510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k111778

B. Purpose for Submission:
New device

C. Measurand:
Target RNA sequences for the respective regions of the Hemagglutinin (HA) gene for seasonal Influenza A/H1, seasonal Influenza A/H3 and 2009 H1N1 Influenza Virus (swine-origin), and for the region of the Nucleocapsid Protein (NP) gene of the 2009 H1N1 Influenza virus, as well as some other Influenza A viruses of swine lineage.

D. Type of Test:
A real-time reverse-transcriptase polymerase chain reaction (Real-time RT-PCR) test intended for the qualitative *in vitro* detection and differentiation of seasonal Influenza A/H1, seasonal Influenza A/H3, and 2009 H1N1 Influenza in nasopharyngeal swab (NPS) and nasopharyngeal wash (NPW) specimens using nucleic acid isolation, amplification, and detection.

E. Applicant:
US Army Office of the Surgeon General, U.S. Army Medical Materiel Development Activity

F. Proprietary and Established Names:
JBAIDS Influenza A Subtyping Kit
Common Name: JBAIDS Influenza A Subtyping rRT-PCR Kit
Real-time PCR assay for differentiation of influenza A subtypes

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
OQW	Class II	21 CFR 866.3332 Reagents for detection of specific novel Influenza A viruses	Microbiology (83)
OEP	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay	Microbiology (83)
OOI	Class II	21 CFR 862.2570 Instrumentation for clinical multiplex test systems	Clinical Chemistry (75)

H. Intended Use:

1. Intended use:

   The Joint Biological Agent Identification and Diagnostic System (JBAIDS) Influenza A Subtyping Kit is intended for the *in vitro* qualitative detection and differentiation of seasonal Influenza A/H1, seasonal Influenza A/H3, and 2009 H1N1 Influenza viral nucleic acids isolated and purified from nasopharyngeal swab (NPS) and nasopharyngeal wash (NPW) specimens from human patients with signs and symptoms of respiratory infection, in conjunction with clinical and epidemiological risk factors. The JBAIDS Influenza A Subtyping Kit contains reverse transcriptase real-time polymerase chain reaction (rRT-PCR) assays for use on the JBAIDS instruments. The Flu A H1, Flu A H3, and Flu A H1 2009 assays of the JBAIDS Influenza A Subtyping Kit target a region of the hemagglutinin (HA) gene of the respective Influenza A virus. The Flu A Sw assay of the JBAIDS Influenza A Subtyping Kit targets a region of the nucleocapsid protein (NP) gene of the 2009 H1N1 Influenza virus, as well as some other Influenza A viruses of swine lineage. This kit is not intended to detect Influenza B or Influenza C Viruses.

   A negative result for all assays in the JBAIDS Influenza A subtyping kit is a presumptive negative result for Influenza A. These results should be confirmed using the JBAIDS Influenza A & B Detection Kit.

   Test results are to be used in conjunction with other clinical and epidemiological information. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.

   Due to low seasonal prevalence, performance characteristics for detection of seasonal Influenza A/H1 were established primarily with retrospective and contrived clinical specimens.

   All users, analysts, and any person reporting diagnostic results from use of this kit should be trained to perform and interpret the results from this procedure by JBAIDS instructors or designees prior to use. Use of this device is limited to designated Department of Defense (DoD) laboratories equipped with the JBAIDS instruments.

   If infection with a novel influenza A virus 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 state or local health departments for testing. Viral culture should not be attempted in these cases unless a biosafety laboratory (BSL) 3+ facility is available to receive and culture specimens.

2. Indication for use:
   Same as Intended Use

3. Special conditions for use statement(s):
   For prescription use only

4. Special instrument requirements:
   Joint Biological Agent Identification and Diagnostic System (JBAIDS) Instrument

I. Device Description:

The JBAIDS Influenza A Subtyping Kit is a real time RT-PCR assay based on the influenza A subtyping assays that are part of the "CDC Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel (rRT-PCR Flu Panel)" and the "CDC Influenza 2009 A (H1N1)pdm Real-Time RT-PCR Panel". These assays have been re-optimized to work with Idaho Technology's proprietary freeze-dried PCR reagent formulation. Purified patient samples (using either the IT 1-2-3 Platinum Path Purification Kit, or the Roche MagNA Pure Compact Nucleic Acid Isolation Kit I in conjunction with the Roche MagNA Pure Compact system) are used to reconstitute the freeze-dried reagents which are then tested using the JBAIDS instrument.

Each JBAIDS Influenza A Subtyping Kit contains sufficient reagents for testing six specimens. There are six vacuum-sealed control pouches of freeze-dried PCR reagents (8 reagent vials each), six vacuum-sealed sample testing pouches of freeze-dried PCR reagents (4 reagent vials each), and six pouches containing one tube each of reconstitution buffer and reagent grade water. Once resuspended, each reagent vial provides enough material for one reaction.

Each control pouch contains the following eight vials:

- **Positive Control (+) vials (Flu A H1, Flu A H3, Flu A H1 2009, and Flu A Sw)**. Once reconstituted, each Positive Control vial contains all reagents necessary for RT-PCR of the appropriate target, including an assay-specific synthetic RNA target. The Positive Controls are resuspended with 10 µL reconstitution buffer and 10 µL reagent grade water before testing in parallel with patient specimens. Amplification in the Positive Control gives assurance that kit reagents are functioning properly and that the assay setup has been performed correctly. A Positive Control must be included for each assay in the JBAIDS run.

  Ingredients: Each Positive Control vial contains <0.001% DNA polymerase complex; <0.001% Reverse Transcriptase; <0.001% target-specific forward and reverse primer; <0.001% target-specific hydrolysis probes; <0.05% dATP, dCTP, dGTP, dTTP; <0.001% assay-specific synthetic target RNA; bovine serum albumin; dithiothreitol; RNase inhibitor; and carbohydrate.

- **Negative Control (-) vials (Flu A H1, Flu A H3, Flu A H1 2009, and Flu A Sw).** Once reconstituted, each Negative Control vial contains all reagents required to perform a RT-PCR reaction except the target RNA, and should therefore give negative results. The Negative Controls are re-suspended with 10 µL reconstitution buffer and 10 µL nuclease-free water that has been purified with a nucleic acid purification kit (NEC). The reconstituted negative control is then tested in parallel with patient specimens to provide assurance that the purification and setup procedures have been performed without contamination. A Negative Control must be included for each assay in the JBAIDS run.

Ingredients: Each Negative Control vial contains <0.001% DNA polymerase complex; <0.001% Reverse Transcriptase; <0.001% target-specific forward and reverse primer; <0.001% target-specific hydrolysis probes; <0.05% dATP, dCTP, dGTP, dTTP; bovine serum albumin; dithiothreitol; RNase inhibitor; and carbohydrate.

Each sample testing pouch contains the following four vials:

- **Unknown vials (Flu A H1, Flu A H3, Flu A H1 2009, and Flu A Sw)**. Once reconstituted, each Unknown vial contains all reagents necessary for RT-PCR of the appropriate target, except target template. The Unknown vials are resuspended with 10 µL reconstitution buffer and 10 µL purified patient sample, and will therefore give negative results unless the appropriate template is found in the patient sample.

  Ingredients: Each Negative Control vial contains <0.001% DNA polymerase complex; <0.001% Reverse Transcriptase; <0.001% target-specific forward and reverse primer; <0.001% target-specific hydrolysis probes; <0.05% dATP, dCTP, dGTP, dTTP; bovine serum albumin; dithiothreitol; RNase inhibitor; and carbohydrate.

Each reconstitution buffer/reagent grade water pouch contains the following two vials:

- **2X Reconstitution Buffer (2X RB).** The buffer solution (purple buffer) is matched to the Influenza assays and is used undiluted. The buffer enhances PCR kinetics and is used to re-suspend JBAIDS freeze-dried reagents. Once reconstituted, all freeze dried reagent vials contain all of the components required for PCR.

  Contents: 600 µL

- **Reagent Grade Water.** Reagent grade water is molecular biology grade water used to reconstitute the Positive Controls.

  Contents: 850 µL

Materials Provided

Kit Contents All vials in the assay pouches contain sufficient reagent for one capillary reaction.		
Qty	Description	Contents
6	Influenza A Subtyping Kit Control Pouch	One Flu A H1 Positive Control (+) One Flu A H1 Negative Control (-) One Flu A H3 Positive Control (+) One Flu A H3 Negative Control (-) One Flu A H1 2009 Positive Control (+) One Flu A H1 2009 Negative Control (-) One Flu A Sw Positive Control (+) One Flu A Sw Negative Control (-)

6	Influenza A Subtyping Kit Sample Pouch	One Flu A H1Unknown (U) One Flu A H3 Unknown (U) One Flu A H1 2009 Unknown (U) One Flu A Sw Unknown (U)
6	Purple Reconstitution Buffer Pouch (purple foil pouch)	One purple reconstitution buffer (600 µL) One reagent grade water (850 µL)
Each kit contains sufficient reagents for testing six specimens.		

Materials Required But Not Provided

Required Equipment	
Minicentrifuge capable of 2000 x g, 5215[1] (Labnet C-1200 or equivalent)	Capillary adaptor for minicentrifuge (Roche Applied Science 1750-1.5[1] or equivalent)
Micropipette: 2 µL–20 µL Micropipette: 20 µL–200 µL Micropipette: 200 µL–1000 µL	Vortex-Genie® 3823[1] (VWR 58810-163 or equivalent)
JBAIDS Instrument	Extra sample carousel JRPD-SUB-0010[1]
Roche MagNA Pure Compact Instrument[2]	PickPen 1-M Magnetic Tool[3] (Bio-Nobile)
Note: See appropriate Sample Purification Kit for additional equipment and materials required for sample purification.	

[1] Available from Idaho Technology
[2] Only required when performing automated sample purification with the Roche MagNA Pure Compact Nucleic Acid Isolation Kit I
[3] Only required when performing manual purification using the IT 1-2-3 Platinum Path Sample Purification Kit

Materials and Reagents Not Provided	
LightCycler glass capillaries and caps (Roche Applied Science 1 909 339) [1]	Aerosol-resistant (filter), nuclease-free pipette tips, appropriate for micropipettes
Microcentrifuge tube rack LABS-SUP-0001[1]	Powder-free latex or nitrile gloves or equivalent
PickPen Tips[3] (Bio-Nobile)	Sodium hypochlorite solution (household bleach)
Molecular biology grade water (Sigma W4502 or equivalent)	
DNAZapTM, (Ambion AM9890 or equivalent DNA degradation solution)	IT 1-2-3 Platinum Path Sample Purification Kit (ASAY-ASY-0120)[1,3] or Roche MagNA Pure Compact Nucleic Acid Isolation Kit I[2]

[1] Available from Idaho Technology
[2] Only required when performing automated sample purification with the Roche MagNA Pure Compact Nucleic Acid Isolation Kit I. Only qualified lots of the MagNA Pure Compact Nucleic Acid Isolation Kit I can be used with the JBAIDS Influenza A & B Detection Kit. Qualified Roche MagNA Pure Compact Nucleic Acid Isolation Kit I lot numbers are posted and viewable on the secure JBAIDS website.
[3] Only required when performing manual purification using the IT 1-2-3 Platinum Path Sample Purification Kit

J. Substantial Equivalence Information:

1. Predicate device name(s):

CDC Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel (rRT-PCR Flu Panel)

CDC Influenza 2009 A (H1N1)pdm Real-Time RT-PCR Panel

2. Predicate k number(s):
   K080570
   K101564

1. Comparison with predicate(s):

Similarities			
Item	Device	Predicate 1	Predicate 2
	JABIDS Influenza A Subtyping Kit	CDC rRT-PCR Flu Panel (K080570)	CDC rRT-PCR 2009 A (H1N1)pdm Flu Panel (K101564)
Intended Use	Qualitative *in vitro* detection and differentiation of seasonal Influenza A/H1, seasonal Influenza A/H3 and 2009 H1N1 Influenza viral nucleic acids from nasopharyngeal swab (NPS) and nasopharyngeal wash (NPW) specimens on the JBAIDS instrument.	Qualitative *in vitro* detection of influenza virus type A or B and for determination of the subtype of seasonal human influenza A virus, as seasonal A/H1 or A/H3, if present, from viral RNA in nasopharyngeal and/or nasal swab specimens, for presumptive identification of virus in patients who may be infected with influenza A subtype A/H5 (Asian lineage) from viral RNA in human respiratory specimens and viral culture on an ABI 7500 Fast Dx Real-time PCR instrument.	Qualitative *in vitro* detection of influenza virus type A and 2009(H1N1) influenza viral RNA from nasopharyngeal swabs (NPS), nasal swabs (NS), throat swabs (TS), nasal aspirates (NA), nasal washes (NW), dual nasopharyngeal / throat swabs (NPS/TS) and lower respiratory tract specimens (LRTS) from human patients with signs and symptoms of respiratory infection and/or from viral culture, on the Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR instrument.
Technology	Real-time PCR using hydrolysis probes	Real-time PCR using hydrolysis probes	Real-time PCR using hydrolysis probes
Assay Results	Qualitative	Qualitative	Qualitative
Nucleic Acid Extraction	Yes	Yes	Yes

Differences			
Item	Device	Predicate 1	Predicate 2
	JABIDS Influenza A Subtyping Kit	CDC rRT-PCR Flu Panel (K080570)	CDC rRT-PCR 2009 A (H1N1)pdm Flu Panel (K101564)
Viruses Detected	Influenza A/H1, Influenza A/H3 and Influenza A/2009 H1N1	Influenza A, Influenza B, Influenza A/H1, Influenza A/H3 and Influenza A/H5	Influenza A and Influenza A/ 2009 H1N1

Specimen Types	Nasopharyngeal swabs, nasopharyngeal washes	Upper respiratory tract specimens (including NPS, NS, TS, NA, NW, NPS/TS) and lower respiratory tract specimens (including BAL, BW, TA, sputum, and lung tissue) and virus culture	Nasopharyngeal swabs, nasal swabs, nasal aspirates, nasal washes, dual nasopharyngeal / throat swabs, broncheoalveolar lavage, tracheal aspirate, bronchial wash and viral culture
Required Instrumentation	JBAIDS instrument	Applied Biosystems 7500 Fast Dx Real-time PCR instrument with SDS software v 1.4	Applied Biosystems 7500 Fast Dx Real-time PCR instrument
Interpretation of Test Results	Automated analysis of test results and controls	User required to interpret test and control results	User required to interpret test and control results
Enzyme Master Mix	Assays come in freeze-dried single use vials that include all components of master mix	Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR Kits	Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR Kits
Reagent Storage	Reagents are stored at room temperature	Reagents are stored at ≤ -20°C	Reagents are stored at ≤ -20°C
Extraction Methods	• IT 1-2-3™ Platinum Path Sample Purification Kit • Roche MagNA Pure Compact Nucleic Acid Isolation Kit I	• Qiagen QIAamp® Viral RNA Mini Kit • Qiagen RNeasy® Mini Kit • Roche MagNA Pure TNA Kit • Roche MagNA Pure LC RNA Isolation Kit II	• Qiagen QIAamp® Viral RNA Mini Kit • Roche MagNA Pure Compact Nucleic Acid Isolation Kit I • Roche MagNA Pure TNA Kit • Roche MagNA Pure LC RNA isolation Kit II • Qiagen QIAcube with QIAamp viral RNA mini kit • bioMerieux NucliSENS easyMAG

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

- Nucleic Acid Based In Vitro Diagnostic Devices for Detection of Microbial Pathogens, FDA Guidance Document (DRAFT: December 8, 2005)
- Establishing Performance Characteristics of In Vitro Diagnostic Devices for Detection or Detection and Differentiation of Influenza Viruses, FDA Guidance Document (DRAFT: February 15, 2008)
- Molecular Diagnostic Methods for Infectious Diseases, CLSI Approved Guideline, MM3-P2 (February 2006)
- Interference Testing in Clinical Chemistry, CLSI Approved Guideline EP7-A2 (November 2005)
- User Verification of Performance for Precision and Trueness, CLSI Approved Guideline EP15-A2 (April 2006, second printing)
- Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline, CLSI Approved Guideline MM13-A (January 2006)
- User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline-Second Edition, CLSI Approved Guideline EP12-A2 (January 2008)
- Protocols for Determination of Limits of Detection and Limits of Quantization, CLSI Approved Guidance EP17-A (2004)
- Evaluation of Precision Performance of Quantitative Measurements Methods; Approved Guidance-Second Edition, CLSI Approved Guidance EP5-A2 (August 2004)

- In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path, Guidance for Industry and FDA Staff (May 1, 2007).
- Class II Special Controls Guidance Document: Reagents for Detection of Specific Novel Influenza A Viruses, Guidance for Industry and FDA Staff (March 22, 2006).

L. Test Principle:

The JBAIDS Influenza A Subtyping Kit is a real time RT-PCR assay based on the influenza A subtyping assays that are part of the "CDC Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel (rRT-PCR Flu Panel)" and the "CDC Influenza 2009 A (H1N1)pdm Real-Time RT-PCR Panel". These assays have been re-optimized to work with Idaho Technology's proprietary freeze-dried PCR reagent formulation. Purified patient samples (using either the IT 1-2-3 Platinum Path Purification Kit or the Roche MagNA Pure Compact Nucleic Acid Isolation Kit I in conjunction with the Roche MagNA Pure Compact system) are used to reconstitute the freeze-dried reagents which are then tested using the JBAIDS instrument.

Real-time RT-PCR involves reverse transcription of specific RNA sequences into complementary DNA sequences, followed by logarithmic amplification and simultaneous detection of those DNA sequences. The JBAIDS Influenza A Subtyping assays use hydrolysis probes to detect amplification of the reverse transcribed RNA sequence of interest. Each hydrolysis probe is labeled on 5' end with a fluorescent reporter moiety (6-FAM) and elsewhere with a quencher (TAMRA), which prevents the probe from emitting fluorescent signal. During PCR, the probe binds to a target sequence in the PCR product. When the Taq polymerase replicates a template to which a hydrolysis probe is bound, the exonuclease activity of the polymerase cleaves the probe, separating the fluorophore from the quencher, and fluorescent signal is generated. This fluorescence is measured and displayed by the JBAIDS instrument during the PCR reaction. The fluorescent signal increases as additional template is amplified and more probes are hydrolyzed.

The JBAIDS Influenza A Subtyping Kit contains four assays: seasonal Influenza A/H1 (Flu A H1); seasonal Influenza A/H3 (Flu A H3); Influenza A, swine lineage (Flu A Sw); and Influenza A/2009 H1 (Flu A H1 2009). The Flu A H1, Flu A H3, and Flu A H1 2009 assays amplify a region of the hemagglutinin gene of the respective influenza A virus. The Flu A Sw assay amplifies a region of the nucleocapsid protein gene of 2009 H1N1 Influenza viruses, as well as some other Influenza A viruses of swine lineage.

The JBAIDS Influenza A Subtyping Kit is based on four major processes:

- Sample collection and purification
- Reconstitution of freeze-dried reagents
- Amplification and detection of target nucleic acid on the JBAIDS instrument
- Automated interpretation of the amplification curves using the JBAIDS software.

