Package Insert

cobas® Influenza A/B

Nucleic acid test for use on the **cobas**® Liat® System

For In Vitro Diagnostic Use

Rx Only

CLIA Complexity: WAIVED*

*For US only

For use with nasopharyngeal swab specimens.

A Certificate of Waiver is required to perform this test in a CLIA Waived setting. To obtain CLIA waiver information and a Certificate of Waiver, please contact your state health department. Additional CLIA waiver information is available at the Centers for Medicare and Medicaid website at www.cms.hhs.gov/CLIA.

Failure to follow the instructions or modification to the test system instructions will result in the test no longer meeting the requirements for waived classification.

Operator should carefully read this entire package insert before using the **cobas**® Influenza A/B assay. Follow all instructions to ensure correct assay performance.

NOTE: For the remainder of this Package Insert, the **cobas**® Liat® Analyzer may be referred to as the Analyzer and the **cobas**® Liat® System may be referred to as the System.

I. Intended Use

The **cobas**® Influenza A/B Nucleic acid test for use on the **cobas**® Liat® System (**cobas**® Influenza A/B) is an automated multiplex real-time RT-PCR assay for the rapid *in vitro* qualitative detection and discrimination of Influenza A virus and Influenza B virus RNA in nasopharyngeal swab specimens from patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors. The test is intended for use as an aid in the differential diagnosis of Influenza A and Influenza B in humans and is not intended to detect Influenza C.

Negative results do not preclude Influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. Conversely, positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

Performance characteristics for Influenza A were established when Influenza A/H1 and A/H3 were the predominant Influenza A viruses in circulation. When other Influenza A viruses are emerging, performance characteristics may vary.

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 department for testing. Viral culture should not be attempted in these cases unless a BSL3+ facility is available to receive and culture specimens.

II. Summary and Explanation

Influenza is an acute respiratory illness caused by infection with the Influenza virus. Influenza viruses consist 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 U.S., Influenza A/H1N1, A/H3N2 and Influenza B are the predominant seasonal viruses.

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

III. Principle of the Procedure

The **cobas®** Influenza A/B assay is a rapid, automated *in vitro* diagnostic test for qualitative detection and differentiation of Influenza type A and type B viral RNA. The assay is performed on the **cobas®** Liat® System. The System automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in biological samples using real-time RT-PCR assays. The **cobas®** Liat® Analyzer consists of an instrument and preloaded software for running tests and viewing the results. The System consists of the Analyzer and a single-use disposable **cobas®** Influenza A/B assay 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 **cobas®** Influenza A/B assay tube, no reagent preparation or additional steps are required. Because each **cobas®** Influenza A/B assay tube is self-contained, cross-contamination between samples is minimized. Turnaround time for a test is ~20 minutes.

The **cobas®** Influenza A/B assay includes reagents for the detection and differentiation of Influenza A and B viral RNA in nasopharyngeal swab (NPS) specimens in universal transport media (UTM) from patients suspected of having Influenza. The assay targets a well-conserved region of the matrix gene of Influenza A viral RNA (Inf A target) and non-structural protein (NS) gene of Influenza B (Inf B target). An Internal Process Control (IPC) is also included. The IPC is present to control for adequate processing of the target viruses through all steps of the assay process and to monitor the presence of inhibitors in the RT-PCR reactions.

IV. Reagents and Instruments

A. Materials Provided

The **cobas®** Influenza A/B assay tube pack (Cat # 07341890190) contains sufficient reagents to process 20 specimens or quality control samples. The pack contains 20 sets of a **cobas®** Influenza A/B assay tube and a transfer pipette. A Package Insert Barcode Card (Cat # 07997060001) with a lot-specific barcode is also included.

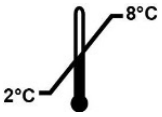
B. Equipment

cobas® Liat® Analyzer, Cat # 07341920190

C. Materials Required but Not Provided

- Acceptable collection kits include Universal Transport Medium (UTM) Swab Collection Kits (BD Cat # 220531* or Copan Cat # 305C), each kit containing a Collection Swab and a tube containing 3 mL of UTM.
  * BD Cat # 220531 is also described as BD universal viral transport (UVT) 3-mL collection kit with flexible minitip flocked swab. This product may not be available in all countries.
- CONTROL **cobas®** Influenza A/B Quality Control Kit, Cat # 07402660190, containing:
  cobas® Influenza A/B Positive Control (Positive Control), Cat # 07758448001
  cobas® Influenza A/B Dilution UTM (Dilution UTM), Cat # 07763794001
  Transfer Pipette, Cat # 07898541001
  Control Kit Barcode Card, Cat # 07945299001
  Negative Control Barcode Label, Cat # 07945248001
  Positive Control Barcode Label, Cat # 07945230001

V. Storage and Handling



- Store the **cobas®** Influenza A/B assay tube and the **cobas®** Influenza A/B Control at 2-8ºC.
- Do not use kits or reagents beyond their expiration dates.
- Do not open individual assay tube packaging until you are ready to perform testing.

VI. Warnings and Precautions

- For *in vitro* diagnostic use only.
- Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.

- Treat all biological specimens, including used **cobas®** Influenza A/B assay tubes and pipettes, 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 and the Clinical and Laboratory Standards Institute.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Do not substitute UTM with other reagents.
- Do not use a damaged **cobas®** Influenza A/B assay tube. Do not use a **cobas®** Influenza A/B assay tube that has been dropped after removal from its foil pouch.
- Do not open individual assay tube packaging until you are ready to perform testing.

