 days did not impact the ability of the Quidel Molecular RSV + hMPV Assay to detect either RSV or hMPV positive near the clinical cut-off. 2x and 5x LoD samples were both detected 100% (90/90) of the time. There was also no statistical difference in Ct value before and after freezing for the 2x LoD and 5x LoD samples. The data of the 99 clinical samples, not shown here, also did not show any significant change in Ct value over time when samples were stored at -70°C.

Stability studies were also performed on rehydrated master mix and extracted RNA samples. hMPV or RSV viruses at 3x LoD were added to negative sample matrix, extracted for RNA and the RT-PCR reaction was performed; the master mix study was performed in triplicate while the RNA extraction study was performed in quadruplicate. The master mix was stable at room temperature and 2° to 8°C for 2 days and for 5 days at -20°C. Extracted RNA stability studies showed that hMPV and RSV extracted RNA was stable for 6 hours at 2°to 8°C, 4 hours at room temperature, one month at -20°C and for 96 hours at -20°C with up to 3 freeze/thaw cycles.

There was no significant change in Ct value during any of the stability studies which supports the stability claims.

*d. Detection limit:*

The LoD was determined for hMPV and RSV using each of the 4 genetic sub-lineages and 2 subtypes of each virus respectively. Virus was diluted in pooled negative NP swab clinical matrix. Each virus sub-lineage/subtype was diluted and each dilution was extracted and run 20 times using both the Cepheid SmartCycler II and ABI 7500 Fast Dx, The LoD was determined to be the lowest titer at which the virus was detected greater than 95% of the time (19/20 or 20/20). For hMPV-A2, hMPV-B2, RSV A and RSV B three separate kit lots were tested and a representative titer was chosen that showed the best repeatability. The final LoD titers were reported for each instrument and all future calculations used the LoD for the specific virus and thermal cycler on which an experiment was being conducted.

Virus	ABI 7500 Fast Dx			Cepheid SmartCycler II		
	Lot 1 (110411)	Lot 2 (2N0020)	Lot 3 (908842)	Lot 1 (110411)	Lot 2 (2N0020)	Lot 3 (908842)
	LoD TCID <sub>50</sub> /mL					
<b>hMPV-A1</b>	1.76E+01			2.65E+01		
<b>hMPV-A2</b>	4.17E+00	4.17E+00	1.25E+00	4.17E+00	4.17E+00	4.17E+00
<b>hMPV-B1</b>	1.05E+00			7.88E+00		
<b>hMPV-B2</b>	3.21E+00	3.21E+00	3.21E+00	3.21E+00	9.63E+00	3.21E+00
<b>RSV A</b>	6.29E-01	5.10E-01	5.10E-01	1.89E+00	1.70E+00	5.10E-01
<b>RSV B</b>	7.5E-01	7.5E-01	7.5E-01	7.5E-01	2.25E+00	2.25E+00

The reported LoD values for each instrument are as follows:

LoD Value Summary for the 7500 Fast Dx & SmartCycler		
Virus	LoD TCID <sub>50</sub> /mL 7500 Fast Dx	LoD TCID <sub>50</sub> /mL SmartCycler
<b>RSV A</b>	6.29E-01	1.89E+00
<b>RSV B</b>	7.50E-01	2.25E+00
<b>hMPV-A1</b>	1.76E+01	2.65E+01
<b>hMPV-A2</b>	4.17E+00	4.17E+00
<b>hMPV-B1</b>	1.05E+00	7.88E+00
<b>hMPV-B2</b>	3.21E+00	9.63E+00

*e. Analytical specificity:*

**Interference:**

To evaluate the potential interference of other common respiratory pathogens the organisms in the table below were tested. Samples were prepared by adding RSV B or hMPV-B2 and the potential interference organism into negative clinical matrix at 3x LoD. The potential interference organisms were tested at one clinically relevant concentration, for viruses 10<sup>4</sup>-10<sup>6</sup> TCID<sub>50</sub>/mL and for bacteria 10<sup>6</sup>-10<sup>8</sup> CFU/mL. Additionally RSV and hMPV were tested for interference when both organisms are in the same sample. For the intra test interference RSV B was tested at 33,300x LoD, 333x LoD, 100x LoD, 30x LoD and 3x LoD. hMPV-B2 was tested at 10,000x LoD, 100x LoD, 30x LoD, 3x LoD. The prepared samples were extracted and run on both the Cepheid SmartCycler II and ABI 7500 Fast Dx.

