510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K182513

B. Purpose for Submission:

New device 510(k) clearance for the FluChip-8G Influenza A+B Assay

C. Measurand:

Influenza A and influenza B viral nucleic acids.

D. Type of Test:

Qualitative multiplex one-step RT-PCR followed by downstream microarray-based hybridization, imaging, and subsequent influenza virus detection and characterization using a pattern recognition-based algorithm

E. Applicant:

InDevR, Inc.

F. Proprietary and Established Names:

FluChip-8G Influenza A+B Assay (FC8G 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(s):

   OZE - Influenza A and influenza B multiplex nucleic acid assay (Primary)
   NSU - Instrumentation for clinical multiplex test systems (Subsequent)
   OEP - Influenza A virus subtype differentiation nucleic acid assay (Subsequent)
   OQW - 2009 H1N1 influenza virus (swine origin), nucleic acid or antigen, detection and

identification (Subsequent)

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The FluChip-8G Influenza A+B Assay is a multiplex RT-PCR *in vitro* diagnostic test intended for the qualitative detection and differentiation of seasonal influenza A/H3N2, seasonal influenza A/H1N1pdm09, and "non-seasonal" influenza A subtypes other than seasonal H1N1pdm09 or H3N2. The assay is also intended for the qualitative detection and differentiation of the genetic lineage of human influenza B viruses as B/Victoria or B/Yamagata. The assay is designed for use on influenza nucleic acids isolated and purified from nasopharyngeal swab and nasal swab specimens from human patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors.

   This assay amplifies the hemagglutinin (HA) gene segment, neuraminidase (NA) gene segment, matrix (M) gene segment, non-structural (NS) gene segment, and nucleoprotein (NP) gene segment for detection and discrimination of influenza A, and amplifies the hemagglutinin (HA) gene segment and neuraminidase (NA) gene segment for detection and discrimination of influenza B. This assay is not intended to detect influenza C viruses.

   FluChip-8G Influenza A+B Assay "non-seasonal" influenza A positive results are for the presumptive detection of influenza A subtypes other than seasonal influenza A/H1N1pdm09 or A/H3N2. The definitive identification of a "non-seasonal" influenza A case requires additional testing and confirmation procedures in consultation with the appropriate public health authorities (e.g., local or state public health departments, etc.) for whom reporting is necessary.

   Negative results do not preclude influenza virus infection. FluChip-8G Influenza A+B Assay "non-seasonal" influenza A negative results, even in the context of a FluChip-8G Influenza A+B Assay positive result for seasonal influenza A/H1N1pdm09 or A/H3N2, or influenza B, do not preclude "non-seasonal" influenza A infection and should not be used as the sole basis for patient management decisions.

   Performance characteristics of the FluChip-8G Influenza A+B Assay for detecting and differentiating seasonal influenza A viruses were established when seasonal influenza A/H3N2 was the predominant influenza A virus circulating in the United States. Performance characteristics may vary with other emerging seasonal influenza A viruses. Performance characteristics of the FluChip-8G Influenza A+B Assay for detecting and differentiating human influenza B genetic lineages were established when influenza

B/Victoria was the predominant influenza B virus circulating in the United States.

Due to low prevalence of "non-seasonal" influenza A viruses, performance characteristics of the FluChip-8G Influenza A+B Assay for detecting "non-seasonal" influenza A viruses and distinguishing "non-seasonal" influenza A from seasonal influenza A H1N1pdm09 and H3N2 were assessed exclusively by conducting cross-validation on a total of 759 microarray images generated from bench testing contrived samples consisting of 352 unique "non-seasonal" influenza A strains representing 62 subtypes, and by bench testing contrived samples and surrogate clinical specimens consisting of 133 unique non-seasonal influenza A strains representing 46 subtypes. FluChip-8G Influenza A+B Assay performance may vary when testing "non-seasonal" influenza A strains not represented in the performance assessment.

If infection with a novel influenza A virus strain 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 local or state health department(s) for testing. Viral culture should not be attempted unless a BSL 3E facility is available to receive and culture specimens.

2. Indication(s) for use:

   Same as Intended Use(s)

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   - BioRad T100 endpoint thermal cycler
   - FluChip-8G Imaging System with FluChip-8G Software v1.0.9.0 or newer

I. Device Description:

Overview

The FluChip-8G Influenza A+B Assay system is a molecular assay system for the detection and differentiation of influenza viruses in which a multiplexed one-step RT-PCR amplification is coupled with downstream microarray-based hybridization, imaging, and subsequent influenza virus detection and characterization using a pattern recognition-based algorithm.

The system consists of the following:

1) A reagent kit comprising the reagents required to conduct RT-PCR and for performing

post-PCR sample processing including PCR product fragmentation, microarray hybridization, washing, fluorescent labeling, and drying.

2) An accessory kit comprising a variety of laboratory equipment and supplies for facilitating execution of the assay.
3) Other general laboratory equipment and supplies required but not provided.
4) The FluChip-8G Imaging System with pre-installed FluChip-8G Software including an image processing module, an underlying neural network-based pattern recognition algorithm for identifying patterns in microarray signals representative of certain influenza target virus groups, and a user interface to facilitate data entry/imaging and provide result reports to the end user.

Materials Provided

FluChip-8G Influenza A+B Assay Reagent Kit (FC-6101)

Reagents	Description	Units Per Kit	Assays Per Unit	Volume Per Unit	Cat. #
FC8G Amplification Reagents:					**FC-6101A**
FC8G Polymerase Mix	Mixture of components necessary for PCR amplification	6 vials	16	500 µL	FC-5015
FC8G RT Enzyme	Enzyme necessary to perform reverse transcription step	6 vials	16	35 µL	FC-5014
FC8G Primer Mix	Mixture of oligonucleotide primers containing sodium azide (0.02%) as a preservative	6 vials	16	40 µL	FC-5004
FC8G Biotin dUTP	Lyophilized biotinylated dUTP	6 vials	16	50 nmol	FC-5008
FC8G Microarray Processing Reagents:					**FC-6101B**
FluChip-8G Microarray Slide	Microarray slide with 16-well silicone chamber. Each well contains a single oligonucleotide microarray	6 slides	16	N/A	FC-5006
Wash Buffer 1 (20x)	Concentrated wash buffer containing sodium azide (0.05%) as a preservative	2 bottles	48	50 mL	MI-5014
Wash Buffer 2 (20x)	Concentrated wash buffer containing sodium azide (0.05%) as a preservative	2 bottles	48	50 mL	MI-5015
FC8G Binding Buffer	Concentrated binding buffer containing sodium azide (0.02%) as a preservative	6 vials	16	1850 µL	FC-5001
FC8G Hyb Mix	Protein-based hybridization solution containing hybridization positive control oligonucleotides	6 vials	16	192 µL	FC-5013

FC8G Label	Lyophilized fluorophore- streptavidin label containing sodium azide (1.5 ng) as a preservative	6 vials	16	15 µg	FC-5002
FC8G Diluent	Diluent containing sodium azide (0.025%) as a preservative	6 vials	16	1500 µL	FC-5012
Slide Drying Sheets	Absorbent sheets to remove wash buffer from the FluChip- 8G Microarray Slide	20 sheets	N/A	N/A	FC-4005
Desiccant Pouches	Desiccant provided to aid in slide drying steps	18 pouches	N/A	N/A	FC-4013

Materials Required but Not Provided

Plasticware and Consumables

Material	**Cat. #**
FluChip-8G Influenza A+B Assay Accessory Kit	**FC-4000**
Humidity Chamber (available in the FluChip-8G Influenza A+B Accessory Kit FC-4000)	MI-4001
Four (4) Wash Bins (available in the FluChip-8G Influenza A+B Accessory Kit FC-4000)	FC-4001
Two (2) Rinse Bottles (available in the FluChip-8G Influenza A+B Accessory Kit FC-4000)	MI-4004
Plastic Forceps (available in the FluChip-8G Influenza A+B Accessory Kit FC-4000)	FC-4002
Slide Drying Box (available in the FluChip-8G Influenza A+B Accessory Kit FC-4000)	FC-4006
Sterile flocked swabs (Copan FLOQSwabs available through VWR, Fisher Healthcare, and Cardinal Healthcare)	--
Universal Transport Media (UTM) consisting of: Hanks' Balanced Salts, Bovine Serum Albumin, L-Cysteine, Gelatin, Sucrose, L-Glutamic Acid, HEPES Buffer, Vancomycin, Amphotericin B, Colistin, and Phenol Red at pH 7.3 ± 0.2 @ 25 °C	--
Bio-Rad 0.2 mL 8-tube PCR strips	TBS0201 (Bio-Rad)
Bio-Rad optical flat 8-cap strips	TCS0803 (Bio-Rad)
Qiagen Collection Tubes	19201 (QIAGEN)
Ice/Ice Bucket or Cold Block	--
Sterile RNase/DNase-free filter micropipettor tips (5 – 1000 µL)	--
Standard micropipettor tips (5 – 1000 µL)	--
Nuclease-free Polypropylene Tubes (1.5 mL)	--
Lint free tissue wipes	--
Clean 1 Liter bottles (2) with lids	--

Reagents and Controls

Material	**Cat. #**
QIAamp DSP Virus Spin Kit	61704 (QIAGEN)
Purified (18 MΩ) water for preparation of wash buffers	--
Nuclease-free/PCR grade water to be used for no-template negative control(s)	--
Ethanol (96 – 100% purity)	--
External positive control (previously characterized influenza positive clinical specimen)	--

External negative control (previously characterized influenza negative clinical specimen)	--

Instrumentation/Equipment

Material	Cat. #
FluChip-8G Imaging System (FluChip-8G Software Package pre-installed)	FC-6000
T100 Thermal Cycler	1861096 (Bio-Rad)
Standard pipettes (5 – 1000 µL)	--
Orbital Shaker (at least 8.5" x 7" stage) - capable of no tilt horizontal movement at 80- 140 RPM	--
Vortex Mixer	--
Centrifuge with rotor for 1.5 and 2 mL tubes capable of achieving 20,000 g (14,000 rpm)	--
Microcentrifuge with rotor adaptor for 0.2 mL 8-tube strips	--
Heating block for lysing samples at 56°C in 1.5 and 2 ml tubes	--
Timer, capable of timing 1 minute intervals over the range from 1 to 30 min	--

Optional Materials Not Provided

Material	Cat. #
Barcode Reader	9029247015 (Zebra)
Slide Storage Box	FC-4024

Quality Control

Assay Controls

- **Internal Control**: Sequences corresponding to portions of the 18s rRNA gene are included on each microarray to monitor adequate specimen collection, and in the absence of influenza, also to indicate the absence of reaction inhibition. Primers to amplify the 18s internal control are included in the Primer Mix so that the sequence complement to these microarray markers is amplified during RT-PCR and subsequently hybridized to the microarray if sufficient human material is present in the collected specimen. This control is expected to fail in samples containing no human cellular material. This control is evaluated in the absence of an influenza positive result and is expected to result in "No Call: Assay Failure (Internal Control)" in samples containing no human cellular material or poorly collected specimens.
- **Hybridization Control:** Oligonucleotides are included on each microarray to monitor the integrity of the hybridization procedure. The complementary sequences are fluorescently tagged single-stranded synthetic DNA sequences included in the Hyb Mix solution that bind specifically to the hybridization control sequences on the microarray. When the hybridization process fails, the FC8G assay result will be "No Call: Assay Failure (Hybridization Control)".
- **Labeling Control:** Each microarray includes a biotin-terminated capture oligonucleotide that bind to the fluorophore-coupled streptavidin solution present in the label applied during the labeling step. This control serves as an indicator of successful microarray labeling. When the labeling process fails, the FC8G assay result will be "No Call: Assay Failure (Labeling Control)".

- **Image Processing Control:** Direct fluorescently tagged sequences are included on each microarray at specific locations. The image processing controls serve as points of reference for microarray placement used by the FluChip-8G software during microarray image analysis. When the FluChip-8G Microarray Slide is improperly positioned in the FluChip-8G Imaging system, the FC8G assay result will be "Error: Image processing failure". A failure of this control may indicate an issue with the microarray quality, placement of the FluChip-8G Microarray Slide in the FluChip-8G Imaging System, or a focus issue of the FluChip-8G Imaging System.

External Quality Control (QC)

- **External Batch Positive Control:** A previously characterized influenza-positive clinical specimen is recommended for inclusion as part of good laboratory practices. This control is user-supplied.
- **External Batch Negative Control:** A previously characterized influenza-negative clinical specimen is recommended for inclusion as part of good laboratory practices. This control is user supplied.
- **Extraction Negative Control:** This control is recommended for inclusion in each testing batch, and is composed of nuclease-free water added by the end user in place of specimen during the nucleic acid extraction step.
- **RT-PCR No Template Control:** This control is recommended for inclusion in each testing batch, and is composed of nuclease-free water added by the end user in place of extracted nucleic acid template during RT-PCR reaction setup.

Workflow

The FluChip-8G Influenza A+B Assay is comprised of the following workflow:

1) Nucleic acid extraction

   First, nucleic acids are extracted from human nasal swab or nasopharyngeal swab specimen(s) stabilized in Universal Transport Media (UTM) employing the QIAamp DSP Virus Spin Kit (catalog #61704). During the nucleic acid extraction step, both DNA and RNA are co-eluted from starting material, and any influenza viral RNA as well as the endogenous 18s rRNA internal control are made accessible for downstream amplification. Extracted nucleic acid is then eluted and utilized as template for downstream amplification.

2) Multiplexed reverse transcription polymerase chain reaction (RT-PCR) amplification followed by heat fragmentation

   The RT-PCR amplification primers designed for the FluChip-8G Influenza A+B Assay (provided as FC8G Primer Mix) provide simultaneous (multiplexed) amplification of several full-length gene segments of the influenza A virus genome (HA, NA, M, NS, and NP), two full length gene segments of the influenza B virus genome (HA, NA), and a segment of the 18s rRNA found in eukaryotic cells as an internal process control. The

FC8G Primer Mix provides amplification of all targeted gene segments for all influenza A and B viruses, regardless of subtype or lineage, within a single reaction. A biotinylated dUTP is supplied, and is used during RT-PCR to incorporate biotin molecules into the amplified product. The FC8G Biotin dUTP supplements the standard dNTPs already present in the FC8G Polymerase Mix. These biotin moieties are utilized for downstream fluorescence labeling with a streptavidin-coupled fluorophore (FC8G Label) after the microarray hybridization step. Post-amplification, RT-PCR products are heat-fragmented for 10 min at 95°C to fragment the amplicons into shorter pieces for more efficient downstream hybridization to the FluChip-8G Microarray Slide.

3) Microarray processing (pre-wash slides, hybridization, post-hybridization slide wash, labeling, and post-label wash)

The FluChip-8G Microarray Slide consists of a 75 mm x 25 mm glass microscope slide onto which 16 replicate microarrays (labeled A1 through H2) are printed. The Microarray Slide is also labeled with a slide number at the top and a barcode at the bottom. Within each replicate microarray, 464 unique synthetic DNA oligonucleotides (capture oligonucleotide sequences) are printed that act as either influenza capture sequences (458, each printed in triplicate) or assay controls (six control sequences). A heat-fragmented post-amplification mixture is added to the microarray and incubated to allow for hybridization between the amplified nucleic acid and the microarray capture sequences. Once hybridization is complete, the microarray is washed to remove any unbound material. The microarray is then labeled with a streptavidin-coupled fluorophore to enable downstream fluorescence imaging.

4) Microarray imaging and data analysis

FluChip-8G Imaging System consists of: a) an optical imaging subsystem used to obtain fluorescence images of FluChip-8G Microarray Slides processed via the FC8G Influenza A+B Assay, b) instrument control software and application programming interface (API), and c) FC8G Software that automates image acquisition, controls the settings and instrument features available to end users through the user interface (UI), and interprets the FluChip-8G Microarray Slide data using a neural network module to provide results to the end users. Image processing algorithms within the FC8G Software locate the microarray pattern within the image and determine the fluorescence intensity of individual oligonucleotide captures on the microarray. Specific captures on the microarray that are intended as controls verify that specific assay processing steps were successfully completed. Trained neural networks are used to recognize the patterns of intensities of the influenza-targeted captures on the microarray as belonging to specific influenza types or subtypes/lineages. The neural network module utilizes seven individual neural networks, with the microarray signals from the 458 influenza capture sequences utilized as inputs. The output of each neural network is a value between 0 and 1, and a cutoff (optimized subsequent to neural network training) is applied to each to determine if each is positive or negative for its trained target. A deterministic decision tree then uses the binary outputs of the controls and neural networks to generate a diagnostic output for each clinical sample. The diagnostic output is then displayed to the

end user in un-editable format for printing and/or saving.

Neural Networks Training and Verification

Neural networks training is an interactive process and includes the following elements:

a) Read in the library of results from well-characterized known samples corresponding to all target virus groups for training.
b) Determine which result files contain sufficient signal to be used for training. Training set inclusion is based on key capture sequences identified for different target virus groups.
c) Use the Fast Artificial Neural Network (FANN) open source library functions to train individual networks. Each individual neural network uses a single hidden layer neuron and a single output neuron trained via supervised learning using backpropagation.
d) Determine thresholds that optimize network performance in terms of positive percent agreement (PPA) and negative percent agreement (NPA) with the known results of a clinically-representative set of naïve samples.
e) Save network files with threshold values for verification and inclusion in FC8G Software.

The database of assay results available for training the neural networks consisted of 3372 microarray images. Inclusion criteria were applied to ensure that the influenza-positive results had detectable signals on key captures of the microarray. This prevented result files with undetectable signal levels from being trained as positives. The final count of microarray images used for training by virus target group is presented in Table 1 below. This includes:

- 478 results from RT-PCR No Template Controls and Extraction Negative Controls.
- Results from 850 unique clinical specimens containing influenza, clinical specimens negative for influenza, and contrived influenza positive samples.
- Results from 1736 replicate analyses from the 850 unique sample strains noted above (primarily from dilution series testing).

Table 1: Neural Network Training Set Composition and Results Excluded from Training

Category	# Trained Images	# Excluded Images	Total Images
Influenza A, H1N1pdm09	446	60	506
Influenza A, Seasonal H3N2	378	30	408
Non-Seasonal Influenza A	**759**	**75**	**834**
Influenza B, Victoria lineage	287	112	399
Influenza B, Yamagata lineage	244	31	275
Clinical Negative	472	0	472
Negative Controls	478	0	478
Overall Totals	3064	308	3372

Regarding training the non-seasonal influenza A neural network, a total of 363 unique non-seasonal influenza A strains (representing 62 subtypes) were used to prepare contrived samples that upon testing generated a total of 834 microarray images. Of these 834 images,

759 microarray images were included in training, and 75 microarray images were excluded from training due to failure to meet the inclusion criteria. Table 2 below lists all 352 unique non-seasonal influenza A strains that were used to prepare contrived samples that upon testing generated the total of 759 microarray images that were included in the training, stratified by subtypes.