- Each single-use **cobas®** Influenza A/B assay tube is used to process one test. Do not reuse a spent **cobas®** Influenza A/B assay tube. If a **cobas®** Influenza A/B assay tube is not housed in a sleeve, or if the assay tube sample compartment already contains liquid, this assay tube has been spent; do NOT use such assay tubes.
- Do not open the cap of the **cobas®** Influenza A/B assay tube during or after the run on the Analyzer.
- Dispose of a used **cobas®** Influenza A/B assay tube, pipette and specimen tubes according to your institution's safety guidelines for hazardous material.
- Due to the high sensitivity of the assays run on the Analyzer, contamination of the work area with previous positive samples may cause false positive results. Handle samples according to standard laboratory practices. Clean instruments and surrounding surfaces according to instructions provided in the cleaning section of the instrument User Manual.
- Sample collection should be performed by specifically trained personnel using the recommended swabs. Inadequate or inappropriate sample collection, storage, and transport may yield false test results.
- Use only the transfer pipettes contained in the **cobas®** Influenza A/B assay tube pack and **cobas®** Influenza A/B Quality Control Kit. Use of alternative transfer pipettes may lead to invalid results.

VII. Specimen Collection, Handling, and Storage

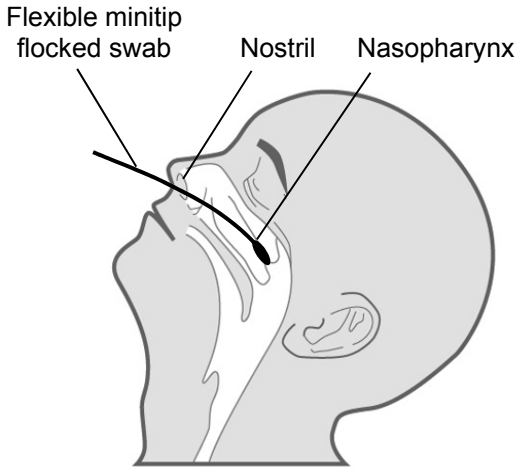
A. Nasopharyngeal Swab Collection

Materials:

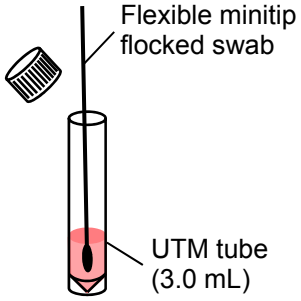
- Sterile flexible minitip flocked swab with a synthetic tip (e.g. Dacron, nylon, or rayon) and an aluminum or plastic shaft. DO NOT use cotton or calcium alginate swabs, or swabs with wood shafts.
- Tube containing 3 mL of Universal Transport Media (UTM).

Procedure:

1. Instruct the patient to blow their nose.
2. Place the patient in a seated position with head against a fixed object (e.g. a wall) to prevent the patient from pulling away during this procedure.
3. Tilt the patient's head back at a 70 degree angle (see Figure).
4. Insert the swab into one nostril straight back (not upwards) and continue along the floor of the nasal passage for several centimeters until reaching the nasopharynx (resistance will be met).
   a. The distance from the nose to the ear gives an estimate of the distance the swab should be inserted.
   b. Do not force the swab, if obstruction is encountered before reaching the nasopharynx, remove the swab and try the other nostril.
5. Rotate the swab gently for 5-10 seconds to loosen the epithelial cells.
6. Remove the swab and immediately insert the swab into the transport media tube. Place the swab head at least ½ inch below the surface of the media, and swirl the swab in the media. Break the swab shaft and leave the swab in the tube. Attach the cap securely.



Nasopharyngeal Swab Area



Place swab into UTM tube

B. Specimen Handling & Storage

- After the nasopharyngeal swab sample from the patient has been collected in UTM, we recommend that specimens should be immediately added to the **cobas®** Influenza A/B assay tube and the **cobas®** Liat® assay run on the Analyzer as soon as possible.
- If specimens cannot be immediately added to the assay tube for testing, nasopharyngeal swab specimens collected in UTM are stable for up to 72 hours in refrigeration (2-8°C).

- Freezing at -70°C or colder (and transportation on dry ice) is required for specimen storage or transportation beyond 72 hours prior to the specimen being added to the assay tube for testing. Ensure all applicable regulations for the transport of biological agents are met.

VIII. Assay Procedure

Note:

- Consult the System User Manual on the detailed operations of the **cobas®** Liat® System.
- To avoid error and sample cross contamination, change gloves between samples and work on one sample at a time. DO NOT add multiple samples into multiple **cobas®** Influenza A/B assay tubes at the same time.

A. Add **cobas®** Influenza A/B assay tube lot

Before using a new lot of **cobas®** Influenza A/B assay tubes, the Add Lot procedure must be performed on the Analyzer to validate the **cobas®** Influenza A/B assay tube lot at your site. The procedure comprises running a negative and a positive control sample.

The Analyzer will prompt you to add the lot if you try to run an assay from a new un-validated lot. You can also compare the lot number on the **cobas®** Influenza A/B assay tube against the list of validated assay tube lots in step 1 below to check if the lot was previously added.

Helpful Hint: 4 barcodes are needed for this procedure. Make sure to scan the right barcode when prompted by the Analyzer.

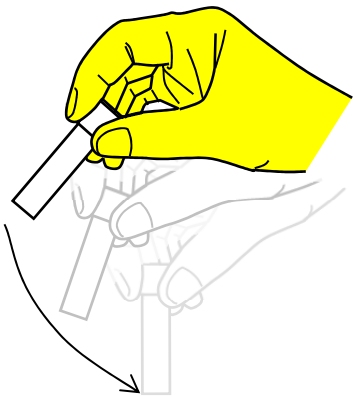
- Package Insert Barcode: On the Package Insert Barcode Card contained in the **cobas®** Influenza A/B assay tube pack. This barcode is lot-specific; match the lot number next to the barcode with the lot number on the **cobas®** Influenza A/B assay tubes.
- **cobas®** Influenza A/B assay tube Barcode: on the **cobas®** Influenza A/B assay tube sleeve.
- Negative Control Barcode: on the Control Kit Barcode Card contained in the QC Kit. Match the lot number next to the barcode with the lot number on the Dilution UTM tube.
- Positive Control Barcode: on the Control Kit Barcode Card contained in the QC Kit. Match the lot number next to the barcode with the lot number on the Positive Control tube.

