

SPECIAL 510(k): Device Modification Review Memorandum

To: Hologic, Inc. (Gen-Probe Prodesse, Inc.)

RE: K132129

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

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

Prodesse ProFlu™+ Assay
510(k) number: K110968

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 and package labeling.
3. A description of the device **MODIFICATION(S)** to demonstrate that the **FUNDAMENTAL SCIENTIFIC TECHNOLOGY** of the modified device **has not changed**.

The 510k submission contained modifications to the Internal Control and Positive Controls as well as expanded Reactivity table to include two additional strains of Influenza A virus, Influenza A/Indiana/10/2011 and Influenza A/Anhui/1/2013. The modifications are summarized as follows:

- a. Outsourcing of the manufacturing of the Internal Control and subsequent minor changes to vector sequence;

The current Internal Control (RIC) in ProFlu+ Assay contains a RNA *in vitro* transcript (IVT). The new Universal Internal Control (UIC-A) will contain a RNA *in vitro* transcript (IVT) and a DNA plasmid to allow users to perform one nucleic acid extraction and test with any combination of the Pro+ Series Assays including ProFlu+, ProhMPV+, ProParaflu+, ProFAST+, and ProAdeno+. Due to the different vector being used in the Universal Internal Control (UIC-A), a minor change was made to the 5' and 3' ends of the UIC-A sequence.

The concentration of the RNA IVT in the Universal Internal Control (UIC-A) is the same as in the current Internal RNA Control (RIC). Handling of Universal Internal Control is identical to that of the current Internal RNA Control (RIC) included in the ProFlu+ Assay.

- b. Outsourcing of the manufacturing of the Positive Control leading to minor changes in the vector sequence, changes to control format and concentration;
The current Positive Controls consist of 4 individual controls for Influenza A (HCT75), Influenza B (HCT76), RSV A (HCT77) and RSV B (JCT78), respectively. The new Positive Controls contain a pooled positive control for Influenza A, Influenza B and RSV A, and a RSV B Control.
 - Due to the change in vector, a minor change was made to the 5' and 3' ends of the Control sequences.
 - The handling of the Positive Controls for the ProFlu+ Assay will be changed to eliminate the customer dilution that occurs immediately prior to RT-PCR setup, effectively raising the testing concentration one log.

c. Revised reactivity table to include two additional strains of Influenza A, Influenza A/Indiana/10/2011 (H3N2v) and Influenza A/Anhui/1/2013 (H7N9);
The Influenza A/H3N2v can be detected at 10² TCID₅₀/mL and Influenza A/H7N9 RNA can be detected at 0.02 pg/μL.

d. Change in Stability Claims.

The stability study demonstrated that the intermediate stock of the Universal Internal Control can stand up to 2 freeze-thaw cycles, the performance of ProFlu+ Supermix can stand up to 5 freeze-thaw cycles (same as the current stability claim), and M-MLV Reverse Transcriptase and RNase Inhibitor II can stand up to 10 freeze-thaw cycles (increased from the current 5 freeze-thaw cycles)

4. Comparison Information (similarities and differences) to applicant's legally marketed predicate device including, labeling, intended use, and physical characteristics.

Similarities		
Element	Modified Prodesse ProFlu+ Assay	Current Prodesse ProFlu+ Assay (K110968)
Organisms Detected	Same	Influenza A virus, Influenza B virus, Respiratory Syncytial Virus
Analyte	Same	RNA
Technological Principles	Same	Multiplex nucleic acid amplification
Specimen Types	Same	Nasopharyngeal Swab
User Complexity	Same	High
Sample Preparation Method	Same	Up front sample processing is required to extract nucleic acid.
Instrumentation	Same	bioMérieux NucliSENS easyMAG or Roche MagNA Pure and Cepheid SmartCycler II Instrument
Time to result	Same	Approximately 4 hours
Controls	Same	Internal control in each sample. External control processed with each batch of samples. (see below for differences)

Differences			
Element		Modified ProFlu+ Assay	Current Prodesse ProFlu+ Assay
Controls	Internal	<ul style="list-style-type: none"> Universal Internal Control - Contains DNA plasmid in addition to RNA IVT Control Stocks outsourced - Change in manufacturer leading to change in control vectors and minor sequence change at the 5' and 3' ends of RNA IVT 	<ul style="list-style-type: none"> Internal RNA Control - Contains RNA IVT Control stocks manufactured in house

