

[Adhere Package Insert Barcode Here]

**Liat™ Influenza A/2009 H1N1 Assay
Package Insert**

Emergency Use Authorization: For *in vitro* Diagnostic Use Only

Proprietary Name

Liat™ Influenza A/2009 H1N1 Assay

Common or Usual Name

Liat Influenza A/2009 H1N1 Assay

Intended Use

The IQuum Liat Influenza A/2009 H1N1 Assay is a rapid, automated, multiplex real-time RT-PCR assay intended for use in laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate complexity tests and in laboratories certified under CLIA to perform high complexity tests using the Liat Analyzer for the *in vitro* qualitative detection and differentiation of 2009 H1N1 influenza viral RNA. The Liat Influenza A/2009 H1N1 Assay uses nasopharyngeal swab (NPS) specimens collected from patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors.

Testing with the Liat Influenza A/2009 H1N1 Assay should not be performed unless the patient meets clinical and epidemiologic criteria for testing suspect specimens. The identification of 2009 H1N1 influenza should be performed along with a clinical and epidemiological assessment.

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

Summary and Explanation

Influenza is an acute respiratory illness caused by infection with the Influenza virus. Influenza viruses consists of three types, influenza A, influenza B and influenza C. Influenza A viruses are further classified by two membrane proteins, hemagglutinin (H) and neuraminidase (N). In the US, influenza A/H1N1, A/H3N2 and influenza B are the predominant seasonal viruses. In March 2009, a novel H1N1 strain was identified in humans in the United States as well as globally.

Symptoms of influenza include fever, chills, headache, malaise, cough, coryza, nausea, vomiting, and diarrhea. Automated and rapid assays that identify and differentiate patients infected with seasonal and novel H1N1 influenza can aid in effective control, proper choice of treatment, and prevention of influenza outbreaks.

Principle of the Procedure

The Liat Influenza A/2009 H1N1 Assay is a rapid, automated *in vitro* diagnostic test for qualitative detection and differentiation of 2009 H1N1 influenza viral RNA. The assay is performed on the IQuum Liat Analyzer. The Liat Analyzer automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in biological samples using real-time RT-PCR assays. The system consists of an instrument and preloaded software for running tests and viewing the results. The system requires the use of a single-use disposable Liat Tube that holds the sample purification, and RT-PCR reagents and hosts the sample preparation and RT-PCR processes. Other than adding the sample to the Liat Tube, no reagent preparation or additional steps are required. Because each Liat tube is self-contained, cross-contamination between samples is minimized. Turnaround time for a test is 26 minutes.

The Liat Influenza A/2009 H1N1 Assay includes reagents for the detection and differentiation of 2009 H1N1 influenza viral RNA in nasopharyngeal swab (NPS) specimens in universal transport media (UTM) or in Liat Swab Dilution Buffer from patients suspected of having influenza. The assay detects a conserved region of the matrix gene of influenza A viral RNA (Inf A target) and the hemagglutinin gene of 2009 H1N1 influenza viral RNA (2009 H1 target). An Internal Process Control (IPC) is also included. The IPC is present to control for adequate processing of the target viruses and to monitor the presence of inhibitors in the RT-PCR reactions.

Reagents and Instruments

Materials Provided



The Liat Influenza A/2009 H1N1 Assay Pack contains sufficient reagents to process 40 specimens or quality control samples. The pack contains the following:

Description	P/N	Qty
Liat Influenza A/2009 H1N1 Assay Tube	20-03701	40
Liat Inf. A/2009 H1N1 Assay Quality Control Kit	20-03643	1
Liat Inf. A/2009 H1N1 Assay Package Insert	34-03649	1

Notes: Material Safety Data Sheets (MSDS) for all reagents provided in this assay are available upon request from IQuum Technical Support.

Storage and Handling



- Store the Liat Influenza A/2009 H1N1 Assay tube at 2 – 8°C.
- Do not open individual tube packaging until you are ready to perform testing.

Materials Required but Not Provided

Liat Analyzer, IQuum, Cat # 40-03352

Materials Available but Not Provided

- Liat NP Swab Collection Kit (Liat Dilution Buffer), IQuum, Cat # 20-03641 containing:
 - Liat Dilution Buffer, IQuum, Cat # 20-03033
 - Swab, Copan, P/N 503C01 (IQuum, Cat # 30-03032)
 - 200 uL Exact Volume Pipette, Poly-Pipets Inc. (IQuum, Cat # 30-03037)

- Liat NP Swab UTM Sample Kit, IQuum, Cat # 20-03642 containing:
 - Liat UTM Dilution Buffer, IQuum, Cat # 20-03640
 - 200 uL Exact Volume Pipette, Poly-Pipets Inc. (IQuum, Cat # 30-03037)
- **CONTROL** Liat Influenza A/2009 H1N1 Assay Quality Control Kit, IQuum, Cat# 20-03643 containing:
 - A/2009 H1N1 Positive Control, IQuum, Cat # 20-03702
 - Liat Dilution Buffer, IQuum, Cat # 20-03033
 - Liat Negative Control Tube, IQuum, Cat # 20-03149
 - 200 uL Exact Volume Pipette, Poly-Pipets Inc. (IQuum, Cat # 30-03037)The Quality Control Kit is provided as a part of the Liat Influenza A/2009 H1N1 Assay Pack but can also be obtained separately from IQuum.
- Universal Transport Media (UTM)
 - Becton Dickinson Cat # 220220 or Copan Cat # 330C

Warnings and Precautions



- Treat all biological specimens, including used Liat Tubes, as if capable of transmitting infectious agents. Because it is often impossible to know which specimens might be infectious, all biological specimens should be treated with universal precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention (1, 2) and the Clinical and Laboratory Standards Institute (3).
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Do not substitute Liat Dilution or UTM Dilution Buffers with other reagents.
- Do not use a damaged Liat Tube.
- Each single-use Liat Influenza A/2009 H1N1 Assay tube is used to process one test. Do not reuse a spent Liat tube.
- Dispose of a used Liat Influenza A/2009 H1N1 Assay tube according to your institution's safety guidelines for hazardous material.