Materials:

- From **cobas®** Influenza A/B assay tube pack:
  - 〈 Package Insert Barcode Card
  - 〈 2 **cobas®** Influenza A/B assay tubes
- From **cobas®** Influenza A/B Quality Control (QC) Kit:
  - 〈 Negative Control: Negative Control Barcode, (see Control Kit Barcode Card), 1 Dilution UTM tube (used as the negative control sample)
  - 〈 Positive Control: Positive Control Barcode, (see Control Kit Barcode Card), 1 Positive Control tube, 1 Dilution UTM tube (used to mix with the positive control), 1 transfer pipette

Procedure:

1. Prepare and test Negative Control sample
   a. Obtain:
      〈 Package Insert Barcode on the Package Insert Barcode Card contained in the **cobas®** Influenza A/B assay tube pack
      〈 Negative Control Barcode on the Control Kit Barcode Card
      〈 1 Dilution UTM tube (used as the negative control sample)
      〈 1 **cobas®** Influenza A/B assay tube from this lot
   b. Select **Assay Menu** on the main menu of an Analyzer.
   c. Select **New Lot** at the bottom of the list.
   d. Select **Scan** and scan the Package Insert Barcode on the Package Insert Barcode Card from the **cobas®** Influenza A/B assay tube pack.
   e. Select **Scan** and scan the Negative Control Barcode on the Control Kit Barcode Card. The Analyzer will prompt "*Add Negative Control & scan Liat Tube ID*."
   f. Take a Dilution UTM tube from the QC Kit; this is used as the negative control sample. Hold the Dilution UTM tube by the tube cap and shake down the liquid in the tube using a quick, sharp, downward wrist motion. Visually check that the Dilution UTM has pooled at the bottom of the tube. If not, repeat the shake down procedure.
   g. Using the Dilution UTM as sample, run the assay following the Running **cobas®** Influenza A/B assay procedure, steps B.2.b-i (Add Sample) and B.3 (Insert **cobas®** Influenza A/B assay tube).
   h. If *"Negative Control Result Accepted"* is displayed at the end of the run, select **OK**. If the result is rejected, repeat the negative control run (step A.1).
   i. Select **Back**.



Shake down the contents in the tube using a quick, sharp, downward wrist motion.

2. Prepare Positive Control sample
   a. Take the following from the QC kit:
      〈 1 transfer pipette
      〈 1 Positive Control tube, containing a pellet of dried chemically-inactivated Influenza A and Influenza B at the bottom of the tube
      〈 1 Dilution UTM tube, containing a unit dose of UTM to be mixed with the positive control
   b. Hold the Dilution UTM tube by the tube cap and shake down the liquid in the tube using a quick, sharp, downward wrist motion. Visually check that the liquid has pooled at the bottom of the tube. If not, repeat the shake down procedure.

c. Using a transfer pipette, transfer the UTM liquid from the Dilution UTM tube into the Positive Control tube:

i. Check that the Positive Control pellet is at the bottom of the tube prior to addition of the Dilution UTM. Do not use the Positive Control if a pellet is not visible prior to rehydration.

ii. Squeeze the bulb of pipette until the bulb is fully flat. While holding the bulb fully flat, insert the pipette tip into the liquid just below the liquid surface in the Dilution UTM tube.

iii. Slowly release the bulb completely while keeping the pipette tip below the liquid surface. You will see the liquid UTM rising into the pipette. After releasing the bulb completely, withdraw the pipette from the Dilution UTM tube. A small volume of liquid may remain in the tube after the bulb is fully released.

iv. Insert pipette into the Positive Control tube until the pipette tip is at the bottom of the tube.

v. Slowly squeeze the bulb to empty the contents of pipette. Avoid creating bubbles in the sample. Do not release the pipette bulb.

vi. While still squeezing the pipette bulb, withdraw the pipette from the tube. Dispose of the transfer pipette according to your institution's guidelines for safe disposal of hazardous material. Do not reuse transfer pipettes.

vii. Cap the Positive Control tube. Hold the Positive Control tube by the cap and shake down the liquid in the tube using a quick, sharp, downward wrist motion.

d. Let the Positive Control tube sit for 5 minutes. During this time, the dried positive control material within the tube will begin to dissolve.

3. Test Positive Control sample

a. Obtain:

〈 Positive Control Barcode on the Control Kit Barcode Card

〈 1 **cobas®** Influenza A/B assay tube from this lot

b. On the Analyzer used for the Negative Control test, select **Scan** and scan the Positive Control Barcode on the Control Kit Barcode Card. The Analyzer will prompt "*Add Positive Control & scan Liat Tube ID*".

c. After the Positive Control tube from Step A.2 has set for 5 minutes, use the transfer pipette from the **cobas®** Influenza A/B assay tube pouch to slowly pipette the sample up and down 10 times to dissolve and mix the positive control sample. Avoid generating bubbles.

d. Using the Positive Control as a sample, run the assay following the Running **cobas®** Influenza A/B assay procedure, steps B.2.b-h (Add Sample) and B.3 (Insert **cobas®** Influenza A/B assay tube).

e. If "*Positive Control Result Accepted. Lot … added*" is displayed at the end of the run, select **OK** to return to the assay menu. If the result is rejected, repeat the positive control run (steps A.2 and A.3).

After Add Lot is completed on one Analyzer use the Advanced Tools Key to transfer the lot information to the other Analyzers at your site. This allows the other Analyzers to use this **cobas®** Influenza A/B assay tube lot without performing Add Lot on each Analyzer. Follow the

Advanced Tools Key Instructions for Use, and perform a "Sync Lot" on the Analyzer on which the Add Lot was performed. Then, repeat the "Sync Lot" procedure on each of the other Analyzers.

B. Running **cobas®** Influenza A/B assay

The recommended environmental operating conditions for the **cobas®** Influenza A/B assay are 15-32°C, 15-80% relative humidity, and ≤2,000m (6,500 feet) above sea level.

Materials: **cobas®** Influenza A/B assay tube from a lot that has been added to the Analyzer. See section A for Add **cobas®** Influenza A/B assay tube lot instructions.