There was no interference seen between any of the organisms tested, all tests were positive for either RSV or hMPV. A decrease in Ct value was seen in the presence of *Neisseria gonorrhoeae*, but sequence analysis showed no homology between the test probe sequence and *Neisseria gonorrhoeae* sequence. At very high levels (at or above 1,000x LoD) RSV may interfere with the detection of hMPV. However, during the clinical trial co-infection was found

to be a rare event, and the concentrations used when interference occurred are much higher than typically present in clinical specimens. Therefore, the risk of RSV/hMPV interference is very low.

Organism	Final Concentration	RSV detection	hMPV detection
hMPV A2	3xLoD	-	+
hMPV B2	3xLoD	-	+
RSV A Long	3xLoD	+	-
RSV B Wash	3xLoD	+	-
A/Mexico/4108/2009	1.4E+07 TCID50/mL	+	+
B/Florida/04/2006	5.24E+05 TCID50/mL	+	+
Adenovirus1/Adenoid 71	5.67E+04 TCID50/mL	+	+
Adenovirus 2	2.51E+09 TCID50/mL	+	+
Adenovirus 3	1.10E+06 TCID50/mL	+	+
Adenovirus 4	9.54E+06TCID50/mL	+	+
Adenovirus 5	4.00E+06 TCID50/mL	+	+
Adenovirus 7	1.25E+07 TCID50/mL	+	+
Adenovirus 11	3.85E+06 TCID50/mL	+	+
Adenovirus 14	8.63E+04 TCID50/mL	+	+
Adenovirus 22	N/A	+	+
Adenovirus 31	1.25E+05 TCID50/mL	+	+
Adenovirus 35	N/A	+	+
Coronavirus NL63	1.41E+04 TCID50/mL	+	+
Coronavirus 229E	1.70E+06TCID50/mL	+	+
Coronavirus OC43	1.67E+06 TCID50/mL	+	+
Coxsackievirus B4	2.43E+06 TCID50/mL	+	+
Coxsackievirus B5/10/2006	2.28E+06 TCID50/mL	+	+
Cytomegalovirus	8.76E+05 TCID50/mL	+	+
Echovirus 7	5.38E+08 TCID50/mL	+	+
Echovirus 9	1.50E+06 TCID50/mL	+	+
Echovirus 6	1.05E+08 TCID50/mL	+	+
Echovirus 11	1.05E+05 TCID50/mL	+	+
Enterovirus 71	2.68E+03 TCID50/mL	+	+
Enterovirus 70	1.66E+05 TCID50/mL	+	+
Epstein Barr Virus	5,000cp/mL	+	+
HSV Type 1 MacInytre	1.95E+06 TCID50/mL	+	+
Human Rhinovirus	1.26E+05 TCID50/mL	+	+
HSV Type 2 G strain	3.67E+06 TCID50/mL	+	+
Measles virus	1.95E+06 TCID50/mL	+	+