Table 2: Unique Non-Seasonal Influenza A Strains that Were Used to Prepare Contrived Samples to Generate the Microarray Images Included in Training the Non-Seasonal Influenza A Neural Network

Strain	Subtype	# of Unique Strain in Subtype	# of Replicate Analyses per Strain	# of Microarray Images per Subtype
A/Arizona/07/2007	H1N1 (pre 2009)	12	1	188
A/Virginia/01/2006			2	
A/Santiago/5248/2008			1	
A/Chelyabinsk/01/2006			1	
A/Denver/1/57			5	
A/Texas/35/2008, E3 (03/12/2009)			2	
A/Mississippi/3/2001 wt 275H			1	
A/Mississippi/3/2001 mut H275Y			1	
Clinical Specimen #1			5	
Clinical Specimen #2			3	
A/New Caledonia/20/1999			74	
A/Brisbane/59/2007			92	
A/Teal/Egypt/677/2004	H1N1 Avian	10	2	24
Unknown #1- from CDC 2014			2	
Unknown #2- from CDC 2014			2	
A/Mallard/Republic of Georgia/4/2010			3	
A/Duck/Alberta/35/76			2	
A/Mallard/Alberta/21/2014			3	
A/Redheaded Duck/MN/SG-00123/2007			2	
A/Gull/DE/428/2009			1	
A/Ruddy Turnstone/DE/274/2009			3	
A/Aqutic Bird/Hong Kong/D125/2002			4	
A/Swine/1976/1931	H1N1 Swine	3	2	5
A/Swine/TN/26/1977			2	
A/Swine/NC/18162/2002			1	
Swine Origin Influenza Virus (soiv)	H1N2	5	2	10
A/Teal/Egypt/431/2003			2	
Unknown #3- from CDC 2014			2	
A/Swine/Ohio/09SW1484E/2009			2	
A/Swine/Ohio/09SW1477/2009			2	
A/Shorebird/Delaware Bay/211/1994	H1N3	1	2	2
A/Red Knot/Delaware Bay/240/1994	H1N8	1	2	2
A/Japan/305/57	H2N2	2	1	3
Unknown #4- from CDC 2014			2	
A/Duck/Germany/1215/73	H2N3	1	2	2
Blue-winged Teal Texas	H2N9	2	2	4
A/Pintail/ALB/293/77			2	
A/Swine/North Carolina/88708/2000, CX/C1 (10/17/2011)	H3N2 Swine	14	2	23
A/Swine/North Carolina/32760/2007, CX/C1 (10/13/2011)			2	

A/Swine/North Carolina/44897/2009, C2/C1 (10/13/2011)			2	
A/Swine/North Carolina/52796/2006, CX/C1 (10/13/2011)			2	
A/Swine/Ohio/09SW73E/2009			2	
A/Swine/Ohio/09SW83E/2009			2	
A/Swine/Ohio/11SW87/2011			2	
A/Swine/Ohio/09SW79M/2009			2	
A/Swine/Texas/4199-2/1998			1	
A/Swine/NC/0668/2011			2	
A/Swine/NC/0666/2011			1	
4D-0114-P29 swine swab			1	
4D-0114-P21 swine swab			1	
4D-0114-P15 swine swab			1	
A/Ohio/20/2012	H3N2v	14	2	28
A/Indiana/16/2012			2	
A/Indiana/14/2012			2	
A/Indiana/65/2012			2	
A/Michigan/20/2012			2	
A/Pennsylvania/17/2012			2	
A/Wisconsin/28/2012			1	
A/Ohio/44/2012			2	
A/Ohio/47/2012			2	
A/Ohio/56/2012			1	
A/Ohio/83/2012			2	
A/Ohio/36/2012			2	
A/Indiana/8/2011			1	
A/Swine/MN/3908-2/11			5	
A/Blue-winged Teal/Texas/G77/2007	H3N6	3	2	6
A/Blue-Winged Teal/Illinois/10OS1546/2010			2	
A/Redhead/Alberta/192/2002			2	
A/Equine/TX/2004	H3N8	21	1	62
A/Equine/New York/452/2003			2	
A/Canine/New York/100525,1/06			2	
A/Canine/Florida/89911,2/06			2	
A/Canine/VA/93653/09			2	
A/Canine/New York/4732/2012			2	
A/Duck/Bangladesh/1293/2008			2	
A/Duck/Bangladesh/1575/2009			2	
Unknown #5- from CDC 2014			2	
Unknown #6- from CDC 2014			2	
A/Camel/Mongolia/335/2012			2	
A/Equine-2/Miami/1963			1	
A/Blue-Winged Teal/Iowa/10OS2411/2010			2	
A/Equine/Pennsylvania/1/2007			2	
A/Duck/Ukraine/63			4	
A/Duck/Chabarovsk/1610/72			2	
A/Equine/KY/4/2011			5	
A/Mallard/Alberta/435/2013			10	
A/Mallard/Alberta/307/2012			8	
A/Laughing Gull/DE/45/2005			5	
A/Songbird/Hong Kong/SB18/2001			2	
A/Mallard/Alberta/54/1993	H3N9	1	2	2
A/Duck/Bangladesh/1746/2010	H4N2	1	2	2
A/Blue-Winged Teal/Alberta/346/2007	H4N3	1	2	2

A/Duck/Bangladesh/1283/2008	H4N6	5	2	10
Unknown #7- from CDC 2014			2	
A/Blue-Winged Teal/Illinois/10OS1563/2010			2	
A/Red Knot/Delaware/541/1988			2	
A/Duck/Czech/56			2	
Unknown #8- from CDC 2014	H4N8	1	2	2
A/Vietnam/JP20-2/2005	H5N1	43	1	63
A/Chicken/Vietnam/NCVDCDC33/2005			2	
A/Chicken/Vietnam/NCVDCDC42/2005			1	
A/Chicken/Vietnam/NCVDCDC37/2005			2	
A/Chicken/Vietnam/NCVDCDC52/2005			2	
A/Chicken/Vietnam/NCVDCDC36/2005			1	
A/Environment/Vietnam/NCVDCDC53/2005			1	
A/Environment/Vietnam/NCVDCDC54/2005			2	
A/Anhui/2/2005			2	
A/Duck/Kulon Progo/Bb Vet/IX/2004			1	
A/Turkey/VA/505477-18/07			2	
A/Mallard/PA/454069-9/2006			9	
A/Ruddy Turnstone/DE/105/2007			3	
A/Duck/MN/1525/81			5	
A/Chicken/Egypt/A10543A/2015			1	
A/Chicken/Egypt/D10565A/2015			1	
A/Chicken/Bangladesh/18247/12			1	
A/Quail/Bangladesh/19250/13			1	
A/Hong Kong/156/97			1	
A/Vietnam/1203/2004			1	
A/Japanese White-Eye/Hong Kong/1038/2006			1	
A/Chicken/Hunan/2246/2006			1	
A/Chicken/Qalubia-Egypt/1/2006			1	
A/Turkey/Egypt/7/2007			1	
A/Egret/Egypt/1162-NAMRU3/2006			1	
A/Common Magpie/Hong Kong/5052/2007			1	
A/Common Buzzard/Bulgaria/38WB/2010			1	
A/Chicken/Egypt/Q1089E/2010			1	
A/Goose/Egypt/M2794A/2011			1	
A/Goose/Hong Kong/631/2009			1	
A/Large-Billed Crow/Hong Kong/497/2011			1	
A/Chicken/Egypt/S3806B/2011			1	
A/Crested Myna/Hong Kong/8381/2012			1	
A/Black Headed Gull/Hong Kong/84/2012			1	
A/Turkey/Egypt/S6405C/2012			1	
A/Chicken/Egypt/M7217B/2013			1	
A/Chicken/Egypt/B9040A/2013			1	
A/Chicken/Egypt/F9514A/2014			1	
A/Chicken/Bangladesh/23974/2014			1	
A/Goose/Bangladesh/25169/2015			1	
A/Chicken/Egypt/A10758D/2015			1	
A/Chicken/Egypt/Q10937B/2015			1	
A/Shorebird/DE/472/2007			1	
A/Duck/Bangladesh/1559/2009	H5N2	18	2	26
A/Mallard/MN/346250/00			2	
Unknown #9- from CDC 2014			1	
A/Mule Duck/Bulgaria/237/2011			3	

A/Ruddy Turnstone/DE/431/2011			2	
A/Mallard/AR/1C/2001			4	
A/Turkey/ND/11419-1/15			1	
A/Turkey/MN/11668-1/2015			1	
A/Turkey/MN/10777/15			1	
A/Turkey/MN/110915-1/15			1	
A/Northern Pintail/WA/40564/14			1	
A/Chicken/Italy/312/97			1	
A/Northern Pintail/Washington/40964/2014			1	
A/Snow Goose/CC15-84B/2015			1	
A/White-Faced Whistling Duck/Colombia/1/2011			1	
A/Shorebird/DE/318/2011			1	
A/Northern Pintail/CA/44242-758/2006			1	
A/Mallard/OH/14OS2758/2014			1	
Unknown #10- from CDC 2014	H5N3	3	2	4
A/Duck/Hokkaido/69/2000			1	
A/Ruddy Turnstone/DE/215/91			1	
A/Mallard/Italy/80/93	H5N4	2	1	2
A/Shorebird/DE/230/2000			1	
A/Mallard/Alberta/383/2009	H5N5	1	1	1
A/Peregrine Falcon/Hong Kong/4955/2015	H5N6	1	1	1
A/Shorebird/DE/75/2004	H5N7	1	1	1
A/Mule Duck/Bulgaria/328/2011	H5N8	7	1	13
A/Quail/CA/K1400794/2014			1	
A/Ruddy Turnstone/DE/237/91			1	
A/Shorebird/DE/192/98			4	
A/Laughing Gull/DE/156/2004			4	
A/Mallard/Korea/W452/14			1	
A/Gyrfalcon/WA/41088-6/14			1	
A/Chicken/Italy/9097/97	H5N9	2	1	2
A/Ruddy Turnstone/DE/117/2011			1	
Unknown #1- from CDC 2013	H6N1	3	2	6
Unknown #11- from CDC 2014			2	
A/Shorebird/Delaware Bay/230/2009			2	
Unknown #12- from CDC 2014	H6N2	2	1	3
A/Turkey/Massachusetts/3740/65			2	
A/Mallard/Alberta/58/1989	H6N4	1	2	2
Blue-winged Teal Texas	H6N5	3	2	6
A/Mallard/Alberta/203/1992			2	
A/Shearwater/Australia/1/73			2	
Unknown #13- from CDC 2014	H6N8	1	1	1
A/Shoveler/Egypt/597/2004	H7N1	8	2	9
Unknown #14- from CDC 2014			1	
Unknown #15- from CDC 2014			1	
A/Turkey/Italy/6423-1/99			1	
A/Parakeet/Netherlands/267497/94			1	
A/Ostrich/Zimbabwe/222/96			1	
A/Mallard/Alberta/34/2001			1	
A/Chicken/Italy/1285/2000			1	
A/Avian/New York/11678-4/2005	H7N2	9	1	19
A/Shorebird/DE/282/2011			3	
A/Chicken/NY/116124/2003			3	
A/Pheasant/NJ/30739-9/2000			3	

A/Ruddy Turnstone/DE/130/99			3	
A/Australian Shelduck/Western Australia/1756/83			3	
A/Mallard/Ramon/79/14T			1	
A/Lesser Noddy/Western Australia/2371/83			1	
A/Ruddy Turnstone/DE/282/2011			1	
A/Mallard/Netherlands/12/2000	H7N3	28	1	28
A/Sanderling/DE/280/2015			1	
A/Mallard/Alberta/27/2001			1	
A/Mallard/Alberta/243/2006			1	
A/Shorebird/DE/552/2006			1	
A/Dunlin/DE/281/2015			1	
A/Red Knot/DE/269/2015			1	
A/Ruddy Turnstone/DE/115/2011			1	
A/Mallard/Alberta/341/2012			1	
A/Ruddy Turnstone/DE/124/2007			1	
A/Ruddy Turnstone/DE/503/2011			1	
A/Ruddy Turnstone/DE/282/2006			1	
A/Ruddy Turnstone/DE/108/2007			1	
A/Shorebird/DE/53/2002			1	
A/Mallard/Alberta/167/2010			1	
A/Red Knot/DE/239/2015			1	
A/Turkey/CA/K1500529/2015			1	
A/Mallard/Alberta/747/2015			1	
A/Mallard/Alberta/174/2010			1	
A/Shorebird/DE/218/2015			1	
A/Laughing Gull/DE/22/2002			1	
A/Chicken/Chile/176822/2002			1	
A/Mallard/Netherlands/12/2000			1	
A/Mallard/OH/14OS823/2015			1	
A/Chicken/Chile/184240-4957/2002			1	
A/Canada/RV444/2004			1	
A/Chicken/Karachi(Pakistan)/NARC-100/2004			1	
A/Chicken/Saskatchewan/HR10/2007			1	
A/Duck/Nanchang/1944/93	H7N4	2	1	2
A/Ruddy Turnstone/DE/284/2006			1	
A/Ruddy Turnstone/DE/262/2006	H7N5	2	1	2
A/Mallard/OH/14OS0821/2015			1	
A/Chilean Teal/Chile/9/2013	H7N6	1	1	1
A/Teal/Egypt/835/2004	H7N7	10	2	23
Unknown #16- from CDC 2014			2	
A/Equine/Prague/56			2	
A/Ruddy Turnstone/NJ/AI11-1678/2011			3	
A/Ruddy Turnstone/DE/134/99			5	
A/Duck/Potsdam/S28716/88			2	
A/Red Knot/DE/259/94			3	
A/Seal/MA/1/80			2	
A/Turkey/Ireland/PV74/95			1	
A/Netherlands/219/2003			1	
A/Mallard/Alberta/579/2010	H7N8	2	1	2
A/Turkey/IN/1573-2/2016			1	
A/Anhui/1/2013	H7N9	9	1	22
A/Turkey/MN/037767/2009			3	
A/Mallard/Alberta/177/2004			3	

A/Mallard/Alberta/114/99			4	
A/Shorebird/DE/28/95			3	
A/Turkey/MN/1/88			2	
A/Hong Kong/5942/2013			2	
A/Taiwan/T1.4/2013			2	
A/Shanghai/1/2013			2	
A/Turkey/Ontario/6118/68	H8N4	1	2	2
A/Shorebird/DE/133/2002			1	
A/Laughing Gull/DE/5/2003	H9N1	3	1	3
A/Ruddy Turnstone/NJ/A107-283/2007			1	
A/Chicken/Hong Kong/g9/1997			1	
A/Hong Kong/1073/99			1	
A/Shorebird/Delaware Bay/127/2003			4	
A/Turkey/Wisconsin/1/66			1	
A/Pheasant/UAE/D1307.B/2011			4	
A/Quail/Lebanon/272/2010			4	
A/Laughing Gull/DE/12/2006			2	
A/Environment/Bangladesh/10306/11 (quail cage)			1	
A/Chicken/Bangladesh/10450/11			1	
A/Chicken/Beijing/1/94			1	
A/ Quail /Hong Kong/G1/97			1	
A/Duck/Hong Kong/Y280/97			1	
A/Sanderling/DE/449/2006			1	
A/Hong Kong/69955/2008			1	
A/Quail/Lebanon/273/2010			1	
A/Hong Kong/33982/2009			1	
A/Shorebird/DE/464/2011			1	
A/Chukar/Shantou/22116/2005			1	
A/Chicken/Hong Kong/NT10/2011			1	
A/Chicken/Hong Kong/CRA45/2010			1	
A/Chicken/Hong Kong/YU341/2008	H9N2	40	1	50
A/American Oystercatcher/Chile/C1307/2105			1	
A/Chicken/Egypt/S4454B/2011			1	
A/Chicken/Bangladesh/25947/2015			1	
A/Chicken/Egypt/S4456B/2011			1	
A/Chicken/Egypt/S5018B/2012			1	
A/Chicken/Egypt/D7663A/2013			1	
A/Duck/Egypt/C9787/2014			1	
A/Quail/Egypt/D9842/2014			1	
A/Chicken/Egypt/S10489C/2015			1	
A/Chicken/Egypt/F10533D/2015			1	
A/Chicken/Egypt/D10553A/2015			1	
A/Chicken/Egypt/D10561/2015			1	
A/Chicken/Egypt/A1093D/2015			1	
A/Chicken/Egypt/S10598E/2015			1	
A/Chicken/Egypt/D10705/2015			1	
A/Chicken/Egypt/D10975/2015			1	
A/Pheasant/Shantou/2785/2015			1	
A/Chukar/Shantou/2777/2015			1	
A/Pigeon/Shantou/3577/2015			1	
A/Mallard/Ireland/PV46B/93	H9N3	1	1	1
A/Shorebird/DE/231/2003	H9N4	1	1	1
A/Shorebird/DE/261/2003	H9N5	2	1	2

A/Mallard/Alberta/162/2007			1	
A/Ruddy Turnstone/DE/268/2011	H9N6	1	1	1
A/Shorebird/Delaware Bay/31/1996	H9N7	3	2	4
A/Shorebird/DE/277/2000			1	
A/Ruddy Turnstone/DE/253/2011			1	
A/Ruddy Turnstone/DE/116/98	H9N8	1	1	1
A/Pheasant/WA/373/49/85	H9N9	4	1	4
A/Ruddy Turnstone/Virginia/2297/1988			1	
A/Shorebird/DE/141/2002			1	
A/Shorebird/DE/554/2007			1	
A/Teal/Egypt/0457/2003	H10N1	4	2	7
Unknown #17- from CDC 2014			2	
A/Mallard/Wisconsin/4230/2009			2	
A/Shorebird/Delaware Bay/338/2009			1	
A/Shorebird/Delaware Bay/63/1996	H10N2	1	2	2
Unknown #1- from CDC 2012	H10N7	8	1	15
Green-winged Teal Texas			2	
A/Shoveler/Egypt/0600/2004			2	
A/Shoveler/Egypt/845/2004			2	
Unknown #18- from CDC 2014			2	
Unknown #19- from CDC 2014			2	
A/Mallard/Illinois/10OS4334/2010			2	
A/Chicken/Germany/N/49			2	
A/Quail/Italy/1117/65	H10N8	1	2	2
Unknown #20- from CDC 2014	H11N1	1	2	2
A/Shorebird/Delaware Bay/216/1999	H11N2	2	2	4
A/Laughing Gull/Delaware Bay/94/1995			2	
A/Duck/Bangladesh/1052/2007	H11N3	2	2	4
A/Ruddy Turnstone/Delaware Bay/39/1994			2	
A/Mallard/Alberta/125/1999	H11N6	2	2	4
A/Duck/England/56			2	
Unknown #21- from CDC 2014	H11N9	5	2	11
Unknown #22- from CDC 2014			2	
A/American Green-Winged Teal/Mississippi/300/2010			2	
A/Common Goldeneye/Iowa/3192/2009			2	
A/Duck/Memphis/546/74			3	
A/Mallard/Wisconsin/4218/2009	H12N5	5	2	10
A/Mallard/Ohio/1688/2009			2	
A/Northern Shoveler/Mississippi/09OS025/2009			2	
A/Northern Pintail/Missouri/319/2009			2	
A/Duck/Alberta/60/76			2	
A/Black-Legged Kittiwake/Quebec/02838-1/2009	H13N6	3	2	5
A/Ring-Billed Gull/Quebec/02434-1/2009			2	
A/Gull/Maryland/704/77			1	
Unknown #23- from CDC 2014	H14N5	2	2	4
A/Mallard/Astrakhan/263/82			2	
A/Shearwater/Australia/2576/79	H15N9	1	2	2
A/Shorebird/Delaware/172/2006	H16N3	1	2	2
Total	**62 Subtypes**	**352**	**759**	**759**

Note: H1N1 (pre 2009), H1N1 Avian, and H1N1 Swine are counted as one subtype; H3N2 Swine and H3N2v are counted as one subtype.

Performance of the neural network algorithm was verified utilizing the technique of k-fold cross-validation (k=10) that allows assessment of how the neural network algorithm will generalize to a completely independent dataset. This mathematical approach was accomplished by dividing the training set randomly into 10 groups. All groups but one are used to train the neural network algorithm, and the algorithm is tested on the remaining group. Then, the groups used for training are rotated until all combinations of nine training groups/one test group have been used. The performance of each test group was then compiled to estimate performance expected on an independent dataset. Therefore, while viruses in the training set were not technically naïve to the training, this approach allowed an estimation of performance as if each portion of the training set had been naïve prior to validation activities.