Procedure:

1. Scan Barcode

   Tear open the foil packaging of the **cobas®** Influenza A/B assay tube and remove the assay tube and the transfer pipette.

   a. Select **Run Assay** on the main menu using the touch screen or function button.

   b. Select **Scan** and scan the **cobas®** Influenza A/B assay tube barcode on the assay tube sleeve by placing the assay tube on the table and sliding the assay tube towards the Analyzer until the red scan light is over the entire barcode.

   c. Select **Scan** again and scan the Patient or Sample barcode, or select **Enter** and type in a Sample or Patient ID. The Analyzer will prompt *"Add UTM sample & re-scan tube ID."*

2. Add Sample

   Use the transfer pipette to load ~200 µL of the sample into the **cobas®** Influenza A/B assay tube. You do not need to measure the sample volume; the Analyzer will adjust the sample volume if more sample was loaded, or output an error if not enough sample was loaded.

   a. Unscrew the sample tube cap. Lift the cap and any attached swab to allow a pipette to be inserted into the sample tube. Avoid lifting the swab completely out of the sample tube.

   b. Obtain the transfer pipette from the **cobas®** Liat® assay tube foil packaging. Firmly squeeze the bulb of the pipette until the bulb is fully flat.

   c. While holding the pipette bulb fully flat, insert the pipette tip into the sample just below the liquid surface.

   d. Slowly release the bulb while keeping the pipette tip below the liquid surface. This will draw up ~200 µL of sample into the pipette. After releasing the bulb completely, withdraw the pipette from the sample.

   e. Unscrew the cap from the **cobas®** Influenza A/B assay tube.

   f. While watching through the viewing window in the sleeve, carefully insert the pipette into the **cobas®** Influenza A/B assay tube. Place the pipette tip near the bottom of the sample compartment.

   g. Slowly squeeze the bulb to empty the contents of the pipette into the **cobas®** Influenza A/B assay tube. Avoid creating bubbles in the sample. Do not release the pipette bulb.

Note: Do not puncture the **cobas®** Influenza A/B assay tube or the seal at the bottom. If you do puncture the seal at the bottom of the sample compartment, discard both the **cobas®** Influenza A/B assay tube and the transfer pipette according to your institution's guidelines for safe disposal of hazardous material and repeat the test starting at Step 2.a. with a new transfer pipette and **cobas®** Influenza A/B assay tube.

h. While still holding the pipette bulb, withdraw the pipette from the assay tube. Screw the cap back on the **cobas®** Influenza A/B assay tube. Dispose of the transfer pipette according to your institution's guidelines for safe disposal of hazardous material. Do not reuse transfer pipettes.

Note: Start the **cobas®** Liat® assay run on the Analyzer as soon as possible, but no later than 4 hours after adding the sample to the **cobas®** Influenza A/B assay tube.

3. Insert **cobas®** Influenza A/B assay tube

a. Select **Scan** and re-scan the **cobas®** Influenza A/B assay tube barcode. The assay tube entry door on top of the Analyzer will open automatically.

b. Remove the **cobas®** Influenza A/B assay tube sleeve.

c. Immediately insert the **cobas®** Influenza A/B assay tube into the Analyzer until the assay tube clicks into place. The **cobas®** Influenza A/B assay tube only fits in one way. If the assay tube is not inserted by the time the door closes, re-scan the **cobas®** Influenza A/B assay tube barcode (step 3a) and insert the **cobas®** Influenza A/B assay tube again. Once the **cobas®** Influenza A/B assay tube is properly inserted, the Analyzer will close the door automatically and begin the test.

4. View Result

During the test, the Analyzer displays the running status and estimated time remaining. Once the test is complete, the Analyzer displays the message, "Please remove the assay tube slowly…" and opens the assay tube entry door automatically.

a. Lift the **cobas®** Influenza A/B assay tube out of the Analyzer.

b. Select **Report** to see the Result Report.

c. Select **Print** to print the report (if applicable).

Select **Back**, and then **Main** to return to the main menu for the next test.

C. Viewing and Interpreting Results

The Analyzer reports results as "Detected", "Not Detected", or "Indeterminate" for each of Influenza A and Influenza B, or "Assay Invalid".

The manual data interpretation and reviewing PCR curves that you may be familiar with from other systems is no longer required. The Analyzer automatically interprets the results from measured fluorescent signals. Embedded calculation algorithms determine the PCR cycle threshold (Ct) and evaluate the Ct and fluorescence endpoint against the valid range to generate a positive or negative PCR result.

Additionally, pattern recognition algorithms inspect the PCR curves to determine if the curve pattern is within specifications or abnormal. For example, if Influenza A is detected but its PCR curve is determined to be abnormal, the result is called "Influenza A Indeterminate. Repeat Assay."

Like the viral targets, the IPC target is also evaluated in every assay run. In the case that Influenza A and Influenza B targets are not detected, the IPC target must be detected for the result to be called "Not Detected"; if the IPC is also not detected or if the IPC PCR curve is abnormal, the result is called "Assay Invalid. Repeat Assay." In some cases, high concentration of target virus may inhibit the amplification of IPC; as such, IPC is not taken into consideration when a target virus is detected.

The table below shows the "Report Results" and the corresponding interpretation.

Interpretation of Results

Result Report		Interpretation
Influenza A	Influenza A Not Detected	Negative test for Influenza A (no Influenza A RNA detected)
	Influenza A Detected	Positive test for Influenza A (Influenza A RNA present)
	Influenza A Indeterminate. Repeat Assay.	Presence or absence of Influenza A cannot be determined. Repeat assay with same sample or, if possible, new sample.
Influenza B	Influenza B Not Detected	Negative test for Influenza B (no Influenza B RNA detected)
	Influenza B Detected	Positive test for Influenza B (Influenza B RNA present)
	Influenza B Indeterminate. Repeat Assay.	Presence or absence of Influenza B cannot be determined. Repeat assay with same sample or, if possible, new sample.
Assay Invalid. Repeat Assay.		Presence or absence of Influenza A and Influenza B cannot be determined. Repeat assay with same sample or, if possible, new sample.
[Error]. Assay Aborted		Presence or absence of target viruses cannot be determined. Repeat assay with same sample or, if possible, new sample.

