AutoGenomics

INFINITI® FLU A-sH1N1 Assay

Directional Package Insert (DPI)

For *In Vitro* Diagnostic Use

CE

FOR EXPORT ONLY

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Authorized EU Agent: Medical Device Safety Service GmbH (MDSS)
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INTENDED USE

The INFINITI FLU A-sH1N1 is an *in vitro* diagnostic test intended for use in conjunction with clinical and epidemiological information:

- to identify patients who may be infected with the swine influenza virus (A/H1N1) to allow public health authorities to respond to and limit transmission of the virus,
- for qualitative detection of influenza virus type A in symptomatic patients from viral RNA in human nasopharyngeal aspirates, and
- for presumptive identification of virus in patients who may be infected with swine influenza A subtype A/H1N1 from viral RNA in human nasopharyngeal aspirates and viral culture in conjunction with clinical and epidemiological risk factors.

The INFINITI FLU A-sH1N1 should not be performed unless the patient meets clinical and epidemiological criteria for testing suspect specimens. The definitive identification of swine influenza A/H1N1 either directly from patient specimens or from virus cultures required additional laboratory testing, along with clinical and epidemiological assessment in consultation with national influenza experts.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.

The results obtained from the INFINITI FLU A-sH1N1 should be used and interpreted only in the context of the overall clinical diagnosis. The information from the INFINITI FLU A-sH1N1 should only be used to supplement other tools for therapeutic decision-making in conjunction with routine monitoring by a physician. Clinicians should use professional judgment when interpreting the results of the test.

DESCRIPTION OF TEST

The INFINITI FLU A-sH1N1 is designed to detect and identify the swine influenza virus (A/H1N1) in human nasopharyngeal aspirates (NPA). The INFINITI FLU A-sH1N1 consists of a multiplex PCR test followed by primer extension and microarray hybridization in an integrated molecular diagnostic device, the INFINITI Analyzer.

The INFINITI FLU A-sH1N1 consists of the following six major processes:

- Reverse transcription of viral RNA.
- Multiplex PCR amplification of cDNA.
- Fluorescent labeling of the amplified product using specific detection primer extension (DPE).
- Hybridization of the labeled amplified product to a microarray.
- Scanning of the microarray.
- Signal detection and analysis.

Multiplex PCR amplification is performed off-line with cDNA. The rest of the processes are automated by the INFINITI Analyzer or INFINITI PLUS Analyzer.

WARNINGS AND PRECAUTIONS

Handling Requirements

- **For *in vitro* diagnostic use. To be used by qualified laboratory personnel.**
- This test is to be used only with human nasopharyngeal aspirates.

- All patient specimens are potentially hazardous and care should be taken when handling materials of human origin. **Follow the CLSI Guidelines (Molecular Diagnostics Methods for Infectious Diseases; Approved Guidelines; MM3-A).**
- Upon receipt of samples, visually inspect sample condition. Specifically, look for abnormal signs that indicate that sample integrity has been compromised (e.g., evaporation, decrease in volume, precipitation, spills, discoloration, sedimentation, separation, turbidity, etc.). If you observe or suspect any sample abnormality, do not perform any test.
- Samples should be handled with extreme caution to prevent contamination, spillage, sample mix-up. Sample containers should be labeled clearly to prevent mix-up.
- Store samples at the specified conditions.
- To minimize the risk of cross contamination, sample preparation, PCR reaction set up and PCR product analysis should be performed according to approved guidelines such as CLSI (Molecular Diagnostic Methods for Genetic Diseases: Approved Guideline).
- Do not pool/mix reagents from different lots.
- Do not use a kit or reagent past its expiration date.
- Store kits and reagents according to the product label.

Laboratory Procedures

- Follow normal precautions for handling laboratory reagents.
- Follow safe laboratory procedures: do not pipette by mouth; wear protective clothing (e.g., disposable gloves laboratory coats) and eye protection; do not eat, drink or smoke in the laboratory work areas; wash hands thoroughly after handling samples and reagents.

Waste Handling

- Dispose of unused reagents, specimens and waste according to applicable country, federal, state and local regulations.
- Safety data Sheets (SDS) are available upon request from AutoGenomics Customer Service.

INFINITI Analyzer and INFINITI Analyzer

- **Read the Operator's Manuals before operating the instruments.** Pay particular attention to "Notes".
- Follow the Caution and Safety Warning in the Operator's Manual.
- Refer to the Installation Requirements Section when installing the instrument.
- Refer to the Errors Section when errors are encountered while operating the instrument.
- Refer to the Help Section when problems are encountered.