The JBAIDS Influenza A Subtyping kit can be used to test NPS and NPW specimens. Samples must be purified prior to testing with the JBAIDS Influenza A Subtyping Kit. The purpose of sample purification is to release nucleic acid contained in the patient sample and to remove extraneous materials (e.g., proteins and chemicals) that can interfere with the PCR reaction. The JBAIDS Influenza A Subtyping kit has been validated for use with either the IT 1-2-3 Platinum Path Sample Purification Kit or the Roche MagNA Pure Compact Nucleic Acid Isolation Kit I on the Roche MagNA Pure Compact system. The Platinum Path kit is a manual purification, while the Roche MagNA Pure system is automated. Both methods have four basic steps: lysis to release the nucleic acids, binding of the nucleic acids to a magnetic substrate, washing away extraneous materials and elution of the purified nucleic acid. A brief outline of the two purification kit methods is provided below:

Method Summary for Purification Kits

Step	IT 1-2-3 Platinum Path Sample Purification Kit	Roche MagNA Pure Compact Nucleic Acid Isolation Kit I*
Cell Lysis to release nucleic acids	Bead-beating (vortexing specimen in the presence of silica beads and binding buffer) is used to physically disrupt the cells.	The samples are lysed by incubation with Proteinase K and a special lysis buffer containing a chaotropic salt.
Nucleic Acid Binding	The bead-beaten sample is transferred to a well of a strip tube containing magnetic beads. The nucleic acid binds to the magnetic beads.	Nucleic acids are immobilized on magnetic glass particles.
Washing to remove extraneous materials	Using a pick-pen with a retractable magnet, the magnetic beads are collected from the first well of the strip tube and transferred to a new well containing a wash buffer. The buffer removes extraneous materials while the nucleic acid remains bound to the magnetic beads. The beads are washed three times (three wells of the strip tube).	Unbound substances are removed by several washing steps.
Elution of nucleic acids	Using the pick-pen, the magnetic beads are transferred to the last well of the strip tube containing elution buffer. The nucleic acids are released from the magnetic beads. The beads are then removed from the sample using the pick pen.	Purified nucleic acids are eluted from the magnetic glass particles.

*All steps are automated within the Roche MagNA Pure Compact instrument.

Negative Extraction Controls (NECs) are processed along with each batch of purified specimens. When processing NECs, the user utilizes the Sample Purification Kit to purify a sample of nuclease-free water. The purified NEC sample is then used to reconstitute the freeze-dried Negative Control vials. The use of an NEC allows for detection of contamination during the entire testing process (purification to reagent setup).

Purified samples are tested using the freeze-dried reagents in the JBAIDS Influenza A Subtyping kit. Reagent vials that are intended for testing patient samples are labeled as Unknowns. Each JBAIDS run requires a Positive Control and a Negative Control for each target assay. The JBAIDS runs should be started within about 30 minutes after

reconstitution of the freeze-dried reagents. If necessary, the reconstituted reagents can be stored for up to four hours on ice or in the refrigerator (2-8 °C).

Reagents are set up in the following order:

1. Unknowns – Reconstitute the reagent pellet in the Unknown vials by adding 10 µL of the provided reconstitution buffer and 10 µL of the purified sample.
2. Positive Controls – After the Unknowns are set up for each sample, set up the Positive Controls by reconstituting the pellet with 10 µL of the provided reconstitution buffer and 10 µL of the provided reagent-grade water.
3. Negative Controls – Set up the Negative Controls last by reconstituting the pellet with 10 µL reconstitution buffer and 10 µL of the purified NEC.
4. For each reagent vial, transfer 19 µL of the reconstituted reagent to a capillary following the same order used for reconstitution. The cap is placed on the capillary tube before proceeding to the next capillary.
5. Centrifuge the capillary tubes to ensure that the PCR reagent is in the tip of the capillary.

Each JBAIDS run can accommodate up to 6 samples with accompanying controls when all subtyping assays are used to test each sample. The Diagnostic Wizard guides the operator through the process of setting up the run in compliance with the JBAIDS Influenza A Subtyping Kit package insert. After selecting the appropriate assays and entering sample information, the operator transfers prepared capillaries to the instrument carousel, guided by the loading pattern displayed in the Diagnostic Wizard. With the capillaries loaded into the instrument, the operator signals the instrument to start the PCR run. The instrument automatically controls the temperature heating cycle in the carousel. At intervals during temperature cycling, fluorescence emission is monitored as the carousel rotates to position each sample above the fluorimeter. Temperature and fluorescence data are displayed in real-time. After starting the run, no input is required and the operator need not supervise the run. At the conclusion of the run, the operator may proceed directly to data analysis and reporting.

Each JBAIDS test is analyzed and assigned a final result by the Detector module of the JBAIDS Software. Possible final results are positive, negative, uncertain, or invalid. To assign a final test result, Detector first analyzes the data from each capillary independently. Then, the software assigns a final result, or a combined call, based on the results of the sample and all of its controls.

Each stage of the analysis is described below:

1. Independent Capillary Call

Detector does not rely on any single aspect of the amplification curve, but rather integrates a number of factors, such as curve shape and signal-to-noise ratio into a combined score. Curves scoring above a threshold are called positive, curves scoring below a threshold are called negative, and curves scoring in a small area in between are called uncertain. The scoring system has been carefully tuned to match human expert calls on real amplification curve data.

Detector calls are characterized as follows:

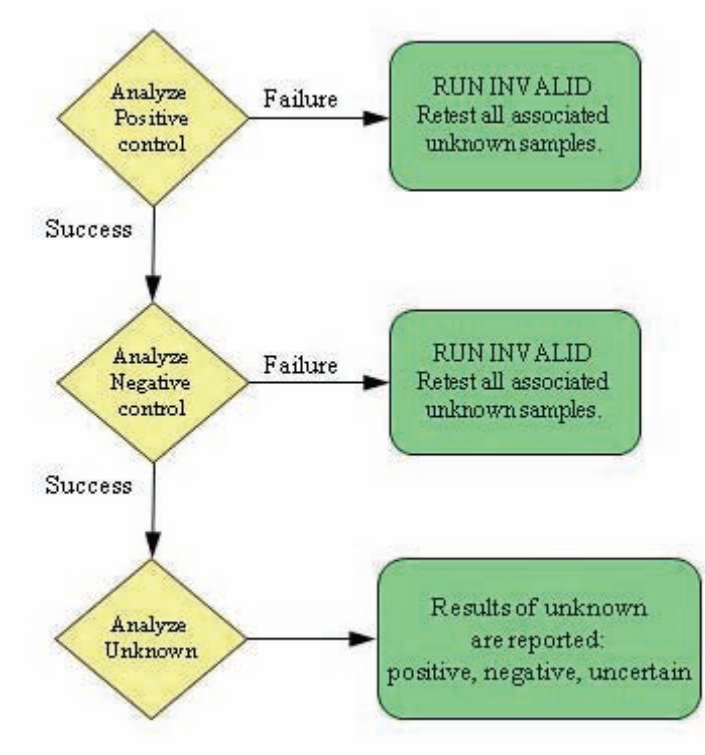
Negative – A curve is called negative if its shape can be closely approximated by a line (or smooth curve), if it has low signal to noise, and if it shows little or no increase in fluorescence during the PCR.

Positive – A curve is called positive if the fluorescence shows exponential growth out of the background, if it has high signal to noise, and if the slope of the exponential region of the amplification is consistent with PCR amplification.

Uncertain – For a small number of curves (about 0.2%) no clear call is possible and the reaction is called uncertain.

2. Test Result or Combined Call

The final test result is a combined call (or meta-call) based on the results of the test sample and its associated controls.



Flow chart outlining how final results are determined

3. Follow-up Testing for Invalid or Uncertain Results

Failure of Positive or Negative Control results in invalid results for all associated samples. Samples with invalid or uncertain results require follow-up testing in order to achieve a valid (positive or negative) result as described below:

- **Invalid –** If either the Positive Control or Negative Control fails to give the expected results, all Unknown samples for that specific assay are called invalid and must be retested using the same purified sample. This testing can be done using the Custom Testing Method.

- **Uncertain –** An uncertain result on a single sample requires retesting with the same purified sample. This testing can be done using the Custom Testing Method.

4. JBAIDS Report

A JBAIDS report is generated automatically for each test run. With the JBAIDS Report, the user integrates the instrument-provided results for the prior performed influenza A test and each of the influenza A subtyping tests to make a final result interpretation.

5. Integrated Result Interpretation

The JBAIDS software automatically interprets the results for each of the target assays. However, the user is required to make the final test interpretation based upon the software test results for each of the four target assays.

Result Interpretation for Use of the JBAIDS Influenza A Subtyping Kit

Subtyping Assay Results				Interpretation	Action
Flu A H1	**Flu A H3**	**Flu A Sw**	**Flu A H1 2009**		
Neg.	Neg.	Neg.	Neg.	Influenza A/H1, Influenza A/H3, and 2009 H1N1 Influenza viral RNA not detected • If an Influenza A test has not yet been performed, this is a presumptive negative result for Influenza A. • If the sample was positive for Influenza A using the JBAIDS Influenza A & B Detection Kit, these results may indicate the presence of a novel strain for Influenza A	If the sample has not previously been tested for Influenza A, these results should be confirmed by the JBAIDS Influenza A & B Detection Kit. If the sample was positive for Influenza A using the JBAIDS Influenza A & B Detection Kit, see "Required Actions for No Subtype Detected Results" section.
Pos.	Neg.	Neg.	Neg.	Seasonal Influenza A/H1 viral RNA detected	No further testing
Neg.	Pos.	Neg.	Neg.	Seasonal Influenza A/H3 viral RNA detected	No further testing
Neg.	Neg.	Pos.	Pos.	2009 H1N1 Influenza viral RNA detected	No further testing
Neg.	Neg.	Pos.	Neg.	Inconclusive for detection of 2009 H1N1 Influenza viral RNA	Retest or re-purify and retest with the Flu A Sw and Flu A H1 2009 assays using the Custom Test Method. If the retest is also inconclusive, obtain a new
Neg.	Neg.	Neg.	Pos.	Inconclusive for detection of 2009 H1N1 Influenza viral RNA	

					sample and retest.
Pos.	Pos.	Neg.	Neg.	Seasonal Influenza A/H1 and Seasonal Influenza A/H3 viral RNAs detected	Multiple infections are possible, but rare. Repeat testing using the residual purified nucleic Acid. If the retest confirms this result, collect and test a new specimen[1]. Contact Idaho Technology if multiple samples provide this result.
Pos.	Neg.	Pos.	Pos.	Seasonal Influenza A/H1 and 2009 H1N1 Influenza viral RNAs detected	
Neg.	Pos.	Pos.	Pos.	Seasonal Influenza A/H3 and 2009 H1N1 Influenza viral RNAs detected	
Pos.	Pos.	Pos.	Pos.	Seasonal Influenza A/H1, Seasonal Influenza A/H3, and 2009 H1N1Influenza viral RNAs detected	

[1]Recent patient exposure to the FluMist® intranasal vaccine may result in false positive results for multiple influenza A subtypes, and should be considered as a possible interferent when multiple influenza infections are detected.

6. Required Actions for No Subtype Detected Results

If the specimen tested positive for influenza A using the JBAIDS Influenza A & B Detection Kit, but none of the subtyping assays are positive, then the interpretation is 'No Subtype Detected'. This result could occur when the titer of the virus in the specimen is low and not detected by the subtyping assays. This result could also indicate the presence of a novel and/or newly emerging influenza A strain. In both cases, the residual purified nucleic acid from the specimen in question, or the re-extracted nucleic acid from the residual specimen in question if residual purified nucleic acid is not available, should be retested with the JBAIDS Influenza A & B Detection Kit (or other FDA cleared nucleic acid-based tests detecting Influenza A) and the JBAIDS Influenza A Subtyping Kit to confirm that the influenza A result is positive and that no influenza A subtype is detected. (Note: the Custom Test Method may be used to include the Influenza A test from the JBAIDS Influenza A & B Detection Kit along with the Influenza A subtyping tests in the same JBAIDS run). If the retest provides a different result, test the sample a third time to ensure the accuracy of the result. If the retest provides the same result, then the function of the JBAIDS Influenza A Subtyping reagents should be verified by testing with appropriate external control materials (known positive samples for seasonal Influenza A/H1, seasonal Influenza A/H3 and 2009 H1N1 Influenza). A negative control (known negative sample or viral transport media) should also be run to test for PCR-product contamination. If the JBAIDS Influenza A Subtyping reagents accurately identify the external positive and negative controls, notify through the chain-of command of the appropriate DoD agencies, or contact the state public health authorities or CDC Influenza Division for confirmatory testing.

7. Custom Test Method

The Custom Test Method can be used to test individual assays from the JBAIDS Influenza A Subtyping Kit. Reasons to use the Custom Test Method include:

- PC or NC failure invalidates the results for only one of the four assays requiring retesting of all samples with only that assay.
- A sample is uncertain for one of the assays, requiring retesting with only that assay.
- A sample is inconclusive for 2009 H1N1 Influenza, requiring retesting with only the Flu A Sw and Flu A H1 2009 assays.
- A "No Subtype Detected" sample is retested using all four influenza A subtype assays and the Influenza A assay from the JBAIDS Influenza A&B Detection Kit.
- During an influenza outbreak, the Department of Defense may choose to recommend use of the custom test method for more efficient and rapid identification of a specific virus. In this case, the relevant subtyping assay(s) from the JBAIDS Influenza A Subtyping Kit may be used in conjunction with the influenza A assay from the JBAIDS Influenza A & B Detection Kit.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A multicenter reproducibility study was performed to determine the overall system reproducibility of the JBAIDS Influenza A Subtyping Kit on the JBAIDS instruments. This study was performed at Idaho Technology Inc. (ITI) and two external sites. The study was conducted with two specimen types: nasopharyngeal wash (NPW) and nasopharyngeal swab (NPS), both collected in viral transport medium (VTM). Both specimen types were purified using two different Sample Purification Kits, the Roche MagNA Pure Nucleic Acid Isolation Kit I and the IT 1-2-3 Platinum Path Sample Purification Kit.

A total of six different panels of nine samples each were prepared for the reproducibility study.

- Panel 1 contained nine NPS specimens that were screened with the Flu A and Flu B assays of the JBAIDS Influenza A & B Detection Kit to ensure that no influenza nucleic acid was present, each spiked with a representative seasonal Influenza A H1N1 virus strain (A/New Caledonia/20/1999) at one of the three different concentrations (LoD/20, LoD, and 3X LoD). Therefore, Panel 1 consisted of three seasonal Influenza A H1N1 virus spiked medium positive NPS specimens (3X LoD), three seasonal Influenza A H1N1 virus spiked low positive NPS specimens (LoD), and three seasonal Influenza A H1N1 virus spiked high negative NPS specimens.
- Panel 2 contained nine NPW specimens that were screened with the Flu A and Flu B assays of the JBAIDS Influenza A & B Detection Kit to ensure that no influenza nucleic acid was present, each spiked with a representative seasonal Influenza A H1N1 virus strain (A/New Caledonia/20/1999) at one of the three different concentrations (LoD/20, LoD, and 3X LoD). Therefore, Panel 2 consisted of three seasonal Influenza A H1N1 virus spiked medium positive

NPW specimens (3X LoD), three seasonal Influenza A H1N1 virus spiked low positive NPW specimens (LoD), and three seasonal Influenza A H1N1 virus spiked high negative NPW specimens.

- Panel 3 contained nine simulated NPS (sNPS) specimens that were screened with the Flu A and Flu B assays of the JBAIDS Influenza A & B Detection Kit to ensure that no influenza nucleic acid was present, each spiked with a representative seasonal Influenza A H3N2 strain (A/New York/55/2004) at one of the three different concentrations (LoD/20, LoD, and 3X LoD). sNPS were generated from diluting HeLa cells into VTM. (Note: refer to the "Validation of Simulated Sample Matrices" section for details). Therefore, Panel 3 consisted of three seasonal Influenza A H3N2 virus spiked medium positive sNPS specimens (3X LoD), three seasonal Influenza A H3N2 virus spiked low positive sNPS specimens (LoD), and three seasonal Influenza A H3N2 virus spiked high negative sNPS specimens.
- Panel 4 contained nine simulated NPW (sNPW) specimens that were screened with the Flu A and Flu B assays of the JBAIDS Influenza A & B Detection Kit to ensure that no influenza nucleic acid was present, each spiked with a representative seasonal Influenza A H3N2 strain (A/New York/55/2004) at one of the three different concentrations (LoD/20, LoD, and 3X LoD). sNPW were generated from diluting HeLa cells into 50% VTM and 50% saline (0.9% NaCl). (Note: refer to the "Validation of Simulated Sample Matrices" section for details). Therefore, Panel 4 consisted of three seasonal Influenza A H3N2 virus spiked medium positive sNPW specimens (3X LoD), three seasonal Influenza A H3N2 virus spiked low positive sNPW specimens (LoD), and three seasonal Influenza A H3N2 virus spiked high negative sNPW specimens.
- Panel 5 contained nine simulated NPS (sNPS) specimens that were screened with the Flu A and Flu B assays of the JBAIDS Influenza A & B Detection Kit to ensure that no influenza nucleic acid was present, each spiked with a representative 2009 H1N1 Influenza strain (A/New York/18/2009) at one of the three different concentrations (LoD/20, LoD, and 3X LoD). sNPS were generated from diluting HeLa cells into VTM. (Note: refer to the "Validation of Simulated Sample Matrices" section for details). Therefore, Panel 5 consisted of three 2009 H1N1 Influenza virus spiked medium positive sNPS specimens (3X LoD), three 2009 H1N1 Influenza virus spiked low positive sNPS specimens (LoD), and three 2009 H1N1 Influenza virus spiked high negative sNPS specimens.
- Panel 6 contained nine simulated NPW (sNPW) specimens that were screened with the Flu A and Flu B assays of the JBAIDS Influenza A & B Detection Kit to ensure that no influenza nucleic acid was present, each spiked with a representative 2009 H1N1 Influenza strain (A/New York/18/2009) at one of the three different concentrations (LoD/20, LoD, and 3X LoD). sNPW were generated from diluting HeLa cells into 50% VTM and 50% saline (0.9% NaCl). (Note: refer to the "Validation of Simulated Sample Matrices" section for details). Therefore, Panel 6 consisted of three 2009 H1N1 Influenza virus spiked medium positive sNPW specimens (3X LoD), three 2009 H1N1 Influenza virus spiked low positive sNPW specimens (LoD), and three 2009 H1N1 Influenza virus spiked high negative sNPW specimens.

Note: Panels 3 and 4 specimens were also co-spiked with a representative Influenza B virus strain (B/Ohio/1/2005) for more efficient reproducibility testing of the JBAIDS

Influenza A Subtyping Kit. Refer to the "Co-spiking Specimen Validation" section for details demonstrating that combining these two viruses in a sample, even at high concentrations, had no detrimental effect on the detection of either virus.