The following failure codes can be displayed in the result report upon completion of a run.

Failure Codes	Sample and External Controls	Negative Control (Add Lot)	Positive Control (Add Lot)
r0*	IPC Not Detected or Indeterminate. Repeat Run	IPC Not Detected or Indeterminate. Repeat Run	IPC Not Detected or Indeterminate. Repeat Run
r1			
r2			
r3			
r4			
r5			

Failure Codes	Sample and External Controls	Negative Control (Add Lot)	Positive Control (Add Lot)
x4	At least one or more Target Indeterminate. Repeat Run	N/A	N/A
FP	N/A	At least one Target Detected or Indeterminate. Repeat Run	N/A
b1	N/A	N/A	At least one Target Not Detected or Indeterminate. Repeat Run
b2			
b3			
b4			
a1	N/A	N/A	At least one Target Not Detected or Indeterminate. Repeat Run
a2			
a3			
a4			

Note*: Failure code r0 goes not appear for Positive Control (Add Lot).

In addition, Pattern Codes may appear. For any questions, please contact your Roche Service representative.

D. Reasons to Repeat the Assay

If the test result is “Indeterminate” for a target virus or “Invalid”, repeat the assay with the same patient specimen, or if possible, collect a new specimen from the patient and repeat the assay using the new specimen. Specimens that have repeat “Indeterminate” or “Invalid” results should be sent to a laboratory for confirmatory testing.

If an “Error” is reported by the Analyzer and/or the assay is aborted, repeat the test with the same patient specimen, or if possible, collect a new specimen from the patient and repeat the assay using the new specimen. Contact your Roche Service Representative if repeat “Errors” are reported.

E. Quality Control **CONTROL**

Internal Process Control (IPC): is an encapsulated RNA that is included in each **cobas®** Influenza A/B assay tube to verify adequate processing of target viruses. The IPC verifies that sample purification of the target viruses 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 is valid if it meets the acceptance criteria.

External Controls: provide additional quality control materials to demonstrate positive or negative assay results using the System and **cobas®** Influenza A/B assay tube. External Controls are run during the Add **cobas®** Influenza A/B assay tube lot procedure (section A). Additional External Controls should be tested in accordance with local, state, federal and/or accrediting organization requirements as applicable. If the controls do not perform as expected, do not test patient specimens; contact your Roche Service Representative.

Negative Control

Materials:

- 1 **cobas®** Influenza A/B assay tube
- From QC Kit: 1 Dilution UTM tube (used as the negative control sample), and Negative Control Barcode on the Control Kit Barcode Card.

Procedure:

The Dilution UTM is used as the sample for the Negative Control run.

1. Take a Dilution UTM tube from the QC Kit.
2. Hold the Dilution UTM tube by the tube cap and shake down the liquid in the tube using a quick, sharp, downward wrist motion (as if shaking down a mercury thermometer). Visually check that the Dilution UTM has pooled at the bottom of the tube. If not, repeat the shake down procedure.
3. Using the Dilution UTM as sample, run the assay following the Running **cobas®** Influenza A/B assay procedure steps B.2.b-h (Add Sample) and B.3 (Insert **cobas®** Influenza A/B assay tube). Scan the Negative Control Barcode on the Control Kit Barcode Card as the Sample ID.
4. View the Results Report by touching or clicking **Report** after the completion of the assay. The Report Result must be “Influenza A Not Detected” and “Influenza B Not Detected” for the negative control to pass.

Positive Control

Materials:

- 1 **cobas®** Influenza A/B assay tube
- From QC Kit: 1 Positive Control tube, 1 Dilution UTM tube (used to mix with the positive control), 1 transfer pipette, and Positive Control Barcode on the Control Kit Barcode Card.

Procedure:

The Positive Control is a unit-dose of dried chemically-inactivated Influenza A and Influenza B. Follow the directions below to dissolve the positive control in Dilution UTM and run it on the **cobas®** Influenza A/B assay.

1. Follow step A.2 of the Add **cobas®** Influenza A/B assay tube lot procedure to prepare the Positive Control sample.
2. After the Positive Control tube from step A.2 has sat for 5 minutes, use a transfer pipette from the **cobas®** Influenza A/B assay tube package to slowly pipette the sample up and down 10 times to dissolve and mix the positive control sample.
3. Using the Positive Control as sample, run the assay following the Running **cobas®** Influenza A/B assay procedure steps B.2.b-h (Add Sample) and B.3 (Insert **cobas®** Influenza A/B assay tube). Scan the Positive Control Barcode on the Control Kit Barcode Card as the Sample ID.
4. View the Results Report by touching or clicking **Report** after the completion of the assay. The Report Result must be “Influenza A Detected” and “Influenza B Detected” for the positive control to pass.

IX. Limitations

- The performance of the **cobas®** Influenza A/B assay was evaluated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- As with other tests, 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 **cobas®** Influenza A/B 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.
- This test has been evaluated for use with human specimen material only.
- False negative results may occur if a specimen is improperly collected, transported or handled. False negative results may occur if inadequate numbers of organisms are present in the specimen.
- If the virus mutates in the target regions, Influenza viruses A or B may not be detected or may be detected less predictably.
- This test has not been evaluated for patients without signs and symptoms of Influenza infection.
- This test is a qualitative test and does not provide the quantitative value of detected organism present.
- Cross-reactivity with respiratory tract organisms other than those tested can lead to erroneous results.
- This assay has not been evaluated for patients receiving intranasal administered Influenza vaccine.
- This assay has not been evaluated for immunocompromised individuals.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

X. Performance Characteristics

A. Clinical Performance

Three sites, including two CLIA waived sites, participated in the clinical study for the **cobas®** Influenza A/B assay. A total of 615 clinical specimens were tested including 435 prospectively collected samples and 180 retrospective samples. Prospective samples were collected from patients with signs and symptoms of Influenza in the Eastern and Southwestern US from 12 February 2009 to 26 March 2009. Viral culture and IFA staining was used as the reference method for these prospectively collected samples. Discordant results were investigated using PCR and bi-directional sequencing.

