510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

k112490

B. Purpose for Submission:

New Device

C. Measurand:

Specific target RNA sequences for the RNA dependant RNA polymerase gene of hMPV.

D. Type of Test:

Qualitative real-time reverse transcription-polymerase chain reaction (RT-PCR) for determination of human metapneumovirus (hMPV) RNA in nasal swabs and nasopharyngeal swabs using the nucleic acid isolation NucliSENS® easyMAG™ System (bioMérieux) and the amplification and detection on the ABI 7500 Fast Dx Instrument with Software version 1.4.

E. Applicant:

Quidel Corporation

F. Proprietary and Established Names:

Quidel Molecular hMPV Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3980, Respiratory viral panel multiplex nucleic acid assay

2. Classification:

   Class II

3. Product code:

   OEM

4. Panel:

   Microbiology

H. Intended Use:

1. Intended use(s):
   The Quidel Molecular hMPV assay is a multiplex Real Time RT-PCR assay for the *in vitro* qualitative detection of human metapneumovirus RNA in nasal and nasopharyngeal swab specimens from patients with signs and symptoms of respiratory infection. This test is intended for use as an aid in the differential diagnosis of human metapneumovirus infections in humans in conjunction with clinical and epidemiological risk factors. This test is not intended to differentiate the four genetic sub-lineages of hMPV.

   Negative results do not preclude 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):

   Not Applicable

4. Special instrument requirements:

   NucliSENS easyMAG automated extraction platform

   Applied Biosystems 7500 Fast Dx platform (software version 1.4)

I. Device Description:

The Quidel Molecular hMPV Assay detects hMPV viral nucleic acids that have been extracted from a patient sample using the NucliSENS® easyMAG® automated extraction platform. A multiplex RT-PCR reaction is carried out under optimized conditions in a single tube generating amplicons for each of the target viruses present in the sample. This reaction is performed utilizing the Applied Biosystems® 7500 Fast Dx platform. Identification of hMPV occurs by the use of target specific primers and a fluorescent-labeled probe that hybridizes to a highly conserved region in the RNA dependent RNA polymerase gene of hMPV.

The following is a summary of the procedure:

1. **Sample Collection:** Obtain Nasal swab and nasopharyngeal swab specimens using standard techniques from symptomatic patients. These specimens are transported, stored, and processed according to established laboratory procedures.

2. **Nucleic Acid Extraction:** Extract Nucleic Acids from the specimens with the NucliSENS easyMAG System following the manufacturer's instructions and using the appropriate reagents. Prior to the extraction procedure add 20 µL of the Process Control (PRC) to each 180 µL aliquot of specimen. The PRC serves to monitor inhibitors in the extracted specimen, assures that adequate amplification has taken place and confirms that the nucleic acid extraction was sufficient.

3. **Rehydration of Master Mix:** Rehydrate the lyophilized Master Mix using the Rehydration Solution. The Master Mix contains oligonucleotide primers, fluorophore and quencher-labeled probes targeting highly conserved regions of hMPV as well as the PRC sequence. The primers are complementary to highly specific and conserved regions in the genome of these viruses. The probes are dual labeled with a reporter dye attached to the 5' end and a quencher attached to the 3' end.

4. **Nucleic Acid Amplification and Detection:** Add 15 µL of the rehydrated Master Mix to each reaction tube or plate well. 5µL of extracted nucleic acids (specimen with PRC) is then added to the plate well. Place the plate into the ABI 7500 FastDx. Once the plate is added to the instrument, the assay protocol is initiated. This protocol initiates reverse transcription of the RNA targets generating complementary DNA, and the subsequent amplification of the target sequences occurs. The Quidel Molecular hMPV assay is based on TaqMan® chemistry, and uses an enzyme with reverse transcriptase, DNA polymerase, and 5'-3' exonuclease activities. During DNA amplification, this enzyme cleaves the probe bound to the complementary DNA sequence, separating the quencher dye from the reporter dye. This step generates an increase in fluorescent signal upon excitation by a light source of the appropriate wavelength. With each cycle, additional dye molecules are separated from their quenchers resulting in additional signal. If sufficient fluorescence is achieved by 35 cycles during the data collection stage of amplification, the sample is reported as positive for the detected target sequences.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Gen-Probe Prodesse hMPV+