- Store the Liat Influenza A/2009 H1N1 Assay tube at 2 – 8°C.
- Do not open individual tube packaging until you are ready to perform testing.

Specimen Collection, Transport and Storage

Nasopharyngeal swab specimens can be collected following the user institution's standard procedures and placed into universal transport media or Liat Dilution Buffer. For sample transport and storage, follow World Health Organization, Instructions for Storage and Transport of Suspected or Confirmed Human and Animal Specimens and Virus Isolates of Pandemic (H1N1) 2009, Published 16 July 2009.

Procedure

Add Liat Assay Tube Lot

1. Power on the Liat Analyzer. Log In using username and password.
2. From the Main Menu, touch “Assay Menu” on the touch screen. The Liat Analyzer shows all recognized tube lot status, including assay name, lot number, validated date and expiration date. Touch “New Lot” on the bottom of the screen.
3. The Liat Analyzer prompts the user to “1. Scan Insert ID.” Obtain the package insert from the new lot. Touch “Scan” or press the corresponding soft key to enable the barcode scanner. Scan the package insert barcode.
4. After the Liat Analyzer recognizes the new package insert ID, it will ask “2. Scan Positive Control ID”. Obtain the positive control ID. Touch “Scan” or press the corresponding soft key to enable the barcode scanner. Scan the positive control barcode.
5. The Liat Analyzer will then ask “3. Add Positive Control & Scan Liat Tube ID”. Take the A/H1N1 Positive Control Tube from the Liat Influenza A/2009 H1N1 Assay Quality Control Kit and load 100 µL into a Liat Influenza A/2009 H1N1 Assay Tube. Scan the tube ID and insert the Liat Tube into the Liat Analyzer. Complete this step within 10 seconds of scanning the tube barcode. The Liat Analyzer will perform the positive control run and evaluate the result against pre-set criteria in the software.
6. Once the assay run is complete, the Liat Analyzer will display the message: “Please remove the tube. Hit <OK> to continue.” Remove the Liat Tube and discard it as biohazardous waste. The Liat Analyzer will show “Positive result accepted” if the result meets the criteria. If the first positive run fails, the Liat Analyzer allows the positive control run to be repeated. The lot will be rejected if the second positive control run also fails.
7. Touch “Back” on the screen, the Liat Analyzer will ask “1. Scan Negative Control ID”. Obtain the negative control ID. Touch “Scan” or press the corresponding soft key to enable the barcode scanner. Scan the negative control barcode.
8. The Liat Analyzer will then ask “2. Add Negative Control & scan Liat Tube ID”. Take the Liat Negative Control Tube from Liat Influenza A/2009 H1N1 Assay Quality Control Kit and load 200 µL into a Liat Influenza A/2009 H1N1 Assay Tube. Scan the tube ID and insert the Liat Tube into the Liat Analyzer. Complete this step within 10 seconds of scanning the tube barcode. The Liat Analyzer will perform the negative control run and evaluate the result against pre-set criteria in the software.
9. Once the assay run is complete, the Liat Analyzer will display the message: “Please remove the tube. Hit <OK> to continue.” Remove the Liat Tube and discard it as biohazardous waste. The Liat Analyzer will show “negative result accepted” if the result meets the criteria. The new lot is now successfully added into the Liat Analyzer. The lot would be rejected if negative control run fails.
10. Use the “Back” button or “Menu” button to return to the Main Menu.

Prepare Sample

To test nasopharyngeal swab samples, elute the swab into Liat Dilution Buffer, following the steps below:

1. Place one swab into the Liat Dilution Buffer.
2. Gently swirl the swab in the Liat Dilution Buffer.
3. Transfer the sample using the EV transfer pipette from the Liat Dilution Buffer tube to the Liat Influenza A/2009 H1N1 Assay Tube.

For nasopharyngeal swab samples already suspended in UTM, dilute the UTM sample using the Liat UTM Dilution Buffer, following the steps below:

1. Transfer the UTM sample into Liat UTM Dilution Buffer using the EV transfer pipette.
2. Pipette up and down using the EV transfer pipette.
3. Transfer the UTM sample using the EV transfer pipette from the Liat UTM Dilution Buffer tube to the Liat Influenza A/2009 H1N1 Assay Tube.

