

SPECIAL 510(k): Device Modification Decision Summary

To: Gen-Probe Prodesse, Inc.

RE: K123838

This 510(k) submission contains information/data on modifications made to the SUBMITTER'S own Class II, Class III or Class I devices requiring 510(k). The following items are present and acceptable (delete/add items as necessary):

- 1) The name and 510(k) number of the SUBMITTER'S previously cleared device:

Trade Name: Pro hMPV™+ Assay

510(k) number: K082688

2. Submitter's statement that the **INDICATION/INTENDED USE** of the modified device as described in its labeling **HAS NOT CHANGED** along with the proposed labeling which includes instructions for use, package labeling, and, if available, advertisements or promotional materials (labeling changes are permitted as long as they do not affect the intended use).

- 3) A description of the device **MODIFICATION(S)** .

- 1- RT-PCR cycling for the New Pro hMPV+ Assay ends after cycle 35 in place of the current endpoint of cycle 50.
- 2- The Pro hMPV+ Supermix has been modified to decrease background fluorescence.
- 3- The hMPV RNA Control III was replaced by the Pro hMPV+ Control which has the following changes:
 - a. The control stock manufacturer is now Asuragen, Inc. instead of Gen-Probe Prodesse.
 - b. The stock concentration (given in copies/μl) of the Pro hMPV + Control is increased by 75%.
 - c. The Pro hMPV+ Control is used at the concentration at which it is supplied, eliminating the need for a 1:10 dilution step prior to assay set up. Combined with the increase in stock concentration (given in copies/μl), the working concentration for the Pro hMPV+ Control is 175% higher than the current hMPV RNA Control III.
 - d. The Pro hMPV+ Control is programmed into the Cepheid SmartCycler software as a defined control (Positive Control – PC).

Select Analytic Studies

Limit of Detection

The analytical sensitivity (limit of detection or LoD) of the Pro hMPV+ Assay was determined using quantified (TCID₅₀/mL) cultures of two hMPV (subtype A2 and subtype B2) strains serially diluted in nasopharyngeal clinical matrix. Each viral strain was processed using the bioMérieux NucliSENS EasyMAG system for extraction and the SmartCycler II Instrument for RT-PCR. The LoD was identical to the original Pro hMPV+ Assay for hMPV strain A2 and 0.5 log lower for hMPV strain B2.

Viral Strain	LoD Concentration (Original Pro hMPV+)	LoD Concentration (Reformulated Pro hMPV+)
hMPV subtype A2	10 ² TCID ₅₀ /mL	10 ² TCID ₅₀ /mL
hMPV subtype B2	10 ¹ TCID ₅₀ /mL	10 ^{0.5} TCID ₅₀ /mL

Positive Control Effectiveness

The Pro hMPV+ Control (PC) is a non-infectious *in vitro* transcribed RNA of the hMPV viral sequence targeted by the New Pro hMPV+ Assay. Its purpose is to test for procedural errors (absence of reagent, instrument failure, etc.) that may result in failure of the assay to detect hMPV. The New Pro hMPV+ Assay uses the PC at a higher concentration than Current Pro hMPV. “Defective” RT-PCR master mixes (e.g. no reverse transcriptase, no Taq polymerase, decreased hMPV primer concentration) were prepared and tested to assess the PC’s ability to detect global errors. Each defective mix was tested using 20 replicates of the New Pro hMPV+ Control. A Negative Control (NC) consisting of Internal Control (IC) in viral transport media, was included in each run. For each defective mix, none of the hMPV PC replicates were detected and thus, the New PC was considered effective.

Clinical Comparison Study

The Pro hMPV+ Assay’s supermix was reformulated and performance characteristics were established by comparing the reformulated assay to the original Pro hMPV+ Assay. One hundred eighty-three retrospective nasopharyngeal swab samples collected during 2011 – 2012 at two sites (Milwaukee, WI and Chicago, IL) were used for this study. NP swab samples positive and negative for hMPV were selected for inclusion based on previous site-specific molecular test results. One sample was not used in the final analysis as it was Unresolved upon initial and repeat testing with both the original and reformulated ProhMPV+ Assays.