STORAGE / STABILITY

BioFilmChip Microarray: 24 months Refrigerated (2 to 8°C)
Intellipac Reagent: 12 months Refrigerated (2 to 8°C)
Note: Remove the Intellipac from the Analyzer and store refrigerated as soon as possible. Do not use after Intellipac has been opened for four weeks.
Amplification Mix: 18 months Frozen (-30 to -15°C)

Note: Specific product expiration date is printed on the product label.

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REAGENTS REQUIRED (SUFFICIENT FOR 48 TESTS)

- AutoGenomics Catalog Number 04-1120-02 INFINITI FLU A-sH1N1 Magazine - BioFilmChip® Microarray: 4 magazines per box
- AutoGenomics Catalog Number 04-2120-02 INFINITI FLU A-sH1N1 Intellipac® Reagent Module: 24 tests per module which contains:
  - dNTPs
  - Labeled-dCTP
  - Detection Specific Primers Mix
  - Extension Reaction Buffer
  - Hybridization Buffer
- AutoGenomics Catalog Number 04-3120-02 INFINITI FLU A-sH1N1 Amplification Mix 4 x 250µl vials of PCR reaction master mix containing:
  - dNTPs
  - Multiplex Primers Mix
  - $MgCl_2$
  - Reaction Buffer
- Product Number 12-0010-02: Wash buffer

REAGENTS REQUIRED BUT NOT PROVIDED BY AUTOGENOMICS

- RNA extraction kit (QIAmp Viral mini kit, Qiagen Catalog Number 52906)
- Armored –RNA: Armored RNA-Hepatitis C Virus (Genotype 1a) in TSM III Buffer, 250 µl (Asuragen Diagnostics, Catalog Number 42004)
- Reverse Transcription kit: SuperScript III Supermix kit for 50 reactions (Invitrogen Catalog Number 18080400).
- Platinum Taq DNA polymerase (Invitrogen, Catalog Number 10966034)
- Titanium Taq DNA polymerase (Clontech Catalog Number 639209)
- SAP (USB Catalog Number 70092Z)
- EXO I (USB Catalog Number 70073Z)
- Distilled water (DNAse and RNAse free)

EQUIPMENT AND CONSUMABLES

The following equipment is required but not provided with the assay reagents

- Pipettors
- Mini Centrifuge
- Pipette tips
- Microfuge tube Racks
- Thermocycler
- Vortex
- 0.2 ml thin wall tubes for PCR
- 1.5 ml microcentrifuge tubes
- 8-well Flat Strip Caps (Genesee Scientific, Catalog No. 22-623)
- AutoGenomics Product Number 11-0060-00: INFINITI Waste tray Stir Bars
- AutoGenomics Product Number 11-0020-00: INFINITI Waste Tray Liners
- AutoGenomics Product Number 11-0080-00: INFINITI® Pipette Tips

- **FOR INFINITI Analyzer***:*
  - AutoGenomics Product Number 10-0010-99: INFINITI Analyzer
  - AutoGenomics Product Number 11-0030-00: 24-Well Plates with Lids
  - AutoGenomics Product Number 11-0050-00: INFINITI Temp Cycle Plate

- **FOR INFINITI PLUS Analyzer***:*

- AutoGenomics Product Number 10-0020-99: INFINITI PLUS Analyzer
- AutoGenomics Product Number 11-0100-00: 48-Well Plates and Product Number 11-0110-00: 48 Well Plate Lid

SPECIMEN
Nasopharyngeal aspirates(NPA) which should be kept at -80°C until RNA extraction**.** Avoid freeze/thaw

RNA EXTRACTION
Before nucleic acid extraction, the NPA should be thawed on ice.

It is required to run a known positive control and a negative control in each test run

Positive Control: To control for RNA extraction, 1.0 µl of Armored RNA is added to 200 µl of the thawed specimen before proceeding with RNA extraction. Use 2.0 µl of armored RNA when using an automated extraction method.

Nucleic acid extraction is performed using the QIAmp Viral RNA Mini Kit following the instructions suggested by the manufacturer.

Final elution volume (40 µl) is stored at -80°C. When working with RNA always keep tubes on ice.

FIRST-STRAND cDNA SYNTHESIS USING SUPERSCRIPT III RT SUPERMIX
The reverse transcription reaction is best performed in a PCR instrument that has been programmed to meet all the required incubation times and temperatures.