Due to the low prevalence of Influenza during the collection period as well as the subsequent emergence of the 2009 H1N1 virus, additional retrospective samples collected between 2008 and 2010, including 2009 H1N1 samples from the 2009-2010 flu season, were also tested. Reference testing for these samples was performed by PCR and bi-directional sequencing based on published methods.

Clinical sample testing was performed at 3 sites in April 2011 using 30 Analyzers in total. The tables below summarize the clinical performance of the **cobas®** Influenza A/B assay.

cobas® Influenza A/B assay Clinical Performance – Prospective Samples

Influenza A		Viral Culture		
		Positive	Negative	Total
cobas® Liat®	Positive	34	13[a]	47
	Negative	0	388	388
	Total	34	401	435

	%	95% CI
Sensitivity	100.0%	(89.8% - 100.0%)
Specificity	96.8%	(94.5% - 98.1%)

[a] Of 13 false positive samples, 8 were Influenza A positive by PCR/sequencing, 4 were negative by PCR/sequencing, and 1 was indeterminate by PCR/sequencing due to poor sequence quality score.

Influenza B		Viral Culture		
		Positive	Negative	Total
cobas® Liat®	Positive	30	24[b]	54
	Negative	0	381	381
	Total	30	405	435

	%	95% CI
Sensitivity	100.0%	(88.6% - 100.0%)
Specificity	94.1%	(91.3% - 96.0%)

[b] Of 24 false positive samples, 13 were Influenza B positive by PCR/sequencing, 3 were negative by PCR/sequencing, and 8 samples were indeterminate by PCR/sequencing due to poor sequence quality score.

cobas® Influenza A/B assay Clinical Performance – Retrospective Samples

Influenza A		PCR/Sequencing		
		Positive	Negative	Total
cobas® Liat®	Positive	74[c]	3	77
	Negative	0	102	102
	Total	74	105	179[d]

	%	95% CI
Positive Agreement	100.0%	(95.1% - 100.0%)
Negative Agreement	97.1%	(91.9% - 99.0%)

[c] Of 74 Influenza A positive retrospective samples, 44 were 2009 H1N1 positive, and 20 were A/H3 positive by PCR/sequencing. The remaining 10 samples were verified to be Flu A positive, no further subtyping was done.

[d] Of 180 samples tested, 179 were valid and one was invalid.

Influenza B		PCR/Sequencing		
		Positive	Negative	Total
cobas® Liat®	Positive	7	1	8
	Negative	0	171	171
	Total	7	172	179[e]

	%	95% CI
Positive Agreement	100.0%	(64.6% - 100.0%)
Negative Agreement	99.4%	(96.8% - 99.9%)

[e] Of 180 samples tested, 179 were valid and one was invalid.

B. Reproducibility

Reproducibility study assesses the total variability of the **cobas®** Influenza A/B assay across operators, study sites, testing days, Analyzers, and **cobas®** Influenza A/B assay tube lots. The **cobas®** Influenza A/B assay was evaluated at 3 sites, including 2 CLIA waived sites. Two operators at each of the 3 sites tested an 8 member reproducibility panel in triplicate on 5 different days, for a total of 720 runs (8 panel members × 3 replicates × 2 operators × 5 days × 3 sites). Fifteen (15) Analyzers and 3 **cobas®** Influenza A/B assay tube lots were used. The reproducibility panel comprises a high negative, a low positive and a moderate positive of each of Influenza A and B, and the assay positive and negative controls. Percent agreement with expected result, mean Ct, and Ct %CV for each site are shown in tables below. %CV for Influenza A ranged between 1.3% and 3.8% and that for Influenza B ranged between 1.1% and 3.4%. Total percent agreement was ≥99.9%.

Influenza A Reproducibility

	Site 1			Site 2			Site 3			Total	
Sample	Agreement w/ expected result	Avg Ct	Total %CV	Agreement w/ expected result	Avg Ct	Total %CV	Agreement w/ expected result	Avg Ct	Total %CV	Agreement w/ expected result	95% CI
Positive Control	30/30	32.0	1.4%	30/30	32.0	2.2%	30/30	31.5	1.3%	90/90 (100%)	95.9%-100%
Negative Control	30/30	39.0	0.0%	30/30	39.0	0.0%	30/30	39.0	0.0%	90/90 (100%)	95.9%-100%
Flu A High Negative*	30/30	39.0	0.0%	30/30	39.0	0.0%	30/30	39.0	0.0%	90/90 (100%)	95.9%-100%
Flu A Low Positive*	30/30	32.1	3.8%	30/30	32.0	2.1%	30/30	31.8	1.8%	90/90 (100%)	95.9%-100%
Flu A Mod. Positive*	30/30	29.9	2.0%	30/30	29.6	2.0%	30/30	29.3	1.9%	90/90 (100%)	95.9%-100%
Flu B High Negative*	30/30	39.0	0.0%	30/30	39.0	0.0%	30/30	39.0	0.0%	90/90 (100%)	95.9%-100%
Flu B Low Positive*	30/30	39.0	0.0%	30/30	39.0	0.0%	30/30	39.0	0.0%	90/90 (100%)	95.9%-100%
Flu B Mod. Positive*	30/30	39.0	0.0%	30/30	39.0	0.0%	30/30	39.0	0.0%	90/90 (100%)	95.9%-100%
Total Agreement	240/240 (100%)			240/240 (100%)			240/240 (100%)			720/720 (100%)	99.5%-100%

*Guidance for Industry and FDA Staff Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses. Document issued on: July 15, 2011