Starting Liat Assay

1. Power on the Liat Analyzer. Log In using username and password.
2. From the Main Menu, touch “Run Assay” on the touch screen
3. The Liat Analyzer prompts the user to “Scan Liat Tube ID.” Obtain a Liat Influenza A/2009 H1N1 Assay Tube from a tube lot that has been added to the Liat Analyzer. NOTE: If the tube lot has not been added for use on the Liat Analyzer, the Analyzer will prompt the user to add the lot to the instrument.
4. Touch “Scan” or press the corresponding soft key to enable the barcode scanner. Scan the Liat Tube barcode. This barcode is located on the Liat Influenza A/2009 H1N1 Assay Tube.
5. The Liat Analyzer prompts the user to “Scan Patient ID.” Touch “Scan” or press the corresponding soft key to enable the barcode scanner. Scan the Patient ID Barcode. If a Positive or Negative control sample is being run, scan the appropriate barcode.
6. The Liat Analyzer prompts the user to “Add Sample to Tube and Rescan Tube ID.” Add the appropriate sample to a Liat Tube as indicated above. Cap the Liat Tube.
7. Re-scan the Liat Tube barcode located on the Liat Tube, and the Liat Analyzer sample entry door opens automatically. Insert the Liat Tube into the Liat Analyzer. Complete this step within 10 seconds of scanning the tube barcode. If this step is not completed within 10 seconds, the Liat Analyzer will close the sample entry door and prompts the use to rescan the tube barcode.
8. Once the Liat Tube is inserted, the Liat Analyzer sample entry door closes automatically and the assay begins.
9. During execution of the assay, the Liat Analyzer displays assay status and progress, as well as an estimate of the remaining assay time.

10. Once the assay run is complete, the Liat Analyzer will display the message: “Please remove the tube. Hit <OK> to continue.” Remove the Liat Tube and discard it as biohazardous waste.
11. Touch or press “OK.” This concludes a Liat assay run. Use the “Back” button or “Menu” button to return to the Main Menu

Viewing Results

View the Results Report by touching or clicking “Report” after the completion of the assay. Quality Control

Internal Process Control (IPC): Included in every assay tube and ensures the sample was correctly processed. The IPC is an encapsulated RNA that is included in each Liat tube to verify adequate processing of the sample virus. The IPC verifies that sample purification of influenza A virus has occurred and verifies that the specimen processing is adequate. Additionally, this control detects specimen-associated inhibition of the RT-PCR reactions. The IPC should be positive in a negative sample and can be negative or positive in a positive sample. The IPC passes if it meets the validated acceptance criteria.

External Controls: Should be used in accordance with local, state, federal accrediting organizations requirements as applicable.

Interpretation of Results

The results are interpreted automatically by the Liat Analyzer from measured fluorescent signals and embedded calculation algorithms, and are clearly shown in the View Results window. Specimen results are interpreted as follows:

Table 1. Interpretation of results from the Liat Analyzer

	Inf A	2009 H1	IPC	Report Results	Details
1	–	–	+	2009 H1 Influenza Viral RNA Not Detected	Influenza A Viral RNA Not Detected 2009H1 Influenza Viral RNA Not Detected
2	+	–	±	2009 H1 Influenza Viral RNA Not Detected	Influenza A Viral RNA Detected 2009H1 Influenza Viral RNA Not Detected
3	+	+	±	2009 H1 Influenza Viral RNA Detected	Influenza A Viral RNA Detected 2009H1 Influenza Viral RNA Detected
4	–	+	±	Assay Invalid*. Repeat test.	Influenza A Viral RNA Not Detected 2009H1 Influenza Viral RNA Detected
5	–	–	–	Assay Invalid*. Repeat test.	Invalid*

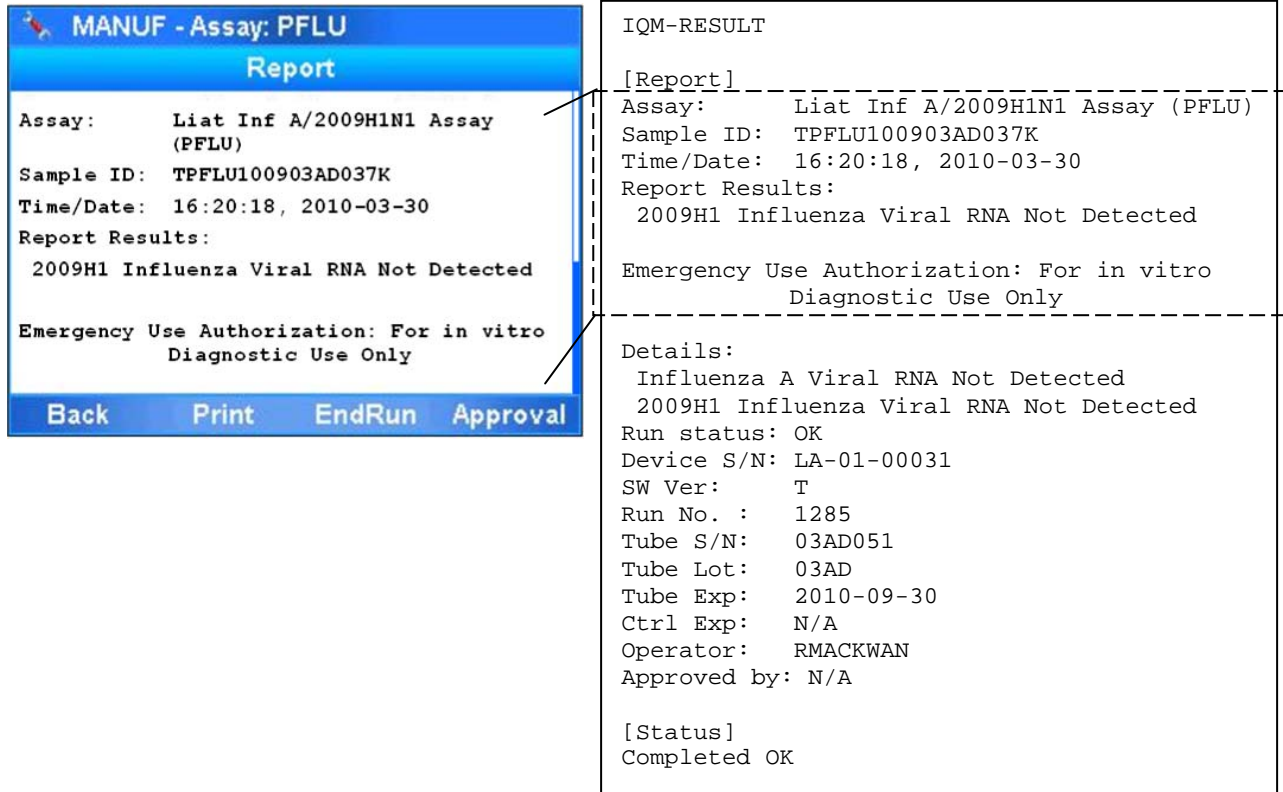
* For invalid specimen results, the specimen should be retested. If upon retest the results remain invalid, then the end user should report the result as indeterminate.