The NPS and sNPS reproducibility panel compositions are presented in the following table:

Level of Seasonal Flu A H1N1 (A/New Caledonia/20/1999) Virus in NPS Specimen	Virus Concentration (EID_{50}/mL)
High Negative (LoD/20) – 3 specimens	2.5
Low Positive (LoD) – 3 specimens	50
Medium Positive (3X LoD) – 3 specimens	150
Level of Seasonal Flu A H3N2 (A/New York/55/2004) Virus in sNPS Specimen	Virus Concentration (EID_{50}/mL)
High Negative (LoD/20) – 3 specimens	0.25
Low Positive (LoD) – 3 specimens	5
Medium Positive (3X LoD) – 3 specimens	15
Level of 2009 H1N1 Influenza (A/New York/18/2009) Virus in sNPS Specimen	Virus Concentration (EID_{50}/mL)
High Negative (LoD/20) – 3 specimens	75
Low Positive (LoD) – 3 specimens	1500
Medium Positive (3X LoD) – 3 specimens	4500

The NPW and sNPW reproducibility panel compositions are presented in the following table:

Level of Seasonal Flu A H1N1 (A/New Caledonia/20/1999) Virus in NPW Specimen	Virus Concentration (EID_{50}/mL)
High Negative (LoD/20) – 3 specimens	2.5
Low Positive (LoD) – 3 specimens	50
Medium Positive (3X LoD) – 3 specimens	150
Level of Seasonal Flu A H3N2 (A/New York/55/2004) Virus in sNPW Specimen	Virus Concentration (EID_{50}/mL)
High Negative (LoD/20) – 3 specimens	0.25
Low Positive (LoD) – 3 specimens	5
Medium Positive (3X LoD) – 3 specimens	15
Level of 2009 H1N1 Influenza (A/New York/18/2009) Virus in sNPW Specimen	Virus Concentration (EID_{50}/mL)
High Negative (LoD/20) – 3 specimens	75
Low Positive (LoD) – 3 specimens	1500
Medium Positive (3X LoD) – 3 specimens	4500

Each panel of 9 samples was tested twice daily for five days at each testing site. Every testing day, two users each tested each of the nine samples. Prior to testing with the JBAIDS assays, one user used the Roche MagNA Pure Nucleic Acid Isolation Kit I and the other user used the IT 1-2-3 Platinum Path Sample Purification Kit to extract viral nucleic acid from each sample. For each panel, each sample was tested a total of 30 times (2 runs/day/site X 5 days X 3 Sites; 15 times after purification with the Platinum Path kit and 15 times after purification with the MagNA Pure kit), and each concentration level per sample type was tested a total of 90 times (3 samples spiked at each concentration level in each of the 6 panels).

Each JBAIDS run performed in this study included Positive Control (PC) and negative extraction control (NEC) reactions. (Note: to ensure that no contamination of virus or amplification product was contracted during sample preparation and reaction setup, a NEC, which was an aliquot of reagent grade water, was prepared and tested with each batch of purified samples.)

Of the 63 Flu A H1 PC reactions tested in this study, all were valid (63/63; 100%). The Flu A H1 PC reactions had a mean Cp of 31.41 ± 0.56 (1.78 % CV). Sixty-one of the 63 Flu A H1 NEC reactions (61/63; 96.8%) tested in this study yielded the expected negative target results and were valid. Two failed NEC reactions were observed and the associated runs were invalid. One failed NEC was likely due to the control capillaries being incorrectly loaded in the JBAIDS carousel, as reported by the user. The purified samples were retested with new controls. The other failed NEC had a positive result for the Flu A H1 assay, possibly due to contamination. The work area was cleaned and fresh samples were re-extracted and tested. All controls were valid on the retest run.

Sixty-two out of 65 Flu A H3 PC and NEC reactions tested in this study were valid (62/65; 95.4%). The mean Cp of the Flu A H3 PC reactions was 30.86 ± 0.20 (0.65% CV). Three invalid runs were successfully detected by the controls. These run failures appear to be due to the carousel being improperly seated by the user. This shifted the capillaries during the test, resulting in a loss of fluorescence detection in all samples. The JBAIDS software correctly assigned failure results to the controls in these runs. The data from these invalid runs were not included in this study. All controls were valid on the retest run.

All 63 Flu A Sw PC and NEC reactions tested in this study were valid. The mean Cp of the Flu A Sw PC reactions was 28.27 ± 0.30 (1.06% CV). All 63 Flu A H1 2009 PC and NEC reactions tested were valid. The mean Cp of the Flu A H1 2009 PC reactions was 32.01 ± 0.39 (1.22% CV).

Although the JBAIDS Influenza A Subtyping Kit does not contain a sample control assay, the Flu SC assay from the JBAIDS Influenza A & B Detection Kit was tested with each sample. This assay targets the human RNaseP sequence and serves as an external control to detect inhibition, inadequate sample extraction, or poor specimen collection. It is recommended that any sample tested with the Influenza A Subtyping Kit first be tested with the Influenza A & B Detection kit; therefore the Flu SC assay would have been previously tested with each sample. Of the 1,628 Flu SC reactions tested in this study, 1,620 Flu SC reactions gave positive results, while 8 negative results were observed (1620/1628; 99.5%). All Flu SC negative results were from NPW or sNPW samples, which may contain fewer human cells per unit volume than swab samples. Because negative Flu SC results only cause a control failure for negative influenza assays, six of the eight negative Flu SC results did not affect the positive metacall; the corresponding influenza assays had the expected positive metacall results. The other two negative Flu SC assays results were called SC Failures, and both occurred with the same sample. On the initial run the sample gave an SC Failure call. According to the protocol the purified sample was retested, both undiluted and diluted 1:10. The retest result for the undiluted sample was again an SC Failure. Therefore a new aliquot of the same sample was re-extracted and tested.

Results for the second retest were positive for the Flu A H3 and Flu SC assays.

For the Flu SC assay, NPS samples exhibited a mean Cp of 30.15 ± 2.36 (7.83% CV) and NPW samples had a mean Flu SC Cp of 33.18 ± 1.88 (5.67% CV). The cell concentrations used to prepare the sNPS and sNPW samples were chosen to mimic Flu SC Cp values of fresh specimens. The sNPS samples had a mean Cp of 32.06 ± 2.00 (6.24% CV) and sNPW samples exhibited a mean Cp of 34.29 ± 2.29 (6.68% CV) for the Flu SC assay. These Cp values are consistent with previous testing of frozen simulated samples prepared at $10^{4.2}$ and $10^{3.7}$HeLa cells/mL for sNPS and sNPW samples, respectively. The 6-8% CV in the Flu SC mean Cp values measured for each sample type arises primarily from differences in Flu SC Cp values observed for the two sample purification kits. Typically MagNA Pure extracted samples exhibit Cp values 4 cycles earlier than respective Platinum Path extracted samples.

Reproducibility Study Summary (Agreement with Expected Positive Results) for the JBAIDS Influenza A Subtyping Kit – Influenza A/H1 Assay

Sample Type	Test Level	IT 1-2-3 Platinum Path Sample Purification Kit # of Positive Samples/# of Total Samples (% Positive Detection)				Roche MagNA Pure Compact Nucleic Acid Isolation Kit I # of Positive Samples/# of Total Samples (% Positive Detection)				All Purification Kits, All Sites	95% CI
		Test Location				Test Location					
		Site 1	Site 2	Site 3	All Sites	Site 1	Site 2	Site 3	All Sites		
NPS	3X LoD	15/15 (100%)	14/15 (93%)	15/15 (100%)	44/45 (98%)	15/15 (100%)	14/15 (93%)	15/15 (100%)	44/45 (98%)	88/90 (98%)	92.2-99.7
	LoD	15/15 (100%)	15/15 (100%)	15/15 (100%)	45/45 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	45/45 (100%)	90/90 (100%)	96.7-99.9
	LoD/20	15/15 (100%)	13/15 (87%)	8/15 (53%)	36/45 (80%)	13/15 (87%)	11/15 (73%)	14/15 (93%)	38/45 (84%)	74/90 (82%)	72.7-89.4
	Detection ≥ LoD	30/30 (100%)	29/30 (97%)	30/30 (100%)	89/90 (99%)	30/30 (100%)	29/30 (97%)	30/30 (100%)	89/90 (99%)	178/180 (99%)	96.0-99.9
	Detection all Levels	45/45 (100%)	42/45 (93%)	38/45 (84%)	125/135 (93%)	43/45 (96%)	40/45 (89%)	44/45 (98%)	127/135 (94%)	252/270 (93%)	90.0-96.0
NPW	3X LoD	15/15 (100%)	15/15 (100%)	15/15 (100%)	45/45 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	45/45 (100%)	90/90 (100%)	96.7-99.9
	LoD	14/15 (93%)	15/15 (100%)	15/15 (100%)	44/45 (98%)	15/15 (100%)	14/15 (93%)	15/15 (100%)	44/45 (98%)	88/90 (98%)	92.2-99.7
	LoD/20	13/15 (87%)	15/15 (100%)	9/15 (60%)	37/45 (82%)	14/15 (93%)	14/15 (93%)	15/15 (100%)	43/45 (96%)	80/90 (89%)	80.5-94.5
	Detection ≥ LoD	29/30 (97%)	30/30 (100%)	30/30 (100%)	89/90 (99%)	30/30 (100%)	29/30 (97%)	30/30 (100%)	89/90 (99%)	178/180 (99%)	96.0-99.9
	Detection All Levels	42/45 (93%)	45/45 (100%)	39/45 (87%)	126/135 (93%)	44/45 (98%)	43/45 (96%)	45/45 (100%)	132/135 (98%)	258/270 (96%)	92.4-97.7

Reproducibility Study Summary (Agreement with Expected Positive Results) for the JBAIDS Influenza A Subtyping Kit – Influenza A/H3 Assay

Sample Type	Test Level	IT 1-2-3 Platinum Path Sample Purification Kit # of Positive Samples/# of Total Samples (% Positive Detection)	Roche MagNA Pure Compact Nucleic Acid Isolation Kit I # of Positive Samples/# of Total Samples (% Positive Detection)	All Purification Kits, All Sites	95% CI

		Test Location				Test Location					
		Site 1	Site 2	Site 3	All Sites	Site 1	Site 2	Site 3	All Sites		
NPS	3X LoD	15/15 (100%)	15/15 (100%)	15/15 (100%)	45/45 (100%)	15/15 (100%)	15/15 (100%)	13/15 (87%)	43/45 (96%)	88/90 (98%)	92.2-99.7
	LoD	15/15 (100%)	15/15 (100%)	15/15 (100%)	45/45 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	45/45 (100%)	90/90 (100%)	96.7-99.9
	LoD/20	12/15 (80%)	10/15 (67%)	9/15 (60%)	31/45 (69%)	14/15 (93%)	13/15 (87%)	14/15 (93%)	41/45 (91%)	72/90 (80%)	70.2-87.7
	Detection ≥ LoD	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	30/30 (100%)	30/30 (100%)	28/30 (93%)	88/90 (98%)	178/180 (99%)	96.0-99.9
	Detection all Levels	42/45 (93%)	40/45 (89%)	39/45 (87%)	121/135 (90%)	44/45 (98%)	43/45 (96%)	42/45 (93%)	129/135 (96%)	250/270 (93%)	88.8-95.4
NPW	3X LoD	15/15 (100%)	15/15 (100%)	14/15 (93%)	44/45 (98%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	45/45 (100%)	89/90 (99%)	94.0-99.9
	LoD	14/15 (93%)	15/15 (100%)	15/15 (100%)	44/45 (98%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	45/45 (100%)	89/90 (99%)	94.0-99.9
	LoD/20	11/15 (73%)	10/15 (67%)	9/15 (60%)	30/45 (67%)	15/15 (100%)	14/15 (93%)	15/15 (100%)	44/45 (98%)	74/90 (82%)	72.7-89.5
	Detection ≥ LoD	29/30 (97%)	30/30 (100%)	29/30 (97%)	88/90 (98%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	178/180 (99%)	96.0-99.9
	Detection All Levels	40/45 (89%)	40/45 (89%)	38/45 (84%)	118/135 (87%)	45/45 (100%)	44/45 (98%)	45/45 (100%)	134/135 (99%)	252/270 (93%)	89.7-96.0

Reproducibility Study Summary (Agreement with Expected Positive Results) for the Combined Results of the Influenza A/2009 H1 and the Influenza A Sw Assays in the JBAIDS Influenza A Subtyping Kit

Sample Type	Test Level	IT 1-2-3 Platinum Path Sample Purification Kit # of Positive Samples/# of Total Samples (% Positive Detection)				Roche MagNA Pure Compact Nucleic Acid Isolation Kit I # of Positive Samples/# of Total Samples (% Positive Detection)				All Purification Kits, All Sites	95% CI
		Test Location				Test Location					
		Site 1	Site 2	Site 3	All Sites	Site 1	Site 2	Site 3	All Sites		
NPS	3X LoD	15/15 (100%)	15/15 (100%)	15/15 (100%)	45/45 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	45/45 (100%)	90/90 (100%)	96.7-99.9
	LoD	15/15 (100%)	15/15 (100%)	15/15 (100%)	45/45 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	45/45 (100%)	90/90 (100%)	96.7-99.9
	LoD/20	15/15 (100%)	15/15 (100%)	15/15[a] (100%)	45/45 (100%)	15/15[a] (100%)	15/15 (100%)	15/15 (100%)	45/45 (100%)	90/90 (100%)	96.7-99.9
	Detection ≥ LoD	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	180/180 (100%)	98.3-99.9
	Detection all Levels	45/45 (100%)	45/45 (100%)	45/45 (100%)	135/135 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	135/135 (100%)	270/270 (100%)	98.9-99.9
NPW	3X LoD	15/15 (100%)	15/15 (100%)	15/15 (100%)	45/45 (100%)	15/15 (100%)	15/15[a] (100%)	15/15 (100%)	45/45 (100%)	90/90 (100%)	96.7-99.9
	LoD	15/15 (100%)	15/15 (100%)	15/15 (100%)	45/45 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	45/45 (100%)	90/90 (100%)	96.7-99.9
	LoD/20	15/15 (100%)	15/15 (100%)	15/15[a] (100%)	45/45 (100%)	15/15 (100%)	15/15[a] (100%)	15/15 (100%)	45/45 (100%)	90/90 (100%)	96.7-99.9

	Detection ≥ LoD	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	180/180 (100%)	98.3-99.9
	Detection All Levels	45/45 (100%)	45/45 (100%)	45/45 (100%)	135/135 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	135/135 (100%)	270/270 (100%)	98.9-99.9

[a]Results of retest shown; first tests were inconclusive.

Note: The rate of positive test results at the high negative level was higher than expected and is most likely the result of variation in the preparation of the samples used for this study, potentially resulting in somewhat higher than expected levels of virus in these samples.

This reproducibility study demonstrated that as expected with all real-time PCR assays, the JBAIDS Influenza A Subtyping Kit may not generate reproducibly positive results when testing samples that have analyte concentrations lower than the LoD concentration, but higher than the assay cutoff concentration. This limitation should be addressed by including the following statement in the Limitation section of the JBAIDS Influenza A & B Detection Kit Instructions for Use: ***"The JBAIDS Influenza A Subtyping Kit may not generate reproducibly positive results when testing samples that have analyte concentrations lower than the LoD concentration, but higher than the assay cutoff concentration."***

The variability of the Cp value can be used as an additional measure of the variability of JBAIDS assays. Therefore, the average and %CV of Cp values obtained in this study were used to further evaluate the precision/reproducibility of the test system.

Reproducibility Study Summary (Mean Cp, SD and %CV) for the Influenza A/H1 Assay in the JBAIDS Influenza A Subtyping Kit

Sample Type	Virus Spike Level	IT 1-2-3 Platinum Path Sample Purification Kit								Roche MagNA Pure Compact Nucleic Acid Isolation Kit I							
		Test Location								Test Location							
		Site 1		Site 2		Site 2		All Sites		Site 1		Site 2		Site 2		All Sites	
		Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV
NPS	3×LoD	32.87 (0.29)	0.88	33.24 (0.47)	1.40	33.54 (0.68)	2.03	33.22 (0.57)	1.71	31.64 (0.65)	2.07	31.91 (0.54)	1.70	31.62 (0.65)	2.06	31.72 (0.62)	1.95
	LoD	34.63 (0.59)	1.72	34.31 (0.71)	2.07	34.84 (0.42)	1.21	34.56 (0.61)	1.77	32.85 (0.81)	2.48	32.82 (0.71)	2.15	32.75 (0.65)	1.98	32.81 (0.71)	2.16
	LoD/20	38.19[a] (2.03)	5.31[a]	37.07 (1.03)	2.77	38.73[a] (1.71)	4.41[a]	37.90[a] (1.74)	4.60[a]	34.69 (0.80)	2.31	35.32 (0.92)	2.61	35.01 (0.85)	2.43	34.99 (0.87)	2.49
NPW	3×LoD	33.12 (0.77)	2.32	33.04[b] (0.97)	2.94[b]	34.03 (0.49)	1.45	33.39 (0.88)	2.63	31.95 (0.53)	1.65	32.38 (0.51)	1.58	31.32 (0.51)	1.61	31.88 (0.67)	2.09
	LoD	34.76 (0.65)	1.87	34.19 (0.35)	1.01	35.57 (0.82)	2.31	34.84 (0.85)	2.44	33.19 (0.48)	1.45	33.54 (0.44)	1.30	32.85 (0.36)	1.10	33.18 (0.50)	1.52
	LoD/20	40.02[a] (2.46)	6.16[a]	38.60[a,b] (2.23)	5.79[a,b]	40.60[a] (2.39)	5.89[a]	39.59[a] (2.44)	6.17[a]	36.05 (0.69)	1.90	36.95 (1.05)	2.85	36.07 (0.69)	1.92	36.35 (0.91)	2.50

[a] One or more capillaries had a Cp of >40.00 and was assigned a Cp of 42.50 cycles.
[b] One medium positive sample and a high negative sample were switched during testing. Based on Cp values they have been assigned to the correct virus spike level during data analysis.

Reproducibility Study Summary (Mean Cp, SD and %CV) for the Influenza A/H3 Assay in the JBAIDS Influenza A Subtyping Kit

Sample Type	Virus Spike Level	IT 1-2-3 Platinum Path Sample Purification Kit								Roche MagNA Pure Compact Nucleic Acid Isolation Kit I							
		Test Location								Test Location							
		Site 1		Site 2		Site 2		All Sites		Site 1		Site 2		Site 2		All Sites	
		Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV
sNPS	3×LoD	34.14 (0.33)	0.96	33.63 (0.84)	2.50	34.09 (0.91)	2.66	33.96 (0.76)	2.23	32.65 (0.36)	1.10	32.57 (0.48)	1.49	31.71 (0.17)	0.54	32.34 (0.55)	1.70
	LoD	35.32 (0.54)	1.52	34.99 (1.07)	3.05	35.64 (0.40)	1.13	35.32 (0.76)	2.15	33.85 (0.33)	0.97	33.89 (0.32)	0.95	32.81 (0.33)	1.02	33.52 (0.60)	1.78
	LoD/20	39.99[a] (1.96)	4.90[a]	39.11[a] (2.60)	6.64[a]	39.70[a] (3.01)	7.57[a]	39.62[a] (2.45)	6.17[a]	36.81 (0.64)	1.74	36.85 (0.60)	1.63	36.01 (0.37)	1.03	36.55 (0.66)	1.81
sNPW	3×LoD	34.29 (0.43)	1.25	34.32 (0.56)	1.64	34.31 (0.49)	1.42	34.31 (0.49)	1.41	31.97 (0.69)	2.15	32.11 (0.61)	1.89	31.46 (0.28)	0.88	31.85 (0.61)	1.91
	LoD	35.99 (0.61)	1.70	36.28[a] (1.85)	5.10[a]	35.90 (0.80)	2.22	36.06[a] (1.21)	3.35[a]	33.36 (0.50)	1.50	34.01 (0.81)	2.38	32.94 (0.23)	0.71	33.44 (0.71)	2.12
	LoD/20	42.14[a] (1.21)	2.87[a]	40.80[a] (2.25)	5.52[a]	41.74a (1.55)	3.72a	41.57[a] (1.75)	4.22[a]	37.20 (1.02)	2.75	37.80[a] (1.88)	4.97[a]	36.52 (0.60)	1.63	37.16[a] (1.34)	3.61[a]

[a] One or more capillaries had a Cp of >40.00 and was assigned a Cp of 42.50 cycles.