Postmarket Surveillance and Software Update Plan

InDevR developed a plan to monitor genetic changes in the circulating strains of influenza annually in order to assess the impact of those changes on the results generated by the FC8G assay. InDevR will test the CDC human influenza panel (includes seasonal influenza A and influenza B strains) and the CDC animal influenza panel (includes select, important non-seasonal strains with pandemic potential) annually, if appropriate and available. If the CDC human and animal influenza panels are not available or not appropriate for testing with the FC8G assay, e.g., virus samples inactivated with Betapropiolactone (BPL) are not compatible with the FC8G assay, etc. InDevR will conduct similar experiments on similar panels compiled from other sources (such as commercial sources and/or collaborators). Such a human influenza panel would consist of two strains of each target virus group, and include strains genetically similar to those included in the annual quadrivalent vaccine formulation for the four components in addition to two pre-2009 H1N1 strains. Composition of such an animal influenza panel will be based on recommendations from the CDC and other surveillance laboratories regarding selecting important non-seasonal strains with pandemic potential, and will be subject to availability limitations. In addition, if testing and reporting from CDC or other surveillance laboratory suggests that a newly emerging strain of influenza is on the rise in the US and it is not represented in the CDC panels, then InDevR will work with CDC and/or surveillance laboratories to test the newly emerging strain with the FC8G assay to determine its reactivity with the newly emerging strain.

If the annual reactivity testing indicates that the assay is not reactive to one or more of the strains tested, InDevR will further investigate by testing other similar strains to confirm that a systematic non-reactivity exists and that this failure to detect the virus was not due to an isolated assay failure or issue. If a change in reactivity is confirmed, the discovery will be reported to the FDA. An update to the training database and re-optimization of the neural network parameters would likely be necessary.

Once data have been generated that indicate an update to the training database is necessary, InDevR will obtain as many strains representing the non-reactive group as possible. If the virus(es) exhibiting low reactivity were initially emerging, it may be difficult to obtain a large number of strains. Even with a relatively low number of strains, it may be possible to

create a robust collection of images by analyzing strains in replicates, as well as analyzing each additional strain at a variety of concentrations by preparing a contrived panel spiked at a variety of concentrations into pooled clinical negative material. These samples will then be analyzed to generate FC8G assay images. Once FC8G images are obtained for these samples, InDevR will utilize the same criteria currently in place for determining suitability for inclusion in the training database. Once the training database update is complete, InDevR will follow the same general procedure used during the initial software development to optimize the neural network parameters and finalize the software update. In brief, InDevR will utilize the FC8G Data Tools software developed for neural network training to optimize the neural network parameters using a k-fold cross-validation approach. Once the neural network parameters have been optimized, InDevR will optimize the thresholds applied to each neural network and sub-net (assay cutoff) to ensure the proper balance of sensitivity and specificity. After the assay thresholds have been optimized, the FC8G Software code would be updated accordingly. Final validation of the updated software with the new neural network parameters and cutoffs will also require bench testing of training-naïve samples of the strain(s) in question.

Once validation of the newly updated software is completed, InDevR will submit a 510(k) to the FDA for the modified device.

Results Interpretation

Interpretation of External Quality Control (QC) Results

- **External Batch Positive Control** (user supplied influenza positive specimen):
  Interpret the External Batch Positive Control results (if included) to determine whether the expected result of influenza positive was obtained and if the batch of samples is valid. Failure to obtain the expected influenza positive result should invalidate the batch, and all samples included in that batch should be retested.

- **External Batch Negative Control** (user supplied influenza negative clinical specimen):
  Interpret the External Batch Negative Control results (if included) to determine whether the expected result of "Influenza Not Detected" was obtained and if the batch of samples is valid. Failure to obtain the expected "Influenza Not Detected" result should invalidate the batch, and all samples included in that batch should be retested.

- **Extraction Negative Control** (nuclease-free water used in place of specimen during extraction):
  Interpret the Extraction Negative Control results to determine whether the expected result of either "Influenza Not Detected" or "No Call: Assay Failure (Internal Control)" was obtained and if the batch of samples are valid. Failure to obtain either "Influenza Not Detected" or "No Call: Assay Failure (Internal Control)" result should invalidate the batch, and all samples included in that batch should be retested.

- **RT-PCR No Template Control** (nuclease-free water used in place of template during RT-PCR reaction setup):

Interpret the RT-PCR No Template Control results to determine whether the expected result of either “Influenza Not Detected” or “No Call: Assay Failure (Internal Control)” was obtained and if the batch of samples are valid. Failure to obtain either “Influenza Not Detected” or “No Call: Assay Failure (Internal Control)” result should invalidate the batch, and all samples included in that batch should be retested.

Interpretation of Assay Results

The table below lists the expected results for the FluChip-8G A+B Assay. The FluChip-8G Software automatically determines the sample result and qualified testing personnel should interpret the sample results as follows:

<table>
<tr><th>If Result is:</th><th>Explanation</th><th>Interpretation of Results</th></tr>
<tr><td>Influenza not Detected</td><td>Valid negative result.</td><td rowspan="6">Report Result</td></tr>
<tr><td>Influenza A Detected, Seasonal H1N1 (2009)</td><td rowspan="4">Valid positive result.</td></tr>
<tr><td>Influenza A Detected, Seasonal H3N2</td></tr>
<tr><td>Influenza B Detected, Victoria Lineage</td></tr>
<tr><td>Influenza B Detected, Yamagata Lineage</td></tr>
<tr><td>Influenza B Detected</td><td>Valid positive result.

Low virus titers can result in detection of influenza B without an associated lineage.</td></tr>
<tr><td>Influenza A Detected: Equivocal Result (no subtyping)</td><td>Low virus titers can result in detection of influenza A without an associated subtype.

Detection of influenza A without a subtype can also in rare cases indicate the presence of a novel strain.</td><td>Retest Specimen to Confirm Result
. If the retest provides a different result, test the sample a third time to ensure the accuracy of the result.
. In the rare instance that all 3 results conflict, the result should be considered inconclusive and a new specimen should be collected and tested if possible.
. If an A/equivocal result is confirmed through retesting described above, contact appropriate public health authorities for confirmatory testing.</td></tr>
<tr><td>Influenza A Detected, Non-Seasonal</td><td>This result indicates the presumptive presence of a novel influenza A virus other than the annually circulating A/H1N1pdm09 and A/H3N2 viruses.

The A/H1N1 virus circulating prior to the appearance of A/H1N1pdm09 is expected to be identified as “Influenza A Detected, Non-Seasonal”.</td><td>Retest Specimen to Confirm Result
. If the retest provides a different result, test the sample a third time to ensure the accuracy of the result.
. In the rare instance that all 3 results conflict, the result should be considered inconclusive and a new specimen should be collected and tested if possible.
. If an A/non-seasonal result is confirmed through retesting described above, contact the appropriate public health authorities for confirmatory testing.</td></tr>
</table>

Multiple Infections Detected (any combination of the individual results above)	This result indicates co-infection with multiple influenza virus types and/or subtypes. Multiple infections are possible but rare. This result may also occur in patients that have recently received an attenuated live intranasal influenza vaccine. If one of the targets detected is 'Influenza A Detected, Non-Seasonal', this result indicates the presumptive presence of a novel influenza A virus other than the annually circulating A/H1N1pdm09 and A/H3N2 viruses.	**Retest Specimen to Confirm Result** . If the retest provides a different result, test the sample a third time to ensure the accuracy of the result. . In the rare instance that all 3 results conflict, the result should be considered inconclusive and a new specimen should be collected and tested if possible. . If a confirmed multiple infection result includes an A/equivocal or A/non-seasonal result, contact appropriate public health authorities for confirmatory testing.
No Call: Assay Failure (Internal Control)	This result indicates the internal control failed to be detected. This result typically indicates poor specimen collection, specimen degradation, or user error in the specimen extraction or RT-PCR steps.	**Retest Specimen** If upon retest a valid result is generated, follow the recommended actions for that result.
No Call: Assay Failure (Hybridization Control)	This result is likely due to an issue in the hybridization step.	**Retest Specimen** If upon retest a valid result is generated, follow the recommended actions for that result.
No Call: Assay Failure (Labeling Control)	This result is likely due to an issue in the post- hybridization labeling step.	
Error: Image Processing Failure	This may indicate an error in microarray processing (See final wash step in assay procedure), in the placement of the FluChip-8G Microarray Slide within the FluChip-8G Imaging System, or the FluChip-8G Imaging System focus setting.	. Visually inspect the back of the slide for artifacts such as dust or salt precipitates. Using a damp lint-free cloth, wipe the back of the slide and **Rescan the Entire Slide**. . Check for correct slide and slide holder orientation, correct any orientation errors and **Rescan the Entire Slide**. . See FluChip-8G Imaging System Operation Manual for instructions on confirming system focus, **Rescan the Entire Slide**. If upon rescan a valid result is generated for the sample that produced the error, follow the recommended actions for that result. For samples that did not produce the error upon initial scan the result from the 1st scan should be used. If upon rescan a valid result is not generated, retest the specimen.
Not Analyzed	Microarray designated as "EMPTY" during sample type selection in FluChip-8G software. The microarray will not be analyzed and no result will be displayed	If the position was unintentionally left empty, rescan the entire slide using the correct sample ID selection. For samples that did not produce the result of "Not Analyzed" in the initial scan the result from the 1st scan should be used.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   CDC Human Influenza Virus Real-Time PCR Diagnostic Panel

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

   K172091

3. Comparison with predicate:

Similarities and Differences		
Item	**Device**	**Predicate**
	FluChip-8G Influenza A+B Assay (K182513)	**CDC Human Influenza Virus Real-Time PCR Diagnostic Panel (K172091)**
Measurand	Influenza RNA	Same
Viruses Detected	Influenza A and B viruses	Same
Influenza A subtype Differentiation	• Seasonal influenza A/H1N1pdm09 • Seasonal influenza A/H3N2 • "Non-seasonal" influenza A	• Same • Same • Influenza A/H5 (Asian Lineage)
Influenza B lineage Differentiation	• Influenza B/Victoria • Influenza B/Yamagata	Same
Assay Type	Qualitative	Same
CLIA Complexity	High	Same
Sample Type	Nasal swab and nasopharyngeal swab specimens	Upper respiratory tract specimens, including nasal swabs and nasopharyngeal swabs; lower respiratory tract specimens
Sample Preparation Method	Nucleic Acid Extraction	Same
Detection Technology	Fluorescence	Same
Technological Principles	RT-PCR followed by endpoint detection by microarray via nucleic acid probes and fluorescence	Real-time RT-PCR via nucleic acid probes and fluorescence
Nucleic Acid Extraction	QIAamp DSP Virus Spin Kit	• QIAamp Viral RNA Mini Kit, Qiagen • QIAcube with QIAamp Viral RNA Mini Kit • MagNA Pure Compact—Total Nucleic Acid Kit, Roche • MagNA Pure Compact—RNA Isolation Kit, Roche • MagNA Pure LC—RNA Isolation Kit II, Roche • NucliSENS easyMAG, bioMerieux
Controls	• Internal Control in each specimen • External positive control processed with each batch • External negative control processed with each batch	Same
Amplification Reagents	Quanta Biosciences qScript One-Step RT-PCR Kit	• Quanta Biosciences qScript One-Step qRT-PCR Kit, low ROX • Invitrogen SuperScript III Platinum One-Step Quantitative RT-PCR Kit

Similarities and Differences		
Item	**Device**	**Predicate**
	FluChip-8G Influenza A+B Assay (K182513)	**CDC Human Influenza Virus Real-Time PCR Diagnostic Panel (K172091)**
Results Interpretation	Automated test interpretation via neural network-based algorithm using fluorescence intensities	Manual test interpretation via C_t value determined from fluorescence intensities
Instrumentation	FluChip-8G Imaging System	Applied Biosystems 7500 Dx Real-Time PCR Instrument

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

None

L. Test Principle:

The FluChip-8G Influenza A+B Assay (FC8G assay) includes a multiplex primer mix to amplify the hemagglutinin (HA), neuraminidase (NA), matrix (M), non-structural (NS), and nucleoprotein (NP) gene segments of all influenza A viruses, as well as the hemagglutinin (HA) and neuraminidase (NA) gene segments of all influenza B viruses, and a portion of the 18s gene present in eukaryotic cells as an endogenous internal control. During a one-step RT-PCR reaction, the primers and RT-PCR enzymes and reagents are utilized to amplify nucleic acids extracted from human nasal specimens while incorporating biotin for downstream fluorescence labeling. The biotinylated amplified products are then heat-fragmented and applied to the FC8G microarray for hybridization, labeling, and detection using the FluChip-8G Imaging System. The system software is trained to identify the patterns of the influenza target virus groups using a large database of thousands of results from viruses of known characterization, and a simple result in the form of a report is returned to the end user via the software user interface.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Two independent studies: within-laboratory repeatability and external multi-site user-to-user reproducibility, were carried out to evaluate the precision and reproducibility of the FluChip-8G Influenza A+B Assay.

   A 45-member panel was used for the two studies. The panel was made with five viral strains that represent the five target influenza virus groups: A/New Caledonia/20/1999 (A/non-seasonal), A/California/07/2009 (A/H1N1pdm09), A/Victoria/361/2011 (A/H3N2), B/Wisconsin/01/2010 (B/Yamagata), and B/Florida/02/2006 (B/Victoria). Each strain was diluted into pooled influenza

negative clinical material at three different target levels: Moderate Positive (MP) (approximately 3xLoD), Low Positive (LP) (approximately 1xLoD), and High Negative (HN) (approximately 0.1xLoD). Influenza negative clinical material consisted of clinical nasopharyngeal swab samples stabilized in universal transport medium (UTM) that were determined to be negative for the presence of influenza virus via an FDA-cleared influenza molecular assay.

External Multi-Site User-to-User Reproducibility Study

Two operators at each of the three separate laboratory sites performed the FluChip-8G Influenza A+B Assay (FC8G assay) according to the assay instructions for use. Performance was evaluated on six unique testing points at the three separate laboratory sites for a total of 18 testing points. Each testing point corresponds to a unique run/execution of the FC8G assay, where each member of the blinded 45-member panel of contrived samples was tested in triplicate. Two unique lots of FC8G assay reagents were evenly divided between the three sites. At each site, each of the two operators executed three testing points. Operators at each site alternated testing until all six test points/site were completed. Each testing point was separated by a minimum of one full day in which no FC8G assay processing for this study occurred.

A total of 810 data points (45 samples/testing point x 18 testing points) were generated in this study. Performance was evaluated by determining the agreement with the expected results, where the MP and LP concentrations were expected to be positive for the intended target, and the HN was expected to be negative.

Table 3 below shows the results of the multi-site user-to-user reproducibility study.

Table 3: Multi-Site User-to-User Reproducibility Study Results

		Site A			**Site** B			**Site C**			**Overall % Agreement and 95% Confidence Interval by Sample (%)**		
		Count and % Agreement		**95% Confidence Interval (%)**	**Count and % Agreement**		**95% Confidence Interval (%)**	**Count and % Agreement**		**95% Confidence Interval (%)**			
A/H1N1 (pre-2009) (A/non-seasonal)	MP (3x LoD)	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	17/18[a]	94%	74.2 - 99.0	53/54	98%	90.2 - 99.7
	LP (1x LoD)	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	54/54	100%	93.4 - 100
	HN (0.1x LoD)	7/18[b]	39%	20.3 - 61.4	11/18[c]	61%	38.6 - 79.7	13/18[d]	72%	49.1 -87.5	31/54	57%	44.2 - 69.7
A/H1N1pdm09	MP (3x LoD)	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	54/54	100%	93.4 - 100
	LP (1x LoD)	18/18	100%	82.4 - 100	14/18[e]	78%	54.8 - 91.0	17/18[f]	94%	74.2 - 99.0	49/54	91%	80.1 - 96.0
	HN (0.1x LoD)	9/18[g]	50%	29.0 - 71.0	11/18[h]	61%	38.6 - 79.7	7/18[i]	39%	20.3 - 61.4	27/54	50%	37.1 - 62.9
A/H3N2	MP (3x LoD)	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	54/54	100%	93.4 - 100
	LP (1x LoD)	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	54/54	100%	93.4 - 100
	HN (0.1x LoD)	17/18[l]	94%	74.2 - 99.0	14/18[m]	78%	54.8 - 91.0	16/18[n]	89%	67.2 - 96.9	47/54	87%	75.6 - 93.6
B/Yamagata	MP (3x LoD)	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	54/54	100%	93.4 - 100
	LP (1x LoD)	18/18	100%	82.4 - 100	17/18[o]	94%	74.2 - 99.0	18/18	100%	82.4 - 100	53/54	98%	90.2 - 99.7
	HN (0.1x LoD)	12/18[p]	67%	43.7 - 83.7	17/18[q]	94%	74.2 - 99.0	15/18[r]	83%	60.8 - 94.2	44/54	81%	69.2 - 89.6
	MP (3x LoD)	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	54/54	100%	93.4 - 100

B/Victoria	LP (1x LoD)	17/18[s]	94%	74.2 - 99.0	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	53/54	98%	90.2 - 99.7
	HN (0.1x LoD)	7/18[t]	39%	20.3 - 61.4	13/18[u]	72%	49.1 -87.5	6/18[v]	33%	16.3 - 56.3	26/54	48%	35.4 - 61.1
Overall	**Moderate Positive (MP) (3x LoD)** **Low Positive (LP) (1x LoD)** **High Negative (HN) (0.1x LoD)**										**269/270** **263/270** **175/270**	**100%** **97%** **65%**	**97.9 - 99.9** **94.7 - 98.7** **58.9 - 70.3**

[a] 1/18 MP replicates was No Call: Assay Failure (Process Control)
[b] 7/18 HN replicates were Flu A non-seasonal; 4/18 HN replicates were Flu A Equivocal
[c] 5/18 HN replicates were Flu A non-seasonal; 2/18 HN replicates were Flu A Equivocal
[d] 4/18 HN replicates were Flu A non-seasonal; 1/18 HN replicates was Flu A Equivocal
[e] 3/18 LP replicates were Flu A/H1N1pdm09 and Flu A non-seasonal dual positive; 1/18 LP replicates was Flu A Equivocal
[f] 1/18 LP replicates were Flu A/H1N1pdm09 and Flu A non-seasonal dual positive
[g] 6/18 HN replicates were Flu A/H1N1pdm09; 3/18 HN replicates were Flu A Equivocal
[h] 2/18 HN replicates were Flu A/H1N1pdm09; 3/18 HN replicates were Flu A Equivocal; 2/18 HN replicates were Flu A non-seasonal
[i] 5/18 HN replicates were Flu A/H1N1pdm09; 5/18 HN replicates were Flu A Equivocal; 1/18 HN replicates was Flu A non-seasonal
[l] 1/18 HN replicates was Flu A/H3N2
[m] 1/18 HN replicates was Flu A/H3N2; 1/18 HN replicates was Flu A Equivocal; 2/18 HN replicates were Flu A/H3N2 and Flu A non-seasonal dual positive
[n] 2/18 HN replicates were Flu A/H3N2
[o] 1/18 LP replicates was Influenza Not Detected
[p] 2/18 HN replicates were Flu B; 2/18 HN were Flu B Yamagata; 2/18 HN were Flu B Victoria
[q] 1/18 HN replicates was Flu B
[r] 1/18 HN replicates was Flu B; 2/18 HN were Flu B Yamagata
[s] 1/18 LP replicates was Error: Image Processing Failure
[t] 1/18 HN replicates was Flu B; 8/18 HN were Flu B Victoria; 2/18 HN were Flu B Yamagata
[u] 1/18 HN replicates was Flu B; 4/18 HN were Flu B Victoria
[v] 12/18 HN replicates were Flu B Victoria

In addition, site-to-site, operator-to-operator, run-to-run, and reagent lot-to-lot reproducibility performance were also assessed using the data generated from this multi-site reproducibility study. There were no statistically significant differences in the observed performance between sites, between operators, between runs, or between reagent lots, as indicated by overlapping confidence intervals for all samples expected to be positive.