	Positive	<ul style="list-style-type: none"> • Pooled Influenza A/ Influenza B/RSV A RNA Control and RSV B RNA Control • Control stocks outsourced. <ul style="list-style-type: none"> - Change in manufacturer leading to change in control vectors and minor sequence changes at the 5' and 3' ends of RNA IVTs • PC does not require dilution; <ul style="list-style-type: none"> - PC is provided as "at use concentration" 	<ul style="list-style-type: none"> • Four individual positive controls (Influenza A RNA Control, Influenza B RNA Control, RSV A RNA Control and RSV B RNA Control) • Control stocks manufactured in house • End user must dilute PC 1:10 prior to use for RT-PCR
Reactivity	Influenza A/ Indiana/10/2011 (H3N2v) Influenza A/ Anhui/1/2013 (H7N9)*	<ul style="list-style-type: none"> • 10^2 TCID₅₀/mL • 0.02 pg/μL 	<ul style="list-style-type: none"> • none
Stability (Freeze-thaw Cycle)	M-MLV Reverse Transcriptase RNase Inhibitor II	<ul style="list-style-type: none"> • 10 cycles • 10 cycles 	<ul style="list-style-type: none"> • 5 cycles • 5 cycles

*Although this test has been shown to detect A/Anhui/1/2013 H7N9 virus RNA and influenza A/ Indiana/10/2011 H3N2v cultured from positive human respiratory specimens, the performance characteristics of this device with clinical specimens that are positive for H7N9 or H3N2v influenza viruses have not been established. The Prodesse ProFlu+™ Assay can distinguish between influenza A and B viruses, but it cannot differentiate influenza A subtypes.

5. A Design Control Activities Summary:

- a. To demonstrate that the modifications in Controls do not change the assay performance, Analytical Studies and a Comparison Study were conducted.
 - Analytical Performances:
 - Analytical Sensitivity Confirmation
LoD, which was established in K110968 in 2011, was confirmed for Influenza A, Influenza B, RSV A and RSV B using one strain of each virus when tested with the UIC-A and modified Positive Controls side by side with the current RIC and Positive Controls. The confirmed LoDs are as follows:
Influenza A 1×10^2 TCID₅₀/mL
Influenza B 1×10^1 TCID₅₀/mL
RSV A 1×10^1 TCID₅₀/mL
RSV B 1×10^2 TCID₅₀/mL
 - IC Interference Study
The IC Interference Study demonstrated that the new control, UIC-A, did not inhibit the detection of target organisms at levels close to LoD.
 - Sample Stability Study
The study demonstrated that the stability of the samples would not be affected by a change in the internal control.
 - Extractor Equivalency Studies

The equivalency of nucleic acid extraction methods between the bioMérieux NucliSENS easyMAG automated extractor and Roche MagNA Pure LC extractor were evaluated by spiking the cultured and titered strain of Influenza A into a negative nasopharyngeal swab (NPS) matrix pool at the confirmed LoD concentration. The study demonstrated the equivalency between the two extraction methods.

- Comparison Study:

The comparison study was conducted for all Pro+ Series Assays including ProFlu+, ProHMPV+, ProParaflu+, ProFAST+, and ProAdeno+ testing 366 positive samples and 66 negative samples. Among the 366 positive samples, 330 were retrospective pre-selected archived NPS specimens with 30 positive samples per target (11 targets total) and 36 were contrived samples, generated by spiking individual negative retrospective NPS samples with whole organism (Influenza A/Seasonal H1 or Parainfluenza 2). Each sample was split into 3 aliquots; one aliquot was tested using the current Internal RNA Control (RIC), one aliquot was tested using the Universal Internal Control (UIC-A), and one aliquot was tested using the current Universal Internal Control (UIA-P) for ProAdeno+ Assay. All samples were then split into 72 panels with 6 samples per panel, extracted and tested by four different operators. Half of the panel samples were extracted using the bioMérieux NucliSENS easyMAG method and the other half using the Roche MagNA Pure LC method. Of the 432 samples utilized in the study, 21 samples were removed from analysis due to the invalid controls or incomplete test results. The results for ProFlu+ Assay are summarized in the following tables:

ProFlu+ Assay Influenza A Results					
		Samples with RIC		Total	
		Positive	Negative	Total	Comments
Samples with UIC-A	Positive	116	1*	117	Percent Positive Agreement 99.2% (95.3% - 99.9%) 95% CI
	Negative	1**	293	294	Percent Negative Agreement 99.7% (98.1% - 99.9%) 95% CI
Total		117	294	411	

*Contrived sample, negative NPS spiked with Influenza A/Seasonal H1

**Sample Influenza B positive with original source laboratory method (culture)

ProFlu+ Assay Influenza B Results					
		Samples with RIC		Total	
		Positive	Negative	Total	Comments
Samples with UIC-A	Positive	26	1*	27	Percent Positive Agreement 100% (87.1% - 100.0%) 95% CI
	Negative	0	384	384	Percent Negative Agreement 99.7% (98.5% - 100.0%) 95% CI
Total		26	385	411	

*Contrived sample, negative NPS spiked with Parainfluenza 2

ProFlu+ Assay RSV Results					
		Samples with RIC		Total	
		Positive	Negative	Total	Comments
Samples with UIC-A	Positive	71	0	71	Percent Positive Agreement 97.3% (90.6% - 99.3%) 95% CI
	Negative	2*	338	340	Percent Negative Agreement 100% (98.9% - 100.0%) 95% CI
Total		73	338	411	

*One contrived sample (negative NPS spiked with Influenza A/Seasonal H1) and one sample Parainfluenza 2 positive with original source method (Luminex RVP).

The results of the analytical studies and the clinical study confirmed the original performance claims of the ProFlu+ Assay and demonstrated that assay performance was not affected by the incorporation of the modified Universal Internal Control (UIC-A) and Positive Controls. The ProFlu+ Assay package insert has been updated to reflect the changes in the controls.

- b. To assess the reactivity of the ProFlu+ Assay with influenza A(H3N2v) virus and influenza A(H7N9) virus, a cultured and tittered strain of H3N2v and purified genomic RNA isolated from a strain of H7N9 were diluted in series to near the assay cutoff. RNA isolated from Flu A/H7N9 instead of a cultured and tittered Flu A/H7N9 virus was used in the reactivity study due to the requirement of biosafety Level 3 unavailable at Gen-Probe Prodesse. The study results showed that the ProFLU+ Assay can detect Influenza A/H3N2v at 10^2 TCID₅₀/mL and Influenza A/H7N9 RNA at 0.02 pg/ μ L.

Although this test has been shown to detect A/Anhui/1/2013 H7N9 RNA and influenza A/Indiana/10/2011 (H3N2v) virus cultured from positive human respiratory specimens, the performance characteristics of this device with clinical specimens that are positive for H7N9 or H3N2v influenza viruses have not been established. The Prodesse ProFlu™+ Assay can distinguish between influenza A and B viruses, but it cannot differentiate influenza A subtypes.

The ProFlu+ Assay package insert has been updated to include the revised reactivity table.

- c. To assess the stability of the Universal Internal Control (UIA) and new Positive Controls, an accelerated Stability study for the Controls stored at -70°C and a freeze-thaw stability study were conducted with one lot of each assay component. The studies demonstrated that the UIC-A and modified Positive Controls can be stored at $\leq -70^{\circ}\text{C}$ for 20 months with up to 2 freeze thaw cycles. Influenza A/Influenza B/RSV Reagent Mix can be frozen and thawed for up to 5 times, and M-MLV Reverse Transcriptase and RNase Inhibitor II up to 10 times, an increase from the current 5 freeze-thaw cycles. The ProFlu+ Assay package insert has been updated to reflect the current stability claims.
- d. A declaration of conformity with design controls was submitted for the manufacturing facility which includes:
- i) A statement signed by the Senior Director of R & D, Gen-Probe Prodesse, Inc., was submitted confirming that, as required by the risk analysis, all verification and validation

activities were performed by the designated individual(s) and the results demonstrated that the predetermined acceptance criteria were met, and

- ii) A “Declaration of Conformity” statement signed by the Associate Director of Quality and Regulatory, Gen-Probe Prodesse, Inc., was submitted stating that 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.

6. 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 (or their preamendment) device.