Influenza B Reproducibility

	Site 1			Site 2			Site 3			Total	
Sample	Agreement w/ expected result	Avg Ct	Total %CV	Agreement w/ expected result	Avg Ct	Total %CV	Agreement w/ expected result	Avg Ct	Total %CV	Agreement w/ expected result	95% CI
Positive Control	30/30	31.2	1.5%	30/30	31.0	2.2%	30/30	30.6	1.1%	90/90 (100%)	95.9%-100.0%
Negative Control	30/30	39.0	0.0%	30/30	39.0	0.0%	30/30	39.0	0.0%	90/90 (100%)	95.9%-100.0%
Flu A High Negative*	30/30	39.0	0.0%	30/30	39.0	0.0%	30/30	39.0	0.0%	90/90 (100%)	95.9%-100.0%
Flu A Low Positive*	30/30	39.0	0.0%	30/30	39.0	0.0%	30/30	39.0	0.0%	90/90 (100%)	95.9%-100.0%
Flu A Mod. Positive*	30/30	39.0	0.0%	30/30	39.0	0.0%	30/30	39.0	0.0%	90/90 (100%)	95.9%-100.0%
Flu B High Negative*	30/30	39.0	0.0%	29/30	38.9	1.7%	30/30	39.0	0.0%	89/90 (98.9%)	94.0%-99.8%
Flu B Low Positive*	30/30	31.3	2.3%	30/30	31.0	3.4%	30/30	30.9	1.7%	90/90 (100%)	95.9%-100.0%
Flu B Mod. Positive*	30/30	29.4	2.6%	30/30	29.5	3.0%	30/30	28.6	3.3%	90/90 (100%)	95.9%-100.0%
Total Agreement	240/240 (100%)			239/240 (99.6%)			240/240 (100%)			719/720 (99.9%)	99.2%-100.0%

*Guidance for Industry and FDA Staff Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses. Document issued on: July 15, 2011

C. Limit of Detection

The Limit of Detection (LOD) of the **cobas®** Influenza A/B assay was tested using 3 strains of Influenza A and 2 strains of Influenza B. The LOD was determined by limiting dilution studies using these titered viruses. The viruses were spiked into nasopharyngeal swab (NPS) sample matrix, and then tested using the **cobas®** Influenza A/B assay. The LOD was determined as the lowest log virus concentration that was detected ≥95% of the time (i.e. log concentration at which at least 19 out of 20 replicates tested positive). The LOD for 3 strains of Influenza A were 10^{-2}-10^{-1} $TCID_{50}$/mL, while those for the 2 strains of Influenza B were 10^{-3}-10^{-1} $TCID_{50}$/mL.

Virus Strain	LOD ($TCID_{50}$/mL)
A/Brisbane/10/2007	10^{-1}
A/Brisbane/59/2007	10^{-2}
A/NY/01/2009	10^{-1}
B/Florida/04/06	10^{-1}
B/Malaysia/2506/04	10^{-3}

D. Analytical Reactivity

Reactivity study evaluates the ability to detect Influenza strains representing temporal and geographical diversity. The **cobas®** Influenza A/B assay was evaluated with 22 Influenza A strains and 10 Influenza B strains. Influenza A strains included 8 seasonal Influenza A/H1 strains, 8 seasonal Influenza A/H3 strains, 3 Influenza A 2009 H1N1 strains and 3 swine origin Influenza A strains. Influenza B strains included that from both the Victoria lineage and Yamagata lineage. The **cobas®** Influenza A/B assay detected all strains tested.

Influenza Strain	Type / Subtype	Test Concentration	Inf A Result	Inf B Result
A/Brisbane/59/2007	Influenza A, seasonal H1	8.0×10^{-3} $TCID_{50}$/mL	+	–
A/New Caledonia/20/99	Influenza A, seasonal H1	1.0×10^{2} $TCID_{50}$/mL	+	–
A/Solomon Island/3/2006	Influenza A, seasonal H1	5.0×10^{-2} $TCID_{50}$/mL	+	–
A/Mal/302/54	Influenza A, seasonal H1	1.0×10^{3} $TCID_{50}$/mL	+	–
A/Denver/1/57	Influenza A, seasonal H1	5.0×10^{2} $TCID_{50}$/mL	+	–
A/FM/1/47	Influenza A, seasonal H1	1.0×10^{2} $TCID_{50}$/mL	+	–
A/PR/8/34	Influenza A, seasonal H1	2.5×10^{1} $TCID_{50}$/mL	+	–
A/Weiss/43	Influenza A, seasonal H1	2.5×10^{3} $TCID_{50}$/mL	+	–
A/Brisbane/10/2007	Influenza A, seasonal H3	1.0×10^{-1} $TCID_{50}$/mL	+	–
A/Alice	Influenza A, seasonal H3	5.0×10^{1} $TCID_{50}$/mL	+	–
A/MRC2	Influenza A, seasonal H3	1.0×10^{2} $TCID_{50}$/mL	+	–
A/Hong Kong/8/68	Influenza A, seasonal H3	2.0×10^{1} $TCID_{50}$/mL	+	–
A/Victoria/3/75	Influenza A, seasonal H3	2.5×10^{1} $TCID_{50}$/mL	+	–
A/Wisconsin/67/05	Influenza A, seasonal H3	5.0×10^{-1} $TCID_{50}$/mL	+	–
A/Port Chalmers/1/73	Influenza A, seasonal H3	5.0×10^{2} $TCID_{50}$/mL	+	–
A/Aichi/2/68	Influenza A, seasonal H3	2.0×10^{2} $CEID_{50}$/mL	+	–
A/NY/01/2009	Influenza A, 2009 H1N1	1.0×10^{-1} $TCID_{50}$/mL	+	–
A/NY/02/2009	Influenza A, 2009 H1N1	2.5×10^{-2} $TCID_{50}$/mL	+	–
A/NY/03/2009	Influenza A, 2009 H1N1	4.0×10^{-1} $TCID_{50}$/mL	+	–
A/New Jersey/8/76	Influenza A, H1N1 non 2009	1.0×10^{1} $TCID_{50}$/mL	+	–
A/Swine/1976/31	Influenza A, H1N1 non 2009	2.0×10^{1} $TCID_{50}$/mL	+	–
A/Swine/Iowa/15/30	Influenza A, H1N1 non 2009	2.0×10^{2} $TCID_{50}$/mL	+	–
B/Florida/04/06	Influenza B (Yamagata lineage)	8.0×10^{-2} $TCID_{50}$/mL	–	+
B/Malaysia/2506/04	Influenza B (Victoria lineage)	2.0×10^{-3} $TCID_{50}$/mL	–	+
B/Florida/7/04	Influenza B (Yamagata lineage)	5.0×10^{-2} $TCID_{50}$/mL	–	+
B/Allen/45	Influenza B	5.0×10^{-1} $CEID_{50}$/mL	–	+
B/GL/1739/54	Influenza B	2.0×10^{1} $TCID_{50}$/mL	–	+
B/Taiwan/2/62	Influenza B	5.0×10^{-2} $TCID_{50}$/mL	–	+
B/Maryland/1/59	Influenza B	5.0×10^{-3} $TCID_{50}$/mL	–	+
B/Mass/3/66	Influenza B	1.0×10^{1} $TCID_{50}$/mL	–	+
B/HongKong/5/72	Influenza B	2.5×10^{-1} $TCID_{50}$/mL	–	+
B/Lee/40	Influenza B	1.0×10^{0} $TCID_{50}$/mL	–	+