Within-Laboratory Precision Study

Two operators at a manufacturer's internal laboratory site performed testing in the within-laboratory precision study. The same panel of 45 contrived samples described previously was utilized to conduct a total of 12 test points, with six test points conducted by each operator. Each testing point was separated by a minimum of one full day in which no FC8G assay processing for this study occurred. The same lot of FC8G reagents, including the QIAamp DSP Virus Spin Kit, and the same imaging system was utilized by both operators for testing.

A total of 540 data points (45 samples/testing point x 6 testing points/operator x 2 operators) were generated in this study. Performance was evaluated by determining the agreement with the expected results, where the MP and LP concentrations were expected to be positive for the intended target, and the HN was expected to be negative.

Table 4 below shows the results of the within-laboratory precision study.

Table 4: Within-Laboratory Precision Study Results

		Operator 1			Operator 2			Overall % Agreement and 95% Confidence Interval by Sample (%)		
		Count and % Agreement		95% Confidence Interval (%)	Count and % Agreement		95% Confidence Interval (%)			
A/H1N1 (pre-2009) (A/non-seasonal)	MP (3x LoD)	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	36/36	100%	90.4 - 100
	LP (1x LoD)	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	36/36	100%	90.4 - 100
	HN (0.1x LoD)	7/18	39%	20.3 - 61.4	4/18	22%	9.0 – 45.2	11/36	31%	18.0 - 46.9
A/H1N1pdm09	MP (3x LoD)	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	36/36	100%	90.4 - 100
	LP (1x LoD)	18/18	100%	82.4 - 100	12/18[a]	67%	43.7 – 83.7	30/36	83%	68.1 - 92.1
	HN (0.1x LoD)	3/18	17%	5.8 – 39.2	10/18	56%	33.7 – 75.4	13/36[b]	36%	22.5 - 52.4
A/H3N2	MP (3x LoD)	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	36/36	100%	90.4 - 100
	LP (1x LoD)	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	36/36	100%	90.4 - 100
	HN (0.1x LoD)	10/18	56%	33.7 - 75.4	9/18	50%	29.0 - 71.0	19/36[c]	53%	37.0 - 68.0
B/Yamagata	MP (3x LoD)	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	36/36	100%	90.4 - 100
	LP (1x LoD)	18/18	100%	82.4 - 100	17/18	94%	74.2 - 99.0	35/36	97%	85.8 - 99.5
	HN (0.1x LoD)	9/18	50%	29.0 – 71.0	17/18	94%	74.2 - 99.0	26/36	72%	56.0 - 84.2
B/Victoria	MP (3x LoD)	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	36/36	100%	90.4 - 100
	LP (1x LoD)	18/18	100%	82.4 - 100	18/18	100%	82.4 - 100	36/36	100%	90.4 - 100
	HN (0.1x LoD)	13/18	72%	49.1 – 87.5	18/18	100%	82.4 -100	31/36	86%	71.3 – 93.9
Overall	**Moderate Positive (MP) (3x LoD)** **Low Positive (LP) (1x LoD)** **High Negative (HN) (0.1x LoD)**							**180/180** **173/180** **100/180**	**100%** **97%** **56%**	**97.9 - 100** **92.2 - 98.1** **48.3 – 62.6**

[a] 6/18 LP replicates were Flu A/H1N1pdm09 and Flu A non-seasonal dual positive on initial testing. All six samples generated the expected Flu A/H1N1pdm09 positive results upon repeat testing per the Instructions for Use (IFU).

[b] 5/36 HN replicates were Flu A non-seasonal on initial testing. Upon repeat testing per the IFU, 2 of 5 samples generated the expected Flu A/H1N1pdm09 positive results, 2 of 5 samples generated Inconclusive results, and 1 of 5 samples generated an Influenza Not Detected result.

2/36 HN replicates were Flu A/H1N1pdm09 and Flu A non-seasonal dual positive on initial testing. All two samples generated Inconclusive results upon repeat testing per the IFU.

1/36 replicates was Flu A Equivocal and Flu A non-seasonal on initial testing. This sample generated an Inconclusive result upon repeat testing per the IFU

[c] 2/36 HN replicates were Flu A non-seasonal on initial testing. Upon repeat testing per the IFU, 1 of 2 samples generated an Inconclusive result, and 1 of 2 samples generated an Influenza Not Detected result.

b. Linearity/assay reportable range:

Not applicable

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

External Quality Control (QC)

The following external controls are recommended to be included in each testing batch, but are not provided in the test kit.

- External Batch Positive Control: A previously characterized influenza-positive clinical specimen is recommended for inclusion as part of good laboratory practices. This control is user-supplied.

- External Batch Negative Control: A previously characterized influenza-negative clinical specimen is recommended for inclusion as part of good laboratory practices. This control is user supplied.
- Extraction Negative Control: This control is recommended for inclusion in each testing batch, and is composed of nuclease-free water added by the end user in place of specimen during the nucleic acid extraction step.
- RT-PCR No Template Control (NTC): This control is recommended for inclusion in each testing batch, and is composed of nuclease-free water added by the end user in place of extracted nucleic acid template during RT-PCR reaction setup.

The following language, pertaining to the external controls, is included in the Instructions for Use (IFU):

"*Good laboratory practice recommends that both an external positive control and external negative control are included alongside clinical specimens in each batch of extractions. These external positive and negative controls should be treated as samples under analysis and interpreted as such. It is also recommended that an extraction negative control (consisting of nuclease-free water) and an RT-PCR No Template Control be run alongside each batch of specimens. These controls should be carried through the entire assay alongside the clinical specimens under analysis.*"

External batch positive controls were prepared for inclusion in each batch of samples analyzed during the prospective clinical study testing. The following four viruses were utilized to prepare the external batch positive controls: Influenza A/Netherlands/2290/2009 (A/H1N1pdm09), Influenza A/Victoria/361/2011 (A/H3N2), Influenza B/Florida/02/2006 (B/Victoria), and Influenza B/Florida/04/2006 (B/Yamagata). Three dilutions of each of the viruses were prepared in viral transport media (VTM) in large volumes to produce twelve overall external positive controls representing high, medium, and low influenza concentrations in each sample, as determined by an influenza molecular assay. Replicate aliquots of each control were prepared and provided to the clinical sites for use in testing. During the prospective clinical study, each site included one of the four external batch positive controls in each batch of specimens tested on a rotating basis.

Replicate aliquots of pooled influenza negative clinical materials that were determined to be negative for the presence of influenza virus via an FDA-cleared influenza molecular assay were also provided to the clinical study sites. During the prospective clinical study, each site included one aliquot of the provided external batch negative control in each batch of specimens tested.

In addition, an Extraction Negative Control and a No Template Control were also included in each batch of specimens tested at each site.

During the prospective clinical study between June 2017 and July 2018 at three sites, upon initial testing, there were one failed external batch negative control, one failed

extraction negative control, and one failed external batch positive control. All controls with initial failed results generated expected results upon repeat testing. The failure rate upon initial testing (including the failed and the invalid results) of the external quality control materials was 1.7% (3/176), with 95% CI: (0.6% - 4.9%).

Nasal and Nasopharyngeal Swab in UTM Specimens Stability

An analytical study was performed to evaluate the stability of clinical nasal swab (NS) and nasopharyngeal swab (NPS) in UTM specimens, when stored at refrigerator temperature (2° - 8°C). Contrived samples consisting of one strain representing each FC8G target virus group (A/H1N1pdm09, A/H3N2, A/non-seasonal, B/Victoria, and B/Yamagata) were prepared in pooled influenza negative clinical matrix at an influenza concentration of approximately 3x LoD. Triplicate extractions of the contrived sample were performed following storage at 10°C ± 2°C for each of the following time points: 0 hour, 24 hours, 36 hours, 48 hours, and 96 hours. For all storage time points tested in this analytical study, an overall agreement of 100% (95%CI, 79.6 – 100) with the expected result was obtained. This data supports a stability claim of 72 hours at refrigerator temperature (2° - 8°C) for clinical nasal swab (NS) and nasopharyngeal swab (NPS) in UTM specimens.

While a formal analytical study was not conducted to determine specimen stability at ≤ -70°C, data from the external controls performed during the clinical study were analyzed to assess stability over time when stored at ≤ -70°C for 427 days. The samples evaluated were those used as external batch controls executed weekly during the course of the clinical study at the InDevR clinical site. Influenza positives in the testing panel were stock influenza viruses spiked into UTM. Influenza negatives in the testing panel were pooled influenza negative clinical material. Based on the results of this analysis, it appears that the nasal swab and nasopharyngeal swab in UTM specimens are stable for up to 14 months when stored at ≤ -70°C.

Nasal and Nasopharyngeal Swab in UTM Specimens Freeze and Thaw Study

An analytical study was conducted to assess the performance of the FC8G Assay on contrived samples that underwent repeated freeze/thaw cycles to establish the equivalency of samples subjected to freeze/thaw and support the clinical study design of testing archived frozen specimens. A panel of 60 contrived influenza samples were utilized in this testing, consisting of 12 unique strains for each of the five target groups (A/H1N1pdm2009, A/H3N2, A/non-seasonal, B/Yamagata, and B/Victoria). Samples were contrived by spiking stock whole virus into pooled influenza negative clinical material at a final concentration of approximately 3x LoD (ranging from 1x to 9x LoD, depending on strain), and five replicate aliquots of each prepared sample were made. The first aliquot of each strain was tested without re-freezing (immediately after preparation), with one aliquot of each strain tested after each of four consecutive freeze-thaw cycles to assess % agreement with expected result. Data from this study demonstrated that the FC8G Assay performance was not affected by up to four freeze and thaw cycles of the specimens.

Extracted RNA Specimens Stability

An analytical evaluation was performed to evaluate the stability of extracted RNA specimens, when stored at ≤ -70°C. A large volume of a representative A/H3N2 stock virus, A/Victoria/361/2011, was utilized to perform this testing by spiking stock virus into pooled influenza-negative clinical material at approximately 3x LoD and creating individual homogeneous aliquots for downstream testing. Sixteen aliquots of the prepared A/H3N2 contrived sample were extracted according to the Instructions for Use (IFU), subsequently pooled to create a homogeneous material, and aliquoted into five aliquots. Each aliquot was of sufficient volume to enable 14 replicate tests. One aliquot was utilized to assess the assay performance at "Day 0", and the remaining four aliquots were stored at ≤ -70°C until tested. Three of the four remaining aliquots were utilized to conduct testing at Days 14, 30, and 40 after storage. The last aliquot was utilized to test the cumulative "worst case" stability of the assay intermediates, including Extracted RNA Specimen, RT-PCR Product, Fragmented RT-PCR product, and Labeled Microarray.

Extracted RNA specimens stored at ≤ -70°C showed 100% agreement with "Day 0" results for the duration of the testing schedule of 40 days, supporting a stability claim of 30 days at ≤ -70°C.

Intact and Heat-Fragmentated RT-PCR Products Stability

An analytical evaluation was performed to evaluate the stability of intact and heat-fragmented RT-PCR products when stored at either +10°C or -10°C.

A large volume of a representative A/H3N2 stock virus, A/Victoria/361/2011, was utilized to perform this testing by spiking stock virus into pooled influenza-negative clinical material at approximately 3x LoD and creating individual homogeneous aliquots for downstream testing. Thirty-five (35) aliquots of the prepared A/H3N2 sample were extracted according to the IFU, and subsequently pooled to create a homogeneous material. This extracted material was then used as template in 254 replicate RT-PCR amplification reactions conducted according to the IFU. Once amplified, all of the replicates of the amplified A/H3N2 sample were pooled to create a homogeneous RT-PCR product which was subsequently aliquoted into approximately 220 individual PCR tubes for downstream processing by the remainder of the FC8G assay.

For the intact (un-fragmented) RT-PCR product testing, 14 replicate aliquots of the amplified A/H3N2 sample were processed through the remainder of the FC8G assay according to the IFU at "Day 0". Eighty-four (84) replicate aliquots of the amplified A/H3N2 sample were divided evenly at two different storage temperatures: 42 aliquots at +10°C and 42 aliquots at -10°C. For the +10°C stored products, 14 aliquots were processed through the remainder of the FC8G assay according to the IFU at Day 3, Day 7, and Day 14 after storage. For the -10°C stored products, 14

aliquots were processed through the remainder of the assay at Days 7, 14, and 30 after storage.

Intact (un-fragmented) RT-PCR products stored at +10°C showed 100% agreement with "Day 0" results for the duration of the testing schedule of 14 days, supporting a stability claim of 7 days at ≤ 8°C. Intact (un-fragmented) RT-PCR products stored at -10°C showed 100% agreement with "Day 0" results for the duration of the testing schedule of 30 days, supporting a stability claim of 14 days at ≤ -15°C.

For the heat-fragmented RT-PCR product testing, 14 replicate aliquots of the amplified A/H3N2 sample were heat-fragmented and processed through the remainder of the FC8G assay according to the IFU at "Day 0". The remaining replicate RT-PCR product aliquots were heat-fragmented according to the IFU, and the fragmented products were then immediately pooled to create a homogeneous material. The homogeneous material was then re-aliquoted. These aliquots were evenly divided between two different storage temperatures, +10°C and -10°C. For the +10°C stored products, aliquots were tested on Days 1, Day 3, Day 7, and Day 14 after storage. For the aliquots stored at -10°C, aliquots were tested on Days 3, 7, 14, and 30 after storage.

Heat-fragmented RT-PCR products stored at +10°C showed 100% agreement with "Day 0" results for the duration of the testing schedule of 14 days, supporting a stability claim of 7 days at ≤ 8°C. Heat-fragmented RT-PCR products stored at -10°C showed 100% agreement with "Day 0" results for the duration of the testing schedule of 30 days, supporting a stability claim of 14 days at ≤ -15°C.

Labeled Microarray Stability

An analytical evaluation was performed to evaluate the stability of labeled microarrays when stored at room temperature (18°C - 25°C).

Two microarray slides processed for other aspects of the assay intermediates stability evaluations (one from the extracted RNA specimen stability evaluation, and one from the RT-PCR product stability evaluation), were imaged on the FC8G Imaging System immediately after assay completion (considered Day 0). A total of 14 replicate A/H3N2 samples were included on each of the two microarray slides. The slides were subsequently stored in a dry microarray slide box in the dark, at room temperature and rescanned using the FC8G Imaging System after Days 3, 7, and 14.

Labeled microarrays stored at room temperature showed 100% agreement with "Day 0" results for the duration of the testing schedule of 14 days, supporting a stability claim of 7 days at room temperature (18°C - 25°C).

Cumulative "Worst Case" Assay Intermediate Storage Stability

To investigate the cumulative "worst" case scenario in which each assay intermediate

was stored for the longest recommended storage time at its highest recommended temperature in the IFU, an aliquot of pooled extracted RNA prepared for the extracted RNA specimens stability testing was separated into 14 identical aliquots, and each aliquot was RT-PCR amplified according to the IFU. The intact double-stranded amplicon RT-PCR products were then stored at +10°C for seven days prior to fragmentation. The products were then heat-fragmented according to the IFU, and the fragmented products were stored at +10°C for seven days prior to hybridization and labeling according to the IFU. After hybridization and labeling, the slide was initially imaged. The imaged slide was then stored for seven days upon which it was re-imaged. All 14 aliquots processed produced the expected A/H3N2 result. The testing data supports the maximum recommended storage times and temperatures presented in the IFU.

d. Detection limit:

The limit of detection (LoD) was determined for each FC8G assay target virus group (influenza A/H3N2, influenza A/H1N1pdm2009, influenza B/Yamagata, influenza B/Victoria, and non-seasonal influenza A) by testing serial dilutions of stock virus strains (used either as intact virus or viral genomic RNA, depending on influenza subtype) spiked into pooled influenza negative clinical material. The LoD was determined for each strain by: 1) performing a 10-fold serial dilution range-finding experiment to determine an appropriate range for testing, 2) analyzing four replicates of a 3-fold limiting dilution series spanning a concentration range both above and below the assay cutoff to approximate the LoD, and 3) confirming the LoD by determining the concentration at which 95% positivity is achieved from testing at least 20 replicates.

LoD for Seasonal Influenza Target Virus Groups

The LoD was established for eight strains of seasonal influenza, including two strains each of A/H1N1pdm09, A/H3N2, B/Yamagata, and B/Victoria. All viral stock materials were intact whole virus of known titer spiked into pooled influenza-negative clinical material. The confirmed LoD in $TCID_{50}$/mL for each strain investigated are shown in Table 5 below, along with associated concentrations in approximate genome copies/mL determined at the LoD.

Table 5: LoD for Seasonal Influenza Target Virus Groups

Target Virus Group	Strain	LoD Concentration ($TCID_{50}$/mL)	Approximate Genome Copies/mL at LoD	Positivity Rate (%)
A/H1N1pdm09	A/New York/01/2009	6.2×10^{0}	1.4×10^{3}	25/25 (100%)
	A/California/07/2009	1.9×10^{-1}	1.8×10^{3}	20/20 (100%)
A/H3N2	A/Perth/16/2009	1.9×10^{0}	1.4×10^{3}	24/25 (96%)
	A/Victoria/361/2011	2.0×10^{1}	1.2×10^{3}	25/25 (100%)
B/Victoria	B/Brisbane/60/2008	1.7×10^{-1}	5.8×10^{2}	24/25 (96%)
	B/Florida/02/2006	8.3×10^{-1}	1.3×10^{3}	20/20 (100%)
B/Yamagata	B/Wisconsin/01/2010	5.6×10^{-2}	6.3×10^{2}	25/25 (100%)
	B/Phuket/3073/2013	1.9×10^{-1}	1.7×10^{3}	20/20 (100%)

LoD for Non-Seasonal Influenza A

The LoD was established for 10 strains of non-seasonal influenza A, representing 10 different subtypes. Due to safety and availability reasons, in this study the A/H5 and A/H7 strains were tested as extracted viral genomic RNA, and the other strains were tested as whole virus. Both types of starting material were spiked into pooled influenza-negative clinical material and processed through the entire assay, including extraction. Specific procedures were followed (i.e., stabilization of RNA with protease/lysis buffer) to ensure that RNA spiked into clinical negative material did not degrade prior to testing. The confirmed LoD in either $TCID_{50}$/mL or approximate genome copies/mL, depending on the starting viral material (i.e., whole virus vs. extracted genomic viral RNA), along with associated concentrations in approximate genome copies/mL determined at the LoD for the whole viruses, are shown in Table 6 below.