Reproducibility Study Summary (Mean Cp, SD and %CV) for the 2009 H1N1 Influenza Assays in the JBAIDS Influenza A Subtyping Kit

Influenza Assay	Sample Type	Virus Spike	IT 1-2-3 Platinum Path Sample Purification Kit								Roche MagNA Pure Compact Nucleic Acid Isolation Kit I							
			Test Location								Test Location							
			Site 1		Site 2		Site 3		All Sites		Site 1		Site 2		Site 3		All Sites	
			Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV	Mean Cp (SD)	% CV
Flu A Sw	sNPS	3×LoD	28.43 (0.44)	1.53	28.78 (0.38)	1.33	28.85 (0.28)	0.99	28.69 (0.41)	1.43	25.60a (0.40)	1.54	26.15 (0.34)	1.29	25.81 (0.29)	1.14	28.85 (0.41)	1.57
		LoD	30.07 (0.60)	2.02	30.26 (0.75)	2.46	30.03 (0.35)	1.15	30.12 (0.59)	1.95	26.98 (0.33)	1.21	27.49 (0.24)	0.87	27.12 (0.28)	1.05	27.20 (0.35)	1.29
		LoD/20	32.81 (0.47)	1.44	32.98 (0.55)	1.67	32.97 (0.62)	1.87	32.92 (0.54)	1.65	29.94 (0.55)	1.83	30.47 (0.39)	1.29	29.99 (0.47)	1.57	30.31 (0.52)	1.73
	sNPW	3×LoD	30.01 (0.72)	2.38	29.85 (0.57)	1.90	29.25 (0.62)	2.13	29.74 (0.70)	2.34	26.32 (0.32)	1.22	26.62 (0.52)	1.96	26.25 (0.34)	1.30	26.40 (0.43)	1.62
		LoD	31.20 (0.56)	1.81	31.28 (0.57)	1.82	30.85 (0.68)	2.20	31.11 (0.62)	2.00	27.70 (0.34)	1.24	28.13 (0.33)	1.18	27.63 (0.31)	1.14	27.82 (0.39)	1.41
		LoD/20	33.80 (0.39)	1.17	33.86 (0.73)	2.16	33.32 (0.54)	1.61	33.66 (0.61)	1.81	30.89 (0.53)	1.72	31.07 (0.42)	1.34	30.48 (0.43)	1.41	30.81 (0.52)	1.68
	sNPS	3×LoD	31.19 (0.48)	1.53	31.13 (0.41)	1.33	31.41 (0.41)	1.32	31.24 (0.44)	1.42	28.84a (0.50)	1.73	29.06 (0.33)	1.15	28.91 (0.23)	0.81	28.93 (0.38)	1.30

		LoD	32.92 (0.52)	1.59	32.86 (0.83)	2.54	32.99 (0.40)	1.21	32.92 (0.60)	1.83	30.34 (0.38)	1.26	30.56 (0.31)	1.01	30.36 (0.30)	0.99	30.42 (0.34)	1.12
		LoD/20	37.11 (0.76)	2.04	36.94 (0.54)	1.46	37.78[a] (1.99)	5.26[a]	37.28[a] (1.29)	3.46[a]	34.24 (0.57)	1.66	34.45 (0.37)	1.09	34.24 (0.31)	0.92	34.31 (0.43)	1.27
	sNPW	3×LoD	31.90 (0.24)	0.74	32.03 (0.52)	1.63	31.67 (0.56)	1.78	31.89 (0.48)	1.51	29.23 (0.36)	1.24	29.44 (0.32)	1.07	29.34 (0.30)	1.04	29.34 (0.33)	1.13
		LoD	33.49 (0.38)	1.15	33.48 (0.60)	1.80	33.33 (0.66)	1.99	33.44 (0.55)	1.66	30.72 (0.39)	1.28	30.92 (0.23)	0.73	30.76 (0.12)	0.38	30.80 (0.28)	0.91
		LoD/20	37.49[a] (1.73)	4.62[a]	37.59[a] (1.89)	5.04[a]	36.88[a] (1.69)	4.57a	37.32[a] (1.76)	4.72[a]	34.28 (0.39)	1.15	34.59 (0.49)	1.41	34.32 (0.36)	1.04	34.40 (0.43)	1.25

[a] One or more capillaries had a Cp of >40.00 and was assigned a Cp of 42.50 cycles.

b. Linearity/assay reportable range:

Not applicable, qualitative assay

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

Assay Controls

The following controls are included in the JBAIDS Influenza A Subtyping Kit:

Negative Control

The NC is used to detect contamination from target-specific amplified product (amplicon), synthetic RNA (as found in the PC vials), or virus. The NC vial is reconstituted using the purified negative extraction control (NEC) (molecular biology grade water). The NEC should be processed along with each batch of specimens purified. The NC detects possible contamination during sample purification and during reconstitution of freeze- dried reagents. Each JBAIDS Influenza A Subtyping Kit run requires one NC for each of the 4 assays (i.e., the Flu A H1, Flu A H3, Flu A H1 2009, and Flu A Sw assays). For each target assay, the NC must be negative, or the JBAIDS software will assign invalid results for that target assay to all of the samples in that run. If the NC fails, the JBAIDS run must be repeated using the same purified samples. If the NC fails again, all associated samples are considered to have invalid results and should be re-purified starting from another aliquot of the original specimens.

Sixty-one of the 63 Flu A H1 NEC reactions (61/63; 96.8%) tested in the multi-site reproducibility study yielded the expected negative target results and were valid. All controls were valid on the retest run. Sixty-two out of 65 Flu A H3 NEC reactions tested in this study were valid (62/65; 95.4%). All controls were valid on the retest run. All 63 Flu A Sw and Flu A H1 2009 NEC reactions tested in this study were valid.

During the prospective clinical study, of 261 runs, there were a total of 8 (3.1%) runs in which the NCs failed. Site 1 and Site 2 had 3 NC failures each, all of which were associated with the Flu A H1 2009 assay. Site 4 and Site 6 each had 1

run failure with the Flu A H1 2009 NC. All associated specimens were retested and no specimens were lost due to these failures.

Positive Control

The PC serves as an amplification and detection control. Each JBAIDS Influenza A Subtyping Kit run requires one PC for each of the 4 assays (i.e., the Flu A H1, Flu A H3, Flu A H1 2009, and Flu A Sw assays). For each target assay, the PC must be positive. If the PC fails, the JBAIDS software will assign invalid results for that target assay to all of the associated samples, and the JBAIDS run must be repeated starting from the same purified specimens. Failure of the PCs may indicate errors in sample setup, degradation of the reagents, or a malfunction of the JBAIDS instrument.

Of the 63 Flu A H1 PC reactions tested in the reproducibility study, all were valid (63/63; 100%). The Flu A H1 PC reactions had a mean Cp of 31.41 ± 0.56 (1.78 % CV). Sixty-two out of 65 Flu A H3 PC reactions tested in this study were valid (62/65; 95.4%). The mean Cp of the Flu A H3 PC reactions was 30.86 ± 0.20 (0.65% CV). All controls were valid on the retest run. All 63 Flu A Sw PC reactions tested in this study were valid. The mean Cp of the Flu A Sw PC reactions was 28.27 ± 0.30 (1.06% CV). All 63 Flu A H1 2009 PC reactions tested were valid. The mean Cp of the Flu A H1 2009 PC reactions was 32.01 ± 0.39 (1.22% CV).

During the prospective clinical study, of 261 runs, there were a total of 2 (0.8%) runs in which the PCs failed. Site 2 had 1 Flu A H1 2009 PC failure. Site 6 had 1 Flu A Sw PC failure. All associated specimens were retested and no specimens were lost due to these failures. The Flu A H1 PC reactions had a mean Cp of 32.1 ± 0.6 (1.8 % CV). The mean Cp of the Flu A H3 PC reactions was 30.3 ± 0.3 (1.0% CV). The mean Cp of the Flu A Sw PC reactions was 28.9 ± 0.4 (1.5% CV). The mean Cp of the Flu A H1 2009 PC reactions was 31.4 ± 0.3 (1.1% CV).

The final Cp cutoff values for the Flu A H1, Flu A H3, Flu A Sw and Flu A H1 2009 positive controls are 37.1, 36.3, 33.4, and 36.7 respectively, and are based on the upper bound of the manufacturing QA Review range for reagent lot release plus 4 x SD. The estimated standard deviation (SD) was set to 0.9 Cp to reflect the amount of variation that is consistent with data obtained in the JBAIDS Influenza A Subtyping Kit and the JBAIDS Influenza A & B Detection Kit analytical and clinical studies. If an amplification curve for a PC fails to amplify or has a Cp value later than the cutoff, the PC will be interpreted as a failure and the associated patient test results will be assigned an invalid test result.

Extraction Control (EC)

Positive Extraction Control (PEC) was not provided with the kit, and inclusion of a PEC with each batch of extracted samples is not a standard part of the testing protocol recommended in the product package insert. However, during the clinical study, a PEC and a NEC were purified with each batch of clinical samples. Inclusion of a NEC with each batch of purified samples is a standard part of the testing protocol recommended in the product package insert. PECs were simulated NPS (sNPS; HeLa cells in viral transport media) spiked with four representative influenza strains (Influenza B,

seasonal Influenza A H1N1, seasonal Influenza A H3N2, and 2009 H1N1 Influenza) at concentrations near the LoD for the influenza assays. The PEC was processed and tested in the same manner as a patient sample. The PEC was considered successful when all tested target assays were positive. A failed PEC was retested using the same purified sample and the results of the retest were accepted as the final result. If the retest was unsuccessful, all associated samples were considered to have invalid results and required re-purification in order to obtain a valid test result.

During the prospective clinical evaluation, 11 out of a total of 194 PECs required retesting. Nine retests (3 at Site 1, 3 at Site 2, 1 at Site 4, and 2 at Site 6) were required due to an invalid assay result (PC or NC). Two PEC failures occurred as a result of one of the four target assays giving a negative result (Flu A H1 one time and Flu A H1 2009 one time). Retesting was performed for one of the failures (Flu A H1 2009) which resolved the failure. There was no retest performed for the other failure. This lack of retesting was a protocol deviation. Five (5) samples associated with this run were excluded from data analysis. The mean Cp of the PEC tested for the Flu A H1 assay was 31.1, with 2.7 Standard Deviation (SD) and 8.6 % CV. The mean Cp of the PEC tested for the Flu A H3 assay was 30.7, with 1.5 Standard Deviation (SD) and 4.7 % CV. The mean Cp of the PEC tested for the Flu A H1 2009 assay was 31.2, with 1.9 Standard Deviation (SD) and 6.1 % CV. The mean Cp of the PEC tested for the Flu A Sw assay was 29.6, with 2.0 Standard Deviation (SD) and 6.8 % CV.

The NEC was a nuclease-free water sample that was processed with each batch of purified specimens. The purified NEC sample was then used to reconstitute the freeze- dried NC.

Specimen Stability

The sponsor recommends that MPW and NPS specimens be collected and placed into viral transport media (VTM). Once collected, specimens may be tested immediately after collection, transported to an off-site testing location, or temporarily stored until testing can be scheduled. Inappropriate handling or storage may compromise the integrity of the specimens and cause inaccurate test results. An analytical study determining the appropriate storage times and temperatures for both unprocessed specimens (e.g., the NPW and NPS specimens) and processed samples (e.g., purified nucleic acid from NPW and NPS specimens) was carried out. The specimen transport and storage conditions that were evaluated are based on common clinical laboratory workflows.

Three Influenza A subtype strains (i.e., Influenza A/H1, Influenza A/H3, and 2009 H1N1 Influenza) were evaluated with the JBAIDS Influenza A Subtyping Kit for both unprocessed and purified samples.

Note: Specimens spiked with a representative Influenza A/H3 virus strain (A/New York/55/2004) were also co-spiked with a representative Influenza B virus strain (B/Ohio/1/2005) for more efficient testing of the JBAIDS Influenza A Subtyping Kit. Refer to the “Co-spiking Specimen Validation” section for details demonstrating that combining these two viruses in a sample, even at high concentrations, had no detrimental effect on the detection of either virus.

For the unprocessed specimen transport and storage evaluation, both negative and positive specimens were assessed. Positive specimens were spiked with appropriate Influenza A viruses at the established LoD for the respective assay. Eight positive (per influenza strain) and four negative specimens were processed at each time point and storage condition per purification kit prior to testing with the freeze-dried reagents. Both specimen types (NPW and NPS) were evaluated independently.

For the purified sample transport and storage evaluation, sets of eight positive samples (per influenza strain) and four negative samples that had been processed with either purification method were tested immediately and also aliquoted for storage under various conditions prior to retesting with the freeze-dried reagents. Purified samples were also tested to determine the storage of reconstituted freeze-dried reagents.

The following table summarizes the final conditions that were evaluated in this study:

Final Storage Conditions Evaluated for each Sample Matrix/Purification Kit Combination

Sample Types	Conditions
Unprocessed Specimens	Day 0, no storage 4 Hours, ambient (18-30°C) Day 3, refrigerated (2-8°C) Day 30, frozen (≤ -15°C)
Purified Samples	Day 0, no storage 4 Hours, ambient (18-30°C) Day 1, refrigerated (2-8°C) Day 30 , frozen (≤ -15°C) Platinum Path only Day 30, frozen (≤ -70°C) MagNA Pure only
Reconstituted Freeze Dried-Reagents	Day 0, no storage 4 Hours, refrigerated (2-8°C)

The performance at each time point was evaluated by comparing the JBAIDS assay result (positive, negative, uncertain, or SC fail) to the expected result and the result at the initial time point. To be acceptable, all negative samples must be negative for the influenza subtyping assays and positive for the Flu SC assay, and seven of eight positive samples must test positive for the relevant assays (Flu A H1, Flu A H3, Flu A H1 2009, and Flu A Sw). In addition, the mean Cp and F_{max} values of the stored sample set were compared to those obtained at the initial time point. For an acceptable storage condition, the mean Cp of the influenza subtyping assays for the positive sample set should not increase by more than 3 cycles, and the mean F_{max} must not decrease by more than 50%.

Unprocessed Specimen Storage Conditions Test Results

At least seven of the eight positive human derived NPS and NPW specimens in each sample set tested positive at all four time points with the respective Influenza A subtyping assay. The mean Cp values for the stored specimens were all within three cycles of the day 0 time point suggesting no decrease in assay performance with storage. In addition, the mean F_{max} values for the time points were never less than 50% of the

F_{max} of the day 0 baseline indicating no reduction of test system performance. All negative specimens tested negative at each time point.

These results indicate that NPS and NPW specimens can be stored at 18-30 °C for up to four hours, at 2-8 °C for up to three days, and at ≤ -15 °C for up to thirty days prior to testing without compromising the results.

Purified Sample Storage Conditions Test Results

At least seven of the eight purified human derived NPS and NPW positive samples in each sample set tested positive at each time point with the respective Influenza A subtyping assay. The average Cp values for the stored samples were all within 3 cycles of the day 0 time point suggesting no decrease in assay performance with storage. In addition, the average F_{max} values for the time points were never less than 50% of the F_{max} of the day 0 baseline indicating no reduction of test system performance. All negative specimens tested negative at each time point.

These results indicate that purified samples can be stored at 18-30 °C for up to four hours, at 2-8 °C for up to one day, and frozen (≤ -15 °C for Platinum Path purified samples, ≤ -70°C for MagNA Pure purified samples) for up to thirty days prior to testing without compromising the results.

Reconstituted Reagent Storage Conditions Test Results

All but one of the positive samples tested positive at both the initial time point and after four hours of refrigeration. The mean Cp values for the stored reactions were all within 3 cycles of the initial time point suggesting no decrease in assay performance with storage. In addition, the mean F_{max} values for the stored reagents were never less than 50% of the F_{max} of the initial baseline indicating no reduction of test system performance. All negative reactions tested negative at both time points.

These results indicate that reconstituted reagents can be stored at 2-8 °C for up to four hours without compromising the results.

d. Detection limit:

Initial estimates for setting the LoD were performed by spiking simulated (s)NPW and sNPS samples with serial dilutions of quantified representative Influenza A viruses (Influenza A H1N1 strain A/New Caledonia/20/1999, Influenza A H1N1 strain A/Hawaii/15/2001, Influenza A H3N2 strain A/New York/55/2004, Influenza A H3N2 strain A/Wisconsin/67/2005, 2009 H1N1 Influenza strain A/New York/18/2009, 2009 H1N1 Influenza strain A/California/7/2009). The spiked samples were then processed using the IT 1-2-3 Platinum Path Sample Purification Kit and the Roche MagNA Pure Compact Nucleic Acid Isolation Kit I, and the purified samples were tested with the associated Influenza A subtyping assays (Flu A H1, Flu A H3, Flu A H12009, and Flu A Sw). An initial estimate of the LoD was made by evaluating the reaction quality at each dilution. Reactions were evaluated using multiple criteria, including the JBAIDS test results, the Cp values, and a visual examination of the amplification curves. LoD estimates were then further evaluated by testing human-derived or simulated samples

spiked with refined serial dilutions of the representative influenza viruses.

Once the LoD was estimated, a set of 20 specimens was collected from 20 unique donors for each combination of specimen type (NPS or NPW) and purification kit (Platinum Path or MagNA Pure). The samples were spiked at the estimated LoD level, processed, and tested with the JBAIDS Influenza A subtyping assays to determine the frequency of positive results. The LoD was confirmed if at least 19 of the 20 samples gave a positive test result.

The LoD levels for detection of each virus subtype were independently established using two representative Influenza A strains of each subtype (seasonal Influenza A H1N1, seasonal Influenza A H3N2, and 2009 H1N1 Influenza). The LoD for detection of the 2009 H1N1 Influenza strain required positive results for both the Flu A Sw and Flu A H1 2009 assays. Specifically, the lowest concentration of 2009 H1N1 Influenza virus where both assays exhibited ≥ 95% positive detection was considered the final LoD.

Based on the titration results for each strain, the LoD levels were set at the concentrations for which ≥95% detection was observed for both sample types and both nucleic acid purification kits. All sets of 20 samples spiked with Influenza A viruses gave at least 19/20 positive results with the appropriate JBAIDS Flu A subtyping assay. The LoD for the Flu A/ H1 assay is 50 EID_{50}/mL for A/New Caledonia/20/1999 and 5,000 EID_{50}/mL for A/Hawaii/15/2001. The LoD for the Flu A H3 assay is 5 EID_{50}/mL for A/New York/55/2004 and 10 EID_{50}/mL for A/Wisconsin/67/2005. The LoD for detection of the Influenza A/2009 H1 virus is 1,500 EID_{50}/mL for A/New York/18/2009 and 5,000 EID_{50}/mL for A/California/7/2009.