Adding Armored RNA Control Post Extraction - **If the Armored RNA control was not added before extraction**, it may be added after extraction. Add 0.6 µl of a 1:3 dilution Armored RNA (in RNAse free water) to 9.4 µl of extracted RNA.

The reverse transcription reaction mixture is prepared as follows:
1 µl of Random Primers
1 µl of annealing buffer
10 µl extracted RNA

The mixture is incubated at 70°C for 5 min, and then the PCR block is cooled to 4°C.

The following reagents are then added to the mixture:
10 µl of 2X first strand reaction buffer
2 µl of enzyme mix

Incubate at 25°C for 5 minutes, then at 50°C for 50 minutes.

Inactivate enzymes at 85°C for 5 minutes. The cDNA is used for PCR.

PCR REACTION
Note:

- Keep Taq DNA polymerase on ice.
- Completely thaw reagents on ice.
- Vortex the amplification mix tube for 2 to 5 seconds then centrifuge briefly to bring the contents to the bottom of the tube.
- To avoid contamination, a separate area is recommended for assembly of the PCR reaction. Decontaminate pipettes and all work surfaces with freshly prepared 10% bleach.

- Filter tips and gloves must be used when handling specimens and controls.
- The PCR product cannot be stored prior to testing. Use immediately.

Note:

- For the INFINTI Analyzer use the 24WP.
- For the INFINTI PLUS Analyzer use the 48WP.

Set up the PCR in the Well Plate as follows:

Amplification Mix	17.4 µl
Platinum Taq	0.1 µl
cDNA sample	2.5 µl
Total	20.0 µl

Place tubes in the thermocycler and cycle using the conditions in the following table:

Step No.	Temperature °C	Time	No. of Cycles
1	94	2 minutes	1
2	94 55 72	30 seconds 30 seconds 1 minute	39
3	72	3 minutes	1
4	4	Hold	1

SAP/EXO PROTOCOL

Post PCR clean-up is a critical step to prevent the remaining PCR substrates from interfering with the signal amplification.

Prepare the enzymes mixture as a master mix with excess. **For example**: if there are 10 PCR reactions, prepare a master mix enough for 12 reactions by pipetting 36 µl of SAP, 9 µl of Exo and 6 µl of Titanium Taq.

SAP	3.00 µl / sample
Exo	0.75 µl / sample
Titanium Taq	0.50 µl / sample
Total	4.25 µl

Dispense 4.25 µl of the enzyme mix into each reaction, gently vortex briefly, then place the well plate in a thermal cycler block using the following conditions:

Step No.	Temperature	Time
1	37°C	20 min
2	94°C	10 min
3	4°C	hold

Sample Loading

1) Carefully remove the 8-well flat strip caps to avoid splashing.
2) Load the well plate in the appropriate orientation (with well A1 in the back left corner) into the Analyzer
   - **INFINITI Analyzer:** Load the assembled 24WP with the associated lid (Catalog # 11-0030-00).
   - **INFINITI PLUS Analyzer:** Load the assembled 48WP with a clean 48WP lid (see instructions in the INFINITI PLUS Analyzer Operations Manual) (Catalog # 11-0110-00, reusable).
3) Load the following: assay specific magazines, Intellipac, INFINITI Static Free Pipette tips, and buffer.
   - **FOR INFINITI Analyzer***:*
     Wash Buffer should be placed in the INFINITI bottle holders. The Wash Buffer goes in the left holder (near the magazine).
   - **FOR INFINITI PLUS Analyzer***:*
     Follow the INFINITI PLUS Analyzer Operator's Manual for checking and replacing Wash buffer.

Operation of the Analyzers

Follow the instructions in the Operator's Manuals

INFINITI Analyzer Operator's Manual (Part Number EM-34000)
INFINITI PLUS Analyzer Operator's Manual (Part Number EM-34041)

QUALITY CONTROL

- Maintain calibration of thermocycler according to manufacturer's specifications.
- Maintain calibration of INFINITI or INFINITI PLUS Analyzer according to AutoGenomics' specifications.
- Maintain calibration of pipettes according to manufacturer's specifications.

INTERPRETATION OF RESULTS

The INFINITI FLU A-sH1N1 is designed to detect and identify the swine influenza virus (A/H1N1). The assay results are reported as "POSITIVE" or "NEGATIVE" for the presence of the specific virus.

If the RNA-D control on the result page is negative, the sample should be repeated.