2. Predicate 510(k) number(s):
   k082688

3. Comparison with predicate:

Subject Device and Comparator Device Comparison		
Item	**Subject Device** Quidel Molecular hMPV Assay	**Predicate Device** Prodesse ProFlu+
Intended Use	The Quidel Molecular hMPV assay is a multiplex Real Time RT-PCR assay for the *in vitro* qualitative detection of human metapneumovirus RNA in nasal and nasopharyngeal swab specimens from patients with signs and symptoms of respiratory infection. This test is intended for use as an aid in the differential diagnosis of human metapneumovirus infections in humans in conjunction with clinical and epidemiological risk factors. This test is not intended to differentiate the four genetic sub-lineages of hMPV. Negative results do not preclude hMPV infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions.	The Pro hMPV+ Assay is a Real Time RT-PCR *in vitro* 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. Negative results do not preclude hMPV infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.
Assay Target	hMPV	hMPV
Sample Types	nasal 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 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

Subject Device and Comparator Device Comparison		
Item	**Subject Device** Quidel Molecular hMPV Assay	**Predicate Device** Prodesse ProFlu+
Detection Techniques/Platform	Multiplex assay using different reporter dyes for each target on the ABI 7500 Fast Dx	Multiplex assay using different reporter dyes for each target on the Cepheid SmartCycler
Viral Targets	RNA polymerase gene	Nucleocapsid

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

Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Testing for Human Metapneumovirus (hMPV) Using Nucleic Acid Assays (October 2009).

Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff; Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable (April 25, 2006).

L. Test Principle:

The real-time PCR process simultaneously amplifies and detects nucleic acid targets in a single closed-tube reaction. Detection of hMPV and the Process Control (PRC) is based on three processes: nucleic acid isolation, reverse transcription, and real time PCR amplification/detection. Human respiratory specimens (nasal swabs and nasopharyngeal swabs) from symptomatic patients are processed initially to isolate and purify viral nucleic acid from the cellular specimen matrix. After initial reverse transcription of RNA into complementary DNA (cDNA), amplification proceeds during which the probe anneals specifically to a region of the template between the forward and reverse primers. As primer extension and amplification occurs, the exonuclease activity of the Taq polymerase cleaves the probe separating the reporter dye away from the quencher. This generates an increase in fluorescent signal upon excitation from a light source of appropriate wavelength. With each cycle, additional reporter dye molecules are cleaved from their respective probes, yielding increased fluorescence signal. The amount of fluorescence at any given cycle is dependent on the amount of PCR product (amplicons) present at that time. Fluorescent intensity is monitored at each PCR cycle by fluorescent detection modules within the real-time instrument.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

The reproducibility of the Quidel Molecular hMPV assay was evaluated at three laboratory sites. The reproducibility panel and controls were tested at each site by two operators for five days in triplicate (2 operators X 5 days X triplicate testing X 3 sites = 90 results per sample). The panels and controls were extracted using the bioMérieux easyMAG system and tested on the ABI 7500Fast Dx.

The reproducibility panel was composed of four simulated samples made by diluting hMPV-A1 into negative nasal matrix. The panel included a medium positive (5x LoD) hMPV sample (2.65E+02 $TCID_{50}$/mL), a low positive (2x LoD) hMPV sample (1.06E+02 $TCID_{50}$/mL), a high negative (0.3X LoD) hMPV sample (1.59E+01 $TCID_{50}$/mL) and a negative. The results of the analysis of reproducibility are summarized below.

Quidel Molecular hMPV Reproducibility										
Panel Member ID	**Site 1**			**Site 2**			**Site 3**			**Total Results**
	Results	**AVE Ct**	**%CV**	**Results**	**AVE Ct**	**%CV**	**Results**	**AVE Ct**	**%CV**	
hMPV High Negative (1.59E+01 $TCID_{50}$/mL)	10/30	33.36*	5.08	10/30	32.76*	3.38	0/30	N/A	N/A	20/90
hMPV Low Positive (1.06E+02 $TCID_{50}$/mL)	30/30	28.73	5.28	30/30	28.18	8.42	29/30	30.18	4.89	89/90
hMPV Medium Positive (2.65E+02 $TCID_{50}$/mL)	30/30	25.09	6.65	30/30	25.18	7.63	30/30	26.58	5.68	90/90
Negative Sample	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/30
hMPV Positive Control	30/30	18.37	3.88	30/30	18.48	4.35	30/30	18.60	3.08	90/90
Negative Control	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/30