Confirmation of Estimated LoD Levels for the Flu A /H1 Assay in the JBAIDS Influenza A Subtyping Kit

Strain	LoD (EID_{50}/mL)	Kit (Sample Type)	Flu A H1		
			Positive /Total	Mean Cp (SD)	Mean Fmax (SD)
A/New Caledonia/ 20/1999	50	MagNA Pure (NPS)	20/20	32.01 (0.72)	19.12 (4.59)
		Platinum Path (NPS)	20/20	33.49 (0.54)	22.34 (4.78)
		MagNA Pure (NPW)	20/20	33.02 (0.99)	21.74 (6.06)
		Platinum Path (NPW)	20/20	34.17 (0.52)	23.69 (3.78)
A/Hawaii/ 15/2001	5,000	MagNA Pure (NPS)	20/20	31.66 (0.61)	24.18 (4.03)
		Platinum Path (NPS)	20/20	33.96 (0.69)	23.30 (5.83)
		MagNA Pure (NPW)	20/20	32.81 (0.55)	26.74 (2.65)
		Platinum Path (NPW)	20/20	34.86 (0.43)	30.72 (3.62)

Confirmation of Estimated LoD Levels for the Flu A /H3 Assay in the

JBAIDS Influenza A Subtyping Kit

Strain	LoD (EID_{50}/mL)	Kit (Sample Type)	Flu A H3		
			Positive /Total	MeanCp (SD)	Mean Fmax (SD)
A/New York/ 55/2004	5	MagNA Pure (NPS)	19/20	32.63 (0.48)	21.52 (4.54)
		Platinum Path (NPS)	20/20	34.45 (0.52)	26.59 (5.87)
		MagNA Pure (NPW)	20/20	33.10 (0.55)	29.08 (7.32)
		Platinum Path (NPW)	20/20	35.50 (0.65)	23.46 (5.31)
A/Wisconsin/	10	MagNA Pure (NPS)	20/20	32.67 (0.70)	27.43 (5.50)
		Platinum Path (NPS)	20/20	34.61 (0.88)	25.84 (6.30)
		MagNA Pure (NPW)	20/20	33.16 (0.47)	35.17 (5.17)
		Platinum Path (NPW)	20/20	34.75 (0.42)	36.86 (5.01)

Confirmation of Estimated LoD Levels detection of 2009 H1N1 Influenza virus with the Flu A H1 2009 and Flu A Sw assays of JBAIDS Influenza A Subtyping Kit

Strain	LoD (EID_{50}/mL)	Kit (Sample Type)	Flu A Sw			Flu A H1 2009		
			Positive /Total	Mean Cp (SD)	Mean Fmax (SD)	Positive /Total	Mean Cp (SD)	Mean Fmax (SD)
A/New York/ 18/2009	1,500	MagNA Pure (NPS)	20/20	27.59 (0.45)	11.40 (1.50)	20/20	30.52 (0.42)	15.95 (1.09)
		Platinum Path (NPS)	20/20	29.85 (0.80)	9.25 (2.76)	20/20	34.01 (0.71)	17.61 (3.15)
		MagNA Pure (NPW)	20/20	28.57 (0.89)	11.68 (2.54)	20/20	31.09 (0.43)	16.05 (1.06)
		Platinum Path (NPW)	20/20	30.15 (0.53)	11.70 (2.63)	20/20	32.68 (0.39)	13.98 (1.34)
A/California/ 07/2009	5,000	MagNA Pure (NPS)	20/20	27.88 (0.53)	9.36 (1.58)	20/20	30.98 (0.40)	16.10 (1.02)
		Platinum Path (NPS)	19/20	29.78 (0.58)	10.15 (2.83)	20/20	32.99 (0.37)	13.40 (1.58)
		MagNA Pure (NPW)	20/20	28.83 (0.47)	13.62 (1.11)	20/20	31.55 (0.52)	17.87 (1.12)
		Platinum Path (NPW)	20/20	30.82 (0.63)	13.74 (1.84)	20/20	33.15 (0.64)	15.13 (0.90)

Note: One possible cause for the variation in LoD concentrations between strains is differences in the method of quantification and detection. Quantification using EID_{50} measures the infectivity and cytotoxicity or lethality of an organism in egg culture, and is therefore subject to many influences (strain-to-strain differences in infectivity, viability of the original material, culturing conditions, etc.). In contrast, the JBAIDS assays detect the presence of target nucleic acid using PCR; differences in detection reflect the quantity of starting target nucleic acid present as well as the complementarities of the assay primers and probes. Consequently, even with closely related/identical target sequences, strains/isolates tested at similar concentrations as quantified by EID_{50}, may contain quite different concentrations of target nucleic acid as

measured by PCR. Therefore, LoD concentrations established for the JBAIDS PCR assays may not reflect significant differences in sensitivity of detection, rather they reflect differences in starting concentration of target nucleic acid. This was the case for the two representative seasonal Influenza A/H1 strains: the A/New Caledonia/20/1999 isolate appears to contain 17 to 45 times more target nucleic acid (as evidenced by a 4.1 to 5.5 Cp shift, assuming amplification efficiency is 2) per EID_{50} than the A/Hawaii/15/2001 isolate.

e. Analytical Reactivity:

For inclusivity testing, sNPS samples were spiked with various strains of influenza target viruses within the specific Influenza A subtyping genera prior to testing with the JBAIDS Influenza A Subtyping Kit. Viruses were spiked at concentrations estimated to be near the LoD of each analyte. Due to differences in quantification methods between primary strains (quantified in EID_{50}/mL) and analytical reactivity panel strains (mostly quantified in $TCID_{50}$/mL), concentrations spanning the primary strain LoD (0.1×, 1×, 10×LoD, assuming 1 EID_{50} was equivalent to 1 $TCID_{50}$) were tested. If a sample containing a particular strain was positive at any of the concentrations, no further testing of that strain was required. If a strain was not detected at any of these concentrations, the strain was retested in 10-fold higher increments until a positive result was obtained or until the maximum concentration possible for that strain stock had been tested.

The following 3 tables provide a complete listing of the Influenza A strains that were used to assess the analytical reactivity or inclusivity of the test system. The 8 Influenza A H1N1 strains tested represents human isolates collected in United States, Australia, Europe, Asia, and the Solomon Islands between 1933 and 2007. The 10 Influenza A H3N2 strains tested represents human isolates collected in South America, North America, Europe and Asia between 1968 and 2009. The 11 Influenza A (swine lineage) 2009 H1N1 strains are representative of human isolates collected throughout the United States in 2009.

Influenza A H1N1 Strains Tested by the Flu A H1 Assay

Strain	Lowest Concentration Detected	Cp
A/PR/8/34	500000 $TCID_{50}$/mL	37.22
A/NWS/33	50 $TCID_{50}$/mL	37.56
A/Weiss/43	500 $TCID_{50}$/mL	42.50[a]
A1/FM/1/47	5 $TCID_{50}$/mL	36.59
A/Mal/302/54	5000 $TCID_{50}$/mL	36.37
A1/Denver/1/57	ND[b]	-
A/Solomon Islands/3/2006	5 $TCID_{50}$/mL	36.32
A/Brisbane/59/07	50 $TCID_{50}$/mL	42.50[a]

[a] Cp calls of >40.00 cycles are assigned a final Cp value of 42.50 cycles.
[b] ND stands for "not detected".

Influenza A H3N2 Strains Tested by the Flu A H3 Assay

Strain	Lowest Concentration Detected	Cp
A/Aichi/2/68	ND[a]	-
A/Hong Kong/8/68	ND[a]	-
A/Port Chalmers/1/73	5 $TCID_{50}$/mL	38.15
A/Victoria/3/75	50 $TCID_{50}$/mL	36.70
A/Brisbane/10/07	5 $TCID_{50}$/mL	38.56
A/Taiwan/760/2007	0.5 $TCID_{50}$/mL	35.99
A/Uruguay/716/2007	0.5 EID_{50}/mL	37.88
A/Perth/16/09	5 $TCID_{50}$/mL	35.84
A/Alice	0.5 $TCID_{50}$/mL	39.65
A/MRC-2 recomb	ND[a]	-

[a] ND stands for "not detected".

2009 H1N1 Influenza (swine lineage) Strains Tested by the Flu A H1 2009 and Flu A Sw Assays

Isolate	Lowest Concentration Detected	Cp	
		Flu A H1 2009	Flu A Sw
A/California/4/2009	1500 $TCID_{50}$/mL	35.71	32.72
A/California/8/2009	150 EID_{50}/mL	35.77	32.06
A/England/195/2009	150 $TCID_{50}$/mL	34.82	31.28
A/Mexico/4108/2009	150 EID_{50}/mL	38.03	32.76
A/North Carolina/18/2009	1500 $TCID_{50}$/mL	33.90	31.97
A/South Carolina/18/2009	150 $TCID_{50}$/mL	33.86	30.85
A/SwineNY/01/2009	150 $TCID_{50}$/mL	30.29	28.01
A/SwineNY/02/2009	150 $TCID_{50}$/mL	27.86	25.79
A/SwineNY/03/2009	150 $TCID_{50}$/mL	31.20	28.59
A/Texas/48/2009	1500 $TCID_{50}$/mL	36.84	33.04
A/Washington/29/2009	150 $TCID_{50}$/mL	37.22	32.78

JBAIDS Influenza A/H1 assay results

The JBAIDS Influenza A/H1 assay accurately identified 7/8 individual Influenza A H1N1 isolates. However, there was high variability in concentrations of virus that was detected (ranging from 5-500,000 $TCID_{50}$/mL). The Flu A H1 assay was unable to detect the A1/Denver/1/57 strain at a final concentration of 5,000 $TCID_{50}$/mL (higher concentrations could not be tested due to low stock concentration).

Sequence alignments of isolates evaluated (or similar isolates) indicated that there were 0-4 mismatches per primer or probe. Older strains for the virus (prior to 1957) tended to have more mismatches than the more contemporary isolates evaluated. A/Solomon Islands/3/2006 and A/Brisbane/59/07 isolates had zero to one mismatch

combined in primers and probe sequences; the Brisbane isolate had 1 mismatch in the forward primer region. Sequence alignments for all available strains isolated between 2006-2011 demonstrated 0-1 mismatches per primer or probe sequence reflecting that this assay was designed to detect contemporary virus strains. Sequence alignment of A/PR/8/34, which was detected at 500,000 $TCID_{50}$/mL, has a mismatch within the first few bases of the 3' end of both the forward and reverse primers. Exact sequences for A/Mal/302/54 and A1/Denver/1/57 were not available. However, sequences from isolates collected in the same geographical region and in the same year were aligned. For both isolates, the sequences had 3 mismatches in both the forward and reverse primers and two consecutive mismatches in the probe. These mismatches may contribute to the decrease in sensitivity of the Flu A H1 assay. Another possible cause for the variation is differences in the quantification methods. LoD concentration differences established in EID_{50} or $TCID_{50}$ can vary dramatically between organism stocks, but may not actually reflect significant differences in target nucleic acid concentration.

JBAIDS Influenza A/H3 assay results

The JBAIDS Influenza A/H3 assay accurately identified 7/10 individual Influenza A H3N2 isolates with a range of virus concentrations from 0.5 to 50 $TCID_{50}$/mL. The following isolates were not detected even at the maximum concentration allowed based upon stock concentration: A/Aichi/2/68 at 114,000 $TCID_{50}$/mL, A/Hong Kong/8/68 at 137,000 $TCID_{50}$/mL, and A/MRC-2 recomb at 7,350 $TCID_{50}$/mL.

Sequence alignments of isolates evaluated (or similar isolates) indicated that there were 0-1 mismatches per primer and 1-5 mismatches with the probe. More contemporary isolates evaluated (strains isolated from 2007-2009) had significantly fewer mismatches in the probe region. In addition, viruses isolated prior to 1975 also had a mismatch within the first 3 bases of the reverse primer binding region. In general, contemporary strains (strains isolated between 2006-2011) have 0-1 mismatches per primer or probe sequence.

Of the isolates with observed decreased sensitivity or missed detection, sequences that were available were aligned with the PCR primers and probe for the Flu A H3 assay to determine whether sequence mismatches may be contributing to the potentially reduced sensitivity of the JBAIDS influenza A/H3 assay. In addition to the mismatch in the 3' end of the reverse primer, both the A/Aichi/2/68 and A/Hong Kong/8/68 isolates had five mismatches in the probe sequence, suggesting that the observed decreased sensitivity for these two isolates may be attributed to the high number of mismatches under the probe. The sequence for A/MRC-2 recomb was not available.

JBAIDS 2009 H1N1 Influenza (Flu A Sw and Flu A H1 2009) assays results

The JBAIDS 2009 H1N1 Influenza (Flu A Sw and Flu A H1 2009) assays accurately identified 11/11 individual 2009 H1N1 Influenza (swine lineage) isolates tested with a range of virus concentration from 150 to 1,500 $TCID_{50}$/mL. Variation in the lowest concentration detected may be due to differences in relative quantification methods as well as virus strain sequence variation.

The analytical inclusivity data for the 4 Influenza A subtyping assays combined with *in silico* analysis indicated that the assays detect contemporary strains of human Influenza A viruses with acceptable analytical sensitivity.

f. Analytical Specificity/Cross-reactivity Evaluation:

Analytical Specificity (Exclusivity) testing involved the following organisms: 1) Non-target influenza types (those not specific to the type assay); 2) Non-influenza viruses; and 3) Other closely related organisms that colonize the upper respiratory tract, cause respiratory symptoms, are common skin flora or laboratory contaminants, or are microorganisms for which much of the population may have been infected.

Each organism was spiked at a high level (when possible, viruses were spiked at $10^5\times$ provided stock units, and other organisms were spiked at $10^6\times$ provided stock units; otherwise organisms were spiked at the highest allowable concentration) into a sNPS sample prior to extraction using the Roche MagNA Pure Nucleic Acid Isolation Kit I and testing with the JBAIDS Influenza A & B Detection Kit.

The following 2 tables provide the exclusivity panels that were used to evaluate the test system analytical specificity or exclusivity:

Non-Target Influenza Viruses Evaluated for Cross reactivity with the Influenza A Subtyping Assays

Type/Subtype		Isolate	Concentration Tested	Subtyping Assays
Influenza A	H2N2 (Avian)	A/chicken/Pennsylvania/298101-4/2004	3.16E+07 $TCID_{50}$/mL	Flu A H1 Flu A H3 Flu A Sw Flu A H1 2009
	H3N8 (Avian)	A/MAL/ALB/16/87	1.72E+03 $TCID_{50}$/mL	
	H4N8 (Avian)	A/chicken/Alabama/1975	1.00E+08 EID_{50}/mL	
	H5N1 (Avian-Human Recombinant)	A/Vietnam/1203/2004(H5N1)-PR8	3.16E+07 EID_{50}/mL	
	H5N1 (Avian)	A/DK/PA/4560069-9/06	1.00E+05 $TCID_{50}$/mL	
	H7N3 (Avian)	A/TY/UT/24721-10/95	3.06E+04 $TCID_{50}$/mL	
	H6N2 (Avian)	A/Chicken/CA/32213-1/2000	1.26E+07 EID_{50}/mL	
	H9N2 (Avian)	A/Turkey/Wisconsin/1966	5.60E+07 EID_{50}/mL	
	H3N8 (Canine)	A/canine/Florida/43/2004	1.00E+05 $TCID_{50}$/mL	
	H3N8 (Equine)	A/Equine/Ohio/01/2009	1.00E+05 $TCID_{50}$/mL	
	H1N1 (Swine)	A/swine/Wisconsin/125/1997	1.00E+05 $TCID_{50}$/mL	
	H1N1 (Swine)	A/SW/GB/19582/92	5.64E+03 $TCID_{50}$/mL	
	H3N2 (Swine)	A/SW/IA/1/99	1.41E+03 $TCID_{50}$/mL	
	H1N1 (Human of swine lineage)	A/Maryland/12/1991	1.00E+05 $TCID_{50}$/mL	
	H1N1 (Human of swine lineage)	A/Iowa/1/2006	1.00E+05 $TCID_{50}$/mL	
	H7N2 (Human)	A/New York/107/2003	30 µl of an unknown concentration into 1mL	
Influenza B		B/Lee/40	7.36E+03 $TCID_{50}$/mL	Flu A H1 Flu A H3 Flu A Sw
		B/Allen/45	1.00E+05 $TCID_{50}$/mL	
		B/GL/1739/54	7.36E+03 $TCID_{50}$/mL	

	B/Maryland/1/59	7.36E+03 $TCID_{50}$/mL	
	B/Taiwan/2/62	4.54E+04 $TCID_{50}$/mL	
	B/Hong Kong/5/72	7.36E+03 $TCID_{50}$/mL	
	B/Malaysia/2506/04	5.09E+03 $TCID_{50}$/mL	
	B/FL/04/06	1.50E+04 $TCID_{50}$/mL	
	B/Brigit	3.14E+04 $TCID_{50}$/mL	
Influenza A H1N1	A/Brisbane/59/07	1.00E+05 $TCID_{50}$/mL	Flu A H3 Flu A Sw Flu A H1 2009
	A1/FM/1/47	4.24E+03 $TCID_{50}$/mL	
	A/PR/8/34	1.00E+05 $TCID_{50}$/mL	
	A/NWS/33	4.24E+03 $TCID_{50}$/mL	
	A1/Denver/1/57	4.24E+03 $TCID_{50}$/mL	
	A/Solomon Islands/3/2006	1.25E+04 $TCID_{50}$/mL	
	A/Weiss/43	4.24E+03 $TCID_{50}$/mL	
	A/Mal/302/54	1.25E+04 $TCID_{50}$/mL	
Influenza A H3N2	A/Port Chalmers/1/73	5.10E+03 $TCID_{50}$/mL	Flu A H1 Flu A Sw Flu A H1 2009
	A/Victoria/3/75	4.24E+03 $TCID_{50}$/mL	
	A/Aichi/2/68	1.00E+05 $TCID_{50}$/mL	
	A/Hong Kong/8/68	1.00E+05 $TCID_{50}$/mL	
	A/Alice (VR-776)	4.24E+03 $TCID_{50}$/mL	
	A/MRC-2 recomb (VR-777)	7.36E+03 $TCID_{50}$/mL	
	A/Brisbane/10/07	7.36E+03 $TCID_{50}$/mL	
2009 H1N1 Influenza (swine lineage)	Swine NY/02/2009	1.25E+04 $TCID_{50}$/mL	Flu A H1 Flu A H3
	Swine NY/03/2009	7.36E+03 $TCID_{50}$/mL	
	Swine NY/01/2009	3.78E+04 $TCID_{50}$/mL	
	A/Mexico/4108/2009	1.00E+05 EID_{50}/mL	
	A/California/8/2009	1.00E+05 $TCID_{50}$/mL	
	A/California/04/2009	1.00E+05 $TCID_{50}$/mL	
	A/Texas/48/2009	1.00E+05 $TCID_{50}$/mL	
	A/Washington/29/2009	1.00E+05 $TCID_{50}$/mL	
	A/South Carolina/18/2009	1.00E+05 $TCID_{50}$/mL	
	A/England/195/2009	4.74E+04 $TCID_{50}$/mL	
	A/North Carolina/39/2009	1.00E+05 $TCID_{50}$/mL	

Exclusivity Panel: Non-influenza Viruses and Organisms

Virus	Concentration Tested	Bacteria/Fungi	Concentration Tested
Adenovirus	1.00E+05 $TCID_{50}$/mL	*Bordetella pertussis*	1.00E+06 CFU/mL
Bocavirus	4.20E+07 copies/mL	*Candida albicans*	1.00E+06 CFU/mL
Coronavirus 229E	7.35E+03 $TCID_{50}$/mL	*Corynebacterium diptheriae*	1.00E+06 CFU/mL
Coronavirus OC43	6.57E+04 $TCID_{50}$/mL	*Escherichia coli*	1.00E+06 CFU/mL
Coronavirus NL63	5.10E+03 $TCID_{50}$/mL	*Haemophilus influenza*	7.80E+04 CFU/mL
Coronavirus HKU1	1.00E+05 copies/mL	*Lactobacillus plantarum*	1.00E+06 CFU/mL
Cytomegalovirus (CMV)	1.50E+04 $TCID_{50}$/mL	*Legionella pneumophila*	1.00E+06 $TCID_{50}$/mL
Enterovirus	1.00E+05 $TCID_{50}$/mL	*Moraxella catarrhalis*	1.00E+06 CFU/mL
Epstein-Barr Virus (EBV)	1.00E+05 copies/mL	*Mycobacterium tuberculosis*	1.00E+06 CFU/mL
Human Metapneumovirus	7.35E+03 $TCID_{50}$/mL	*Mycoplasma pneumonia*	1.69E+05 $TCID_{50}$/mL
Human Rhinovirus	5.10E+03 $TCID_{50}$/mL	*Neisseria elongata*	1.00E+06 CFU/mL
Measles Virus (Rubeola)	1.00E+05 $TCID_{50}$/mL	*Neisseria meningitidis*	1.00E+06 CFU/mL

Mumps	4.53E+04 $TCID_{50}$/mL	*Pseudomonas aeruginosa*	1.00E+06 CFU/mL
Parainfluenza virus 1	1.25E+04 $TCID_{50}$/mL	*Staphylococcus aureus*	1.00E+06 CFU/mL
Parainfluenza virus 2	1.50E+04 $TCID_{50}$/mL	*Staphylococcus epidermidis*	1.00E+06 CFU/mL
Parainfluenza virus 3	1.00E+05 $TCID_{50}$/mL	*Streptococcus pneumonia*	1.00E+06 CFU/mL
Parainfluenza virus 4	1.00E+05 $TCID_{50}$/mL	*Streptococcus pyogenes*	1.00E+06 CFU/mL
Respiratory Syncytial Virus	1.25E+04 $TCID_{50}$/mL	*Streptococcus salivarius*	7.59E+05 CFU/mL

For the non-target influenza virus exclusivity panel, the expected negative results were obtained for all of the assays with the following exceptions. Three Influenza A viruses were detected by the Flu A Sw assay: A/Maryland/12/1991, A/Iowa/1/2006, A/swine/Wisconsin/125/1997. These results were not unexpected since these isolates were all of subtype H1N1 with the first two isolated from humans but containing swine lineage sequences and the third isolated from swine. BLAST evaluation of the Flu A Sw assay primers and probes against recent influenza sequences (from 2009-2011) indicated that of the top 500 sequence alignments (from 2009-2011), 8 percent of the isolates that aligned within an E value range of 7e-06 to 3e-05 were of isolates from swine.