Table 6: LoD for Non-Seasonal Influenza A Target Virus Group

Subtype	**Strain**	**LoD Concentration ($TCID_{50}$/mL)**	**Approximate Genome Copies/mL at LoD**	**Positivity Rate (%)**
A/H1N1pre2009	A/New Caledonia/20/1999	1.2×10^{2}	5.6×10^{3}	20/20 (100%)
A/H1N1v	A/Swine/Illinois/4L013/2015	7.5×10^{3}	1.8×10^{5}	24/25 (96%)
A/H3N2v	A/Indiana/08/2011	9.7×10^{1}	2.9×10^{3}	19/20 (95%)
A/H3N8	A/Blue-Winged Teal/Iowa/10OS2411/2010	9.3×10^{1}	8.7×10^{2}	20/20 (100%)
A/H5N1	A/Anhui/01/2005*	N/A	3.2×10^{3}	20/20 (100%)
A/H5N2	A/Mule Duck/Bulgaria/237/2011*	N/A	1.5×10^{3}	20/20 (100%)
A/H5N8	A/Mule Duck/Bulgaria /328/2011*	N/A	4.9×10^{3}	20/20 (100%)
A/H7N7	A/Ruddy Turnstone/New Jersey/AI11- 1678/2011*	N/A	1.2×10^{3}	20/20 (100%)
A/H7N9	A/Mallard/Alberta/177/2004*	N/A	5.8×10^{2}	20/20 (100%)
A/H9N2	A/Shorebird/Delaware Bay/127/2003	1.8×10^{-1}	7.7×10^{2}	20/20 (100%)

* Extracted viral genomic RNA was used as starting material.

The A/H1N1v virus strain tested (A/Swine/IL/4L013/2015) was consistently misidentified as an A/H1N1pdm09 virus during the study. For samples at and above the LoD, this virus was correctly identified as non-seasonal influenza A, but was also identified incorrectly as an A/H1N1pdm09 (a dual positive result). At concentrations below the LoD, the A/H1N1v samples were misidentified as A/H1N1pdm09 only. The H3N2v virus strain tested (A/Indiana/08/2011) resulted in correct identification as non-seasonal influenza A at concentrations at and above the LoD, but was misidentified at concentrations below the LoD as an A/H1N1pdm09. The misidentification of A/H1N1v and A/H3N2v as seasonal A/H1N1pdm09 or as non-seasonal influenza A and A/H1N1pdm09 dual infections is likely due to close genetic similarities among these viruses. The assay Instructions for Use (IFU) includes limitation language indicating these possible results for variant viruses, and follow-up retesting requirement for all dual positive results.

e. Analytical Reactivity

Analytical reactivity (inclusivity) was evaluated for 52 additional temporally and geographically diverse influenza strains representing all FC8G assay target virus groups. The FC8G assay was executed on either titered stock whole virus or extracted RNA (depending on subtype) spiked into pooled clinical negative material at close to the LoD concentration. Each strain was tested in triplicate. Any virus that was not correctly identified in triplicate was tested again at a higher concentration until detected.

Analytical Reactivity of Seasonal Influenza Target Virus Groups

Analytical reactivity (inclusivity) was determined for 32 seasonal influenza strains near the limit of detection as shown in Table 7 below. In this study, whole viruses were utilized as starting material for the testing. The testing concentrations are shown in both $TCID_{50}$/mL and approximate genome copies/mL. The multiple of the LoD tested was determined based on the approximate genome copies/mL values.

Table 7: Analytical Reactivity of Seasonal Influenza Target Virus Groups

Target Virus Group	Strain	Concentration ($TCID_{50}$/mL, unless specified)	Concentration (approximate genome copies/mL)	Approximate Multiple of LoD Detected
A/H1N1pdm09	A/South Carolina/18/2009	1.5×10^0	2.5×10^3	1x
	A/Canada/6294/2009	1.9×10^0	1.3×10^3	1x
	A/Netherlands/2290/2009	2.9×10^0	5.0×10^3	3x
	A/New York/18/2009	1.1×10^0	9.9×10^2	1x
	A/Utah/20/2009	1.2×10^0	1.0×10^4	6x
	A/Massachusetts/15/2013	4.0×10^0	7.9×10^3	4x
	A/Mexico/4108/2009	2.5×10^{-1}	1.9×10^3	1x
	A/New Hampshire/02/2010	7.7×10^0	3.2×10^3	2x
	A/California/04/2009	1.1×10^1	4.7×10^3	3x
	A/Dominican Republic/7293/2013	2.2×10^2	3.9×10^3	2x
	A/Michigan/45/2015	1.1×10^3 (EID_{50}/mL)	6.5×10^3	4x
A/H3N2	A/Stockholm/6/2014	2.6×10^0	2.4×10^3	2x
	A/South Australia/55/2014	1.1×10^0	2.2×10^2	0.2x
	A/Switzerland/9715293/2013	1.4×10^0	5.7×10^2	0.4x
	A/Norway/466/2014	1.5×10^0	5.3×10^2	0.4x
	A/Texas/71/2007	1.9×10^0	4.2×10^3	3x
	A/Uruguay/716/2007	1.2×10^0	5.2×10^3	4x
	A/Texas/50/2012	2.3×10^0	3.9×10^3	3x
	A/Santiago/7981/2006	2.1×10^0	2.9×10^3	2x
	A/Wisconsin/67/2005	7.9×10^0	5.3×10^2	0.4x
	A/Hong Kong/4801/2014	4.6×10^2 (EID_{50}/mL)	2.1×10^3	2x

B/Victoria	B/Nevada/03/2011	3.1×10^{-1}	1.3×10^{3}	1x
	B/Malaysia/2506/2004	5.4×10^{-1}	1.7×10^{3}	1x
	B/New Jersey/01/2012	4.6×10^{-1}	2.6×10^{3}	2x
	B/Victoria/304/2006	1.6×10^{1}	4.7×10^{3}	4x
	B/Hong Kong/330/2001	1.4×10^{1}	3.4×10^{3}	3x
	B/Texas/02/2013	3.8×10^{3} (EID_{50}/mL)	1.6×10^{3}	1x
B/Yamagata	B/Texas/06/2011	6.9×10^{-1}	3.6×10^{3}	2x
	B/Pennsylvania/07/2007	1.6×10^{0}	3.8×10^{3}	2x
	B/Massachusetts/02/2012	1.7×10^{0}	5.3×10^{3}	3x
	B/Brisbane/3/2007	5.9×10^{-1}	2.8×10^{3}	2x
	B/Florida/07/2004	2.3×10^{1}	1.2×10^{3}	1x

Analytical Reactivity of Non-Seasonal Influenza A Target Virus Group

Analytical reactivity (inclusivity) was determined for 20 virus strains belonging to the non-seasonal influenza A target virus group (i.e., all influenza A subtypes other than A/H1N1pdm09 or A/H3N2). For A/H5 and A/H7 strains, due to safety and availability reasons, starting material used was extracted genomic viral RNA in this study. All other strains utilized whole virus as starting material in this study. Both types of starting material were spiked into pooled influenza-negative clinical material at close to the LoD concentrations and processed through the entire assay, including extraction. Results are shown in Table 8 below. The multiple of the LoD tested was determined based on the approximate genome copies/mL values.

Table 8: Analytical Reactivity of Non-Seasonal Influenza A Target Virus Group

Subtype	**Strain**	**Concentration (EID_{50}/mL, unless specified)**	**Concentration (approximate genome copies/mL)**	**Approximate Multiple of LoD Detected**
A/H1N1pre2009	A/Fujian Gulou/1896/2009	7.5×10^{4}	3.4×10^{3}	1x
A/H1N1sw	A/Swine/1976/1931	6.2×10^{3}	6.9×10^{3}	2x
A/H1N1v	A/Swine/Illinois/4L036/2015	No Titer Available	6.7×10^{4}	23x*
A/H1N8	A/Red Knot/Delaware Bay/240/1994	2.9×10^{5} $TCID_{50}$/mL	4.5×10^{3}	2x
A/H2N2	A/Chicken/New York/SG-00425/2004	No Titer Available	5.6×10^{3}	2x
A/H3N2sw	A/Swine/Ohio/11SW87/2011	9.1×10^{3} $TCID_{50}$/mL	7.9×10^{3}	3x
A/H3N2v	A/Minnesota/11/2010	3.0×10^{4}	1.0×10^{4}	4x
A/H3N8	A/Blue-Winged Teal/Alberta/221/1978	No Titer Available	1.6×10^{3}	1x
A/H3N8	A/Camel/Mongolia/335/2012	2.5×10^{3}	5.6×10^{3}	2x
A/H3N9	A/Mallard/Alberta/54/1993	2.9×10^{4}	2.1×10^{3}	1x
A/H4N6	A/Blue-Winged Teal/Illinois/10OS1563/2010	4.6×10^{4}	5.6×10^{3}	2x
A/H5N1	A/Goose/Yunnan/5539/2005**	N/A	4.5×10^{3}	2x
A/H5N1	A/Chicken/Vietnam/NCVD-016/2008**	N/A	7.9×10^{3}	3x
A/H5N2	A/Northern Pintail/California/44242-758/2006**	N/A	2.6×10^{4}	9x*
A/H5N8	A/Duck/England/36254/2014**	N/A	7.3×10^{3}	3x
A/H6N4	A/Mallard/Alberta/58/1989	4.9×10^{3}	2.1×10^{3}	1x
A/H7N7	A/Italy/3/2013**	N/A	1.7×10^{4}	6x*
A/H7N9	A/Hong Kong/5942/2013**	N/A	6.9×10^{3}	3x

A/H9N2	A/Turkey/Wisconsin/1/1966	3.8×10^3	3.0×10^3	1x
A/H11N2	A/Laughing Gull/Delaware Bay/94/1995	7.0×10^5	1.4×10^4	5x*

* Not correctly detected at lower concentrations.
** Extracted viral genomic RNA was used as starting material.

f. Analytical specificity

Analytical Specificity for Influenza Viruses

Analytical specificity for all target virus groups was evaluated with the FC8G assay to determine whether strains of a known target influenza virus group were misidentified as another influenza virus group targeted by the assay when run at high concentration. High concentrations of stock influenza virus or extracted genomic viral RNA (depending on subtype) were spiked into pooled influenza-negative clinical material in triplicate and analyzed by the FC8G assay to determine if any mis-identification occurred.

Analytical Specificity for Seasonal Influenza Target Virus Groups

Analytical specificity (exclusivity) was demonstrated for 32 strains of seasonal influenza at high concentrations. Whole virus was utilized as starting material in this study and spiked into influenza-negative clinical material at concentrations $>10^6$ approximate genome copies/mL. Results of this study are shown in Table 9 below.

Table 9: Analytical Specificity of Seasonal Influenza Target Virus Groups

Target Virus Group	Virus Strain	Concentration ($TCID_{50}$/mL, unless specified)	Concentration (approximate genome copies/mL)	Mis-Identification
A/H1N1pdm09	A/Canada/6294/2009	9.3×10^3	3.0×10^7	Not observed
	A/Michigan/45/2015	2.6×10^7 (EID_{50}/mL)	2.4×10^7	Not observed
	A/Dominican Republic/7293/2013	5.0×10^6	1.3×10^8	Not observed
	A/New York/18/2009	4.1×10^3	1.4×10^7	Not observed
	A/New Hampshire/02/2010	8.5×10^4	4.0×10^7	Not observed
	A/Massachusetts/15/2013	1.4×10^4	1.5×10^7	Not observed
	A/Mexico/4108/2009	8.1×10^3	8.0×10^6	Not observed
	A/Utah/20/2009	4.1×10^3	1.8×10^7	Not observed
	A/South Carolina/18/2009	2.8×10^3	1.1×10^7	Not observed
	A/Netherlands/2290/2009	4.7×10^3	5.7×10^6	Not observed
	A/California/04/2009	1.2×10^5	1.5×10^7	Not observed

A/H3N2	A/Wisconsin/67/2005	1.4×10^5	3.9×10^8	Not observed
	A/Texas/50/2012	8.5×10^3	8.0×10^6	Not observed
	A/Switzerland/9715293/2013	4.7×10^3	8.6×10^6	Not observed
	A/South Australia/55/2014	1.6×10^4	2.4×10^7	Not observed
	A/Stockholm/6/2014	1.2×10^4	8.6×10^6	Not observed
	A/Norway/466/2014	5.9×10^3	4.6×10^6	Not observed
	A/Texas/71/2007	1.8×10^4	3.0×10^7	Not observed
	A/Santiago/7981/2006	1.7×10^4	1.3×10^7	Not observed
	A/Hong Kong/4801/2014	1.6×10^6 (EID_{50}/mL)	2.8×10^7	Not observed
	A/Uruguay/716/2007	8.5×10^3	1.3×10^7	Not observed
B/Victoria	B/Hong Kong/330/2001	7.2×10^4	2.1×10^7	Not observed
	B/Nevada/03/2011	6.8×10^3	9.1×10^7	Not observed
	B/Malaysia/2506/2004	1.9×10^3	2.1×10^7	Not observed
	B/Victoria/304/2006	2.5×10^4	2.4×10^8	Not observed
	B/New Jersey/01/2012	1.6×10^3	4.6×10^7	Not observed
	B/Texas/02/2013	8.3×10^7 (EID_{50}/mL)	9.8×10^7	Not observed
B/Yamagata	B/Brisbane/3/2007	2.5×10^4	7.4×10^7	Not observed
	B/Massachusetts/02/2012	1.7×10^4	6.0×10^7	Not observed
	B/Texas/06/2011	2.3×10^3	4.6×10^7	Not observed
	B/Florida/07/2004	7.2×10^5	3.2×10^7	Not observed
	B/Pennsylvania/07/2007	2.8×10^3	8.0×10^6	Not observed

There were no misidentifications observed at the concentrations tested in this study.

Analytical Specificity for Non-Seasonal Influenza A Target Virus Group

Analytical specificity (exclusivity) was determined for 19 non-seasonal influenza A viruses. In this study the A/H5 and A/H7 strains were tested as extracted genomic viral RNA for safety and availability reasons, and all other strains were tested as whole virus. Starting materials were spiked into pooled influenza negative clinical material at concentrations $>10^6$ approximate genome copies/mL. Results of this study are shown in Table 10 below.

Table 10: Analytical Specificity of Non-Seasonal Influenza A Target Virus Group

Subtype	**Strain**	**Concentration (EID_{50}/mL, unless specified)**	**Concentration (approximate genome copies/mL)**	**Mis- Identification**
A/H1N1pre2009	A/Fujian Gulou/1896/2009	2.0×10^8	4.6×10^7	Not observed
A/H1N1sw	A/Swine/1976/1931	1.1×10^6	4.0×10^7	Not observed
A/H1N1v	A/Swine/Illinois/4L036/2015	No Titer Available	1.6×10^8	Not observed
A/H1N8	A/Red Knot/Delaware Bay/240/1994	1.1×10^9	8.5×10^7	Not observed
A/H2N2	A/Chicken/New York/SG-00425/2004	No Titer Available	1.2×10^8	Not observed
A/H3N2sw	A/Swine/Ohio/11SW87/2011	2.9×10^6 $TCID_{50}$/mL	2.0×10^7	Not observed
A/H3N2v	A/Minnesota/11/2010	2.8×10^7	6.4×10^7	Not observed

A/H3N8	A/Blue-Winged Teal/Alberta/221/1978	No Titer Available	7.0×10^6	Not observed
A/H3N8	A/Camel/Mongolia/335/2012	6.3×10^5	1.8×10^7	Not observed
A/H3N9	A/Mallard/Alberta/54/1993	1.1×10^8	8.5×10^7	Not observed
A/H4N6	A/Blue-Winged Teal/Illinois/ 10OS1563/2010	1.1×10^8	1.5×10^8	Not observed
A/H5N1	A/Goose/Yunnan/5539/2005*	N/A	3.5×10^6	Not observed
A/H5N2	A/Northern Pintail/Washington/ 40964/2014*	N/A	1.8×10^7	Not observed
A/H5N8	A/Duck/England/36254/2014*	N/A	3.8×10^6	Not observed
A/H6N4	A/Mallard/Alberta/58/1989	9.1×10^6	2.0×10^8	Not observed
A/H7N7	A/Italy/3/2013*	N/A	1.9×10^6	Not observed
A/H7N9	A/Hong Kong/5942/2013*	N/A	2.3×10^6	Not observed
A/H9N2	A/Turkey/Wisconsin/1/1966	1.1×10^8	2.8×10^8	Not observed
A/H11N2	A/Laughing Gull/DelawareBay/94/ 1995	1.4×10^9	9.8×10^7	Not observed

* Extracted genomic viral RNA.

There were no misidentifications observed at the concentrations tested in this study.

Analytical Specificity with Non-Influenza Respiratory Pathogens

Analytical specificity of the FC8G assay with non-influenza organisms that may be present in the respiratory tract of patients presenting with influenza-like illness was conducted testing a panel of 34 non-influenza organisms (19 bacteria isolates and 15 viruses). A stock of each organism in culture media at known titer was spiked into pooled influenza-negative clinical material at concentrations $\geq 10^6$ CFU/mL for bacteria and $\geq 10^5$ $TCID_{50}$/mL or CCU/mL for viruses and atypical bacteria, whenever possible. Each organism was extracted once and tested in triplicate. For any organisms producing a false positive result of influenza, additional replicates were tested as needed to evaluate consistency of assay results in the presence of potentially cross-reactive organisms. Final results of this study are shown in Table 11 below.

Table 11: Analytical Specificity of Non-Influenza Organisms

Organism	Concentration Tested	Cross-Reactivity
Adenovirus 71	8.0×10^6 $TCID_{50}$/mL	Not observed
Adenovirus 7A	3.0×10^7 $TCID_{50}$/mL	Not observed
Coronavirus 229E	2.5×10^5 $TCID_{50}$/mL	Not observed
Coronavirus OC43	1.4×10^5 $TCID_{50}$/mL	Not observed
Cytomegalovirus	1.2×10^5 $TCID_{50}$/mL	Not observed
Enterovirus 71	8.0×10^5 $TCID_{50}$/mL	Not observed
Epstein Barr Virus	2.2×10^8 $TCID_{50}$/mL	Not observed
Human parainfluenza 1	9.5×10^6 $TCID_{50}$/mL	Not observed
Human parainfluenza 2	4.5×10^5 $TCID_{50}$/mL	Not observed
Human parainfluenza 3	2.5×10^5 $TCID_{50}$/mL	Not observed
Measles	1.3×10^5 $TCID_{50}$/mL	Not observed
Human metapneumovirus	1.3×10^5 $TCID_{50}$/mL	Not observed
Mumps virus	5.0×10^5 $TCID_{50}$/mL	Not observed
Human respiratory syncytial virus	1.3×10^5 $TCID_{50}$/mL	Not observed

Rhinovirus 1A	7.0×10^4 $TCID_{50}$/mL	Not observed
Bordetella pertussis	3.8×10^9 CFU/mL	Not observed
Chlamydophila pneumoniae	8.0×10^5 $TCID_{50}$/mL	Not observed
Corynebacterium diphtheriae	2.3×10^6 CFU/mL	Not observed
Escherichia coli	4.7×10^9 CFU/mL	Not observed
Hemophilus influenza	2.7×10^8 CFU/mL	Not observed
Lactobacillus acidophilus	1.3×10^8 CFU/mL	Not observed
Legionella pneumophila	9.0×10^9 CFU/mL	Not observed
Moraxella catarrhalis	6.5×10^6 CFU/mL	Not observed
Mycobacterium tuberculosis	7.0×10^6 CFU/mL	Not observed
Mycoplasma pneumoniae	1.0×10^6 CCU/mL	Not observed
Neisseria meningitidis	1.0×10^6 CFU/mL	Not observed
Neisseria sicca	1.8×10^8 CFU/mL	Not observed
Pseudomonas aeruginosa	1.0×10^6 CFU/mL	Not observed
Pseudomonas aeruginosa	1.0×10^6 CFU/mL	Not observed
Staphylococcus aureus	7.5×10^8 CFU/mL	Not observed
Staphylococcus epidermidis	7.5×10^8 CFU/mL	Not observed
Streptococcus pneumoniae	3.4×10^7 CFU/mL	Not observed
Streptococcus pyogenes	8.5×10^6 CFU/mL	Not observed
Streptococcus salivarius	3.8×10^6 CFU/mL	Not observed

None of the organisms tested in this study were found to be cross-reactive with the FC8G assay at the concentrations tested.