(1) 2009 H1N1 Viral RNA Not Detected

Influenza A Viral RNA Not Detected; 2009 H1 Influenza Viral RNA Not Detected.

- Inf A and 2009 H1 target RNAs are not detected.
- Internal process control has a Ct and amplitude within the valid range and endpoint above the minimum setting. The amplification curve of the internal process control fits the pattern recognition setting.

Figure 1. Screenshot, 2009 H1N1 Viral RNA Not Detected



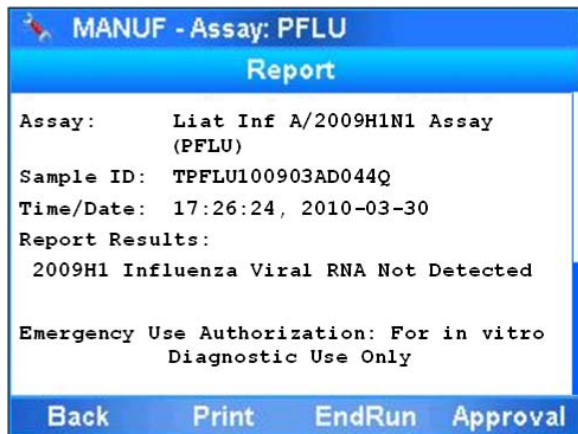
The screenshot displays a diagnostic report interface. On the left is a blue-bordered window titled 'MANUF - Assay: PFLU Report'. It contains the following text: 'Assay: Liat Inf A/2009H1N1 Assay (PFLU)', 'Sample ID: TPFLU100903AD037K', 'Time/Date: 16:20:18, 2010-03-30', 'Report Results: 2009H1 Influenza Viral RNA Not Detected', and 'Emergency Use Authorization: For in vitro Diagnostic Use Only'. At the bottom of this window are buttons for 'Back', 'Print', 'EndRun', and 'Approval'. On the right is a larger white window titled 'IQM-RESULT'. It contains a dashed-line header '[Report]' followed by: 'Assay: Liat Inf A/2009H1N1 Assay (PFLU)', 'Sample ID: TPFLU100903AD037K', 'Time/Date: 16:20:18, 2010-03-30', 'Report Results: 2009H1 Influenza Viral RNA Not Detected', 'Emergency Use Authorization: For in vitro Diagnostic Use Only', a dashed-line header 'Details:', 'Influenza A Viral RNA Not Detected', '2009H1 Influenza Viral RNA Not Detected', 'Run status: OK', 'Device S/N: LA-01-00031', 'SW Ver: T', 'Run No. : 1285', 'Tube S/N: 03AD051', 'Tube Lot: 03AD', 'Tube Exp: 2010-09-30', 'Ctrl Exp: N/A', 'Operator: RMACKWAN', 'Approved by: N/A', a dashed-line header '[Status]', and 'Completed OK'. Two lines connect the report content in the left window to the corresponding text in the right window.

(2) 2009 H1N1 Viral RNA Not Detected

Influenza A Viral RNA Detected; 2009 H1 Influenza Viral RNA Not Detected.

- The Inf A target has a Ct and amplitude within the valid range and endpoint above the minimum setting. The amplification curve of Inf A target fits the pattern recognition setting.
- High concentration of Inf A target may inhibit the amplification of the internal process control. The internal process control will not be taken into consideration in these cases.