Mumps virus	2.75E+08 TCID50/mL	+	+
Parainfluenza Type 1	2.50E+05 TCID50/mL	+	+
Parainfluenza Type 2	2.20E+04 TCID50/mL	+	+
Parainfluenza Type 3	9.10E+05 TCID50/mL	+	+
Parainfluenza Type 4	1.36E+07 TCID50/mL	+	+
Varicella Zoster Virus	7.5E+02TCID50/mL	+	+
<i>Bordetella pertussis</i>	7.6E+08 CFU/mL	+	+
<i>Bordetella bronchiseptica</i>	1.65E+07 CFU/mL	+	+
<i>Chlamydophila pneumoniae</i>	1.0E+3 DNA copies/ul	+	+
<i>Chlamydia trachomatis</i>	2.10E+05 TCID50/mL	+	+
<i>Legionella pneumophila</i>	1.36E+07 CFU/mL	+	+
<i>Mycobacterium intracellulare</i>	4.30E+06 CFP/mL	+	+
<i>Mycobacterium tuberculosis</i>	1.10E+07 CFU/mL	+	+
<i>Mycobacterium avium</i>	3.07E+05 CFU/mL	+	+
<i>Mycoplasma pneumoniae</i>	4.64E+06 CCU/mL	+	+
<i>Haemophilus influenzae</i>	3.60E+06 CFU/mL	+	+
<i>Pseudomonas aeruginosa</i>	2.71E+06 CFU/mL	+	+
<i>Proteus vulgaris</i>	5.20E+06 CFU/mL	+	+
<i>Proteus mirabilis</i>	2.39E+06 CFU/mL	+	+
<i>Neisseria gonorrhoeae</i>	6.90E+02 CFU/mL	+	+
<i>Neisseria meningitidis</i>	3.60E+06 CFU/mL	+	+
<i>Neisseria mucosa</i>	7.60E+06 CFU/mL	+	+
<i>Klebsiella pneumoniae</i>	3.90E+06 CFU/mL	+	+
<i>Escherichia coli</i>	3.30E+06 CFU/mL	+	+
<i>Moraxella catarrhalis</i>	2.98E+03 CFU/mL	+	+
<i>Corynebacterium diphtheriae</i>	2.39E+06 CFU/mL	+	+
<i>Lactobacillus plantarum</i>	1.06E+06 CFU/mL	+	+
<i>Streptococcus pneumoniae</i>	9.50E+05 CFU/mL	+	+
<i>Streptococcus pyogenes</i>	2.43E+06 CFU/mL	+	+
<i>Streptococcus salivarius</i>	1.49E+06 CFU/mL	+	+
<i>Staphylococcus epidermidis</i>	7.50E+05 CFU/mL	+	+
<i>Staphylococcus aureus</i>	7.80E+06 CFU/mL	+	+
<i>Candida albicans</i>	1.79E+05 CFU/mL	+	+

**Cross Reactivity:**

To evaluate the potential cross reactivity of the test with other common respiratory pathogens, the organisms in the table above were tested. Samples were prepared by adding the potential interference organism into negative clinical matrix at 3x LoD. The potential interference organisms were tested at one clinically relevant concentration, for viruses 10<sup>4</sup>-10<sup>6</sup> TCID50/mL

and for bacteria  $10^6$ - $10^8$  CFU/mL (the same titers as in the Interference table above). The prepared samples were extracted and run on both the Cepheid SmartCycler II and ABI 7500 Fast Dx.

There was no cross-reactivity seen between any of the organisms tested, all tests were negative for both RSV or hMPV.

**Inclusivity:**

Each virus was diluted into negative clinical matrix at 1x-3x LoD. Samples were extracted and tested in triplicate on either the ABI 7500 Fast Dx or Cepheid SmartCycler II. Virus was detected in all positive samples in all but one virus strain, hMPV B1 Peru3. Only 2/3 samples were positive during testing of hMPV B1 Peru 3. This result was probably due to a combination of small differences in LoD among different genetic sub-lineage and titer variability near the LoD. The study adequately shows that the assay can detect the two RSV subtypes and four hMPV genetic sub-lineages.