* CV of positive results

b. Linearity/assay reportable range:

Not Applicable

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

Freeze-Thaw Equivalency

The impact of using fresh clinical samples versus frozen clinical specimens on the Quidel Molecular hMPV assay ability to detect hMPV was evaluated. Briefly, 220 clinical specimens were aliquoted and processed fresh (< 72-hours post collection) and frozen (a minimum of 7-days storage at -70°C). Twelve (12) samples were discarded from analysis due to invalid results. The invalid rate of the fresh samples was 7/220 (3%) and the invalid rate of the frozen samples was 11/220 (5%). Six of the invalids overlapped between fresh and frozen. There were two hMPV positive samples that did not overlap

between fresh and frozen in the comparison study. The positive samples evaluated in the study spanned virus concentrations detected from 11 Ct to 30 Ct. The results are summarized in the 2x2 table below and indicate that the use of fresh specimens and frozen specimens, as defined above, are equivalent.

Quidel Molecular hMPV Assay – Fresh vs. Frozen Analysis			
Nasopharyngeal Swab	**Fresh Specimen**		
Frozen Specimen	Positive	Negative	Total
Positive	26	1	27
Negative	1	180	181
Total	27	181	208
PPA			96.3%
NPA			99.4%

Kit Stability

An evaluation of real-time stability using 3 lots of the Quidel Molecular hMPV kits at 2°C - 8°C and room temperature was done over the course of 73 days, with additional real time results to be collected for 460 days (15 months). The following information summarizes the acceptance criteria; furthermore these criteria will also be used throughout the stability study:

1. Quidel Molecular hMPV kit stability demonstrates 15 months real time stability at 2°C to 8°C.
2. Quidel Molecular hMPV kit stability demonstrates 15 months equivalent accelerated stability at 2°C to 8°C.
3. Quidel Molecular hMPV kit stability demonstrates 15 months real time stability at room temperature. (RT)
4. hMPV tests positive at each time point tested, 3x, 100x and 1000x LoD ($\geq$ 5 cycles and $\leq$35 cycles for 7500 Dx).
5. The NTCs test negative at each time point tested (undetermined).

No significant failure of the assay was detected at any of the experimental conditions to date (real time stability data collected to day 73) when compared to the t=0 time point. This was calculated by assuming a maximum CV% of 5% and calculating the 95% upper confidence limit (UCL) for each t=0 sample. Also, all NTCs tested negative at each time point tested.

Master Mix Stability after Rehydration

An evaluation of master mix stability after rehydration was carried out over a 5 day period using various storage conditions, including -20°C, 4°C, and 25°C

(room temperature). Mock specimens at 100X, 10X, and 3X LoD were used in the analysis. Results show that the rehydrated master mix can be stored for up to 24-hours at room temperature without any significant affect on the Ct values for the hMPV or the PRC. The data demonstrates that for times longer than 24-hours the rehydrated master mix can be stored at 4°C or -20°C for up to 5 days.

Extracted Specimen Stability

An evaluation of the stability of extracted specimens was carried out using the easyMAG extraction system followed by real-time RT-PCR analysis. Parallel aliquots of samples that were hMPV A (3X LoD) and hMPV B (3X LoD) positive were used for this study. Samples were analyzed at time 0 (immediately after extraction), after 2h at room temperature, after 8h at 2-8°C, after 1 month at -20°C, and after 1 month at -20°C with up to three freeze thaw cycles. There was no indication of degradation of the extracted specimen in the conditions tested.

d. Detection limit:

The limit of detection (LoD) of the Quidel Molecular hMPV assay on the ABI 7500 Fast Dx instrument was determined using limiting dilutions of re-cultured and re-titered viral stocks. The following isolates were used for the analysis and represent both lineages and subtypes: A1 Italy #6621 MK/2 LLCMK2/25, A2 Vanderbilt TN 94-49, B1 Italy #4702 LLCMK2/18, and B2 Italy #3817 LLCMK2/30.