Figure 2. Screenshot, 2009 H1N1 Viral RNA Not Detected



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IQM-RESULT

[Report]
Assay: Liat Inf A/2009H1N1 Assay (PFLU)
Sample ID: TPFLU100903AD044Q
Time/Date: 17:26:24, 2010-03-30
Report Results:
  2009H1 Influenza Viral RNA Not Detected

Emergency Use Authorization: For in vitro
  Diagnostic Use Only

Details:
  Influenza A Viral RNA Detected
  2009H1 Influenza Viral RNA Not Detected
Run status: OK
Device S/N: LA-01-00034
SW Ver: T
Run No. : 558
Tube S/N: 03AD044
Tube Lot: 03AD
Tube Exp: 2010-09-30
Ctrl Exp: N/A
Operator: YTIAN
Approved by: N/A

[Status]
Completed OK
```

(3) 2009 H1N1 Viral RNA Detected

Influenza A Viral RNA Detected; 2009 H1 Influenza Viral RNA Detected.

- The Inf A target has a Ct and amplitude within the valid range and endpoint above the minimum setting. The amplification curve of Inf A target fits the pattern recognition setting.
- The 2009 H1 target has a Ct and amplitude within the valid range and endpoint above the minimum setting. The amplification curve of 2009 H1 target fits the pattern recognition setting.
- High concentration of Inf A and 2009 H1 targets may inhibit the amplification of internal process control. The internal process control will not be taken into consideration in these cases.

Figure 3. Screenshot, 2009 H1N1 Viral RNA Detected

The screenshot displays a software interface for a laboratory assay. The top bar is blue with the text 'MANUF - Assay: PFLU' and 'Report' below it. The main display area is white with black text. It shows the following information: Assay: Liat Inf A/2009H1N1 Assay (PFLU); Sample ID: TPFLU100903AD047N; Time/Date: 16:35:54, 2010-03-30; Report Results: 2009H1 Influenza Viral RNA Detected; Emergency Use Authorization: For in vitro Diagnostic Use Only. At the bottom of the interface are four buttons: Back, Print, EndRun, and Approval. To the right of the interface is a text window titled 'IQM-RESULT' containing a detailed report. The report includes: [Report] Assay: Liat Inf A/2009H1N1 Assay (PFLU); Sample ID: TPFLU100903AD047N; Time/Date: 16:35:54, 2010-03-30; Report Results: 2009H1 Influenza Viral RNA Detected; Emergency Use Authorization: For in vitro Diagnostic Use Only; Details: Influenza A Viral RNA Detected, 2009H1 Influenza Viral RNA Detected; Run status: OK; Device S/N: LA-01-00030; SW Ver: T; Run No.: 1404; Tube S/N: 03AD047; Tube Lot: 03AD; Tube Exp: 2010-09-30; Ctrl Exp: N/A; Operator: RMACKWAN; Approved by: N/A; [Status] Completed OK.

```
MANUF - Assay: PFLU
Report
Assay:      Liat Inf A/2009H1N1 Assay
            (PFLU)
Sample ID:  TPFLU100903AD047N
Time/Date:  16:35:54, 2010-03-30
Report Results:
2009H1 Influenza Viral RNA Detected

Emergency Use Authorization: For in vitro
Diagnostic Use Only

Back      Print      EndRun     Approval

IQM-RESULT
[Report]
Assay:      Liat Inf A/2009H1N1 Assay (PFLU)
Sample ID:  TPFLU100903AD047N
Time/Date:  16:35:54, 2010-03-30
Report Results:
2009H1 Influenza Viral RNA Detected

Emergency Use Authorization: For in vitro
Diagnostic Use Only

Details:
Influenza A Viral RNA Detected
2009H1 Influenza Viral RNA Detected
Run status: OK
Device S/N: LA-01-00030
SW Ver:     T
Run No. :   1404
Tube S/N:   03AD047
Tube Lot:   03AD
Tube Exp:   2010-09-30
Ctrl Exp:   N/A
Operator:   RMACKWAN
Approved by: N/A