Microbial Interference Study

Microbial interference was assessed for the FC8G assay by testing a 10-member test panel that represented an abbreviated version of the test panel used in the Analytical Specificity Study above. The non-influenza organism at high concentration (i.e., $\geq 10^6$ CFU/mL for bacteria and $\geq 10^5$ $TCID_{50}$/mL or CCU/mL for viruses and atypical bacteria) was mixed with an influenza virus at close to the LoD concentration in the same sample to determine if the presence of the non-target organism would interfere with the detection of the influenza analyte using the FC8G assay.

Contrived samples with one representative virus strain from each of the five target virus groups, A/California/07/2009 (A/H1N1pdm09), A/Victoria/361/2011 (A/H3N2), B/Florida/02/2006 (B/Victoria), B/Wisconsin/01/2010 (B/Yamagata), and A/New Caledonia/20/1999 (A/H1N1 pre-2009, non-seasonal influenza A), were prepared at a concentration of approximately 3x to 5x LoD. Influenza samples were prepared in pooled influenza-negative clinical material, and the diluted non-influenza organisms were added to the extraction buffer to yield the desired concentration for testing. Each contrived sample was extracted once and tested in triplicate.

Results of the Microbial Interference Study are summarized in Table 12 below.

Table 12: Summary Results of the Microbial Interference Study

Non-Influenza Organism	Concentration Tested	# of Correct FC8G Assay Results					Overall Agreement	Interference
		A/H1N1 pdm09	A/H3N2	B/Victoria	B/Yamagata	A/H1N1 (pre-2009)		
None	N/A	3/3	3/3	3/3	3/3	3/3	100%	N/A
Adenovirus 71	1.6×10^6 $TCID_{50}$/mL	3/3	3/3	3/3	3/3	3/3	100%	Not observed
Coronavirus 229E	1.0×10^5 $TCID_{50}$/mL	3/3	3/3	3/3	3/3	3/3	100%	Not observed
Human parainfluenza 3	1.0×10^5 $TCID_{50}$/mL	3/3	3/3	3/3	3/3	3/3	100%	Not observed
Human metapneumovirus	1.2×10^5 $TCID_{50}$/mL	3/3	3/3	3/3	3/3	3/3	100%	Not observed
Human respiratory syncytial virus	8.5×10^6 $TCID_{50}$/mL	3/3	3/3	3/3	3/3	3/3	100%	Not observed
Rhinovirus 1A	1.1×10^5 $TCID_{50}$/mL	3/3	3/3	3/3	3/3	3/3	100%	Not observed
Hemophilus influenza	3.3×10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	100%	Not observed
Mycoplasma pneumoniae	1.1×10^6 CCU/mL	3/3	3/3	3/3	3/3	3/3	100%	Not observed
Staphylococcus aureus	1.7×10^6 CFU/mL	3/3	3/3	3/3	3/3	3/3	100%	Not observed
Streptococcus pneumoniae	6.8×10^6 CFU/mL	3/3	3/3	3/3	2/3*	3/3	93%	Not observed

* One Influenza B Yamagata sample was identified only as influenza B only (without Yamagata lineage).

The results showed that the evaluated non-influenza organisms at the concentrations tested in this study did not interfere with the detection of influenza virus samples at close to the LoD concentrations.

g. Influenza Competitive Interference Study

To assess performance of the FC8G assay for samples containing two different influenza viruses, samples containing one representative virus from each of two different target virus groups were spiked into pooled influenza negative clinical material and analyzed by the FC8G assay. Mock dual infection samples that represented all permutations of dual infections of the following five influenza strains, A/South Carolina/18/2009, A/Victoria/361/2011, A/Ruddy Turnstone/Delaware Bay/39/1994, B/New Jersey/01/2012, and B/Texas/06/2011, representing the five target virus groups, A/H1N1pdm09, A/H3N2, A/H11N3 (non-seasonal influenza A), B/Victoria, and B/Yamagata, respectively, at three different concentrations of each included virus (high, medium, and low, corresponding to approximately 500x, 60x, and 4x LoD, respectively, based on the assessment of Ct values generated by an FDA-cleared influenza molecular assay), were analyze for a total of 90 samples, as outlined in Table 13 below.

Results of the Competitive Interference Study are summarized in Table 13 below.

Table 13: Competitive Interference Study Testing Scheme and Results

Sample	Source 1		Source 2		Source 1 Virus Identified	Source 2 Virus Identified	Both Sources of Viruses Identified
	Virus	Concentration	Virus	Concentration			
1	A/H1N1pdm09	High	A/H3N2	High	No	Yes	No
2		High		Medium	Yes	No	No
3		High		Low	Yes	No	No
4		Medium		High	No	Yes	No
5		Medium		Medium	No	Yes	No
6		Medium		Low	Yes	No	No
7		Low		High	No	Yes	No
8		Low		Medium	No	Yes	No
9		Low		Low	No	Yes	No
10		High	A/H11N3	High	No	Yes	No
11		High		Medium	Yes	Yes	Yes
12		High		Low	Yes	No	No
13		Medium		High	No	Yes	No
14		Medium		Medium	Yes	Yes	Yes
15		Medium		Low	Yes	No	No
16		Low		High	No	Yes	No
17		Low		Medium	No	Yes	No
18		Low		Low	Yes	Yes	Yes
19		High	B/Victoria	High	Yes	Yes	Yes
20		High		Medium	Yes	Yes	Yes
21		High		Low	Yes	No	No
22		Medium		High	Yes	Yes	Yes
23		Medium		Medium	Yes	Yes	Yes
24		Medium		Low	Yes	No	No
25		Low		High	No	Yes	No
26		Low		Medium	Yes	Yes	Yes
27		Low		Low	Yes	Yes	Yes
28		High	B/Yamagata	High	Yes	No	No
29		High		Medium	Yes	No	No
30		High		Low	Yes	No	No
31		Medium		High	Yes	Yes	Yes
32		Medium		Medium	Yes	Yes	Yes
33		Medium		Low	Yes	No	No
34		Low		High	No	Yes	No
35		Low		Medium	Yes	Yes	Yes
36		Low		Low	Yes	Yes	Yes
37	A/H3N2	High	A/H11N3	High	Yes	Yes	Yes
38		High		Medium	Yes	No	No
39		High		Low	Yes	No	No
40		Medium		High	No	Yes	No
41		Medium		Medium	Yes	Yes	Yes
42		Medium		Low	Yes	No	No
43		Low		High	No	Yes	No
44		Low		Medium	No	Yes	No
45		Low		Low	Yes	Yes	Yes
46		High	B/Victoria	High	Yes	Yes	Yes
47		High		Medium	Yes	Yes	Yes
48		High		Low	Yes	No	No
49		Medium		High	Yes	Yes	Yes

50		Medium		Medium	Yes	Yes	Yes
51		Medium		Low	Yes	No	No
52		Low		High	No	Yes	No
53		Low		Medium	No	Yes	No
54		Low		Low	Yes	Yes	Yes
55		High		High	Yes	No	No
56		High		Medium	Yes	No	No
57		High		Low	Yes	No	No
58		Medium		High	Yes	Yes	Yes
59		Medium	B/Yamagata	Medium	Yes	Yes	Yes
60		Medium		Low	Yes	No	No
61		Low		High	No	Yes	No
62		Low		Medium	No	Yes	No
63		Low		Low	Yes	Yes	Yes
64		High		High	Yes	Yes	Yes
65		High		Medium	Yes	Yes	Yes
66		High		Low	Yes	No	No
67		Medium		High	No	Yes	No
68		Medium	B/Victoria	Medium	Yes	Yes	Yes
69		Medium		Low	Yes	No	No
70		Low		High	No	Yes	No
71		Low		Medium	No	Yes	No
72	A/H11N3	Low		Low	No	Yes	No
73		High		High	Yes	Yes	Yes
74		High		Medium	Yes	Yes	Yes
75		High		Low	Yes	No	No
76		Medium		High	No	Yes	No
77		Medium	B/Yamagata	Medium	Yes	Yes	Yes
78		Medium		Low	Yes	No	No
79		Low		High	No	Yes	No
80		Low		Medium	No	Yes	No
81		Low		Low	No	Yes	No
82		High		High	Yes	No	No
83		High		Medium	Yes	No	No
84		High		Low	Yes	No	No
85		Medium		High	No	Yes	No
86	B/Victoria	Medium	B/Yamagata	Medium	Yes	No	No
87		Medium		Low	Yes	No	No
88		Low		High	No	Yes	No
89		Low		Medium	No	Yes	No
90		Low		Low	No	No	No

The FC8G assay resulted in a correct dual infection identification for 33% (30/90) of samples analyzed, and correctly identified at least one of the two viruses in the sample 66% (59/90) of the time. One sample, containing B/Victoria and B/Yamagata at low concentrations, resulted in an “influenza B detected (no lineage)” result.

Results of this mock dual infection testing, organized by relative concentrations of the two viruses, are presented in Table 14 below.

Table 14: Results of the Mock Dual Infection Testing, Organized by Relative Concentrations of the Two Viruses

Relative Concentration	Concentration (Source 1 + Source 2)	Total # of Samples Tested	Correctly Identified as Dual Infection		Correctly Identified a Single Infection Only		Identified Influenza Type Only	
			#	%	#	%	#	%
Equal Viral Load	High + High	10	5	50%	5	50%	0	0%
	Medium + Medium	10	8	80%	2	20%	0	0%
	Low + Low	10	6	60%	3	30%	1	10%
Equal Viral Loads Summary		30	19	63%	10	33%	1	3%
Unequal Viral Load	High + Medium	20	9	45%	11	55%	0	0%
	Medium + Low	20	2	10%	18	90%	0	0%
	High + Low	20	0	0%	20	100%	0	0%
Different Viral Loads Summary		60	11	18%	49	82%	0	0%

This analysis showed that samples for which both viruses were at the same concentration had a 63% rate of correct dual infection identification. This was significantly higher than the 18% rate of correct dual identification when samples contained viruses at two different concentrations. Regarding the samples that contained two viruses at different relative concentrations, 82% of samples resulted in a correctly identified single infection result by the FC8G assay only.

An additional analysis of samples with different viral concentrations that resulted in a correctly identified single infection only by the FC8G assay is presented in Table 15 below.

Table 15: Analysis of Samples with Unequal Viral Concentrations that Resulted in a Correctly Identified Single infection Only by the FC8G Assay

Relative Concentrations	# of Single Infections Identified Correctly from Table 14	"High" Correctly Identified	"Medium" Correctly Identified	"Low" Correctly Identified
High + Medium	11	11 (100%)	0 (0%)	N/A
Medium + Low	18	N/A	18 (100%)	0 (0%)
High + Low	20	20 (100%)	N/A	0 (0%)

This analysis showed that when the samples contained two viruses at unequal relative concentrations, the virus at higher concentration was identified correctly 100% of the time.

Furthermore, an analysis of each co-infection by influenza subtype and lineage is presented in Table 16 below.

Table 16: Analysis of Co-infections by Influenza Subtype and Lineage

Co-Infection by Subtype or Lineage		Dual Infection Correctly Identified	Correctly Identified Single Infection Only	Identified Influenza Type Only
Same Influenza Type or Lineage	B/Victoria + B/Yamagata	0	8	1
	A/H1N1pdm09 + A/H3N2	0	9	0
	A/H1N1pdm09 + A/H11N3	3	6	0
	A/H11N3 + A/H3N2	3	6	0
Same Influenza Type or Lineage		6 (17%)	29 (81%)	1 (3%)
Different Influenza Types or Lineage	A/H1N1pdm09 + B/Victoria	6	3	0
	A/H1N1pdm09 + B/Yamagata	4	5	0
	A/H3N2 + B/Victoria	5	4	0
	A/H3N2 + B/Yamagata	3	6	0
	A/H11N3 + B/Victoria	3	6	0
	A/H11N3 + B/Yamagata	3	6	0
Different Influenza Type or Lineage		24 (44%)	30 (56%)	0 (0%)

This analysis demonstrated that the FC8G assay was less likely to identify a dual infection on samples containing viruses from the same influenza type or lineage (17%) compared to a co-infection of samples including one influenza A and one influenza B virus (44%). Further, a co-infection of both influenza B lineages (B/Victoria + B/Yamagata), as well as a co-infection of both the seasonal influenza A viruses (A/H1N1pdm09 + A/H3N2) resulted in zero dual infections correctly identified. The only dual infections identified in co-infection of the same influenza virus types were influenza A seasonal viruses mixed with an influenza A non-seasonal virus (either A/H1N1pdm09 or A/H3N2 with A/H11N3) in 33% of such samples tested.

h. Potentially Interfering Substances Study

The effects of 17 potentially interfering substances that may be encountered in respiratory specimens were assessed in an analytical study. To evaluate the potential for interfering substances to cause false negative results in influenza positive samples, one representative virus strain from each of the five target virus groups, A/California/07/2009 (A/H1N1pdm09), A/Perth/16/2009 (A/H3N2), B/Florida/02/2006 (B/Victoria), B/Wisconsin/01/2010 (B/Yamagata), and A/Chicken/NY/SG-00425/2004 (A/H2N2, non-seasonal influenza A), was analyzed in triplicate at approximately 5x the LoD in the presence of the interferent. Samples were prepared by spiking each potentially interfering substance into a contrived specimen of pooled influenza negative clinical material containing one of the virus strains. To evaluate the potential for false positive results due to the presence of potentially interfering substances in influenza negative samples, representative influenza negative samples were analyzed in triplicate in the presence of each substance.

If a substance was found to interfere in an influenza positive sample (i.e., caused a false negative result), samples were prepared with the interferent at a lower

concentration and retested until a concentration of interferent was tested that resulted in the correct/expected result for all three replicates. If a substance was found to interfere in an influenza negative sample (i.e., caused a false positive result), the existing extracted sample was re-analyzed in triplicate to assure reproducible results. Any substance that resulted in consistent false positive or false negative results are reported as an interfering substance with the FC8G assay.

The results of the study are summarized in Table 17 below.

Table 17: Summary Results of the Interfering Substances Study

Substance	Active Interferent	Concentration Tested	# of Correct FC8G Assay Results					
			A/H1N1 pdm09	A/H3N2	B/ Victoria	B/ Yamagata	A/H2N2	Influenza Negative Samples
Mucin	Mucin	0.1 mg/mL	3/3	3/3	3/3	3/3	3/3	3/3
Blood	Blood	0.25% v/v	3/3	3/3	3/3	3/3	3/3	3/3
Chloraseptic Cough Drops	Benzocaine, Menthol	1.0 mg/mL (w/v of drop)	3/3	3/3	3/3	3/3	3/3	3/3
Halls Cough Drops	Menthol	1.0 mg/mL (w/v of drop)	3/3	3/3	3/3	3/3	3/3	3/3
Neo-Synephrine nasal spray	Phenylephrine	10% v/v	3/3	3/3	3/3	3/3	3/3	3/3
Anefrin nasal spray	Oxymetaxoline	10% v/v	3/3	3/3	1/3[a]	0/3[a]	3/3	3/3
		5% v/v	N/A	N/A	3/3	3/3	N/A	N/A
Saline Nasal Spray	Sodium Chloride	10% v/v	3/3	3/3	3/3	3/3	3/3	3/3
Tobramycin	Tobramycin	0.2 mg/mL	3/3	3/3	3/3	3/3	3/3	3/3
Mupirocin	Mupirocin	0.1 mg/mL	3/3	3/3	3/3	3/3	3/3	3/3
Beclomethasone	Beclomethasone	16 µg/ml	3/3	3/3	3/3	3/3	3/3	3/3
Dexamethasone	Dexamethasone	0.1 mg/ml	3/3	3/3	3/3	3/3	3/3	3/3
Allergy Nasal Spray	Triamcinolone	1 % v/v	3/3	3/3	3/3	3/3	3/3	3/3
Mometasone	Mometasone	5 µg/mL	3/3	3/3	3/3	3/3	3/3	3/3
Budesonide Nasal Spray	Budesonide	25 µg/mL	3/3	3/3	3/3	3/3	3/3	2/3[b]
Zicam	Galphimia Glauca, Luffa Operculata, Sabadilla	5% v/v	3/3	3/3	3/3	3/3	3/3	3/3
Zanamivir	Zanamivir	0.1 mg/mL	3/3	3/3	2/3[c]	3/3	3/3	3/3
FluMist	FluMist	0.1% v/v	3/3	3/3	3/3	3/3	3/3	0/3[d]

[a] Resulted in false negative results.
[b] One false positive result for seasonal A/H3N2, but was not reproducible upon retesting of three additional replicates.
[c] One dual positive call for B/Victoria and A/H1N1pdm09, but the A/H1N1pdm09 false positive result was not reproducible upon retesting of three additional replicates.
[d] 3/3 false positive results for seasonal A/H1N1pdm09.

The study results showed that no interference with the FC8G assay was observed in the presence of potentially interfering substances at the concentrations tested in this study with the following exceptions:

Anefrin nasal spray interfered with the FC8G assay by resulting in false negatives on both B/Yamagata and B/Victoria samples at a concentration of 10% (v/v). The influenza B virus samples were reanalyzed in the presence of 5% (v/v) Anefrin nasal

spray, and were correctly identified in all three replicates. In addition, as expected, FluMist (an attenuated live intranasal vaccine) interfered with the assay by causing false positive results in influenza negative samples. Limitations regarding these observations were developed and included in the assay Instructions for Use (IFU).

i. Carry-Over Study

Potential sample-to-sample carry-over contamination was investigated by analyzing influenza positive samples at high concentration (>10^6 approximate genome copies/mL) next to pooled clinical negative samples in a single run, and conducting the microarray hybridization portion of the assay in a checkerboard pattern of alternating positives and negatives. Run-to-run carry-over contamination was evaluated by executing a run of the alternating positive/negative samples used for the sample-to-sample carry-over contamination testing followed by a run containing only influenza negatives to assess risk of carry-over. This cycle of testing was repeated three times. An A/H3N2 virus strain A/Uruguay/716/2007 was utilized to prepare positive samples in this testing. The influenza negative sample tested was pooled influenza negative clinical material.

In addition, as an alternative assessment of sample-to-sample contamination during hybridization, a mock hybridization study was performed. Two solutions, one containing blue dye and one without dye, were added to eight FluChip-8G Microarray Slides in an alternating, checkboard pattern (i.e., 64 wells with dye, 64 wells without dye). The FluChip-8G Microarray Slides were then placed on orbital shaker at 125-140 RPM for one hour to mimic the hybridization step. Triplicate absorbance measurements were then taken of the solution in all wells without dye to determine whether any dye contaminated neighboring wells.

The FC8G assay results from the carry-over contamination study are in Table 18 below.

Table 18: Carry-over Contamination Study Results

		Number of Correct FC8G Assay Calls			**Total**
	Sample	**Round 1**	**Round 2**	**Round 3**	
Sample-to-Sample Contamination	A/H3N2	14/14	13/14*	12/14*	39/39 (100%)
	Negative	14/14	14/14	13/14**	41/42 (98%)
Run-to-Run Carry-over	Negative	14/15**	15/15	15/15	44/45 (98%)

* Three A/H3N2 samples resulted in "image processing failure," and were removed from final analysis.
** Resulted in 1 FP for A/H1N1pdm09.

Three out of 42 A/H3N2 samples analyzed during the study resulted in image processing failures and were removed from analysis. All other 39 high positive H3N2 samples resulted in the expected result. While two pooled clinical negative samples did produce a false positive result for A/H1N1pdm09, follow up analysis of these

RNA extracts using an FDA-cleared influenza molecular assay resulted in no influenza detected indicating no evidence of cross-contamination during extraction. It is expected that these two incidents are not systematic, as this rate of false positivity is consistent with assay performance in other studies. The possibility does exist, however, that this was due to cross-contamination during the microarray processing steps.