Each of the viral stocks were spiked in negative nasal matrix and serially diluted. The replicate dilutions were extracted using the NucliSENS easyMAG. In total there were 21 extraction runs per dilution level tested to establish the LoD. Each extraction was analyzed on the ABI 7500 Fast Dx and the LoD was established as the lowest concentration that yielded positive result rate of ≥95%. The LoD established for hMPV A1, A2, B1, and B2 are summarized below.

hMPV Type	Sub-Type	Strain	LoD $TCID_{50}$/mL
A	A1	Italy #6621 MK/2 LLCMK2/25	**5.29E+01**
A	A2	Vanderbilt TN 94-49	**1.39E+01**
B	B1	Italy #4702 LLCMK2/18	**3.15E+00**
B	B2	Italy #3817 LLCMK2/30	**1.07E+01**

hMPV A1	$TCID_{50}$/mL			hMPV A2	$TCID_{50}$/mL		
$TCID_{50}$/mL	9.76E+01	**5.29E+01**	2.65E+01	**$TCID_{50}$/mL**	4.14E+01	**1.39E+01**	4.14E+00
Replicate-1	27.5898	29.5677	34.0142	Replicate-1	19.2623	22.7783	28.8796
Replicate-2	26.751	29.7919	33.7657	Replicate-2	18.5254	20.0743	Und.
Replicate-3	27.3281	28.0972	Und.	Replicate-3	17.7657	22.3149	28.9211
Replicate-4	27.9529	28.0159	33.6861	Replicate-4	17.8513	23.882	22.644
Replicate-5	27.952	29.6624	34.2712	Replicate-5	18.2482	24.1073	33.8105
Replicate-6	27.3933	29.3138	32.1021	Replicate-6	18.3644	23.7096	33.5286
Replicate-7	26.1488	28.1323	Und.	Replicate-7	18.3857	20.2891	34.5839
Replicate-8	27.2575	29.8472	34.2665	Replicate-8	18.1594	23.2851	29.7116
Replicate-9	27.05	27.7465	34.7051	Replicate-9	19.0474	20.1477	28.5702
Replicate-10	27.8662	29.1535	33.9066	Replicate-10	18.2692	22.5548	25.644
Replicate-11	26.6194	30.3058	Und.	Replicate-11	17.624	20.8038	31.4981
Replicate-12	27.2965	29.4415	Und.	Replicate-12	17.7512	19.8295	33.0868
Replicate-13	25.976	33.4114	Und.	Replicate-13	18.7787	26.462	23.3888
Replicate-14	24.6651	28.5803	33.6673	Replicate-14	17.9668	22.0258	25.1626
Replicate-15	26.5171	26.5084	Und.	Replicate-15	17.6512	21.6152	24.8982
Replicate-16	26.9674	26.9903	Und.	Replicate-16	18.3541	20.1346	Und.
Replicate-17	27.9973	29.0467	31.7243	Replicate-17	18.0115	24.3418	Und.
Replicate-18	27.0961	27.772	32.9554	Replicate-18	17.8084	22.4488	26.1243
Replicate-19	26.7276	29.9229	Und.	Replicate-19	18.331	20.4704	30.8669
Replicate-20	27.7816	28.555	33.8992	Replicate-20	18.1839	19.8139	26.2264
Replicate-21	25.9937	28.1505	34.0623	Replicate-21	18.0977	19.3523	25.6508
NTC	Und.	Und.	Und.	NTC	Und.	Und.	Und.
# (35>Ct>5)	21	21	13/21	**# (35>Ct>5)**	21	21	18
percentage	100%	100%	61.9%	**percentage**	100%	100%	86%
average Ct	26.996543	28.95301	33.839688	**average Ct**	18.21131	21.925771	28.510911
STDEV	0.8276129	1.4379573	0.7746443	**STDEV**	0.4355696	1.9106898	3.7537431

hMPV B1	$TCID_{50}$/mL			hMPV B2	$TCID_{50}$/mL		
$TCID_{50}$/mL	1.05E+01	**3.15E+00**	2.36E+00	**$TCID_{50}$/mL**	3.21E+01	**1.07E+01**	3.21E+00
Replicate-1	22.7485	29.1931	Und.	Replicate-1	19.849	25.1686	31.8105
Replicate-2	23.9339	30.8059	Und.	Replicate-2	21.5502	25.777	32.6886
Replicate-3	24.0032	30.3606	Und.	Replicate-3	21.3323	25.6818	31.2016
Replicate-4	24.5789	28.9146	32.9987	Replicate-4	20.6455	25.4246	34.2754
Replicate-5	25.8666	28.8763	Und.	Replicate-5	19.425	27.2705	31.7572