[Status]
Completed OK
```

(4) Assay Invalid

Influenza A Viral RNA Not Detected; 2009 H1 Influenza Viral RNA Detected.

- Inf A target RNAs are not detected.
- The 2009 H1 target has a Ct and amplitude within the valid range and endpoint above the minimum setting. The amplification curve of 2009 H1 target fits the pattern recognition setting.
- Internal process control has a Ct and amplitude within the valid range and endpoint above the minimum setting. The amplification curve of internal process control fits the pattern recognition setting.
- Result is invalid, Repeat test

Figure 4. Screenshot, Invalid Result

MANUF - Assay: PFLU
Report

Assay: Liat Inf A/2009H1N1 Assay (PFLU)
Sample ID: TPFLU100903AD048M
Time/Date: 16:41:40, 2010-03-30
Report Results:
Assay Invalid. Repeat test.
Failure code: r0
Emergency Use Authorization: For in vitro Diagnostic Use Only

Back Print EndRun Approval

IQM-RESULT

[Report]
Assay: Liat Inf A/2009H1N1 Assay (PFLU)
Sample ID: TPFLU100903AD048M
Time/Date: 16:41:40, 2010-03-30
Report Results:
Assay Invalid. Repeat test.
Failure code: r0
Emergency Use Authorization: For in vitro Diagnostic Use Only

Details:
Influenza A Viral RNA Not Detected
2009H1 Influenza Viral RNA Detected
Run status: OK
Device S/N: LA-01-00036
SW Ver: T
Run No. : 748
Tube S/N: 03AD048
Tube Lot: 03AD
Tube Exp: 2010-09-30
Ctrl Exp: N/A
Operator: YTIAN
Approved by: N/A

[Status]
Completed OK

(5) Assay Invalid

- Presence or absence of Inf A and 2009 H1 target RNAs cannot be determined.
- Internal process control result is negative, internal process control Ct and amplitude is not within the valid range, or the amplification curve of internal process control does not fit pattern recognition setting; and Inf A target and 2009 H1 target are not detected.
- The Result 5 “Report” screen and result report are similar to that of Result 4, except without the text “Influenza A Viral RNA Not Detected” and “2009H1 Influenza Viral RNA Detected” in the “Details” section.

Reasons to Repeat the Assay

If any of the test results mentioned below occur, repeat the test according to instructions in Starting Liat Assay section below using a new Liat Tube (do not reuse Liat Tubes).

- An “Invalid” result indicates that (1) the IPC failed and the sample was not properly processed or PCR was inhibited, or (2) that Influenza A viral RNA was not detected but 2009 H1 Influenza viral RNA was detected.
- An “Error” is reported and the assay is aborted. Possible causes include temperature control, motion control failure and power failure.

Limitations

- The performance of the Liat Influenza A/2009 H1N1 Assay was evaluated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.
- Results from the Liat Influenza A/2009 H1N1 Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
- Analyte targets (viral nucleic acid) may persist in vivo, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious, or are the causative agents for clinical symptoms.
- Detection of the Influenza A matrix sequence without detection of the 2009 H1N1 sequence (i.e., Influenza A: Detected result) does not rule out the possibility of 2009 H1N1 influenza.
- This test has been evaluated for use with human specimen material only.
- The detection of viral nucleic acid is dependent upon proper specimen collection, handling, transportation, storage and preparation, including extraction. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
- False negative results may occur if inadequate numbers of organisms are present in the specimen.
- If the virus mutates in the target region, influenza virus may not be detected or may be detected less predictably.
- This test is a qualitative test and does not provide the quantitative value of detected organism present.
- This test has not been evaluated for patients without signs and symptoms of influenza infection.
- This test has not been evaluated for monitoring treatment of Influenza infection.
- This test has not been evaluated for screening of blood or blood product for the presence of influenza.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- This test has not been evaluated for affects of potentially interfering substances on performance.
- Cross-reactivity with respiratory tract organisms others than those included in the analytical studies can lead to erroneous results.

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- This assay has not been evaluated for patients receiving intranasal administered influenza vaccine.
- This assay has not been evaluated for immunocompromised individuals.
- The prevalence of infection will affect the test's predictive value.

Performance Characteristics

All studies were performed in accordance with *Guidance for Industry and FDA Staff, In Vitro Diagnostic 2009 H1N1 Tests for Use in the 2009 H1N1 Emergency*. See attached Tables for data.

Analytical Performance

1. Analytical Sensitivity / Limit of Detection (LoD)