When the assay is not completed, and no results are reported, the assay should be repeated. If the report displays a message which indicates the reason why no results are reported, then this message is recorded in the Error Log. Please refer to the Trouble Shooting section of the INFINITI Analyzer Operator's Manual where the error may be described, or contact AutoGenomics technical support.

LIMITATIONS

The INFINITI FLU A-sH1N1 is designed to detect and identify only the swine influenza virus (A/H1N1). The test does not report other respiratory viruses and does not claim either the absence or the presence of these viruses. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.

PERFORMANCE CHARACTERISTICS

Analytical Specificity

Studies related to specificity were conducted during assay development. There are three levels of specificity required in the assay:

- PCR primers
- ASP – target binding region
- ASP – chip binding region

PCR primer specificity was determined by amplicon size on a gel and sequencing the amplicon. ASP primer specificity was determined by the correct calls made by the assay using known samples (e.g., Coriell). Capture probe specificity is determined by hybridizing different oligos and demonstrating that only the correct oligo hybridizes to the known spot.

Analytical Reactivity; Cross-Reactivity

- Analytical reactivity (inclusivity) studies were performed for the various influenza strains to demonstrate specificity of the INFINITI FLU A-sH1N1 in detecting the swine flu virus. Nine (9) influenza A and five (5) influenza B strains were used in the study. All influenza A samples were positive for the FluA probe and internal control RNA-D probe, and negative for the H1-s probe. All influenza B samples were negative for both FluA and H1-s probes and positive for internal control RNA-D.

- Cross reactivity studies were performed to demonstrate that the presence of the bacterial species and respiratory viruses listed below does not affect the specificity of the INFINITI FluA-sH1N1 Assay. Both FluA and H1-s probes were negative for all samples. The internal RNA-D control was positive for all samples.

Bacteria	
Streptococcus pneumoniae	Corynbacter jeikeium
Bordetella pertussis	Moraxella catarrhalis
Streptococcus salivarius	Staphylococcus aureus
Streptococcus pyogenes	Pseudomonas aeruginosa
Neisseria meningitidis	Escherichia coli
Haemophilus influenzae	Staphylococcus epidermidis

Virus	
Adenovirus C	Parainfluenza virus 3
Coronavirus NL63	Respiratory syncytial virus A
Enterovirus B	Respiratory syncytial virus B
Influenza B	Rhinovirus A
Metapneumovirus A	Rhinovirus B
Metapneumovirus B	

Limit of Detection (LoD)

Analytical sensitivity (LoD) for the INFINITI FLU A-sH1N1 was determined for the overall test system, from nucleic acid extraction to detection and identification of the virus strain. LoD determination was performed using the QIAmp Viral RNA Mini Kit (QIAGEN, Cat #52906) for the nucleic acid extraction. This is the recommended extraction method for the INFINITI FLU A-sH1N1. Extraction was performed following the manufacturer's instructions for use.

- The analytical sensitivity (LoD) of the INFINITI FLU A-sH1N1 for the novel H1N1 strain was determined, using serial dilutions of two (2) samples, to be 500 copies. The analytical sensitivity (LoD) of the INFINITI FLU A-sH1N1 for the seasonal flu A was determined to be 100 copies. A summary of the results is provided in the following table.

- Additional LoD studies were conducted. In this LoD study, the re-grown isolates were first diluted serially using 1:10 dilutions, and then viral RNA extracted from each dilution level. Two SOIV samples and one seasonal Flu A sample were used in the study. The minimal virus concentration which provides 100% correct results using a ratio cut-off of 3 was 500 PFU/ml.

- A 20-replicate validation was performed starting with RNA extraction. Two samples at the 500 PFU/ml level were used in this validation: Sample #843 (SOIV) and A/Brisbane/59/2007 (seasonal Flu A). The 20 replicates were independently processed from the dilution step, i.e., extraction, and the assay were independently done for each sample.
    - Nineteen (19) of the 20 replicates (95%) of the SOIV sample gave the correct result; one sample had a negative RNA indicating an invalid assay, and therefore, no result was reported. Upon repeat, the assay gave the correct result.
    - All 20 replicates (100%) of the seasonal FluA sample gave the correct result.
    - Overall, 39 of 40 replicates (97.5%) gave correct results.

Assay Precision and Reproducibility

- A three-site reproducibility study was conducted to demonstrate inter-site, inter-operator, and inter-instrument reproducibility. One lot of reagents was used; each site had one operator and one instrument. Each site received the same twelve (12) contrived nasopharyngeal samples. RNA was extracted from the reproducibility samples as soon as received. For each assay day, the extracted RNA was assayed in duplicate following the instructions in the package insert. A total of 262 tests were run. Table 1 provides a summary of the three-site reproducibility study.