In addition, Influenza A H3N2 virus isolate A/SW/IA/1/99 (isolated from swine) was detected by both the Flu A H3 and Flu A Sw assays. Detection of swine Influenza A/H3 viruses by the Flu A H3 assay is not unexpected as the hemagglutinin sequences for swine and human isolates are very similar.

All 36 non-influenza organisms, tested at high concentrations, (bacteria or fungi spiked at 10^6 CFU/mL or $TCID_{50}$/mL and viruses spiked at 10^3 - 10^5 copies/mL or $TCID_{50}$/mL) gave the expected negative results for all Influenza A subtyping assays. This evaluation indicated that the primers and probes in the JBAIDS Influenza Subtyping assays do not cross-react with the non-influenza exclusivity organisms tested.

g. Assay cut-off:

Each JBAIDS test is analyzed with the data analysis software module. The module is called Detector. The module determines the outcome of the tests conducted on the samples. The module first analyzes the data from each capillary independently. Then the replicates of the samples are analyzed together (if applicable). Finally the module interprets the outcome of these analyses for the sample and its controls.

The analysis module algorithm consists of two basic parts. The first identifies samples that are obviously positive or negative (Obvious Tests), and second that computes a refined estimate of the samples status when the sample is not called an obvious positive or obvious negative (Expert Test).The algorithms are based on an Expert System approach to determining positive and negative samples, but the approach is not learning after release, and is deterministic, meaning that every sample will have the same outcome every time the algorithm evaluates the sample. The purpose of Obvious Tests is to call those samples that are clearly positive or negative. In this way, only non-obvious samples will need to be processed by the more computationally involved detector algorithms. Obvious tests produce internal scores and compare them against fixed thresholds to return a positive/negative/uncertain call. Expert Tests algorithm is based on mathematically modeling the expected shapes of the amplification curves of

positive and negative samples. The model itself is based on tests for nine distinct characteristics in the amplification curves and it assigns nine test scores to each curve. After the tests are scored, the amplification curve is scored as a weighted sum of the tests.

Thresholds for the Obvious Tests and weights in the Expert Tests for Detector were determined using training data sets and numerical optimization to pick parameters that ensure Detector minimizes the number of error observed in real-world data.

Validation data from JBAIDS Customer Validation Testing, and Dugway Operational Assessment were gathered and evaluated with Detector to validate the analysis module algorithm. These data contain samples from five separate data sets: typical data, atypical data, negative dominated, RNA data and Dugway field data. The data were gathered from varied sources, including a variety of assays, instruments and users, to obtain examples of all possible JBAIDS data. There are 28,467 samples in the data sets. 99.6% of the samples were correctly called by the Detector. 0.19% of the samples were called "Uncertain" and 0.21% of the samples were incorrectly called.

h. Interfering Substances:

An interfering substances study was carried out to examine whether a panel of endogenous and exogenous potential RT-PCR inhibitors and technique-specific substances (substances that could be introduced into the PCR reaction as contaminants during sample purification or during reaction setup) affect the performance of the JBAIDS Influenza Subtyping Kit. The concentration of substances tested represented a relevant concentration in accordance with CLSI EP7-A2 *Interference Testing in Clinical Chemistry Approved Guideline*, or for test substances not listed in the CLSI document, concentrations were determined by evaluating the scientific literature or references from other IVD package inserts. For each endogenous, exogenous and technique specific test substance, sNPW specimens containing a representative Influenza A/H1, Influenza A/H3, or 2009 H1N1 Influenza virus (A/New Caledonia/20/1999, A/New York/55/2004, or A/New York/18/2009) at a concentration equivalent to 5×LoD were spiked with the appropriate amount of test substance.

Note: sNPW specimens spiked with a representative seasonal Influenza A H3N2 virus strain (A/New York/55/2004) were also co-spiked with Influenza B virus (B/Ohio/1/2005) for more efficient interfering substances testing of the JBAIDS Influenza A & B Detection Kit. Refer to the "Co-spiking Specimen Validation" section for details demonstrating that combining these two viruses in a sample, even at high concentrations, had no detrimental effect on the detection of either virus.

In each round of purifications, an influenza-containing sNPW sample that had not been spiked with the interfering substance was also included. This sample served as a control to which the other specimens were compared. All specimens, except those containing Roche MagNA Pure Compact Nucleic Acid Isolation Kit I technique specific substances (i.e., MagNA Pure buffers), were purified using the Roche MagNA Pure Compact Nucleic Acid Isolation Kit I on the Roche MagNA Pure Compact System. Each purified sample was tested with the Influenza A Subtyping assays (Flu A H1, Flu A H3, or Flu A H1 and Flu A Sw) corresponding to the virus with which it

was spiked, and also tested with the Flu SC assay. The Roche MagNA Pure Compact Nucleic Acid Isolation Kit I technique-specific substances that could be introduced during sample purification (i.e., MagNA Pure buffers) were added to the purified samples immediately prior to influenza assay reaction setup. Substances were considered to be potential inhibitors if the results for the tested assays were negative.

Note: Only sNPW samples purified using the Roche MagNA Pure Compact Nucleic Acid Isolation Kit I on the Roche MagNA Pure Compact System were evaluated. NPS samples purified with the IT 1-2-3 Platinum Path Sample Purification Kit were previously evaluated with the FDA cleared JBAIDS Influenza A/H5 (Asian lineage) Detection System (k100287). The Platinum Path kit was shown to remove both endogenous and exogenous substances from NPS and throat swab samples. In addition, the wash and elution buffers contained in the IT 1-2-3 Platinum Path Sample Purification Kit (technique-specific substances) did not interfere with detection of the JBAIDS Influenza A/H5 assays.

List of Potentially Interfering Substances

Endogenous Substances	Exogenous Substances	
Blood (with Na Citrate) Mucin (bovine submaxillary gland, type I-S) Human Genomic DNA	Tobramycin Mupirocin FluMist® intranasal vaccine Dexamethasone Fluticasone propionate Snuff (nasal tobacco) Zicam Nasal Gel	Nasal Decongestant Spray (Phenylephrine HCl 1.0%) Nasal Decongestant spray (Oxymetazoline HCl 0.05%) Saline nasal spray with preservatives (0.65% NaCl Phenylcarbinol Benzalkonium Chloride) Analgesic Ointment (Vicks®VapoRub®) Petroleum Jelly Petroleum and Glycerin topical gel (Chlorseptic®)
Solvents Used[1]	Technique-specific Substances	
Water Acetone DMSO	Disinfecting Wipes Ethanol RNAseOut MagNA Pure Buffers from cartridge wells #1-6	Swabs • Copan 168C (rayon/twisted aluminum shaft) • Copan 503CS01 (flocked nylon/plastic shaft) • Copan 175KS01 (polyester/aluminum shaft) • Copan 502CS01 (flocked nylon/plastic shaft) • Millipore 519CS01M (flocked nylon/plastic shaft) Viral Transport Media • Remel M5 • Remel M6

[1] These are solvents used to dissolve potentially interfering substances in preparation for testing.

Test Concentrations for Endogenous Substances

IS#	Test Substance	Test Concentration	Solvent
EN1	Blood (with Na Citrate)	1% v/v	sNPW sample
EN2	Mucin (bovine submaxillary gland, type I-S)	1% w/v	Water

Test Concentrations for Exogenous Substances

IS#	Test Substance	Test Concentration	Solvent
EX1	Tobramycin (systemic antibiotic)	0.6 mg/mL	Water

EX2	Mupirocin (active ingredient in anti-bacterial ointment)	2% w/v	sNPW sample
EX3 Blank	FluMist intranasal vaccine (2009-2010 Formula)	1% v/v	sNPW Blank
EX4	Saline Nasal Spray with Preservatives 0.65% NaCl Phenylcarbinol Benzalkonium Chloride	1% v/v	sNPW sample
EX5	Nasal Decongestant Spray Oxymetazoline HCl 0.05% (also contains Benzalkonium chloride, menthol, eucalyptol, camphor, benzyl alcohol and phosphate buffers)	1% v/v	sNPW sample
EX6	Analgesic ointment (Vicks®VapoRub®)	1% w/v	sNPW sample
EX7	Petroleum Jelly (Vaseline®)	1% w/v	sNPW sample
EX8	Zicam® Nasal Gel	5% v/v	sNPW sample
EX9	Snuff (nasal tobacco)	1% w/v	sNPW sample
EX10	Dexamethasone	0.084 μg/mL	Acetone
EX11	Fluticasone propionate	0.22 μg/mL	DMSO
EX12	Nasal Decongestant Spray Phenylephrine HCl 1.0% (also contains benzalkonium chloride, anhydrous citric acid, purified water, sodium chloride, and sodium citrate)	1% v/v	sNPW sample
EX13	Petroleum and Glycerin topical gel (Chlorseptic®)	1% v/v	sNPW sample

Test Concentrations for Technique-Specific Substances

IS#	Test Substance		Test Concentration
TS1	Disinfecting Wipes		1/4 – 1/2 in^2
TS2	Ethanol		7%
TS3	RNaseOut		1 % v/v
TS4	MagNA Pure Reagent Cartridge Contents	Well 1: Proteinase K solution	10 % v/v
TS5		Well 2: Lysis Buffer solution	10 % v/v
TS6		Well 3: Magnetic Glass Particles	10 % v/v
TS7		Wells 4,5: Wash Buffer I	10 % v/v

TS8		Well 6: Wash Buffer II	10 % v/v
TS9		Well 7: Wash Buffer III	10 % v/v
TS10	Swabs	Copan 168C (rayon/twisted aluminum shaft)	1 swab
TS11		Copan 503CS01 (flocked nylon/plastic shaft)	1 swab
TS12		Copan 175KS01 (polyester/aluminum shaft)	1 swab
TS13		Millipore 519CS01M (flocked nylon/plastic shaft)	1 swab
TS14		Copan 502CS01 (flocked nylon/plastic shaft)	1 swab
TS15	Viral Transport Medium	Remel M5	100%
TS16		Remel M6	100%
TS17		Copan UTM	100%

Test Concentrations for Human Genomic DNA

IS#	Test Substance	Dilution	Test Concentration	Solvent
DNA1	Human genomic DNA 263 ng/µL	None	20 ng/µL (500 ng)	sNPW sample
DNA2		200 ng/µL	2 ng/µL (50 ng)	water
DNA3		20 ng/µL	0.2 ng/µL (5 ng)	water
DNA4		2 ng/µL	0.02 ng/µL (0.5 ng)	water

Of all of the endogenous and exogenous substances tested, only snuff (nasal tobacco) has the potential to cause inaccurate negative test results due to inhibition. Of the technique- specific substances tested, improper introduction of contents from four of the MagNA Pure reagent cartridge wells to purified samples have the potential to cause inaccurate negative test results due to inhibition:

- MagNA Pure Proteinase K Solution (well 1)
- MagNA Pure Lysis Solution (well 2)
- MagNA Pure Magnetic Glass Particles (well 3)
- MagNA Pure Wash Buffer I (well 4)

These substances, along with ethanol, bleach, and DNA*Zap*™, which are known PCR inhibitors, are included in the package insert as potentially interfering substances.

In addition, the study also demonstrated that the JBAIDS Influenza A Subtyping assays react with the viral material contained in the 2009-2010 version of the FluMist intranasal influenza vaccine (MedImmune). When NPS and NPW specimens are collected from individuals who have recently received a dose of the nasal vaccine, the JBAIDS Influenza A Subtyping assays may react with the vaccine material to give inaccurate positive results.

Note: The material being tested (FluMist® Influenza Vaccine Live, Intranasal; Intranasal Spray, 2009-2010 Formula) was provided by the manufacturer (MedImmune) in individual sprayers, each containing a single 0.2 mL dose. Each dose contained approximately $10^{6.5\text{-}7.5}$ FFU (fluorescence focus units) each of three live,

attenuated influenza virus reassortants including:

- A/South Dakota/6/2007 (H1N1) (A/Brisbane/59/2007-like)
- A/Uruguay/716/2007 (H3N2) (A/Brisbane/10/2007-like)
- B/Brisbane/60/2008.

Non-viral components of the vaccine listed in the provided materials include: monosodium glutamate, gelatin, arginine, sucrose, gentamicin sulfate, monobasic potassium phosphate and dibasic potassium phosphate.

i. Carry-Over Contamination:

An analytical study was carried out to evaluate the frequency of carryover in the JBAIDS Influenza A Subtyping Kit when it is used as instructed by the product insert. Significant levels of carryover will result in false positive test results for negative samples that are contaminated by adjacent positive samples. For the JBAIDS Influenza A Subtyping Kit, carryover could occur during sample purification or reagent setup.

To assess the potential for carryover, sample sets consisting of negative and high positive samples were processed in an alternating pattern using the Roche MagNA Pure Compact Nucleic Acid Isolation Kit I on the Roche MagNA Pure Compact system and then tested with the JBAIDS Influenza A Subtyping Kit.

Note: The potential for carryover in samples purified with the IT 1-2-3 Platinum Path Purification Kit was previously evaluated with JBAIDS Influenza A/H5 (Asian lineage) Detection Kit (k100287) and demonstrated that significant levels of carryover do not occur when samples are processed and tested according to the instructions in the package insert.

High positive sNPS samples were spiked with Influenza A H3N2 strain A/New York/55/2004 at approximately 2000× LoD (~1.0×10^4 EID_{50}/mL). Five independent sample sets were tested for a total of 20 positive and 20 negative samples. Positive and negative samples were purified in an alternating pattern, and the PCR reactions for the positive and negative samples were set up in the order in which the samples were processed. The frequency of false positive and uncertain results for the negative samples was evaluated using the Flu A H3 assay as the representative assay from the JBAIDS Influenza A Subtyping Kit.

All positive samples resulted in early Flu A H3 assay Cp values (< 23.00 cycles) and robust F_{max} values (> 49.00). All negative samples produced negative results, with no detectable Cp values. No carryover was observed in this evaluation. The test results are shown in the following table:

Flu A H3 Assay Carryover Results for sNPS Samples Purified with the Magna Pure Compact Nucleic Acid Isolation Kit I Purification Kit

Trial Number	Number of Positive Samples	Flu A H3 Sample Results for Positive Samples		Number of Negative Samples	Flu A H3 Sample Results for Negative Samples	
		Mean Cp (SD)	Mean Fmax (SD)		Positive	Negative
1	4	22.60 (0.10)	50.78 (3.61)	4	0	4
2	4	22.74 (0.18)	50.20 (2.42)	4	0	4
3	4	22.17 (0.35)	51.79 (2.24)	4	0	4
4	4	22.22 (0.40)	56.45 (3.79)	4	0	4
5	4	22.41 (0.22)	49.67 (2.62)	4	0	4
Overall	20	22.43 (0.33)	51.78 (3.66)	20	0	20

Overall, sample purification and reaction setup in this carryover study resulted in no false positive results when samples were processed in accordance with the purification kit and reaction setup directions. PCR is a very sensitive technique and false positives caused by contamination are a recognized risk. Users will be reminded that failure to follow protocol and improper technique may result in false positive results.

j. Validation of Simulated Sample Matrices

Nasopharyngeal wash (NPW) and nasopharyngeal swab (NPS) specimens are the two sample matrices evaluated with the JBAIDS Influenza A Subtyping Kit and used for most of the non-clinical evaluations. However, given the need for large numbers of individual samples without influenza virus, simulated NPS (sNPS) and simulated NPW (sNPW) samples were developed and validated to prevent inaccurate test results arising from the presence of influenza viruses in human derived nasopharyngeal specimens. Simulated samples were comprised of human HeLa cells diluted to concentrations in viral transport medium (VTM) that produced similar Cp values as the Flu SC assay produced when testing human derived NPS and NPW samples.

HeLa cell concentrations of $10^{3.2}$ cells/mL and $10^{4.3}$ cells/mL for sNPW and sNPS sample matrix types, respectively, resulted in similar Cp values for the Flu SC assay as human derived NPW and NPS specimens. To confirm that the simulated sample matrix types did not have any advantageous or detrimental effects on the performance of the system, multiple aliquots of simulated and human derived samples were spiked at the LoD with a representative seasonal influenza A virus (H3N2 strain A/New York/55/2004) and tested with the applicable influenza and control assays (Flu A, Flu A H3, and Flu SC). Simulated and human derived samples exhibited equivalent rates of detection. Mean Cp and F_{max} values were also comparable between the human derived and simulated samples.

k. Co-spiked Specimen Validation

For efficient use of resources during non-clinical testing of the JBAIDS Influenza A & B Detection and Influenza A Subtyping Kits, Influenza A H3N2 and Influenza B viruses were co-spiked in some studies. Specifically, co-spiked samples were used in the following analytic evaluations: Reproducibility, Sample Transport and Storage, and Interfering Substances.

An analytical study was carried out to validate the use of specimens co-spiked with Influenza A H3N2 and Influenza B viruses. This study was performed by evaluating single-spiked and co-spiked samples containing Influenza A H3N2 A/New York/55/2004 and/or Influenza B B/Ohio/01/2005. NPW specimens purified with the Platinum Path kit were evaluated in this study because the Cp values for the Flu A assay in NPW samples, purified with Platinum Path kit, were greater than Cp values for the other sample type/purification kit combinations. The first part of this study evaluated freshly collected NPW samples spiked with an influenza A H3N2 virus at LoD only (single spike) or additionally spiked with influenza B virus at 500×LoD (co-spiked). The second part of this study evaluated the opposite, NPW samples spiked with influenza B virus at 1×LoD (single spike) or further spiked with influenza A H3N2 at 500×LoD (co-spiked).

For each part of the study, 60 single and 60 co-spiked samples were prepared and evaluated. In particular, pooled fresh NPW samples were spiked with virus at 1×LoD. Half of this pool was co-spiked with the alternate virus at 500×LoD, resulting in one single spiked pool and one co-spiked pool. Following spiking, the samples were purified using the Platinum Path kit and then tested with appropriate freeze-dried reagent test assays (Flu A, Flu B, and Flu A H3). A total of 240 samples were evaluated.