Analytical sensitivity / LoD studies determine the lowest detectable concentration of influenza virus at which greater than or equal to 95% of all (true positive) replicates test positive. The LoD of Influenza A and Influenza A/2009 H1N1 were determined for the Liat Influenza A/2009 H1N1 Assay. Briefly, characterized samples were adjusted to the highest log₁₀ TCID₅₀/mL before making serial 10-fold dilutions and spiking into nasopharyngeal swab collected UTM. A small scale LOD study was done to establish the tentative LOD by measuring triplicate results. The highest dilution yielding 3/3 positive calls was used to establish the concentration used for LOD confirmation.

To confirm LOD the sample with the lowest concentration of analyte generating 3/3 positives was used and analyzed by testing 20 replicates. The final LOD was established by determining the lowest concentration that yielded 19/20 positive results, or 95%.

The analytical sensitivity of the Liat Influenza H1N1 Assay was tested using titrated cultures of 2009 H1N1 Influenza A viruses (A/Swine NY/01/2009 H1N1, A/Swine NY/02/2009 H1N1) and seasonal Influenza A viruses (A/Brisbane/59/07). The LoD of the Liat Influenza A/2009 H1N1 Assay for the tested A/Swine NY/01/2009 H1N1 strain was determined as 10⁻² TCID₅₀/mL, and for A/Swine NY/02/2009 H1N1 strain was determined to be 10⁻¹ TCID₅₀/mL, and for A/Brisbane/59/07 strain was determined to be 10⁻¹ TCID₅₀/mL, at which ≥ 95% of the replicates tested positive.

Table 2. Summary of LoD results for 2009 H1N1 Influenza virus by Liat Influenza A/2009 H1N1 Assay using nasopharyngeal swab samples in UTM and Liat Dilution Buffer

Influenza Virus Tested	Influenza Strain Designation	TCID ₅₀ /mL	Swab Elution Buffer	InfA Call Rate	2009 H1 Call Rate
2009 H1	A/Swine NY/01/2009 H1N1	10 ⁻²	UTM	20/20	20/20
			Liat Dilution Buffer	20/20	20/20
2009 H1	A/Swine NY/02/2009 H1N1	10 ⁻¹	UTM	20/20	20/20*
			Liat Dilution Buffer	20/20	20/20
Seasonal A	A/Brisbane/59/07	10 ⁻¹	UTM	20/20	0/20
			Liat Dilution Buffer	20/20	0/20

* Twenty-one (21) runs were performed within this group, of which 1 run gave an "Invalid" result.

2. Analytical Specificity

The analytical specificity of Liat Influenza A/2009 H1N1 Assay was evaluated with respect to:

Reactivity (Inclusivity). The Liat Influenza A/2009 H1N1 Assay was evaluated for Reactivity/Inclusivity with a number of Influenza viral strains, which include 9 seasonal Influenza A subtype H1, 8 seasonal Influenza A subtype H3, three (3) Influenza A 2009 H1N1 strains and three (3) swine origin Influenza A strains, near LoD level. The results of the Liat Influenza A/2009 H1N1 Assay demonstrate that all seasonal and swine origin Influenza A strains showed positive results for Influenza A and negative results for 2009 H1. All 2009 H1N1 Influenza A strains showed positive results for Influenza A and positive results for 2009 H1N1. This indicates that Liat Influenza A/2009 H1N1 assay is specific for the tested Influenza A and 2009 H1N1 viruses (Table 3).

Potential cross-reactivity with seasonal human influenza viruses and other respiratory pathogens. Cross-reactivity of the Liat Influenza A/2009 H1N1 Assay was evaluated using other human pathogens and seasonal Influenza B viruses at medically relevant levels. The test panel was comprised of 25 viral and 16 bacterial strains. Three (3) to four (4) selected viruses or bacteria were pooled together to form a group and spiked into nasopharyngeal swab specimen in UTM and Liat Dilution buffer matrices, respectively. The tested concentrations of viruses or bacteria were selected at medically relevant levels or the highest titers obtained. The Liat Influenza A/2009 H1N1 Assay showed no cross reactivity for the tested organisms. Bacteria were tested at 10⁶ to 10⁸ cfu/mL or 10⁴ TCID₅₀/mL (Chlamydia). Viruses were tested at 10³ to 10¹⁰ TCID₅₀/mL. The Liat assay results for the tested strains were 100% concordant with the expected results (Table 4).

Table 3. Reactivity of Influenza virus strains

Specimen Tested	Titer	Target	UTM			Liat Dilution Buffer		
			# Tested	# detected Inf A	# detected 2009 HI	# Tested	# detected Inf A	# detected 2009 HI
A/Swine NY 03/2009 H1N1	10 ² TCID ₅₀ /mL	Human 2009 H1N1	3	3	3	3	3	3
A/Denver/1/57	10 ² CEID ₅₀ /mL	Human Seasonal H1	3	3	0	3	3	0
A/FM/1/47	10 ² CEID ₅₀ /mL	Human Seasonal H1	3	3	0	3	3	0
A/Mal/302/54	10 ² CEID ₅₀ /mL	Human Seasonal H1	3	3	0	3	3	0
A/New Caledonia/20/99	10 ² TCID ₅₀ /mL	Human Seasonal H1	3	3	0	3	3	0
A/NWS/33	10 ² CEID ₅₀ /mL	Human Seasonal H1	3	3	0	3	3	0
A/PR/8/34	10 ² TCID ₅₀ /mL	Human Seasonal H1	3	3	0	3	3	0
A/Solomon Island/3/2006	10 ² TCID ₅₀ /mL	Human Seasonal H1	3	3	0	3	3	0
A/Weiss/43	10 ⁴ CEID ₅₀ /mL	Human Seasonal H1	3	3	0	3	3	0
A/Aichi/2/68	10 ² CEID ₅₀ /mL	Human Seasonal H3	3	3	0	3	3	0
A/Alice	10 ² CEID ₅₀ /mL	Human Seasonal H3	3	3	0	3	3	0
A/Brisbane/10/07	10 ¹ TCID ₅₀ /mL	Human Seasonal H3	3	3	0	3	3	0
A/HongKong/8/68	10 ² TCID ₅₀ /mL	Human Seasonal H3	3	3	0	3	3	0
A/MRC2	10 ² CEID ₅₀ /mL	Human Seasonal H3	3	3	0	3	3	0
A/Port Chambers/1/73	10 ² CEID ₅₀ /mL	Human Seasonal H3	3	3	0	3	3	0