Replicate-6	24.5199	27.4292	31.2083	Replicate-6	20.9775	25.3866	30.7778
Replicate-7	23.11	29.7065	27.3465	Replicate-7	20.8207	24.3929	31.0495
Replicate-8	24.3543	26.5085	32.5957	Replicate-8	20.9963	24.7287	33.045
Replicate-9	21.7705	30.205	34.5152	Replicate-9	19.6393	24.6584	31.4825
Replicate-10	24.3884	28.6596	30.3947	Replicate-10	20.4821	25.6951	34.2537
Replicate-11	24.6276	27.079	Und.	Replicate-11	19.9232	18.9505	30.3872
Replicate-12	24.2194	29.6216	33.2577	Replicate-12	20.4557	22.0397	28.6635
Replicate-13	24.4698	28.6556	30.0068	Replicate-13	17.9482	25.1784	Und.
Replicate-14	24.6023	29.9063	31.1036	Replicate-14	20.6474	23.4714	34.253
Replicate-15	24.4274	29.3225	34.2523	Replicate-15	19.6833	22.6455	Und.
Replicate-16	23.083	27.9436	34.3973	Replicate-16	20.2427	24.4066	Und.
Replicate-17	25.26	28.0269	29.7855	Replicate-17	19.4036	25.0521	Und.
Replicate-18	24.1283	28.7603	30.4092	Replicate-18	20.5268	23.1831	32.2944
Replicate-19	24.5536	30.0526	Und.	Replicate-19	20.7651	24.6162	29.8554
Replicate-20	24.2826	28.4218	Und.	Replicate-20	21.292	25.6423	31.0606
Replicate-21	24.4037	30.1913	Und.	Replicate-21	20.2716	26.1634	30.0698
NTC	Und.	Und.	Und.	NTC	Und.	Und.	Und.
# (35>Ct>5)	21	21	13	**# (35>Ct>5)**	21	21	17
percentage	100%	100%	61.9%	**percentage**	100%	100%	81.0%
average Ct	24.158662	28.982895	31.50985	**average Ct**	20.3275	24.54921	31.701512
STDEV	0.876732	1.1404107	2.4938263	**STDEV**	0.8259372	1.7634874	1.6164972

e. Analytical specificity (Cross-reactivity):

The analytical specificity of the Quidel Molecular hMPV assay was evaluated by testing a panel consisting of 26 viruses, 24 bacteria, and one yeast strain that represent common respiratory pathogens or flora commonly present in nasopharynx. The bacteria and yeast were tested at concentrations of 10^5 to 10^{10} CFU/mL with the actual test concentration indicated for each organism in the following table. Viruses were tested at concentrations of 10^3 to 10^6 $TCID_{50}$/mL. All samples were extracted using the NucliSENS easyMAG instrument and tested in triplicate. The results are presented below. Briefly, the analytical specificity of the Quidel Molecular hMPV assay was 100% with no cross reactivity observed with the tested organisms.

Cross-reactivity		
Organism ID	**TCID50/mL or CFU/mL.**	**hMPV Result**
RSV Long	4.40E+04	Negative
RSV Washington	1.75E+04	Negative