“True” hMPV positives were considered as any sample that tested positive for hMPV by the original Pro hMPV+ Assay. “True” hMPV negatives were considered as any sample that tested negative for hMPV by the original Pro hMPV+ Assay. Discrepant analysis for samples where the reformulated Pro hMPV+ Assay and the original Pro hMPV+ results were in disagreement was performed using RT-PCR with hMPV specific primers targeting the hMPV phosphoprotein gene followed by bi-directional genetic sequencing.

hMPV Comparison Results

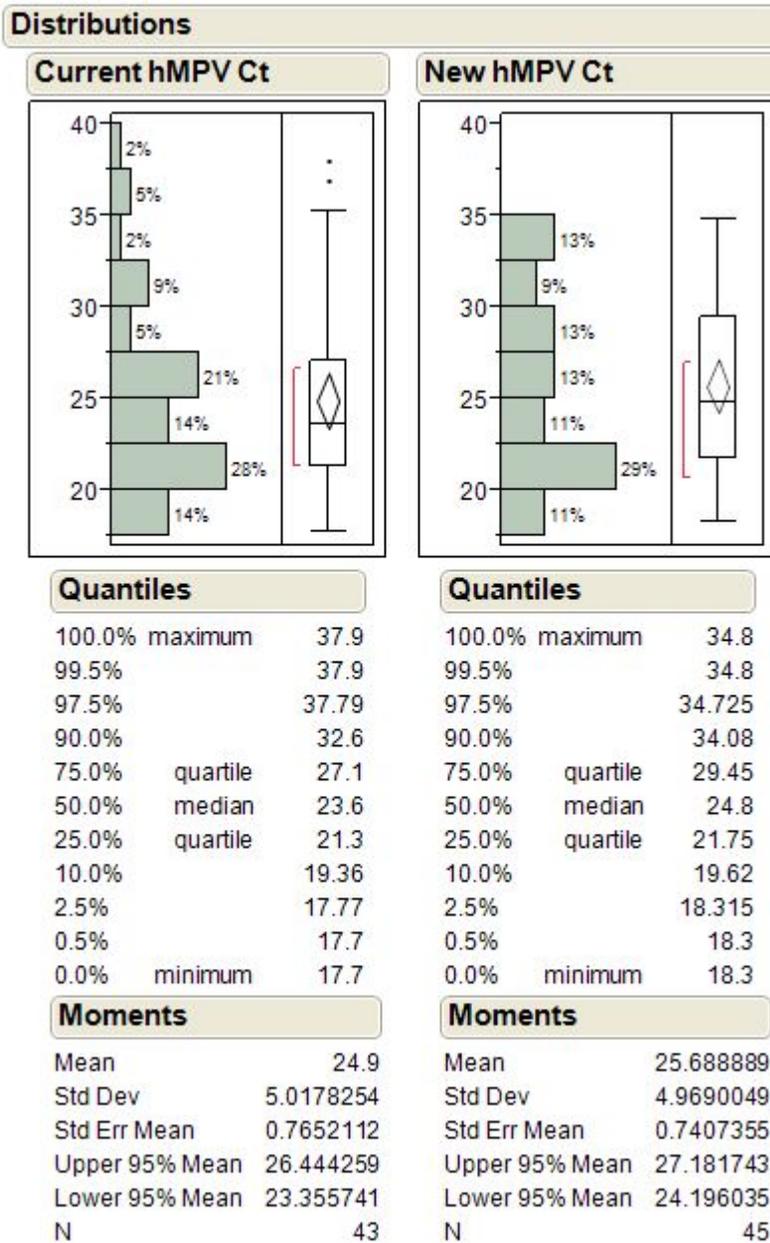
		Current Pro hMPV+ Assay			Comments
		Positive	Negative	Total	
New Pro hMPV+ Assay	Positive	43	2*	45	Percent Positive Agreement 100% (91.80%-100%) 95% CI
	Negative	0	137	137	Percent Negative Agreement 98.6% (94.91%-99.61%) 95% CI
Total		43	139	182	

* Two samples tested positive for hMPV by bi-directional sequencing.