For the samples spiked with influenza B at 1×LoD, all single (60) and co-spiked (60) samples were detected with the Flu B assay. In addition, the influenza A H3N2 virus at 500×LoD was detected in 60/60 samples by the Flu A and Flu A H3 assays. These results demonstrate that 100% detection was achieved for single (influenza B at 1×LoD) and co- spiked (influenza B at 1×LoD/ influenza A H3N2 at 500×LoD) samples. For the Flu A assay, samples spiked with influenza A H3N2 at 1×LoD were detected in 59/60 single- spiked and 58/60 co-spiked samples, resulting in 98.3 and 96.7% detection rates, respectively. For the Flu A H3 assay the same samples were positive for 60/60 single- spiked and 59/60 co-spiked samples, resulting in 100 and 98.3% detection rates, respectively. In addition, the influenza B virus at 500×LoD was detected in 60/60 samples by the Flu B assay.

Differences in mean Cp values between single- and co-spiked samples did not vary by more than 0.19 cycles for the three assays, and mean F_{max} values for co-spiked samples were reduced by less than 5% from mean F_{max} values for single-spiked samples. The quantitative differences in mean Cp and F_{max} values between single- and co-spiked samples were lower than the standard deviations for each assay and spike set.

The study results demonstrate that there is no significant quantitative or qualitative difference between specimens single spiked or co-spiked with influenza B and influenza A H3N2 viruses.

l. PCR followed by Bi-directional Sequencing Comparator Assays

The comparator assay for the JBAIDS Influenza A Subtyping Kit is PCR followed bi-directional sequencing of amplicons. Comparator assays for the JBAIDS Influenza A Subtyping Kit were designed by Idaho Technology and validated with Limit of Detection (LoD) and reactivity studies.

The research and development group designed the Influenza A/H1, Influenza A/H3, and the 2009 H1N1 Influenza assays such that they recognized different locations on the hemagglutinin gene(s) than targeted by the JBAIDS Influenza A Subtyping assays.

JBAIDS Influenza A Subtyping and Comparator Assay Gene Targets

Test Panel	Purification Method	Organism	Subtype	JBAIDS Target	Comparator Assay Target	Chemistry
Subtyping	Qiagen QIAamp Viral RNA	Influenza A	Seasonal H1	Hemagglutinin (HA) gene	Hemagglutinin (HA) gene	Hydrolysis probe
		Influenza A	Seasonal H3	Hemagglutinin (HA) gene	Hemagglutinin (HA) gene	Hydrolysis probe [1]
		Influenza A	2009 H1N1	Nucleocapsid protein (NP) and Hemagglutinin (HA) gene	Hemagglutinin (HA) gene	Hydrolysis probe [1]

[1] Nested assays.

The LoD confirmation results for the comparator assays are listed in the following table:

Comparator Assays LoD Confirmation Results

Comparator Assay	Organism	Sample Type	LoD Concentration	# Pos/ Total	% Positive
Big Flu H1 Assay	Seasonal Influenza A/H1	NPS	50 EID_{50}/mL	20/20	100%
Big Flu H1 Assay	Seasonal Influenza A/H1	NPW	50 EID_{50}/mL	20/20	100%
Big Flu H1-2009 Assay	2009 H1N1 Influenza	NPS	1500 EID_{50}/mL	20/20	100%
Big Flu H1-2009 Assay	2009 H1N1 Influenza	NPW	1500 EID_{50}/mL	20/20	100%

Big Flu H3 Assay	Seasonal Influenza A/H3	NPS	5 EID_{50}/mL	20/20	100%
Big Flu H3 Assay	Seasonal influenza A H3N1	NPW	5EID50	19/20	95%

The inclusivity and exclusivity validation results are listed in the following tables:

Inclusivity Results

Organism	Strain	Concentration (EID_{50}/ml)	Big FluA H1 Assay	Big FluA H1-2009 Assay	Big FluA H3 Assay
Influenza A/H1	A/Brisbane/59/07	5	Positive	Negative	Negative
	A/Solomon Islands/3/2006		Positive	Negative	Negative
2009 H1N1 Influenza	Swine NY/02/2009	1500	Negative	Positive	Negative
	Swine NY/03/2009		Negative	Positive	Negative
	Swine NY/01/2009		Negative	Positive	Negative
	A/Mexico/4108/2009		Negative	Positive	Negative
	A/California/8/2009		Negative	Positive	Negative
	A/California/04/2009		Negative	Positive	Negative
	A/Texas/48/2009		Negative	Positive	Negative
	A/Washington/29/2009		Negative	Positive	Negative
	A/South Carolina/18/2009		Negative	Positive	Negative
	A/England/195/2009		Negative	Positive	Negative
	A/North Carolina/39/2009		Negative	Positive	Negative
Influenza A H3	A/Port Chalmers/1/73	50	Negative	Negative	Positive
	A/Victoria/3/75		Negative	Negative	Positive
	A/Aichi/2/68		Negative	Negative	Positive
	A/Hong Kong/8/68		Negative	Negative	Positive
	A/Alice (VR-776)		Negative	Negative	Positive
	A/MRC-2 recomb (VR-777)		Negative	Negative	Positive
	A/Brisbane/10/07		Negative	Negative	Positive

Exclusivity Results

Organism	Strain	Concentration (EID_{50}/ml)	Big FluA H1 Assay	Big FluA H1 2009	Big FluA H3 Assay
Adenovirus	Type 1 (Zeptometrix	1.02E+08	Negative	Negative	Negative
Bocavirus	Clinical Sample (ProvLab)	1.40E+09	Negative	Negative	Negative
Coronavirus 229E	ATCC VR-740	1.70E+05	Negative	Negative	Negative
Coronavirus OC43	ATCC VR-759	2.19E+06	Negative	Negative	Negative

Coronavirus NL63	NR-470 (BEI resources)	1.70E+05	Negative	Negative	Negative
Coronavirus HKU1	PCMC 6213	4.17E+10	Negative	Negative	Negative
Human Metapneumovirus	hMPV-16/IA10-2003 (Type A1)	2.45E+05	Negative	Negative	Negative
Human Rhinovirus	1A	1.70E+05	Negative	Negative	Negative
Enterovirus	Echovirus 6	1.02E+08	Negative	Negative	Negative
Parainfluenza virus 1	Zeptometrix #0810014CF	4.17E+05	Negative	Negative	Negative
Parainfluenza virus 2	Zeptometrix #0810015CF	5.01E+05	Negative	Negative	Negative
Parainfluenza virus 3	Zeptometrix #0810016CF	6.61E+06	Negative	Negative	Negative
Parainfluenza virus 4	Zeptometrix #0810060CF	2.82E+08	Negative	Negative	Negative
Respiratory Syncytial Virus	A (Zeptometrix #0810040ACF)	4.17E+05	Negative	Negative	Negative
Cytomegalovirus (CMV)	AD-169 ATCC VR-538	5.01E+05	Negative	Negative	Negative
Epstein-Barr Virus (EBV)	B95-8	6.04E+09	Negative	Negative	Negative
Measles Virus (Rubeola)	Zeptometrix #0810025CF	1.26E+06	Negative	Negative	Negative
Mumps	Zeptometrix #0810079CF	1.51E+06	Negative	Negative	Negative
Bordetella pertussis	A639	3.50E+09	Negative	Negative	Negative
Candida albicans	Zeptometrix # 0801504	1.00E+08	Negative	Negative	Negative
Corynebacterium diptheriae	ATCC 14779 CDC [NCTC	4.20E+09	Negative	Negative	Negative
Escherichia coli	O157:H7	2.34E+10	Negative	Negative	Negative
Haemophilus influenza	MinnA	2.60E+06	Negative	Negative	Negative
Lactobacillus plantarum	17-5	1.75E+09	Negative	Negative	Negative
Legionella pneumophilia	Philadelphia Strain	2.63E+09	Negative	Negative	Negative
Moraxella catarrhalis	Ne 11- type strain	6.83E+08	Negative	Negative	Negative
Mycobacterium tuberculosis	H37Ra-1	2.20E+08	Negative	Negative	Negative
Mycoplasma pneumonia	M129 (Type 1)	5.63E+06	Negative	Negative	Negative
Neisseria elongate	type strain	1.99E+09	Negative	Negative	Negative
Neisseria meningitides	M1027-type strain	1.63E+09	Negative	Negative	Negative
Pseudomonas aeruginosa	Clinical isolate	1.05E+10	Negative	Negative	Negative
Staphylococcus aureus	COL	8.40E+09	Negative	Negative	Negative
Staphylococcus epidermidis	RP62A	6.20E+08	Negative	Negative	Negative
Streptococcus pneumonia	type 59	5.54E+08	Negative	Negative	Negative
Streptococcus pyogenes	Zeptometrix # 0801512	7.57E+08	Negative	Negative	Negative
Streptococcus salivarius	ATCC 13419	2.53E+07	Negative	Negative	Negative

m. Fresh vs. Frozen Specimen Study

The JBAIDS Influenza A Subtyping Kit includes assays for the detection of Influenza A subtypes in NPS and NPW specimens collected from individuals exhibiting influenza-like-illness. A prospective clinical evaluation of this test kit was performed in order to establish the clinical sensitivity and specificity of the assays contained in the JBAIDS Influenza A Subtyping Kit. The prospective clinical evaluation was performed from December 2010 through April 2011 at five geographically diverse locations. During this study, sufficient positive and negative samples were obtained to establish the clinical sensitivity and specificity for detection of 2009 H1N1 Influenza

and Influenza A/H3 viruses. However, seasonal Influenza A/H1 did not circulate during the trial period.

To account for the possibility that frozen archived samples might be required to supplement the prospective clinical trial data, an analytical study was performed to establish that the results obtained with frozen samples are equivalent to those obtained when testing freshly collected NPS or NPW samples.

Pools of freshly collected NPS and NPW specimens were spiked at 100×LoD, 10×LoD, 3×LoD, and 1×LoD with a representative Influenza A/ H1 virus strain (A/New Caledonia/20/1999). The spiked pools were then aliquoted. Samples that were to be tested after freezing were aliquoted from each pool and stored at ≤ -70°C for at least 24 hours. The remainder of each pool was aliquoted for immediate analysis. Testing of sample sets consisted of purifying individual aliquots with either the Platinum Path or the MagNA Pure kits and assaying with the Influenza A subtyping assays. The following table lists the number of samples tested at each virus concentration:

Virus Concentrations and Numbers of Fresh and Frozen Samples Evaluated with both Purification Methods

Virus Spike Level	Influenza Virus Concentration (Total Fresh and Frozen Replicates)
	A/New Caledonia/20/1999
Unspiked	N/A (6)
100×LoD	5000 EID50/mL (6)
10×LoD	500 EID50/mL (6)
3×LoD	150 EID50/mL (24)
1×LoD	50 EID50/mL (24)

Successful validation of frozen samples required that the JBAIDS test results were equivalent to the results obtained when testing freshly collected samples. Frozen samples were considered acceptable to use in the clinical evaluation if the JBAIDS results are at least 90% in agreement between fresh and frozen samples with a lower 95% CI bound of 86%. If this statistical confidence was not reached with 60 samples, additional samples were evaluated. For informational purposes only, the crossing point (Cp) and relative maximum fluorescence (F_{max}) values were evaluated for the fresh and frozen samples. Ideally the Cp values should not vary by more than 3 cycles and the F_{max} values should not be more than 50% different between the two data sets. The 3 cycle Cp and 50% F_{max} differences allow for intrinsic system variability in JBAIDS Influenza A Subtyping Kit. If the observed Cp and F_{max} differences were greater than these, the data were reviewed to determine if using frozen samples interferes with detection.

For the Flu A H1 assay, JBAIDS test results for fresh and frozen spiked NPS samples were in 100% agreement (95% CI 96-100%). Test results for fresh and frozen NPW samples were also in 100% agreement (95% CI 96-100%) for all virus spike levels. More NPW samples were tested than originally planned due to a user error in which

samples were lost. While loading frozen NPW samples into the MagNA Pure kit, the user mishandled four samples (two at 3×LoD and two at 1×LoD). Additional samples were prepared and tested, both fresh and frozen, to replace the lost samples.

The Cp and F_{max} values were also compared between fresh and frozen NPS and NPW samples. Differences between fresh and frozen Cp values ranged between 0.76 cycles earlier and 0.80 cycles later for the Flu A H1 assay. Differences in F_{max} values in frozen samples ranged from 23% less to 38% greater than fresh samples tested with the Flu A H1 assay.

The results of this study demonstrate that there are no significant qualitative differences between JBAIDS test results for fresh or frozen NPS and NPW samples spiked with seasonal Influenza A H1N1 virus and evaluated with the Flu A H1 assay. Accurate test results were obtained from fresh specimens as well as specimens that had been subjected to freezing. The results of this study support the use of frozen archived seasonal Influenza A H1N1 NPS and NPW samples to supplement the performance claims of the JBAIDS Influenza A Subtyping Kit.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable. Refer to the Clinical Studies Section of this document.

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Prospective Clinical study

The clinical performance of the JBAIDS Influenza A Subtyping Kit was established during prospective studies at 5 geographically separated clinical study sites in the U.S. The study sites were located in the West (Fairfield, CA, and San Diego, CA), the Southwest (San Antonio, TX), the Midwest (Dayton, OH), and the Southeast (Fayetteville, NC). NP swab or wash samples were collected and tested during the 2010/2011 influenza season (December 2010 to April 2011). Subjects with signs and symptoms of influenza-like illness were invited to participate. Upon obtaining informed consent, an NPW or NPS specimen was collected for JBAIDS and comparator assay testing.

Written informed consent (or assent, if appropriate) was acquired from each subject and/or their parent/guardian (if under 18) at the time of enrollment into the study. After informed consent was acquired, the subject was assigned a Volunteer Identification Number (VIN). The VIN was used to de-identify the specimen used for JBAIDS testing and to provide data to the sponsor.

Patient specimens were divided into single use aliquots for testing immediately after collection. Typically, one sample aliquot was placed at 4°C to be purified within one day and tested using the JBAIDS Influenza A Subtyping Kit. Specimens collected on Fridays were placed at < -15°C over the weekend and thawed for testing on Monday. Aliquots for comparator testing were placed at < -15°C and sent to Idaho Technology, Inc., on a weekly basis. Once received at Idaho Technology, the samples were placed at < -70ºC until they were tested using the comparator assays.

Patient samples were purified using either the IT 1-2-3 Platinum Path Purification Kit (3 testing sites), or the Roche MagNA Pure Compact Nucleic Acid Isolation Kit I in conjunction with the Roche MagNA Pure Compact system (4 testing sites), and tested with the JBAIDS Influenza A Subtyping Kit.

All samples were tested for Influenza A subtypes according to the instructions in the clinical trial protocol. All collected samples, regardless of the Influenza A test result generated by the JBAIDS Influenza A & B Detection Kit, were tested with all assays contained in the JBAIDS Influenza A Subtyping kit. Frozen aliquots of NPS or NPW specimens were shipped to ITI on a weekly basis for comparator PCR testing using the CDC rRT-PCR Flu Panel Assays for Influenza A & B viruses, as well as additional subtype-specific PCR assays followed by bi-directional sequencing of amplicons. Refer to "PCR followed by Bi-directional Sequencing Comparator Assays" section for details regarding assays targets and analytical validation results. Comparator PCR testing was performed by ITI research associates in a blinded manner; specimens were labeled only with the subject's VIN and the JBAIDS test results were not available to them. Specimen testing with the CDC rRT- PCR Flu Panel assays was performed in accordance with the package insert. PCR followed by bi-directional sequencing comparator assays were performed on all specimens that were tested positive for Influenza A by the CDC rRT- PCR Flu Panel. Bi-directional sequence data was used to verify the identity of each amplicon. "True" seasonal Influenza A/H1, seasonal Influenza A/H3, and 2009 H1N1 Influenza positives, respectively, were considered as any sample that had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched seasonal Influenza A/H1, seasonal Influenza A/H3, or 2009 H1N1 Influenza sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov), respectively, with acceptable E-values.

The E-value ranges generated from the prospective clinical trial per Influenza A subtype were presented in the following table:

Influenza A Subtype	E-value Low	E-value High
Influenza A/H1[1]	N/A	N/A
2009 H1N1 Influenza	2.28E-55	1.62E-35
Influenza A/H3	1.2E-133	5.25E-105

[1] The seasonal Influenza A/H1 virus was not circulating during the 2010-2011 influenza season (http://www.cdc.gov/flu/) and was not detected during the prospective clinical evaluation of the JBAIDS Influenza A Subtyping Kit, except for 1 false positive A/H1 result.

A total of 269 runs were performed during the JBAIDS Influenza A Subtyping Kit clinical trial and 261 (97.0%) JBAIDS runs were completed successfully. Of the 8 (3.0%) runs that failed to complete, 6 occurred due to a software defect. The remaining 2 instrument errors were due to a "case blower error" causing the run to abort. There

were a total of 9 (3.4%) additional runs in which the run controls (NCs and or PCs) failed. Eight failures were associated with the Flu A H1 2009 NC and (or) PC, and one additional failure occurred with the Flu A SW PC. All associated specimens were retested and no specimens were lost due to these failures.

During the prospective clinical study, positive and negative nucleic acid extraction controls (PEC and NEC, respectively) were processed with each batch of purification specimens to ensure that the purification process was effectively performed. The PEC was considered successful when all tested target assays were positive. A failed PEC was retested using the same purified sample and the results of the retest were accepted as the final result. If the retest was unsuccessful, all associated samples were considered to have invalid results and required re-purification in order to obtain a valid test result. During the clinical evaluation, 11 out of a total of 194 PECs required retesting. Nine retests were required due to an invalid assay result (PC or NC). Two PEC failures occurred as a result of one of the four target assays giving a negative result (Flu A H1 one time and Flu A H1 2009 one time). Retesting was performed for one of the failures (Flu A H1 2009) which resolved the failure. There was no retest performed for the other failure. This lack of retesting was a protocol deviation. Five (5) samples associated with this run were excluded from analysis.

A total of 833 subjects were enrolled in the prospective study initially. Eighteen (18) (2.2%) withdrew or were withdrawn from the study for various reasons, including discrepancy in consent documents (n=7), patient refusing sample collection or withdrawal from study (n=7), or improper specimen collection (n=4). Seven (7) specimens were withdrawn from the study due to invalid testing results from the comparator assay. Four (4) specimens were withdrawn due to a contamination event at Site 5. An additional 4 samples were excluded due to inconclusive JBAIDS Influenza A Subtyping Kit results. All data collected from these individuals were removed from the study analyses. Five (5) additional specimens were removed from analysis due to a batch control failure that was not retested. The remaining 795 subjects (483 NPW specimens and 312 NPS specimens) were included in the demographic and clinical performance analyses.

Of the 795 prospective specimens that were included in the clinical performance analyses, 94% (751/795) of these specimens were successful on the first attempt (Site 1: 359/370 =97%; Site 2: 191/206 =93%; Site 3: 54/56 =96%; Site 4: 110/118 =93%; Site 5: 37/45 =98%). The remaining 6% (44/795) required retesting: “Invalid” (32/44), “Inconclusive” (11/44), and “Unsubtypeable” (1/44) (11 samples from Site 1; 15 samples from Site 2; 2 samples from Site 3; 8 sample from Site 4; and 8 sample from Site 5). Forty (40) out of 44 samples resolved upon a 1st retest and the remaining 4 samples required a re-extraction and retest and resolved.