<b>RSV Inclusivity Panel</b>				
<b>Subtype</b>	<b>Strain</b>	<b>TCID<sub>50</sub>/mL</b>	<b>(ABI 7500 Fast Dx)</b>	<b>(Cepheid SmartCycler II)</b>
A (NIBSC)	A-2	N/A	Positive	Positive
A	A-2	5.67E+00	Positive	Positive
B	Washington	2.25E+00	Positive	Positive
B	9320	2.25E+00	Positive	Positive
B	CH9318(1B)	2.25E+00	Positive	Positive

<b>hMPV Inclusivity Panel</b>				
<b>Subtype</b>	<b>Strain</b>	<b>TCID<sub>50</sub>/mL</b>	<b>(ABI 7500 Fast Dx)</b>	<b>(Cepheid SmartCycler II)</b>
A1	IA3-2002 G Gene	7.95E+01	Positive	Positive
A1	IA10-2003	7.95E+01	Positive	Positive
A2	IA14-2003 G Gene	1.25E+1	Positive	Positive
A2	Clinical Isolate	1.25E+1	Positive	Positive
B1	Peru2-2002 G Gene	2.36E+01	Positive	Positive
B1	Peru3-2003 G Gene	2.36E+01	Positive	Positive

B2	Peru1-2002 G Gene	9.63E+00	Positive	Positive
B2	Peru6-2003	9.63E+00	Positive	Positive
B2	IA18-2003 G Gene	9.63E+00	Positive	Positive
B2	IA18-2003 G Gene	9.63E+00	Positive	Positive
hMPV (NIBSC)	N/A	N/A	Positive	Positive

The Quidel Molecular RSV + hMPV assay detected 100% of the RSV and hMPV on both the ABI 7500 Fast Dx and Cepheid SmartCycler® II platforms.

*f. Assay cut-off:*

Threshold: The threshold for the ABI 7500 Fast Dx was established using the background fluorescence from cycle 3-15. The threshold is set to above the background fluorescence, below the plateau and linear regions of the amplification curve, within the exponential phase of the amplification curve. The threshold is designed to maximize sensitivity and specificity. The threshold settings for each analyte were determined individually using positive and negative clinical specimens.

The threshold for the Cepheid SmartCycler II was established using the background subtraction performed by the software algorithm and adjusting the threshold based on amplification curves obtained from positive and negative clinical samples.

Assay cut-off: The Ct cut-off was determined after testing clinical specimens and choosing a Ct value that was higher than the Ct value of the lowest positive concentration of analyte.

<b>Summary of Threshold Settings for the Quidel Molecular RSV + hMPV Assay by Instrument</b>		
	<b>ABI 7500 Fast Dx</b>	<b>SmartCycler</b>
hMPV	5.4e+04	10
RSV	8.0e+04	20
MS2	2.4e+04	10

<b>Recommended Ct Cut-Off Values by Instrument</b>				
	<b>ABI 7500 Fast Dx</b>		<b>SmartCycler</b>	
	<b>Latest Ct Value Observed*</b>	<b>Recommended Ct Cut-Off</b>	<b>Latest Ct Value Observed</b>	<b>Recommended Ct Cut-Off</b>

hMPV	24.66	35	38.00	50
RSV	30.08	35	49.70	50

\*These values reflect subtraction of first the 10 cycles

## 2. Comparison studies:

### a. *Method comparison with predicate device:*

Method comparison was performed against the gold standard of culture for RSV testing and a FDA cleared device for hMPV. The testing description and data are listed in the Clinical studies section.

### b. *Matrix comparison:*

A transport media study was conducted to show efficacy of 5 different transport media (UTM, M4, M4-RT, M5 and M6). RSV and hMPV were spiked into the media at 3x LoD. Each condition was extracted in triplicate, and each extraction was tested twice. There was not statistical difference in Ct value among the media tested on either the ABI 7500 Fast Dx or the Cepheid SmartCycler II.

## 3. Clinical studies:

### a. *Clinical Sensitivity and Specificity:*

The clinical study for this device was performed during the 2012 respiratory virus season, January – March, 2012. All 1014 nasal swab and nasopharyngeal swap specimens were collected prospectively. One specimen per patient was collected at four US study sites. A total 414 samples were tested fresh and 600 were frozen and tested at a later date. Reference testing for RSV was DSFA or culture with DFA, performed immediately after collection. A FDA cleared hMPV molecular test was used as the hMPV comparator for the Quidel Molecular RSV + hMPV Assay.