Cross-reactivity		
Organism ID	**TCID50/mL or CFU/mL.**	**hMPV Result**
Influenza A/Mexico/4108/2009	1.40E+07	Negative
Influenza B/Florida/04/2006	5.25E+05	Negative
Adenovirus 1/Adenoid 71	5.67E+04	Negative
Coronavirus 229E	1.70E+06	Negative
Coronavirus OC43	1.67E+06	Negative
Coxsackievirus B4	2.43E+06	Negative
Coxsackievirus B5/10/2006	2.28E+06	Negative
Cytomegalovirus	8.76E+05	Negative
Echovirus 7	5.38E+08	Negative
Echovirus 9	1.50E+06	Negative
Echovirus 6	1.05E+08	Negative
Echovirus 11	1.50E+05	Negative
Enterovirus 71	2.68E+03	Negative
Enterovirus 70	1.66E+05	Negative
Epstein Barr Virus	5,000cp/mL	Negative
HSV Type 1 Maclnytre strain	1.95E+06	Negative
HSV Type 2 G strain	3.67E+06	Negative
Rubeola	3.78E+05	Negative
Mumps virus	8.43E+04	Negative
Parainfluenza Type 1	2.50E+05	Negative
Parainfluenza Type 2	2.20E+04	Negative
Parainfluenza Type 3	9.10E+05	Negative
Parainfluenza Type 4	9.57E+06	Negative
Varicella Zoster Virus	7.50E+02	Negative
Bordetella pertussis	1.04E+07	Negative
Bordetella bronchiseptica	2.55E+07	Negative
Chlamydia trachomatis	2.10E+05	Negative
Legionella pneumophila	2.05E+08	Negative
Mycobacterium intracellulare	6.90E+08	Negative
Mycobacterium tuberculosis	6.60E+07	Negative
Mycobacterium avium	1.36E+10	Negative
Haemophilus influenzae	5.90E+07	Negative
Pseudomonas aeruginosa	5.15E+07	Negative
Proteus vulgaris	2.65E+08	Negative

Cross-reactivity		
Organism ID	**TCID50/mL or CFU/mL.**	**hMPV Result**
Proteus mirabilis	2.75E+07	Negative
Neisseria gonorrhoeae	2.15E+07	Negative
Neisseria menigitidis	1.85E+08	Negative
Neisseria mucosa	1.85E+08	Negative
Klebsiella pneumoniae	3.30E+07	Negative
Escherichia coli	6.80E+07	Negative
Moraxella catarrhalis	5.85E+07	Negative
Corynebacterium diptheriae	6.0E+05	Negative
Lactobacillus plantarum	1.03E+08	Negative
Streptococcus pneumoniae	4.5E+07	Negative
Streptococcus pyogenes	2.05E+08	Negative
Streptococcus salivarius	2.50E+06	Negative
Staphylococcus epidermidis	2.6E+07	Negative
Staphylococcus aureus	5.15E+08	Negative
Candida albicans	1.07E+06	Negative

f. Analytical specificity (Interfering Substances):

The performance of the Quidel Molecular hMPV in the presence of potential inhibitors that may be found in nasopharyngeal specimens was evaluated. The potentially interfering substances were evaluated using hMPV A1, hMPV B1 at a concentration of 3x LoD. The results of the analysis are presented in the following table. No evidence of interference caused by the substances tested was observed.

Substance Name	**Concentration Tested**	**hMPV A1 Result (3x LoD)**	**hMPV B1 Result (3x LoD)**
Mucin (Bovine Submaxillary Gland, type I-S)	60 µg/mL	Positive	Positive
Blood (human), EDTA anticoagulated	2% (vol/vol)	Positive	Positive
Neo-Synephrine	15% (vol/vol)	Positive	Positive
Afrin Nasal Spray	15% (vol/vol)	Positive	Positive
Zicam Homeopathic Non-Drowsy Allergy Relief No Drip Liquid Nasal Gel	5% (vol/vol)	Positive	Positive
Saline Nasal Spray	15% (vol/vol) of dose	Positive	Positive
Throat Lozenges	0.68g/mL; 1/18 drop, crushed; active ingredients: 1.7 mg/mL menthol	Positive	Positive

Substance Name	Concentration Tested	hMPV A1 Result (3x LoD)	hMPV B1 Result (3x LoD)
Zanamivir	3.3-5 mg/mL	Positive	Positive
Tobramycin	4.0 µg/mL	Positive	Positive
Mupirocin	6.6-10 mg/mL	Positive	Positive
Oseltamivir phosphate	7.5-25 mg/mL	Positive	Positive

g. Assay cut-off:

The "cutoff value" represents the fluorescent intensity signal (reported in Relative Fluorescent Units) at which a "positive" reaction reaches a relative fluorescent intensity above the background or baseline of a "negative" reaction. If a sample exceeds the threshold in a detection channel during PCR, the sample is considered positive for that channel. If the sample does not exceed the threshold for a detection channel by the last PCR cycle, the sample is considered negative for that channel.