The mock hybridization analysis resulted in none of the wells having absorbance measurements that were significantly above background, indicating no cross-contamination event during microarray hybridization.

There was no evidence of systematic sample-to-sample carryover or run-to-run cross contamination. However, any open platform assay based on PCR and endpoint detection has the potential for carry-over and/or cross-contamination if careful measures to avoid contamination are not taken. Therefore, the FC8G assay IFU includes the warnings and limitations language below which stresses the importance of good technique to obtain proper results:

"*Limit use of this product to personnel trained in the techniques of PCR-based molecular diagnostic assays. PCR-based assays require effort by the operator to reduce potential for cross-contamination during all steps of the procedure, including: nucleic acid extraction, transfer of nucleic acid, and RT-PCR reaction preparation. In addition, care should be taken not to introduce cross-contamination during the microarray hybridization step.*"

"Laboratory workflow should proceed in a unidirectional manner, starting with nucleic acid extraction and ending with microarray detection. Change gloves whenever contamination is suspected. Use dedicated areas and separate supplies/equipment for pre and post amplification processes."

j. Assay cut-off

The threshold value or cutoff for each of the neural networks was selected separately and subsequently to the neural network training and architecture lock down in order to optimize the performance of the neural network over a given set of samples. Initially, the entire training database of samples was used as the set over which performance was optimized. The thresholds were then determined for each neural network as the value which would minimize the total number of errors (false positives plus false negatives) over this dataset.

It was later observed that the dataset used to select the thresholds differed significantly from what would be expected with real clinical data. The training dataset had a much higher percentage of influenza-positive samples than influenza-negative samples. This was good for training the various influenza-positive patterns, but resulted in a higher False Negative/False Positive ratio than would be expected in real-world testing. In addition, the training dataset included many samples processed as part of dilution

series used to determine the limit of detection, and thus had a much higher percentage of influenza-positive samples below the limit of detection than would be expected in real-world data. Identifying these samples as true positive rather than false negative required lower thresholds. These biases in the dataset used to determine the original thresholds biased them toward sensitivity over specificity.

To optimize the thresholds for real-world testing, a set of 910 training naïve clinical samples, consists of 251 influenza A positive samples, 163 influenza B positive samples, and 496 influenza negative samples, was compiled from an existing in-house archive comprised of material received from various vendors. The clinical samples were processed by the FC8G assay and used to revise the thresholds. These samples were of unknown concentration, and thus considered to be clinically-representative of both influenza concentrations and human background material. This set of 910 samples was entirely independent from the clinical study data presented in the Clinical Studies section. A Receiver Operating Characteristic (ROC) method was used to optimize the thresholds.

The revised and optimized thresholds were further validated during the prospective clinical study conducted between June 2017 and July 2018.

2. Comparison studies:

   a. Method comparison with predicate device:

   Not applicable.

   b. Matrix comparison :

   Not applicable.

3. Clinical Studies :

   Prospective Clinical Study

   Clinical performance characteristics of the FC8G assay were evaluated in a multi-center clinical study between June 2017 and July 2018 in the U.S.

   Nasal swab (NS) or nasopharyngeal swab (NPS) specimens were prospectively collected between 2013-2017 from consented patients meeting a febrile respiratory illness (FRI) case definition. Patients were enrolled at seven geographically distributed clinical sites across the United States under a standing influenza surveillance protocol. Upon specimen collection, the nasal swab and nasopharyngeal swab specimens were stabilized in Copan Universal Transport Media (UTM) and shipped frozen to a reference laboratory as part of this ongoing influenza surveillance effort. Remaining volume of each specimen was archived and stored at ≤ -70°C at the reference laboratory.

To assess the clinical performance of the FC8G assay, the prospectively collected archived specimens were thawed and tested with an FDA-cleared influenza molecular assay (the comparator assay) by the reference laboratory, and then blinded and shipped to one of the three participating testing sites. Each clinical study testing site performed the FC8G assay on approximately one third of the blinded specimens. After all specimens were tested with the FC8G assay, the specimens were un-blinded and the FC8G assay result was compared to the comparator assay result for each specimen. Specimens with discordant results were reflex tested using bi-directional sequencing of the hemagglutinin (HA) and neuraminidase (NA) genes.

Of the 1065 subjects enrolled in this study, there were seven specimens excluded from the performance analyses due to deviation from the study protocol, leaving a total of 1058 prospectively collected swab specimens to be included in the evaluation of the assay performance. There were an additional 36 specimens that did not generate a final result with the FC8G assay due to insufficient sample volume as a result of an external batch control failure (user error) during initial or retesting efforts, resulting in a total of 1022 specimens with a final valid FC8G assay result.

Patient age and gender distribution for all the 1022 prospective specimens (700 NS and 322 NPS specimens) are presented in Table 19 below.

Table 19: Patient Demographics - Prospective Clinical Study

Age Group	**Number**	**Percent of Patients**
≤ 5 years	432	42.3%
6 to 21 years	299	29.2%
22 to 59 years	230	22.5%
≥ 60 years	61	6.0%
Total	1022	100%
Sex	**Number**	**Percent of Patients**
Male	467	45.7%
Female	552	54.0%
Not Reported	3	0.3%
Total	1022	100%

Of the total of 1022 samples with a final valid FC8G assay result, 26 specimens resulted in a failure of the FC8G assay internal control to amplify above the threshold value, i.e., “No Call: Assay Failure (Internal Control)”, and were excluded from the performance analyses. In addition, there were 12 specimens that resulted in a comparator assay result of “inconclusive” due to internal control failures, and were also excluded from the performance analyses.

The performance of the FC8G assay testing the remaining 984 specimens (664 NS and 320 NPS specimens) against the comparator assay is presented in Table 20 below.

Table 20: FC8G Assay Performance Testing NS and NPS Specimens against the Comparator Assay – Prospective Clinical Study

Virus Group (Analyte)	Sensitivity			Specificity		
	TP/(TP+FN)	%	95% CI	TN/(TN+FP)	%	95% CI
Influenza A (Overall)	51/58	87.9%	77.1 – 94.0	915/926	98.8%	97.9 – 99.3
Influenza A/H1N1pdm09	3/3	100.0%	43.9 – 100.0	975/981	99.4%	98.7 – 99.7
Influenza A/H3N2	48/55	87.2%	76.0 – 93.7	924/929	99.5%	98.7 99.8
Influenza A/Non-seasonal	0/0	--	--	983/984 *	99.9%	99.4 – 100.0
Influenza B(Overall)	22/22	100.0%	85.1 – 100.0	960/962	99.8%	99.2 – 99.9
Influenza B/Victoria	14/14	100.0%	78.5 – 100.0	970/970	100.0%	99.6 – 100.0
Influenza B/Yamagata	8/8	100.0%	67.6 – 100.0	976/976	100.0%	99.6 – 100.0

*One dual infection was detected by the FC8G assay, resulting in both an influenza A/H3N2 call and influenza A/non-seasonal call. Flu A/H3N2 only was detected by the comparator. This was the only FC8G assay false positive for non-seasonal influenza A observed during the study.

The performance of the FC8G assay against the comparator assay stratified by patient age groups are presented in Table 21 below.

Table 21: FC8G Assay Performance Testing NS and NPS Specimens against the Comparator Assay Stratified by Patient Age Groups – Prospective Clinical Study

Influenza Virus Group	≤ 5 Years of Age (n=417)		6 to 21 Years of Age (n=278)		≥ 22 Years of Age (n=289)	
Influenza A (Overall)	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI
	90.0%	98.3%	93.3%	98.4%	77.8%	100.0%
	(9/10)	(400/407)	(28/30)	(244/248)	(14/18)	(271/271)
	59.6% - 98.2%	96.5% -99.2%	78.7% - 98.2%	95.9% - 99.4%	54.8% - 91.0%	98.6% - 100.0%
Influenza A/H1N1pdm09	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI
	0%	99.0%	100.0%	99.3%	100.0%	100.0%
	(0/0)	(413/417)	(1/1)	(275/277)	(2/2)	(287/287)
	N/A	97.6% - 99.6%	20.7% - 100.0%	97.4% - 99.8%	34.2% -100.0%	98.7% - 100.0%
Influenza A/H3N2	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI
	90.0%	99.3%	93.1%	99.2%	75.0%	100.0%
	(9/10)	(404/407)	(27/29)	(247/249)	(12/16)	(273/273)
	59.6% - 98.2%	97.9% - 99.7%	78.0% - 98.1%	97.1% - 99.8%	50.5% - 89.8%	98.6% - 100.0%
Influenza A/Non-seasonal	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI
	0%	100.0%	0%	99.6%	0%	100.0%
	(0/0)	(417/417)	(0/0)	(277/278)	(0/0)	(289/289)
	N/A	99.1% - 100.0%	N/A	98.0% - 99.9%	N/A	98.7% - 100.0%
Influenza B (Overall)	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI
	100.0%	100.0%	100.0%	99.3%	100.0%	100.0%
	(7/7)	(827/827)	(10/10)	(266/268)	(5/5)	(284/284)
	64.6% - 100.0%	99.5% - 100.0%	72.2% - 100.0%	97.3% - 99.8%	56.6% - 100.0%	98.7% - 100.0%

Influenza B/Victoria	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI
	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	(6/6)	(411/411)	(6/6)	(272/272)	(2/2)	(287/287)
	61.0% - 100.0%	99.1% - 100.0%	61.0% - 100.0%	98.6% - 100.0%	34.2% - 100.0%	98.7% - 100.0%
Influenza B/Yamagata	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI
	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	(1/1)	(416/416)	(4/4)	(274/274)	(3/3)	(286/286)
	20.7% - 100.0%	99.1% - 100.0%	51.0% - 100.0%	98.6% - 100.0%	56.6% - 100.0%	98.7% - 100.0%

Compared to the comparator assay, the performance of the FC8G assay in this prospective clinical study was also analyzed by testing site, and by specimen type (NS vs. NPS), demonstrating similar performance across the three testing sites and across the two specimen types.

The invalid rate observed during this clinical study was 5.9% (62/1058), 95% CI: 4.6% - 7.4%. This rate includes 26 specimens for which the internal control did not produce a result (a “no call” invalid result), and 36 specimens for which inadequate specimen volume existed to generate a final valid result due to an external batch control failure (user error) either during initial or recommended retesting.

One dual infection was detected by the FC8G assay during this study, resulting in both a Flu A/H3N2 call and a Flu A/non-seasonal call by the FC8G assay. A Flu A/H3 result was generated by the comparator assay. This specimen was retested in accordance with the follow-up recommendations in the FC8G assay instructions for use. Upon retest, this specimen produced the same dual infection result. Reflexive bi-directional sequencing for this specimen produced a result of Influenza A/H3N2, matching the comparator assay result.

Supplemental Clinical Study

As the prevalence of influenza in the prospective clinical study was expected to be lower than needed to obtain sufficient numbers of influenza positive specimens to establish FC8G assay performance, archived nasopharyngeal swab (NPS) specimens that were prospectively collected from 500 subjects from November 16, 2014 to December 13, 2014 and from January 28, 2016 to April 6, 2016, were acquired from a vendor to be enrolled in a supplemental clinical study. These NPS specimens represented all specimens archived during a defined period that met the same inclusion criteria as the prospective clinical study described previously, and were available in the vendor’s possession with a sufficient volume.

The acquired archived specimens were thawed and tested with an FDA-cleared influenza molecular assay (the comparator assay) by the reference laboratory, and then blinded and shipped to one of the three participating testing sites. Each clinical study testing site performed the FC8G assay on approximately one third of the blinded specimens. After all

specimens were tested with the FC8G assay, the specimens were un-blinded and the FC8G assay result was compared to the comparator assay result for each specimen. Specimens with discordant results were reflex tested using bi-directional sequencing of the hemagglutinin (HA) and neuraminidase (NA) genes.

Patient age and gender distribution for all the 500 NPS specimens are presented in Table 22 below.

Table 22: Patient Demographics – Supplemental Clinical Study

Age Group	**Number**	**Percent of Patients**
≤ 5 years	224	44.8%
6 to 21 years	274	54.8%
22 to 59 years	2	0.4%
≥ 60 years	0	0.0%
Total	500	100%
Sex	**Number**	**Percent of Patients**
Male	235	47.0%
Female	265	53.0%
Total	500	100%

The performance of the FC8G assay testing the 500 NPS specimens against the comparator assay is presented in Table 23 below.

Table 23: FC8G Assay Performance Testing NPS Specimens against the Comparator Assay – Supplemental Clinical Study

Virus Group (Analyte)	**Sensitivity**			**Specificity**		
	TP/(TP+FN)	**%**	**95% CI**	**TN/(TN+FP)**	**%**	**95% CI**
Influenza A (Overall)*	189/204	92.6%	88.2 – 95.5	293/296	98.9%	97.1 – 99.7
Influenza A/H1N1pdm09	24/26	92.3%	75.9 – 97.9	469/474	98.9%	97.6 – 99.5
Influenza A/H3N2	163/175	93.1%	88.4 – 96.0	325/325	100.0%	98.8 – 100.0
Influenza A/Non-seasonal	0/0	--	--	500/500	100.0%	99.2 – 100.0
Influenza B (Overall)	52/53	98.1%	90.1 – 99.7	444/447	99.3%	98.0 – 99.8
Influenza B/Victoria	46/46	100.0%	92.3 – 100.0	452/454	99.6%	98.4 – 99.9
Influenza B/Yamagata	6/7	85.7%	48.7 – 97.4	492/493	99.8%	98.9 – 100.0

* Influenza A (Overall) category includes two specimens that resulted in a FC8G assay result of "Influenza AH1N1pdm09". The comparator assay generated a result of "Influenza A (Equivocal), No subtyping". Both samples were correctly identified as influenza A by the FC8G assay, therefore, counted as true positives in the Influenza A (Overall) category, but were both counted as false positives for the Influenza A/H1N1pdm09 subtype.

The performance of the FC8G assay against the comparator assay in the supplemental clinical study stratified by patient age groups are presented in Table 24 below.

Table 24: FC8G Assay Performance Testing NS Specimens against the Comparator Assay Stratified by Patient Age Groups – Supplemental Clinical Study

Influenza Virus Group	≤ 5 Years of Age (n=224)		6 to 21 Years of Age (n=274)		≥ 22 Years of Age (n=2)	
Influenza A (Overall)	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI
	92.3%	100.0%	92.8%	97.8%	0.0%	100.0%
	(60/65)	(159/159)	(129/139)	(132/135)	(0/0)	(2/2)
	83.2% - 96.7%	97.6% - 100.0%	87.3% - 96.0%	93.7% - 99.2%	N/A	34.2% - 100.0%
Influenza A/H1N1pdm09	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI
	81.8%	99.5%	100.0%	98.5%	0.0%	100.0%
	(9/11)	(212/213)	(15/15)	(255/259)	(0/0)	(2/2)
	52.3% - 94.9%	97.4% - 99.9%	79.6% - 100.0%	96.1% - 99.4%	N/A	34.2% - 100.0%
Influenza A/H3N2	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI
	94.2%	100.0%	92.6%	100.0%	100.0%	0.0%
	(49/52)	(172/172)	(112/121)	(153/153)	(2/2)	(0/0)
	84.4% - 98.0%	97.8% - 100.0%	86.5% - 96.0%	97.6% - 100.0%	34.2% - 100.0%	N/A
Influenza A/Non-seasonal	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI
	0.0%	100.0%	0.0%	100.0%	0.0%	100.0%
	(0/0)	(224/224)	(0/0)	(274/274)	(0/0)	(2/2)
	N/A	98.3% - 100.0%	N/A	98.6% - 100.0%	N/A	34.2% - 100.0%
Influenza B (Overall)	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI
	92.3%	99.5%	100.0%	99.1%	0.0%	100.0%
	(12/13)	(210/211)	(40/40)	(232/234)	(0/0)	(2/2)
	66.7% - 98.6%	97.4% - 99.9%	91.2% - 100.0%	96.9% - 99.8%	N/A	34.2% - 100.0%
Influenza B/Victoria	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI
	100.0%	99.5%	100.0%	99.6%	0.0%	100.0%
	(10/10)	(213/214)	(36/36)	(237/238)	(0/0)	(2/2)
	72.2% - 100.0%	97.4% - 99.9%	90.4% - 100.0%	97.7% - 99.9%	N/A	34.2% - 100.0%
Influenza B/Yamagata	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI	Sensitivity 95% CI	Specificity 95% CI
	66.7%	100.0%	100.0	99.6%	0.0%	100.0%
	(2/3)	(221/221)	(4/4)	(269/270)	(0/0)	(2/2)
	20.8% - 93.9%	98.3% - 100.0%	51.0% - 100.0%	97.9% - 99.9%	N/A	34.2% - 100.0%

Compared to the comparator assay, the performance of the FC8G assay in this supplemental clinical study was also analyzed by testing site. The FC8G assay demonstrated similar performance across the three testing sites.

No invalid result or dual infection result was generated by the FC8G assay during this study.

Retrospective Clinical Studies

A retrospective clinical study was conducted to supplement the prospective performance studies, since there were low numbers of positive specimens for A/H1N1pdm09 and

B/Yamagata in the prospectively collected cohorts. A total of 98 retrospective blinded NPS or NS specimens, collected from 2010 to 2018, were analyzed by the FC8G assay against the same comparator assay utilized in the prospective clinical studies, to increase the number of influenza positive specimens per target virus group. One specimen was removed from the performance analysis due to an internal control failure in the FC8G assay, resulting in 97 specimens being included in the performance analysis.

The performance of the FC8G assay testing the 97 NS or NPS retrospective specimens against the comparator assay is presented in Table 25 below.

Table 25: FC8G Assay Performance Testing NS or NPS Specimens against the Comparator Assay – Retrospective Clinical Study #1

Virus Group (Analyte)	Sensitivity			Specificity		
	TP/(TP+FN)	%	95% CI	TN/(TN+FP)	%	95% CI
Influenza A (Overall)*	24/25	96.0%	80.5 – 99.3	72/72	100.0%	94.9 – 100.0
Influenza A/H1N1pdm09	24/24	100.0%	86.2 – 100.0	73/73	100.0%	95.0 – 100.0
Influenza A/H3N2	0/0	--	--	97/97	100.0%	96.2 – 100.0
Influenza A/Non-seasonal	0/0	--	--	97/97	100.0%	96.2 – 100.0
Influenza B (Overall)	63/65	96.9%	89.5 – 99.2	32/32	100.0%	89.3 – 100.0
Influenza B/Victoria	0/0	--	--	97/97	100.0%	96.2 – 100.0
Influenza B/Yamagata	62/64	96.8%	89.3 – 99.1	33/33	100.0%	89.6 – 100.0

* Influenza A (Overall) category includes one specimen that resulted in a FC8G assay result of "Influenza B/Yamagata". The comparator assay generated a result of "Influenza A (Equivocal), No subtyping" and "Influenza B/Yamagata". This sample was counted as a false negative in the Influenza A (Overall) category, but was counted as a true positive for the Influenza B (Overall) and the Influenza B/Yamagata categories.

An additional retrospective clinical study was also conducted to supplement the prospective performance studies, since there were lower than expected numbers of specimens that were obtained from patients 65 years of age or older in the prospectively collected cohorts. One hundred and ten (110) NPS specimens were sourced retrospectively to increase the number of specimens in the 65+ age bracket. All these retrospective specimens for the 65+ age group were collected between 2016 and 2018, with 51 specimens collected from males and 59 collected from females. The specimens were analyzed by the FC8G assay against the same comparator assay utilized in the prospective clinical studies. Two specimens resulted in an assay failure by either the FC8G assay or the comparator assay and were not included in the performance analysis. The performance of the FC8G assay on the 108 retrospective NPS samples with a valid result for the 65+ age bracket is shown in Table 26 below.