A/Victoria/3/75	10 ² CEID ₅₀ /mL	Human Seasonal H3	3	3	0	3	3	0
A/Wisconsin/67/05	10 ¹ TCID ₅₀ /mL	Human Seasonal H3	3	3	0	3	3	0
A/Swine/1976/31	10 ² CEID ₅₀ /mL	Swine H1N1, non 2009	3	3	0	3	3	0
A/Swine/Iowa/15/30	10 ² CEID ₅₀ /mL	Swine H1N1, non 2010	3	3	0	3	3	0
A/New Jersey/8/76	10 ² CEID ₅₀ /mL	Human H1N1, non 2009	3	3	0	3	3	0

Table 4. Cross reactivity of various pathogens			UTM			Liat Dilution Buffer		
Groups	Organisms	Concentration	# Tested	# Inf A Negative	# 2009 HI Negative	# Tested	# Inf A Negative	# 2009 HI Negative
1	Adenovirus Type 1	10 ⁶ TCID ₅₀ /mL	3	3	3	3	3	3
	Adenovirus Type 7	10 ⁴ TCID ₅₀ /mL						
	Human Coronavirus 229E	10 ³ TCID ₅₀ /mL						
	Human Coronavirus OC43	10 ⁴ TCID ₅₀ /mL						
2	Enterovirus	10 ⁴ TCID ₅₀ /mL	3	3	3	3	3	3
	Human Parainfluenza Type I	10 ³ TCID ₅₀ /mL						
	Human Parainfluenza Type 2	10 ⁴ TCID ₅₀ /mL						
	Human Parainfluenza Type 3	10 ⁶ TCID ₅₀ /mL						
3	Measles	10 ⁴ TCID ₅₀ /mL	4	4	4	4	4	4
	Human metapneu-movirus	10 ³ TCID ₅₀ /mL						
	Mumps virus	10 ⁴ TCID ₅₀ /mL						
	Respiratory syntial virus type B	10 ³ TCID ₅₀ /mL						
4	Rhinovirus Type 1A	10 ⁵ TCID ₅₀ /mL	3	3	3	3	3	3
	Cytomegalovirus	10 ⁶ TCID ₅₀ /mL						
	Epstein Barr virus	10 ⁵ TCID ₅₀ /mL						
5	<i>Bordetella pertussis</i>	10 ⁶ cfu/mL	3	3	3	3	3	3
	<i>Chlamydia pneumoniae</i>	10 ⁴ TCID ₅₀ /mL						
	<i>Corynebactrium</i>	10 ⁷ cfu/mL						
	<i>Escherichia coli</i>	10 ⁶ cfu/mL						
6	<i>Haemophilus influenzae</i>	10 ⁶ cfu/mL	3	3	3	3	3	3
	<i>Lactobacillus</i>	10 ⁸ cfu/mL						
	<i>Legionella pneumophila</i>	10 ⁷ cfu/mL						
	<i>Moraxelaa Catarrhalis</i>	10 ⁸ cfu/mL						
7	<i>Neisseria meningitides</i>	10 ⁶ cfu/mL	3	3	3	3	3	3
	<i>Neisseria</i>	10 ⁶ cfu/mL						
	<i>Pseudomonas aeruginosa</i>	10 ⁸ cfu/mL						
	<i>Staph aureus</i>	10 ⁸ cfu/mL						
8	<i>Staph epidermidis</i>	10 ⁷ cfu/mL	3	3	3	3	3	3
	<i>Streptococcus pneumoniae</i>	10 ⁶ cfu/mL						
	<i>Streptococcus pyogens</i>	10 ⁸ cfu/mL						
	<i>streptococcus salivarius</i>	10 ⁶ cfu/mL						
9	B/Florida/7/04	10 ³ TCID ₅₀ /mL	4	4	4	4	4	4
	B/Allen/45	10 ¹⁰ TCID ₅₀ /mL						
	B/GL/1739/54	10 ⁵ TCID ₅₀ /mL						
	B/Taiwan/2/62	10 ³ TCID ₅₀ /mL						
10	B/Maryland/1/59	10 ³ TCID ₅₀ /mL	4	4	4	4	4	4
	B/Mass/3/66	10 ⁶ TCID ₅₀ /mL						
	B/HongKong/5/72	10 ⁵ TCID ₅₀ /mL						
11	B/Lee/40	10 ⁷ TCID ₅₀ /mL	3	3	3	3	3	3
	B/Malaysia/2506/04	10 ³ TCID ₅₀ /mL						
	B/Florida/04/06	10 ³ TCID ₅₀ /mL						

3. Clinical Studies

The Liat Influenza A/2009 H1N1 Assay performance characteristics were established by comparing clinical study results to the Focus Diagnostic Influenza A H1N1 (2009) Real Time RT-PCR EUA test.

The archived clinical samples tested were comprised of a total of 85 clinical samples collected in Universal Transport Medium (UTM) or Viral Transport Medium (VTM). Of the 85 samples, 35 samples were Influenza A and 2009 H1N1 positive confirmed with an emergency use authorized test, 50 samples were Influenza A negative and H1N1 negative confirmed by an emergency use authorized test.

Table 5 summarizes the results of clinical performance of Liat Influenza A/2009 H1N1 Assay with the comparator assay. The data indicate 100% agreement with the comparator method for positive and negative clinical samples. The two-sided 95% confidence interval was calculated and is presented in the table below.

Table 5. Evaluation of the Liat Influenza A/2009 H1N1 Assay with EUA Comparator Assay















		Comparator Assay		
		Positive	Negative	Total
Liat Influenza A/2009 H1N1 Assay	Positive	35	0	35
	Negative	0	50	50
	Total	35	50	85

	Positive	Negative
% agreement	100.0%	100.0%
95% CI Lower	90.1%	92.9%
95% CI Upper	100.0%	100.0%

References

- Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories. Richmond JY and McKinney RW (eds) (1993). HHS Publication number (CDC) 93-8395.
- Interim Biosafety Guidance for All Individuals Handling Clinical Specimens or Isolates Containing 2009-H1N1 Influenza A Virus (Novel H1N1), including Vaccine Strains, August 15, 2009; (http://www.cdc.gov/h1n1flu/guidelines_labworkers.htm).
- Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline. Document M29 (refer to latest edition).

Table of Symbols

Symbol	Used for	Symbol	Used for
	Do not reuse		Use by YYYY-MM-DD or YYYY-MM
	Contains sufficient for < n > tests		Serial number
	<i>In vitro</i> diagnostic medical device		Batch code
	Consult instructions for use		Catalog number
	Control		Temperature limitation
	Positive control		Biological risks
	Negative control		Manufacturer



Manufacturer

IQum, Inc.
 700 Nickerson Road
 Marlborough, MA 01752
 Tel: 508-970-0099
 Fax: 508-970-0119

For assistance, contact IQum using one of the following contact details. Make sure you provide the Liat Analyzer serial number and Liat Tube lot number when you call or email.

For technical support, use the following contact details:
 Tel: 508-970-0099 x 502
 Email: techsupport@iquum.com

IQum Technical Support can be reached by telephone Monday through Friday, from 8 AM to 8 PM Eastern Time.