Summary of Included Subject Demographics

		Overall	Site 1	Site 2	Site 3	Site 4	Site 5
NPS		312	50	206	56	0	0
NPW		483	320	0	0	118	45
Total		795	370	206	56	118	45
Sex	Female	405 (50.9%)	188 (50.8%)	122 (59.2%)	23 (41.1%)	56 (47.5%)	16 (35.6%)

	Male	390 (49.1%)	182 (49.2%)	85 (40.8%)	33 (58.9%)	62 (52.5%)	29 (64.4%)
Age[a]	Mean	26.4	23.4	24.5	30.3	23.1	30.8
	Median	24.0	24.0	18	27.5	17.0	26.0
	Min	0.5	0.5	0.5	2.0	0.5	18.0
	Max	92.0	92.0	69.0	81.0	68.0	62.0
Age Range[b]	≤5	149 (18.7%)	88 (23.8%)	40 (19.4%)	4 (7.1%)	17 (14.4%)	0 (0%)
	6-21[c]	229 (28.8%)	79 (21.4%)	74 (35.9%)	10 (17.9%)	54 (45.8%)	12[c] (26.7%)
	22-49	330 (41.6%)	178 (48.1%)	54 (26.2%)	35 (62.5%)	34 (28.8%)	30 (66.7%)
	≥50	86 (10.8%)	25 (6.8%)	38 (18.4%)	7 (12.5%)	13 (11.0%)	3 (6.7%)

[a] 0.5 was used for all ages under 1 year for these calculations.
[b] The age groups ≤ 5 years and ≥ 50 years correspond to high risk groups for which the CDC strongly recommends seasonal Influenza vaccination (http://www.cdc.gov/flu/protect/keyfacts.htm).
[c] Site 5 enrolled adults only; this category reflects participants 18 to 21 years of age.

The following table shows the summary of positive percent agreement (PPA) and negative percent agreement (NPA) for the JBAIDS Influenza A Subtyping Kit comparing to the comparator RT- PCR followed by bi-directional sequencing assays, stratified by specimen type and extraction method:

JBAIDS Influenza A Subtyping Kit Clinical Performance Summary

Influenza A Subtype	Sample Matrix	Purification Kit	PPA			NPA		
			TP/(TP+FN)	Percent	95% CI	TN/(TN+FP)	Percent	95% CI
Seasonal A/H1	NPW	Platinum Path	0/0	-	-	277/278	99.6%	98.0-100%
		MagNA Pure	0/0	-	-	205/205	100.0%	98.2-100%
		Combined	**0/0**	**-**	**-**	**482/483**	**99.8%**	**98.9-100%**
	NPS	Platinum Path	0/0	-	-	132/132	100.0%	97.2-100%
		MagNA Pure	0/0	-	-	180/180	100.0%	98.0-100%
		Combined	**0/0**	**-**	**-**	**312/312**	**100.0%**	**98.8-100%**
2009 H1N1	NPW	Platinum Path	50/50	100.0%	92.9-100%	227/228	99.6%	97.6-100%
		MagNA Pure	16/16	100.0%	79.4-100%	187/189	98.9%	96.2-99.9%
		Combined	**66/66**	**100.0%**	**94.6-100%**	**414/417**	**99.3%**	**97.9-99.9%**
	NPS	Platinum Path	24/24	100.0%	85.8-100%	108/108	100.0%	96.6-100%
		MagNA Pure	10/10	100.0%	69.2-100%	169/170	99.4%	96.8-100%
		Combined	**34/34**	**100.0%**	**89.7-100%**	**277/278**	**99.6%**	**98.0-100%**
Seasonal A/H3	NPW	Platinum Path	14/14	100.0%	76.8-100%	264/264	100.0%	98.6-100%
		MagNA Pure	19/19	100.0%	82.4-100%	186/186	100.0%	98.0-100%
		Combined	**33/33**	**100.0%**	**89.4-100%**	**450/450**	**100.0%**	**99.2-100%**
	NPS	Platinum Path	18/18	100.0%	81.5-100%	115/115	100.0%	96.8-100%
		MagNA Pure	8/8	100.0%	63.1-100%	171/171	100.0%	97.9-100%
		Combined	**26/26**	**100.0%**	**86.8-100%**	**286/286**	**100.0%**	**98.7-100%**

The following two tables show JBAIDS Influenza A Subtyping Kit clinical performance by site for NPW and NPS specimens, respectively. Due to similar performance when samples were purified with the Platinum Path and MagNA Pure

nucleic acid extraction kits, data obtained for samples purified by both methods were combined.

JBAIDS Influenza A Subtyping Kit Clinical Performance by Site for NPW Specimens

Assay	Site	PPA		95% CI	NPA		95% CI
Flu A H1	1	0/0	0%	-	320/320	100%	98.9-100%
	4	0/0	0%	-	117/118	99.2%	95.4-100%
	5	0/0	0%	-	45/45	100%	92.1-100%
Flu A H1 2009 and Flu A Sw	1	26/26	100%	86.8-100%	293/294	99.7%	98.1-100%
	4	37/37	100%	90.5-100%	80/81	98.8	93.3-99.97%
	5	3/3	100%	29.2-100%	41/42	97.6%	87.4-99.9%
Flu A H3	1	20/20	100%	83.2-100%	300/300	100%	98.8-100%
	4	3/3	100%	29.2-100%	115/115	100%	96.8-100%
	5	10/10	100%	69.2-100%	35/35	100%	90.0-100%

JBAIDS Influenza A Subtyping Kit Clinical Performance by Site for NPS Specimens

Assay	Site	PPA		95% CI	NPA		95% CI
Flu A H1	1	0/0	N/A	N/A	50/50	100%	92.9-100%
	2	0/0	N/A	N/A	206/206	100%	98.2-100%
	3	0/0	N/A	N/A	56/56	100%	93.6-100%
Flu A H1 2009 and Flu A Sw	1	0/0	N/A	N/A	50/50	100%	92.9-100%
	2	30/30	100%	88.4-100%	175/176	99.4%	96.9-99.99%
	3	4/4	100%	39.8-100%	52/52	100%	93.2-100%
Flu A H3	1	0/0	N/A	N/A	50/50	100%	92.9-100%
	2	21/21	100%	83.9-100%	185/185	100%	98.0-100%
	3	5/5	100%	47.8-100%	51/51	100%	93.0-100%

b. Retrospective Clinical study

The seasonal Influenza A/H1 virus was not circulating during the 2010-2011 influenza season (http://www.cdc.gov/flu/) and was not detected during the prospective clinical evaluation of the JBAIDS Influenza A Subtyping Kit, except for 1 false positive A/H1 result. Therefore, performance of the JBAIDS Influenza A Subtyping Kit testing seasonal Influenza A/H1 could not be established during the prospective clinical study. A retrospective clinical study was carried out at two different clinical sites to supplement the prospective evaluation data by analyzing pre-selected, archived specimens that were known to be positive for seasonal Influenza A/H1. Specimens were evaluated using both the Platinum Path and MagNA Pure nucleic acid purification kits.

A total of 56 NPS specimens were received for testing in this study. Thirty- five (35)

of the specimens had previously tested positive for seasonal Influenza A/H1. These specimens were obtained from the CDC and were originally collected from medical facilities and reference laboratories where they were previously tested by CDC and FDA-cleared laboratory methods and found to contain seasonal Influenza A/H1. No demographic information was available for these specimens. Specimens may have experienced as many as three freeze/thaw cycles prior to this study. The specimens were selected only for their previous test result; there was no effort to choose specimens with specific analyte levels. An additional 21 Influenza A negative NPS specimens from patients with respiratory illness, but known to be negative for influenza, were obtained from ARUP Laboratories. Upon arrival at ITI, a 4-digit study number (VIN) was assigned to each sample and a key was created in order to randomize the specimens such that the users testing the samples were blinded as to the expected test result. Specimens were divided approximately evenly for testing between both purification kits.

Because it is possible that the provided samples had been misidentified or had degraded during storage or previous handling, the presence or absence of the expected analyte was confirmed using "validation" PCR assays. The validation PCR assays were identical to the comparator assays that were used for the prospective clinical evaluation study. Briefly, specimens were first evaluated with the CDC Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel (rRT-PCR Flu Panel) for Influenza A and B. Specimens positive for Influenza A were then subject to additional subtype-specific PCR, in this case using the comparator validation assay used for the prospective clinical evaluation of the JBAIDS Influenza A Subtyping Kit, an assay specifically designed to detect seasonal Influenza A/H1. Positive PCR reactions for the seasonal Influenza A/H1 comparator validation assay were analyzed using bi-directional sequencing for definitive confirmation. If this validation testing did not confirm the presence or absence of the expected organism in the sample, the sample was not analyzed further as this result may have reflected that the sample had been improperly labeled or handled. Validation assay operators were blinded as to the expected result for each sample. Validated specimens with confirmed identities were then tested. Specimens were purified using either the Platinum Path or MagNA Pure sample purification kits then analyzed with the JBAIDS. The Influenza A/H1 assay from the JBAIDS Influenza A Subtyping Kit was run in a Custom Test Method with the Influenza A (Flu A) and influenza sample control (Flu SC) assays from the JBAIDS Influenza A & B Detection Kit.

Validation testing confirmed all 21 influenza-negative specimens and 30 of the 35 seasonal Influenza A/H1 samples. Validation PCR testing revealed that Ct values ranged from 19.2 to 32.0 (median 25.1) out of 45 cycles, demonstrating that the specimen population contained a diverse level of analyte. Half of the samples were extracted using the Platinum Path purification kit and half using MagNA pure.

One (1) seasonal Influenza A/H1 sample did not have a valid test result due to SC failure, and was excluded from data analysis. The remaining 29 seasonal Influenza A/H1 samples and the 21 influenza-negative specimens were included in the performance analysis.

The following table presents the PPA and NPA for the archived clinical specimens. Data from both extraction kits are combined due to identical performance.

Performance Summary of Seasonal Influenza A/H1 Archived Clinical NPS Specimens

Influenza Assay	Specimen Type	PPA	Percent	95% CI	NPA	Percent	95% CI
Flu A H1	NPS	29/29	100%	88.1-100%	21/21	100%	83.4-100%

c. Surrogate Clinical Specimen Testing for Seasonal Influenza A/H1

The seasonal Influenza A/H1 virus was not circulating during the 2010-2011 influenza season (http://www.cdc.gov/flu/) and was not detected during the prospective clinical evaluation of the JBAIDS Influenza A Subtyping Kit, except for one false positive A/H1 result. In addition, archived clinical NPW samples that had previously tested positive for the seasonal Influenza A/H1 virus were not available. Therefore, a clinical study was performed to evaluate the performance of the JBAIDS Influenza A Subtyping Kit using residual clinical NPS and NPW specimens spiked with a seasonal Influenza A H1N1 strain.

One hundred thirty-six (136) surrogate seasonal Influenza A H1N1 NPS and NPW clinical specimens (68 of each specimen type) were prepared using residual influenza-negative specimens that were collected during the prospective clinical trial of the JBAIDS influenza kits. These specimens had been previously evaluated with both the JBAIDS as well as comparator assays and shown to be influenza- negative. Residual specimens were spiked at ITI with a representative seasonal influenza A H1N1 (A/New Caledonia/20/1999) strain as follows:

NPS and NPW Sample Spike Levels and Replicate Numbers

Spike Level	NPS and NPW Replicates	
	Platinum Path	MagNA Pure
Negative	8	8
1×LoD	18	18
3×LoD	18	18
10×LoD	8	8
100×LoD	8	8
1,000×LoD	8	8
Total	**68**	**68**

A study number (VIN) was assigned to each sample and a key was created in order to randomize the samples such that the users testing the samples were blinded as to the expected test result. Frozen samples were then sent to 2 different clinical trial sites for testing. Samples were purified using either the Platinum Path or MagNA Pure sample purification kits, and then analyzed with the JBAIDS Flu A subtyping assays.

Of the 136 surrogate samples included in this study, a valid Influenza A test result was obtained for 128 samples (62 NPW and 66 NPS). The remaining 8 samples with invalid Influenza A test results were not included in the analysis.

The following table presents the PPA and NPA for the surrogate clinical specimens. Half of the samples were extracted using the Platinum Path purification kit and half

using the MagNA pure kit. Data from both extraction kits are combined due to identical performance.

Performance Summary of Seasonal Influenza A/H1 Surrogate Clinical Specimens

Assay	Sample Type	PPA			NPA		
		TP/(TP+FN)	Percent	95% CI	TN/(TN+FP)	Percent	95% CI
Flu A H1	NPW	54/54	100%	93.4-100%	8/8	100.0%	63.1-100%
	NPS	59/59	100%	93.9-100%	7/7	100.0%	59.0-100%
Flu A H1 2009 and Flu A Sw	NPW	0/0	-	-	62/62	100.0%	94.2-100%
	NPS	0/0	-	-	66/66	100.0%	94.6-100%
Flu A H3	NPW	0/0	-	-	62/62	100.0%	94.2-100%
	NPS	0/0	-	-	66/66	100.0%	94.6-100%

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

The prospective trial of the JBAIDS Influenza A Subtyping Kit tested nasopharyngeal wash (NPW) and nasopharyngeal swab (NPS) specimens obtained from individuals with influenza-like illness (ILI). Specimens were collected from volunteers at five sites across the United States from December 2010 through April 2011. A total of 795 patient specimens were analyzed. The number and percentage of positive cases as determined with the JBAIDS Influenza A Subtyping Kit by testing site and sample type or by age group and sample type are presented in the following tables. Seasonal Influenza A/H1 was not circulating while this study was conducted.

Expected Values (as Determined by the JBAIDS) Summary by Site and Sample Type

Positive Results by Influenza Assay	Sample Type	Overall (NPS: n=312; NPW: n=483)		Site 1 (NPS: n=50; NPW: n=320)		Site 2 (n=206)		Site 3 (n=56)		Site 4 (n=118)		Site 5 (n=45)	
		Number	Expected Value	Number	Expected Value	Number	Expected Value	Number	Expected Value	Number	Expected Value	Number	Expected Value
Seasonal Influenza A/ H1	NPS	0	0%	0	0%	0	0%	0	0%	-	-	-	-
	NPW	1	0.2%	0	0%	-	-	-	-	1	0.8%	0	0%
TOTAL		1	1.2%	0	0%	0	0%	0	0%	1	0.8%	0	0%
2009 H1N1 Influenza	NPS	35	11.2%	0	0%	31	15.0%	4	7.1%	-	-	-	-
	NPW	68	14.1%	26	8.2%	-	-	-	-	38	32.2%	4	8.9%
TOTAL		103	13.0%	26	7.0%	31	15.0%	4	7.1%	38	32.2%	4	8.9%
Seasonal Influenza	NPS	26	8.3%	0	0%	21	10.2%	5	8.9%	-	-	-	-

	NPW	33	6.8%	20	6.3%	-	-	-	-	3	2.5%	10	22.2%
TOTAL		59	7.4%	20	5.4%	21	10.2%	5	8.9%	3	2.5%	10	22.2%

Expected Value (as Determined by the JBAIDS) Summary by Age Group and Sample Type

Analyte	Sample Type	Total (Expected Value)	≤5 years	6-21 years	22-49 years	≥50 years
Seasonal Influenza A/H1	NPS (n=312)	0 (0%)	0	0	0	0
	NPW (n=483)	1 (0.2%)	0	1	0	0
2009 H1N1 Influenza	NPS (n=312)	35 (11.2%)	1	14	14	6
	NPW (n=483)	68 (14.1%)	12	21	27	8
Seasonal Influenza A/H3	NPS (n=312)	26 (8.3%)	3	5	11	7
	NPW (n=483)	33 (6.8%)	3	5	22	3

N. Instrument Name:

Joint Biological Agent Identification and Diagnostic System (JBAIDS) Instrument.

O. System Descriptions:

1. Modes of Operation:

The JBAIDS Instrument is used to perform real time reverse transcription, PCR amplification and detection of nucleic acid. Three other nucleic acid amplification tests that use the JBAIDS Instrument have received 510(k) clearance: JBAIDS Anthrax Detection System (k051713), JBAIDS Plague Detection System (k072631), JBAIDS Tularemia Detection System (k072547), JBAIDS Influenza A/H5 (Asian Lineage) Detection Kit (k100287), and JBAIDS Q Fever Detection System (k103207). The JBAIDS Instrument is a ruggedized, portable real-time PCR instrument designed to withstand the conditions of transport and use likely to be encountered in a military field laboratory. The instrument is comprised of an air thermocycler that amplifies specific DNA sequences using PCR and a fluorimeter that measures fluorescence signals associated with production of PCR product (amplicon) during the course of the reaction. For thermocycling, samples contained in glass capillaries are placed in the sample chamber where they are heated and cooled by the JBAIDS instrument.

The JBAIDS Software is preloaded on a ruggedized laptop computer. The software controls the instrument's thermal cycling functions, acquires the fluorescence data from the instrument, and displays the fluorescence data for the user during the run. When the run is finished, the software's Detector module analyzes the data and displays test results. The instrument and software have the ability to perform either diagnostic (IVD) or surveillance (environmental) testing.

The IVD software includes the following diagnostic components:

- A traceable database that limits the data that the user can change in a test run and that requires the user to enter change notes for any changes that are allowed.
- A Diagnostic Wizard that guides the operator through the process of setting up the JBAIDS run in compliance with the package insert. IVD testing is always performed using the Diagnostic Wizard and the traceable database. The wizard initially presents to the operator a predefined selection of FDA cleared JBAIDS IVD assays. JBAIDS assays under evaluation for FDA clearance are also available in limited release software. Once the user has selected the IVD assays to test, the Wizard guides the user through the process of setting up and loading each PCR reaction into the instrument. The wizard requires each run to contain a Positive Control and a Negative Control for each target included in the run as well as the inclusion of additional controls (e.g., inhibition controls, sample controls), when they are required.
- A Detector module that automatically analyzes the fluorescence amplification curves and displays the final test results to the user. The Detector module ensures that the correct rules are applied both to the interpretation of each amplification curve and to the final interpretation (e.g., metacall), based on the results of the samples and the relevant controls.
- A report certification that reflects the current regulatory status of the test being performed. When an assay is being evaluated for FDA clearance, the JBAIDS test report includes the statement 'For Investigational Use Only. The performance characteristics of this product have not been established.' Once the assay has received FDA clearance, the software is modified and the report certification statement becomes 'For *In Vitro* Diagnostic Use Only'.

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:

   User enters Patient ID/Sample ID by typing it in.

4. Specimen Sampling and Handling:

   Not applicable

5. Calibration:
   Not applicable

6. Quality Control:

   The following controls are included in the JBAIDS Influenza A Subtyping Kit

Negative Control

The NC is used to detect contamination from target-specific amplified product (amplicon), synthetic RNA (as found in the PC vials), or virus. The NC vial is reconstituted using the purified negative extraction control (NEC) (molecular biology grade water). The NEC should be processed along with each batch of specimens purified. The NC detects possible contamination during sample purification and during reconstitution of freeze- dried reagents. Each JBAIDS Influenza A Subtyping Kit run requires one NC for each of the 4 assays (i.e., the Flu A H1, Flu A H3, Flu A H1 2009, and Flu A Sw assays). For each target assay, the NC must be negative, or the JBAIDS software will assign invalid results for that target assay to all of the samples in that run. If the NC fails, the JBAIDS run must be repeated using the same purified samples. If the NC fails again, all associated samples are considered to have invalid results and should be re-purified starting from another aliquot of the original specimens.

Positive Control

The PC serves as an amplification and detection control. Each JBAIDS Influenza A Subtyping Kit run requires one PC for each of the 4 assays (i.e., the Flu A H1, Flu A H3, Flu A H1 2009, and Flu A Sw assays). For each target assay, the PC must be positive. If the PC fails, the JBAIDS software will assign invalid results for that target assay to all of the associated samples, and the JBAIDS run must be repeated starting from the same purified specimens. Failure of the PCs may indicate errors in sample setup, degradation of the reagents, or a malfunction of the JBAIDS instrument.

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

Not applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

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