Table 26: FC8G Assay Performance Testing NPS Specimens against the Comparator Assay – Retrospective Clinical Study #2

Virus Group (Analyte)	Sensitivity			Specificity		
	TP/(TP+FN)	%	95% CI	TN/(TN+FP)	%	95% CI
Influenza A (Overall)	17/18	94.4%	74.2 – 99.0	90/90	100.0%	95.9 – 100.0
Influenza A/H1N1pdm09	3/3	100.0%	43.9 – 100.0	105/105	100.0%	96.5 – 100.0
Influenza A/H3N2	14/15	93.3%	70.2 – 98.8	93/93	100.0%	96.0 – 100.0
Influenza A/Non-seasonal	0/0	--	--	108/108	100.0%	96.6 – 100.0
Influenza B (Overall)	11/12	91.6%	64.6 – 98.5	96/96	100.0%	96.2 – 100.0
Influenza B/Victoria	0/0	--	--	108/108	100.0%	96.6 – 100.0
Influenza B/Yamagata	11/12	91.6%	64.6 – 98.5	96/96	100.0%	96.2 – 100.0

Non-Seasonal Influenza A Virus Challenge Study

Due to the rarity of non-seasonal influenza A infections in humans and therefore the lack of non-seasonal influenza A in the prospectively collected clinical study cohorts or in the retrospective specimens, a FC8G assay non-seasonal influenza A challenge study was executed.

The non-seasonal influenza A challenge study was performed testing 220 blinded surrogate clinical samples representing 41 unique influenza A subtypes, including an equal number of influenza positive and influenza negative samples. One hundred (100) surrogate clinical samples were contrived by spiking each of the 100 unique non-seasonal influenza A strains into unique individual clinical negative NS or NPS collected in VTM (i.e., influenza negative clinical specimen from a single patient). The 100 non-seasonal strains were selected to represent diversity in host-origin and subtype, with a focus on subtypes with pandemic potential. Fifty (50) of the 100 unique non-seasonal influenza A viruses were tested with the FC8G assay at concentrations from approximately 0.5x to 3x LoD, and the other 50 non-seasonal influenza A viruses were analyzed at concentrations greater than 3x LoD (see Table 27 below). For safety reasons, all H5 and H7 subtypes were utilized as extracted RNA and not intact whole virus. To reduce potential bias, 10 contrived surrogate clinical specimens from seasonal target virus groups, and 110 influenza-negative clinical specimens were also included in the sample set. All specimens included in this study were blinded to the analyst, and all testing occurred at a single site.

Overall, this challenge study included testing 62 non-seasonal influenza A strains representing subtypes that were included in the training set. The challenge study also included testing 38 non-seasonal influenza A strains that were not included in the training set (i.e., naïve to training) as a challenge to the assay. All the 100 non-seasonal influenza A strains tested in this challenge study were contrived in unique individual influenza negative clinical specimens, and therefore, had a unique clinical background compared to other analyses of the FC8G microarray images generated by testing the same strain that may have been included in training.

Table 27: Non-Seasonal Influenza A Virus Strains Included in the Challenge Study

Strain	Host of Isolation	Subtype	Material Input into Extraction	Concentration (approximate genome copies/mL)	Approximate Multiples of LoD Tested
A/Canvasback/Alberta/276/2005	Avian	A/H1N1	Whole Virus	8.3×10^4	28x
A/Redheaded Duck/Minnesota/SG-00123/2007	Avian		Whole Virus	6.9×10^3	2x
A/Mallard/Republic of Georgia/4/2010	Avian		Whole Virus	2.5×10^5	84x
A/Duck/Alberta/35/1976	Avian		Viral Genomic RNA	2.9×10^4	10x
A/South Dakota/06/2007	Human	A/H1N1 pre-2009	Whole Virus	6.4×10^3	2x
A/Florida/03/2006	Human		Whole Virus	6.2×10^5	208x
A/Solomon Islands/3/2006	Human		Whole Virus	3.4×10^3	1x
A/Fukushima/141/2006	Human		Whole Virus	4.5×10^3	2x
A/St. Petersburg/8/2006	Human		Whole Virus	5.5×10^4	18x
A/Swine/Ohio/09SW1477/2009	Swine	A/H1N2	Whole Virus	6.7×10^4	23x
A/Swine/Ohio/09SW1484E/2009	Swine		Whole Virus	3.7×10^3	1x
A/Shorebird/Delaware Bay/211/1994	Avian	A/H1N3	Whole Virus	2.5×10^5	84x
A/Japan/305/1957	Human	A/H2N2	Viral Genomic RNA	8.9×10^3	3x
A/Duck/Germany/1215/1973	Avian	A/H2N3	Viral Genomic RNA	8.7×10^3	3x
A/Swine/North Carolina/32760/2007	Swine	A/H3N2sw	Viral Genomic RNA	6.3×10^3	2x
A/Swine/North Carolina/44897/2009	Swine		Viral Genomic RNA	5.4×10^3	2x
A/Swine/North Carolina/52796/2006	Swine		Viral Genomic RNA	4.0×10^3	1x
A/Swine/North Carolina/88708/2000	Swine		Viral Genomic RNA	3.7×10^3	1x
A/Indiana/10/2011	Avian	A/H3N2v	Whole Virus	2.2×10^5	74x
A/Michigan/20/2012	Human		Viral Genomic RNA	1.1×10^4	4x
A/Ohio/20/2012	Human		Viral Genomic RNA	7.1×10^3	2x
A/Ohio/36/2012	Human		Viral Genomic RNA	5.7×10^3	2x
A/Ohio/44/2012	Human		Viral Genomic RNA	5.5×10^3	2x
A/Blue-Winged Teal/Illinois/10OS1546/2010	Avian	A/H3N6	Whole Virus	1.3×10^5	42x
A/Redhead/Alberta/192/2002	Avian		Whole Virus	4.8×10^3	2x
A/Equine/Pennsylvania/1/2007	Equine	A/H3N8	Whole Virus	1.4×10^3	0.5x
A/Equine/Miami/1/1963	Equine		Whole Virus	1.4×10^3	0.5x
A/Duck/Chabarovsk/1610/1972	Avian		Whole Virus	1.6×10^3	0.5x
A/Duck/Ukraine/1963	Avian		Whole Virus	4.1×10^5	137x
A/Blue-Winged Teal/Alberta/346/2007	Avian	A/H4N3	Whole Virus	3.4×10^3	1x
A/Mallard/Alberta/35/2001	Avian	A/H4N6	Whole Virus	3.9×10^3	1x
A/Shorebird/Delaware/309/2008	Avian		Whole Virus	3.3×10^5	111x
A/Red Knot/Delaware/541/1988	Avian		Whole Virus	1.3×10^5	42x
A/Common Magpie/Hong Kong/645/2006	Avian	A/H5N1	Viral Genomic RNA	3.1×10^4	11x
A/Vietnam/1194/2004	Avian		Viral Genomic RNA	3.1×10^4	11x
A/Chicken/Egypt/M7217B/2013	Avian		Viral Genomic RNA	3.8×10^3	1x
A/Chicken/Egypt/Q1089E/2010	Avian		Viral Genomic RNA	8.6×10^3	3x
A/Cambodia/X0810301/13	Human		Viral Genomic RNA	4.2×10^3	1x
A/Duck/Bangladesh/19097/13	Avian		Viral Genomic RNA	4.4×10^3	2x
A/Egypt/N04915/2014	Human		Viral Genomic RNA	3.3×10^3	1x
A/Indonesia/NIHRD11771/2011	Human		Viral Genomic RNA	1.0×10^4	4x
A/Chicken/Bangladesh/11rs1984-30/2011	Avian		Viral Genomic RNA	3.6×10^3	1x
A/Duck/Pennsylvania/10218/1984	Avian	A/H5N2	Viral Genomic RNA	8.4×10^3	3x
A/Chicken/Vietnam/NCVD-14-A324/2014	Avian	A/H5N6	Viral Genomic RNA	2.4×10^4	8x
A/Shorebird/Delaware/101/2004	Avian	A/H5N7	Viral Genomic RNA	3.6×10^5	124x

A/Shorebird/Delaware Bay/230/2009	Avian	A/H6N1	Whole Virus	3.0×10^3	1x
A/Ruddy Turnstone/Delaware/293/2006	Avian	A/H6N2	Whole Virus	2.5×10^5	84x
A/Shorebird/Delaware/124/2001	Avian		Whole Virus	4.2×10^3	1x
A/Mallard/Alberta/203/1992	Avian	A/H6N5	Whole Virus	7.2×10^4	24x
A/Chicken/Italy/1285/2000	Avian	A/H7N1	Viral Genomic RNA	2.9×10^3	1x
A/Mallard/Alberta/34/2001	Avian		Viral Genomic RNA	2.0×10^5	68x
A/Mallard/Netherlands/12/2000	Avian	A/H7N3	Viral Genomic RNA	3.3×10^5	109x
A/Chicken/Jalisco/12283/12	Avian		Viral Genomic RNA	1.6×10^4	5x
A/Laughing Gull/Delaware Bay/42/2006	Avian		Viral Genomic RNA	3.1×10^6	1024x
A/Chicken/Chile/176822/2002	Avian		Viral Genomic RNA	4.1×10^5	133x
A/Ruddy Turnstone/Delaware Bay/290/2006	Avian	A/H7N4	Viral Genomic RNA	6.1×10^3	2x
A/Mallard/Alberta/26/2001	Avian	A/H7, N1, N3, N5*	Viral Genomic RNA	3.9×10^3	1x
A/Netherlands/33/2003	Avian	A/H7N7	Viral Genomic RNA	3.6×10^5	124x
A/Seal/Massachusetts/1/1980	Seal		Viral Genomic RNA	6.9×10^3	2x
A/Shanghai/1/2013	Human	A/H7N9	Viral Genomic RNA	4.8×10^4	16x
A/Mallard/Alberta/194/92	Avian	A/H8N4	Whole Virus	5.2×10^3	2x
A/Mallard Duck/Alberta/743/1983	Avian	A/H9N1	Whole Virus	3.0×10^3	1x
A/Shorebird/Delaware Bay/133/2002	Avian		Whole Virus	1.4×10^5	49x
A/Quail/Hong Kong/G1/1997	Avian	A/H9N2	Whole Virus	2.1×10^3	1x
A/Quail/Lebanon/272/2010	Avian		Viral Genomic RNA	4.7×10^3	2x
A/Chicken/Beijing/1/1994	Avian		Viral Genomic RNA	2.2×10^4	8x
A/Hong Kong/33982/2009	Human		Viral Genomic RNA	5.1×10^4	17x
A/Chukar/Shantou/22116/2005	Avian		Viral Genomic RNA	2.1×10^4	7x
A/Chicken/Hong Kong/NT10/2011	Avian		Viral Genomic RNA	5.5×10^4	18x
A/Duck/Hong Kong/Y280/1997	Avian		Whole Virus	2.3×10^5	79x
A/Hong Kong/308/14	Human		Viral Genomic RNA	7.0×10^6	2320x
A/Shorebird/Delaware Bay/246/2003	Avian	A/H9N5	Whole Virus	6.0×10^3	2x
A/Ruddy Turnstone/Delaware/510/1988	Avian	A/H9N6	Whole Virus	3.3×10^5	111x
A/Gray Plover/Chile/C1313/2015	Avian	A/H9N7	Whole Virus	4.4×10^5	147x
A/Shorebird/Delaware Bay/31/1996	Avian		Whole Virus	3.9×10^3	1x
A/Shorebird/Delaware Bay/277/2000	Avian		Whole Virus	4.2×10^3	1x
A/Ruddy Turnstone/Virginia/2297/1988	Avian	A/H9N9	Whole Virus	3.4×10^3	1x
A/Mallard/Wisconsin/4230/2009	Avian	A/H10N1	Whole Virus	2.9×10^5	97x
A/Shorebird/Delaware Bay/338/2009	Avian		Whole Virus	1.5×10^4	5x
A/Shorebird/Delaware Bay/63/1996	Avian	A/H10N2	Whole Virus	3.2×10^3	1x
A/Shorebird/Delaware/260/2000	Avian	A/H10N4	Whole Virus	1.2×10^5	39x
A/Chicken/Germany/N/49	Avian	A/H10N7	Whole Virus	3.0×10^3	1x
A/Mallard/Illinois/10OS4334/2010	Avian		Whole Virus	1.5×10^4	5x
A/Quail/Italy/1117/1965	Avian	A/H10N8	Whole Virus	5.1×10^4	17x
A/Shorebird/Delaware Bay/216/1999	Avian	A/H11N2	Whole Virus	2.5×10^5	84x
A/Ruddy Turnstone/Delaware Bay/39/1994	Avian	A/H11N3	Whole Virus	5.8×10^5	194x
A/Duck/England/56	Avian	A/H11N6	Whole Virus	3.9×10^3	1x
A/Mallard/Alberta/125/1999	Avian		Whole Virus	3.0×10^3	1x
A/Laughing Gull/Delaware/2/2002	Avian	A/H11N9	Whole Virus	4.8×10^3	2x
A/Shorebird/Delaware/6/2002	Avian		Whole Virus	5.4×10^5	181x
A/American Green-Winged	Avian		Whole Virus	2.2×10^5	74x
A/Common Goldeneye/Iowa/3192/2009	Avian		Whole Virus	1.4×10^5	49x
A/Mallard/Ohio/1688/2009	Avian	A/H12N5	Whole Virus	3.2×10^3	1x
A/Mallard/Wisconsin/4218/2009	Avian		Whole Virus	7.3×10^3	3x

A/Northern Pintail/Missouri/319/2009	Avian		Whole Virus	3.0×10^3	1x
A/Northern Shoveler/Mississippi/09OS025/2009	Avian		Whole Virus	3.7×10^3	1x
A/Ring-Billed Gull/Quebec/02434-1/2009	Avian	A/H13N6	Whole Virus	2.3×10^5	79x
A/Black-Legged Kittiwake/Quebec/02838-1/2009	Avian		Whole Virus	5.9×10^4	20x
A/Wedge-Tailed Shearwater/Western	Avian	A/H15N9	Whole Virus	3.7×10^3	1x
A/Shearwater/Australia/2576/79	Avian		Whole Virus	9.5×10^4	32x

* This sample is a mixed infection of A/H7N1, A/H7N3, and A/H7N5.

The non-seasonal influenza A strains utilized to build contrived specimens in this study were characterized by a combined method comprised of virus characterization provided on Certificates of Analysis (COA) for commercially obtained strains, sequencing information for the hemagglutinin (HA) and the neuraminidase (NA) genes, and/or an FDA-cleared influenza molecular assay. Performance of the FC8G assay in this study was assessed against the expected results of the contrived specimens. One negative sample produced an inconclusive (internal control failure) result by the FDA-cleared influenza molecular assay, and was removed from the performance analysis. Six negative samples resulted in FC8G assay internal control failures, and were also excluded from the performance analysis.

FC8G assay performance for the remaining 213 contrived specimens, are presented in Table 28 below.

Table 28: FC8G Assay Performance Testing Contrived Specimens – Challenge Study

Virus Group (Analyte)	Sensitivity			Specificity		
	TP/(TP+FN)	%	95% CI	TN/(TN+FP)	%	95% CI
Influenza A (Overall)	105/105	100%	96.5 - 100	107/108	99%	94.9 – 99.8
Influenza A/H1N1pdm09	2/2	100%	34.2 - 100	209/211	99%	96.6 – 99.7
Influenza A/H3N2	3/3	100%	43.9 - 100	210/210	100%	98.2 - 100
Influenza A/Non-seasonal	**99/100**	**99%**	**94.6 – 99.8**	**113/113**	**100%**	**96.7 - 100**
Influenza B (Overall)	5/5	100%	56.6 - 100	208/208	100%	98.2 - 100
Influenza B/Victoria	2/2	100%	34.2 - 100	211/211	100%	98.2 - 100
Influenza B/Yamagata	3/3	100%	43.9 - 100	210/210	100%	98.2 - 100

The overall sensitivity of the assay for the non-seasonal influenza A target virus group was 99% (95% CI of 96.4%- 99.8%) and specificity was 100% (95% CI of 96.7% - 100.0%). All 10 seasonal influenza viruses included in this study were correctly identified.

One pre-2009 H1N1 strain (A/Fukushima/141/2006) analyzed at approximately 1.5x LoD produced a false positive result for A/H1N1pdm09. Based on the results of the FDA-cleared influenza molecular assay, the concentration of human cellular DNA appeared to be orders of magnitude higher than the concentration of the influenza A RNA in this specimen, indicating there might have been a competition between 18s and influenza A amplifications in this specific specimen. In addition, one negative sample resulted in a false positive for A/H1N1pdm09.

4. Clinical cut-off**:**

Not applicable.

5. Expected values/Reference range:

In the FC8G assay prospective clinical study (described in the "Clinical Studies" section above), a total of 984 nasal or nasopharyngeal swab specimens prospectively collected from the 2013-2014 through 2016-2017 influenza seasons were determined to be evaluable. The number and percentage of influenza cases per specified age group, as determined by the FC8G assay, stratified by the virus groups, are presented in Table 29 and Table 30 below.

Table 29: Seasonal Influenza A Positives by the FC8G Assay per Patient Age Group

Age Group	Number of Nasal or Nasopharyngeal Swab Specimens	Number of Influenza A/H3N2 Positives	Influenza A/H3N2 Positivity Rate	Number of Influenza A/H1N1pdm09 Positives	Influenza A/H1N1pdm09 Positivity Rate	Number of Seasonal Influenza A Positives	Seasonal Influenza A Positivity Rate
≤ 5 years	417	12	2.9%	4	1.0%	16	3.8%
6 to 21 years	278	29	10.4%	3	1.1%	32	11.5%
22 to 59 years	228	10	4.4%	2	0.9%	12	5.3%
≥ 60 years	61	2	3.3%	0	0.0%	2	3.3%
Total	984	53	5.4%	9	0.9%	62	6.3%

Table 30: Influenza B Positives by the FC8G Assay per Patient Age Group

Age Group	Number of Nasal or Nasopharyngeal Swab Specimens	Number of Influenza B/Victoria Positives	Influenza B/Victoria Positivity Rate	Number of Influenza B/Yamagata Positives	Influenza B/Yamagata Positivity Rate	Number of Influenza B Positives	Influenza B Positivity Rate
≤ 5 years	417	6	1.4%	1	0.2%	7	1.7%
6 to 21 years	278	6	2.2%	4	1.4%	12*	4.3%
22 to 59 years	228	2	0.9%	1	0.4%	3	1.3%
≥ 60 years	61	0	0.0%	2	3.3%	2	3.3%
Total	984	14	1.4%	8	0.8%	24	2.4%

* Two samples were Influenza B positive with no lineage identified by the FC8G assay.

N. Instrument Name:

BioRad T100 endpoint thermal cycler
FluChip-8G Imaging System

O. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ___X_____ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No __X_____

2. Software:

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

   Yes _____X___ or No _____

3. Specimen Identification:

   Operator manually label each specimen container with patient ID.

4. Specimen Sampling and Handling:

   Manually handled using pipettes.

5. Calibration:

   To be qualified annually by InDevR.

6. Quality Control:

   Quality control is addressed for each specific FDA-cleared assay to be run on the instruments.

P. Other Supportive Instrument Performance Characteristics Data Not Covered in the "Performance Characteristics" Section above:

Not applicable.

Q. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

R. Conclusion:

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