The performance of the Quidel assay in detecting RSV and hMPV was acceptable. The Quidel assay detected an additional 21 and 28 positive RSV samples not detected by DSFA and cell culture with DFA respectively. This was confirmed by an FDA-cleared RT-PCR assay. The detection of hMPV was equivalent to a FDA cleared hMPV molecular test. Discrepant samples were not further tested for hMPV.

<b>Cepheid Smart Cycler II Combined Site - Respiratory syncytial virus</b>								
		DSFA & Cell Culture w/DFA					95% CI	
		POS	NEG	Total	Sensitivity	<b>97.9%</b>	93.9%	99.3%
QM RSV + hMPV	POS	137	21*	158	Specificity	<b>97.6%</b>	96.3%	98.4%
	NEG	3	849	852				
	Total	140	870	1010				

\* All originally discordant specimens were positive for RSV by an FDA-cleared RT-PCR assay.

<b>Cepheid SmartCycler II Combined Site - Human metapneumovirus</b>								
		FDA Cleared hMPV Molecular Test					95% CI	
		POS	NEG	Total	Positive percent agreement	<b>96.7%</b>	92.4%	98.6%
QM RSV + hMPV	POS	145	3	148	Negative percent agreement	<b>99.6%</b>	98.9%	99.9%
	NEG	5	798	803				
	Total	150	801	951				

<b>ABI 7500 Fast Dx Combined Site - Respiratory syncytial virus</b>								
		DSFA & Cell Culture w/DFA					95% CI	
		POS	NEG	Total	Sensitivity	<b>98.6%</b>	94.9%	99.6%
QM RSV + hMPV	POS	138	28*	166	Specificity	<b>96.8%</b>	95.4%	97.8%
	NEG	2	840	842				
	Total	140	868	1008				

\* All originally discordant specimens were positive for RSV by an FDA-cleared RT-PCR assay.

<b>ABI 7500 Fast Dx Combined Site - Human metapneumovirus</b>								
		FDA Cleared hMPV Molecular Test					95% CI	
		POS	NEG	Total	Positive percent agreement	<b>98.0%</b>	94.3%	99.3%
QM RSV + hMPV	POS	147	9	156	Negative percent agreement	<b>98.9%</b>	97.9%	99.4%
	NEG	3	790	793				
	Total	150	799	949				

4. Clinical cut-off:  
N/A

5. Expected values/Reference range:

The prevalence detected for RSV and hMPV during the 2012 respiratory virus season was approximately 16% and 15% respectively. Prevalence was higher for both viruses in children under 5 years of age. The table below shows the overall prevalence and the prevalence for all sites segregated by age group. The prevalence detected by this device is appropriate for expected values for RSV and hMPV during the winter months.

Applied BioSystem 7500 Fast Dx						Cepheid SmartCycler II				
Age Group (years)	RSV Positive	Prevalence	hMPV Positive	Prevalence	total tested	RSV Positive	Prevalence	hMPV Positive	Prevalence	total tested
<1	72	28.3%	38	15.0%	254	71	27.5%	35	13.6%	258
1 to 5	72	19.9%	76	21.5%	354	67	19.0%	73	20.7%	352
6 to 10	6	4.5%	17	12.7%	134	6	4.5%	16	11.9%	134
11 to 15	3	4.8%	7	11.3%	62	3	4.8%	6	9.7%	62
16 to 21	3	9.1%	1	3.0%	33	3	9.1%	1	3.0%	33
>21	10	5.7%	18	10.3%	175	8	4.6%	17	9.7%	175
<b>Total</b>	<b>166</b>	<b>16.4%</b>	<b>157</b>	<b>15.5%</b>	<b>1012*</b>	<b>158</b>	<b>15.6%</b>	<b>148</b>	<b>14.6%</b>	<b>1014</b>

**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.