The cut-off for the Quidel Molecular hMPV Assay was determined and confirmed through a phased approach. The preliminary threshold was established using data obtained from the LoD studies and from the analysis of a set of clinical specimens. Data from the analysis of multiple replicates near the LoD of the assay were used to establish the threshold such that sensitivity was maximized. Similarly, the latest Ct value form the LoD data and those observed in the analysis of the set of clinical specimens were used the set the cut-off. Using the parameters summarized in the table below, a verification study was done to confirm the threshold and cut-off values.

Quidel hMPV Molecular Assay on the ABI 7500 Fast Dx Instrument		
Analyte	**Confirmed Threshold (RFU)**	**Confirmed Ct Cut-Off***
hMPV A1	5.4e4	35
hMPV B1	5.4e4	35

*These values reflect subtraction of the first 10 cycles. Fluorescence is monitored after the first 10 cycles during the data collection stage of amplification.

Results from the comparison to an FDA-cleared molecular device to validate the proposed cut-off and threshold are:

- The Quidel Molecular hMPV assay showed 100% (7/7) PPA with an FDA cleared molecular comparator device.
- The Quidel Molecular hMPV assay showed 100% (58/58) NPA with an FDA cleared molecular comparator device.

These results demonstrated that the preliminary threshold and Ct cut-off settings determined have been confirmed and effective for maximizing

sensitivity of the Quidel hMPV Molecular Assay.

g. Comparison of transport media:

Analytical performance of the Quidel Molecular hMPV assay was evaluated with five different transport media. A stock of hMPV-B1 was used to evaluate performance across the different transport media at a level close to the LoD (3X). Briefly, dilutions of hMPV-B1 were made and each of the dilutions was extracted in triplicate. Each extraction was then tested in duplicate on the ABI 7500 Fast Dx using the Quidel Molecular hMPV standard protocol. No differences between the tested transport media were observed and the results are summarized below.

Evaluation of Transport Media in the Quidel Molecular hMPV Assay					
		Average		**STDEV**	
Test Media	**Template**	**MPV**	**PRC**	**MPV**	**PRC**
MS4	3x LoD hMPV	21.86	16.29	0.90	0.9
	Media + PrC	Und	16.51	Und	0.07
MS4-RT	3x LoD hMPV	21.40	16.37	0.98	0.10
	Media + PrC	Und	16.51	Und	0.18
MS5	3x LoD hMPV	21.37	16.46	0.33	0.11
	Media + PrC	Und	16.56	Und	0.11
MS6	3x LoD hMPV	21.21	16.32	0.51	0.08
	Media + PrC	Und	16.75	Und	0.49
UTM Lot 49L606	3x LoD hMPV	22.56	16.57	0.91	0.37
	Media + PrC	Und	16.48	Und	0.17
UTM Lot 51N618	3x LoD hMPV	20.96	16.47	0.56	0.25
	Media + PrC	Und	16.70	Und	0.19
UTM Lot 49C600	3x LoD hMPV	21.98	16.07	0.98	0.18
	Media + PrC	Und	16.54	Und	0.15
Overall Av		21.62	16.36	0.74	0.28

h. Carry-over Contamination Analysis:

An evaluation of potential carry–over contamination during extraction and/or amplification was carried out. High negative and positive samples were run in alternating series to evaluate performance of the device and to monitor for potential carry-over contamination. The evaluation included alternating extractions high and low concentration of virus on the indicated platform. During the amplification stage the plate was setup in a checkerboard approach locating negative and positive samples in adjoining wells. No cross contamination was observed in the evaluation of the Quidel Molecular hMPV kit.

2. Comparison studies:

a. Method comparison with predicate device:

Not Applicable

b. Matrix comparison:

Not Applicable

3. Clinical studies:

a. Prospective Clinical Studies:

Performance characteristics of the Quidel Molecular hMPV assay was established in a prospective study during the 2010-2011 respiratory virus season (January to March 2011). A total of 1116 specimens were evaluated in this study (742-fresh, 374-frozen). Of the fresh specimens, 399 specimens were nasal swabs, and 343 were nasopharyngeal swabs. The fresh specimens were collected for routine respiratory virus testing at thirteen sites across the United States. The frozen specimens were collected and tested at two geographically distinct locations and were comprised of 374 nasopharyngeal swabs.