Of the 183 samples chosen, 53 were previously reported to be positive and 130 were reported to be negative by initial testing. Of the 53 positive 13 were negative upon retesting with the Current and New Pro hMPV+ Assay. This was most likely due to low levels of analyte in the sample, indicated by high Ct during initial testing. There were 4 samples that tested positive among the chosen 130 negative samples; this was probably due to the fact that the initial screening assay for those samples did not detect hMPV.

To support the lowering of the Ct cut-off to 35 cycles a histogram of the Ct values for all positive clinical samples was generated. The distribution of Ct values shows that the maximum Ct detected in a clinical sample was below the 35 Ct threshold. In addition, the clinical data shows that the new version of the

assay has the same PPA as the current assay and was able to detect two additional hMPV positive samples. This data supports the lower Ct threshold.



- 4) The **FUNDAMENTAL SCIENTIFIC TECHNOLOGY** of the modified device has not changed.
- 5) **Comparison Information** (similarities and differences) to applicant's legally marketed predicate device including, labeling, intended use, and physical characteristics.

Features	New Device	Predicate Devices
	New Pro hMPV+ Assay	Current Pro hMPV+ Assay
510(k)	K123838	K082688
Regulation	866.3980	866.3980
Product Code	OEM	OEM
Device Class	Class II	Class II
Intended Use	For the <i>in vitro</i> qualitative detection of human metapneumovirus nucleic acids.	For the <i>in vitro</i> qualitative detection of human metapneumovirus nucleic acids.
Technology/ Detection	Real-Time RT-PCR Detection	Real-Time RT-PCR Detection
Specimen Types	NP swabs	NP swabs
Nucleic Acid Isolation	Roche MagNA Pure LC System and bioMérieux NucliSENS easyMAG	Roche MagNA Pure LC System and bioMérieux NucliSENS easyMAG
Instrument /Assay Platform	Cepheid SmartCycler II System	Cepheid SmartCycler II System
Assay Controls	hMPV positive RNA transcript control and an Internal RNA control provided	hMPV positive RNA transcript control and an Internal RNA control provided

Element	New Device: Pro hMPV+ Assay	Predicate: Current Pro hMPV+ Assay (K082688)
Assay Cutoff Cycle for hMPV detection	35	40
hMPV Positive RNA Control	Provided "at use" concentration for RT-PCR; no dilution prior to RT-PCR required.	Dilute 1:10 prior to use for RT-PCR

6) Design Control Activities Summary:

a) Risk Analysis:

A Failure Mode and Effect Analysis (FMEA) method was used to evaluate risk for the proposed changes to the labeling. The methods of risk analysis were consistent with 21 CFR 860, ISO: 14971. The following table is a summary of the risk analysis:

Device Modification	Cause of Risk	Hazardous Situation(s)	Consequences	Risk Control Measure(s)	Risk Acceptability Criteria	Verification/Validation Methods	Summary Conclusion
Cycling for the New Pro hMPV+ Assay will	Adjustment of the cycling parameters	False Negative Result	Improper patient management	Analytical Sensitivity and Clinical Performance	The LoD for New Pro hMPV+ was required to	Analytical Sensitivity and Clinical performance	Analytical sensitivity of the New Pro hMPV+ Assay is identical to the