Table 1

Sample ID	Sample Type[a]	# samples tested	1st time run				
			samples with calls[b]	samples with correct calls[c]	samples with incorrect calls[d]	samples with no calls[e]	correct call rate[f] (%)
1	Neg	22	22	22	0	0	100%
2	Neg	22	22	22	0	0	100%
3	Neg	22	22	22	0	0	100%
4	Neg	22	22	22	0	0	100%
5	Neg	22	22	22	0	0	100%
6	Neg	22	22	22	0	0	100%
7	Neg	22	22	22	0	0	100%
8	Neg	22	22	22	0	0	100%
9	Neg	22	22	22	0	0	100%
10	FLU A	22	21	17	4	1	81.0%
12	Neg	22	22	22	0	0	100%
13	sH1N1	20[g]	20	20	0	0	100%
Total		262	261	257	4	1	98.5%

a Neg = no FLU A/sH1N1 detected
FLU A = FLU A virus detected
sH1N1 = 2009 swine flu detected
b Samples for which a Pos or a Neg call was made.
c Samples for which the call was correct. This does not include samples with no calls.
d Samples for which the call was incorrect, e.g., false positive and false wrong negative results. This does not include samples with no calls.
e Samples which had no results
f Samples with correct calls/samples with calls. This does not include samples with no calls.
g On day 1, Site 1 used the wrong sample.

- A lot-to-lot reproducibility study was conducted at one site using three lots. One operator and one instrument were used in the study. The same 12 reproducibility samples were used in this study. Table 2 provides a summary of the lot-to-lot reproducibility study.

Table 2

Lot	# samples tested	1st time run				
		samples with calls[a]	samples with correct calls[b]	samples with incorrect calls[c]	samples with no calls[d]	correct call rate[e] (%)
Lot 1	118	118	118	0	0	100%
Lot 2	24	24	24	0	0	100%
Lot 3	95	83	82	1	12	98.8%

a Samples for which a Pos or a Neg call was made.
b Samples for which the call was correct. This does not include samples with no calls.
c Samples for which the was incorrect, e.g., false positive and false wrong negative results. This does not include samples with no calls.
d Samples which had no results.
e Samples with correct calls/samples with calls. This does not include samples with no calls.

Clinical Performance – Method Comparison

- 127 samples from patients suspected with the swine flu virus infection were tested using the INFINITI FLU A-sH1N1 test. Twenty-eight (28) of the 127 patients tested positive and 99 tested negative for the swine flu virus infection. The results of the INFINITI FLU A-sH1N1 were compared to the results obtained using the RT-PCR protocol for Swine H1N1 Influenza authorized by the Public Health Agency of Canada. The INFINITI positive results were further confirmed by sequencing. The sequence data (in the H1 gene) was obtained using the primers from the National Microbiology Laboratory.

  The following summarizes the results of the comparison studies:
  - 27 of the positive samples (96%) were confirmed positive by bi-directional sequencing. Bi-directional sequencing result for one positive sample was not available.
  - All 28 positive samples tested positive with the Canada RT-PCR method.
  - Of the 99 negative samples, 98 tested negative by the Canada RT-PCR, and 1 tested positive by the Canada RT-PCR. The one sample which tested negative by the INFINITI FLU A-sH1N1, but tested positive by the RT-PCR ("false negative") had suspect low copy number for the sample.

		RT-PCR Reference assay				
		Positive	Negative			
INFINITI assay	Positive	28	0	28		
	Negative	1	98	99		
		29	98	127		

Positive agreement :	0.97
Negative agreement :	1.00
Positive predictive value :	1.00
Negative predictive value :	0.99

- Sixty-six (66) patient samples were tested at a US clinical laboratory. Results were compared to those obtained by PCR.
  - On initial testing of the 66 samples, one sample tested positive for FLU A-sH1N1, 57 were negative for Flu A and eight (8) had "no call" results.
  - Of the eight (8) "no calls", six were repeated. One was positive for Flu A, one was positive for FLU A-sH1N1, one was Negative and three were "no calls". Two of the initial "no calls" were not repeated for unknown reasons.
  - There were no incorrect calls, i.e., all calls (61 samples) made by the INFINITI FLU A-sH1N1 were correct.
  - There were 5 no calls (8.2%).

  The following summarizes the comparison study.

  58 negative samples
  2 sH1N1 positive
  1 FluA positive
  5 No calls