The 1116 specimens were tested by both the subject and a FDA cleared molecular comparator device for human metapneumovirus RNA. Sixteen of these specimens were invalid on initial testing with the subject device (1.4%). Re-testing of the specimens according to the Interpretation algorithm described above also yielded invalid results. Thirty-six specimens were invalid on initial and repeat testing (as per the device's package insert) on the comparator device (3.2%). Eight specimens were invalid in both devices, therefore, a total of 44 invalid specimens were removed from additional analysis. The remaining 1072 specimens were used in the 2x2 analysis of clinical performance.

The evaluation of the Quidel Molecular hMPV Assay was conducted at two external laboratory sites and one internal site. Each site used the ABI 7500Fast Dx platform for amplification/detection and the bioMerieux easyMAG for nucleic

acid extraction. Each site included in the study was capable of performing highly complex laboratory tests.

Each specimen included in the study had the following testing performed. Prospective testing performed at the trial sites was done within 72-hours post-collection using the Quidel Molecular hMPV Assay with the bioMerieux easyMAG for nucleic acid extraction. Archival reference testing performed at a central testing location using samples that have been frozen within 72-hours post-collection and stored at -70°C. The frozen specimens were analyzed with a FDA cleared molecular device for hMPV according to the instructions for use provided at a central location and were not preselected based on previous results and are representative of an all-comers based study.

For inclusion in the study the specimens meet the following criteria:

- Approximately 1-mL of leftover or waste specimens in viral transport media that were submitted for respiratory virus culture.
- Specimens were tested in a prospective manner.
- Acceptable specimen types used for the evaluation: nasal swab, nasopharyngeal swab
- Specimens transported, received and stored at 2° to 8°C for up to 72 hours.
- No gender restrictions.
- No age restrictions.
- No results from the investigational device were made available to the patient's physician.
- All patient identifiers were removed and replaced with study identifiers prior to inclusion in the study.

Exclusion of specimens from the study was based on the following criteria:

- There were no patient exclusion criteria since the specimens were from patients that the treating physician has deemed qualified for diagnostic testing.
- Inadequate specimen volume to perform the three devices as required.
- Specimen inadequately stored or shipped.
- Minimal patient information consisting of age or and gender was inadequate
- Samples not received within 72 hours of collection, or stored at >8°C.

The following table summarizes the gender and age distribution of all patients enrolled in the evaluation.

Age and Gender Distribution		
Sex	F	M
Total	539	577
≤ 5 years	233	268

6 – 21 years	118	157
22 – 59 years	141	121
≥ 60 years	47	31
Total	539	577

The overall clinical performance data are summarized in the following 2x2 table:

Nasal/nasopharyngeal swab (N=1072)	**FDA Cleared RT-PCR**		
Quidel Molecular	Positive	Negative	Total
Positive	60	2*	62
Negative	3	1007	1010
Total	63	1009	1072
			95% CI
Positive Percent Agreement	60/63	95.2%	86.7 to 99.0%
Negative Percent Agreement	1007/1009	99.8%	99.3% to 100%

* Specimens negative for hMPV by sequence analysis

4. Clinical cut-off**:**

   Not Applicable

5. Expected values/Reference range:

In the Quidel Molecular hMPV Assay clinical studies, a total of 1100 (1.4%) eligible nasopharyngeal (NP) swab and nasal swabs (NS) specimens were tested from 15 clinical laboratories across the United States during the in the winter of 2011 (January 2011 – March 2011). Originally 1116 specimens were available for the study, sixteen of these specimens were invalid on initial testing with the subject device, resulting in 1100 specimens. The number and percentage of the hMPV RNA positive cases by the Quidel Molecular hMPV Assay, calculated by age group, are presented in the following table:

hMPV RNA Positive by the Quidel Molecular hMPV Assay (N=1100)			
Age Group	**Number of Patients**	**Number of Positives**	**Prevalence**
≤ 1 year	131	10	7.6%
1 to 5 years	358	25	7.0%
6 to 10 years	147	7	4.8%
11 to 15 years	72	1	1.4%
16 to 21 years	53	0	0%
> 21 years	339	19	5.6%
Total	1100	62	5.6%

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.