end after cycle 35 instead of cycle 50.	may result in an incorrect result.			of the New Pro hMPV+ Assay was compared to the current Pro hMPV+ Assay	be within 0.5 log of the LoD for Current Pro hMPV+ for both strains tested.	of the New Pro hMPV+ Assay was compared to the current Pro hMPV+ Assay.	Current Pro hMPV+ Assay for hMPV strain A2 and 0.5 log lower for hMPV strain B2. IN a retrospective clinical study, New Pro hMPV+ Assay demonstrated 100% positive percent agreement (95%CI=91.80%-100%) and 98.6% negative percent agreement (95%CI=94.91%-99.61%) as compared to the Current Pro hMPV+ Assay.
The Positive Control concentration will be increased by 0.75 log.	The Positive Control may not detect global failure of the assay in the FAM channel.	False Negative Result due to undetected malfunction of the assay.	Improper patient management.	The Positive Control concentration was designed to be detected at ~75% of the way through the SmartCycler protocol (Ct range =26-30). At this point in the assay, it should detect global failure if the assay is not performing as intended.	The Positive Control for New Pro hMPV+ should be detected between 13 and 35 cycles.	The Positive Control for New Pro hMPV+ was run with the Analytical Sensitivity and Clinical studies. A Positive Control Effectiveness Study was also performed.	The Positive Control for the New Pro hMPV+ Assay met all acceptance criteria and resulted in zero run failures in both Analytical and Clinical Studies. The Effectiveness Study concluded that the new Positive Control is effective at detecting global errors in the FAM channel.
The Positive Control will be run at the supplied concentration instead of in diluted form.	Customers may forget to run the Positive Control undiluted.	The Positive Control could fail invalidating the results.	Unnecessary re-testing and longer turnaround time to result.	The New Pro hMPV+ Package Insert will detail how to handle the Positive Control specifying that the Positive Control should not be diluted prior to assay set-up.	The Positive Control for New Pro hMPV+ should be detected between 13 and 35 cycles.	The Positive Control for New Pro hMPV+ was run with the Analytical Sensitivity and Clinical Performance studies.	The Positive Control for the New Pro hMPV+ Assay met all acceptance criteria and resulted in zero run failures in both Analytical and Clinical Studies.
The Positive Control will be programmed into the software as	Software errors in the Positive Control will	Customers may need to interpret the results of the Positive Control	Improper interpretation of results.	Programming the Positive Control into the SmartCycler software is designed to	The Positive Control for New Pro hMPV+ should be detected	The Positive Control for New Pro hMPV+ was run	The Positive Control for the New Pro hMPV+ Assay met all acceptance criteria and resulted in

a defined control.	invalidate the run.	themselves.		reduce the amount of interpretation performed by the customer. Inclusion of the internal quencher on the hMPV probe will reduce, if not eliminate, Smart Cyclor errors due to high fluorescence.	between 13 and 35 cycles.	with the Analytical Sensitivity and Clinical Performance studies.	zero run failures in both Analytical and Clinical Studies.
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b) Analytical Reactivity Testing was conducted as described in section 3, Device Modifications.

c) Declaration of Conformity

A "Declaration of Conformity" statement was submitted for the manufacturing facility and validation activities and signed by the Director of Quality Assurance and the Senior Director of Technical Operations respectively. The statements indicate that;

- i) The manufacturing facility is in conformance with design control procedure requirements as specified in 21 CFR 820.30 and the records are available for review.
- ii) The validation activities, as required by the risk analysis, for the modification were performed by the designated individuals and the results demonstrated that the predetermined acceptance criteria were met.

In conclusion, based on both the results of the analytical reactivity testing and the risk management report, the modified labeling is truthful and accurate. The changes do not affect the performance of the test and it is therefore substantially equivalent to the current cleared test.

7) A Truthful and Accurate Statement, a 510(k) Summary or Statement and the Indications for Use Enclosure.

The labeling for this modified subject device has been reviewed to verify that the indication/intended use for the device is unaffected by the modification. In addition, the submitter's description of the particular modification(s) and the comparative information between the modified and unmodified devices demonstrate that the fundamental scientific technology has not changed. The submitter has provided the design control information as specified in The New 510(k) Paradigm and on this basis, I recommend the device be determined substantially equivalent to the previously cleared device.