E. Cross Reactivity

Cross reactivity study evaluates potential cross-reactivity with non-influenza respiratory pathogens and other microorganisms with which the majority of the population may have been infected. The **cobas®** Liat® assay was evaluated against a panel of 31 human pathogens. Bacteria were tested at 10^5–10^6 CFU/mL. Viruses were tested at 10^3–10^5 $TCID_{50}$/mL and genomic DNA at 10^5 copies/mL. The **cobas®** Influenza A/B assay showed no cross reactivity for the tested organisms.

Viral/Bacterial pathogen	Test concentration	Inf A Result	Inf B Result
Adenovirus Type 1	8.9×10^5 $TCID_{50}$/mL	–	–
Adenovirus Type 7	4.5×10^4 $TCID_{50}$/mL	–	–
Human Coronavirus 229E	1.4×10^3 $TCID_{50}$/mL	–	–
Human Coronavirus OC43	7.9×10^4 $TCID_{50}$/mL	–	–
Enterovirus	1×10^5 $TCID_{50}$/mL	–	–
Human Parainfluenza Type 1	2.8×10^3 $TCID_{50}$/mL	–	–
Human Parainfluenza Type 2	1.4×10^5 $TCID_{50}$/mL	–	–
Human Parainfluenza Type 3	1.6×10^5 $TCID_{50}$/mL	–	–
Measles	7.9×10^4 $TCID_{50}$/mL	–	–
Human Metapneumovirus	7×10^3 $TCID_{50}$/mL	–	–
Mumps virus	7.9×10^4 $TCID_{50}$/mL	–	–
Respiratory syncytial virus type B	1.4×10^4 $TCID_{50}$/mL	–	–
Rhinovirus Type 1A	1.6×10^5 $TCID_{50}$/mL	–	–
Cytomegalovirus	4.5×10^4 $TCID_{50}$/mL	–	–
Epstein Barr virus	1.9×10^5 copies/mL	–	–
Bordetella pertussis	1.8×10^5 CFU/mL	–	–
Chlamydia pneumoniae	8×10^4 $TCID_{50}$/mL	–	–
Corynebacterium sp.	5.0×10^6 CFU/mL	–	–
Escherichia coli	6.6×10^6 CFU/mL	–	–
Haemophilus influenzae	3×10^6 CFU/mL	–	–
Lactobacillus sp.	1.6×10^6 CFU/mL	–	–
Legionella pneumophila	7×10^6 CFU/mL	–	–
Moraxella catarrhalis	5.8×10^6 CFU/mL	–	–
Neisseria meningitidis	3.2×10^6 CFU/mL	–	–
Neisseria sp.	1.8×10^6 CFU/mL	–	–
Pseudomonas aeruginosa	1.6×10^6 CFU/mL	–	–
Staphylococcus aureus	4.5×10^6 CFU/mL	–	–
Staphylococcus epidermidis	6×10^6 CFU/mL	–	–
Streptococcus pneumoniae	1.9×10^6 CFU/mL	–	–
Streptococcus pyogenes	3.7×10^6 CFU/mL	–	–
Streptococcus salivarius	4.3×10^6 CFU/mL	–	–

F. Inhibition by other Microorganisms

Interfering microorganism study evaluates whether non-influenza respiratory pathogens and other microorganisms with which the majority of the population may have been infected can interfere in the detection of Influenza A or B by the **cobas®** Influenza A/B assay. The panel of 31 human pathogens tested in the cross-reactivity study was tested for potential interference. Bacteria were tested at 10^5–10^6 CFU/mL, viruses were tested at 10^3–10^5 $TCID_{50}$/mL and genomic DNA at 10^5 copies/mL in the presence of either A/Brisbane/59/2007 or B/Malaysia/2506/04 at 3x LOD concentration in negative NPS matrix. Results show that the presence of the tested microorganisms did not interfere with the detection of Inf A or Inf B.

Pathogen	Pathogen Concentration	A/Brisbane/59/07		B/Malaysia/2506/04	
		Inf A Result	Inf B Result	Inf A Result	Inf B Result
Adenovirus Type 1	8.9×10^5 $TCID_{50}$/mL	+	–	–	+
Adenovirus Type 7	4.5×10^4 $TCID_{50}$/mL	+	–	–	+
Human Coronavirus 229E	1.4×10^3 $TCID_{50}$/mL	+	–	–	+
Human Coronavirus OC43	7.9×10^4 $TCID_{50}$/mL	+	–	–	+
Enterovirus	1×10^5 $TCID_{50}$/mL	+	–	–	+
Human Parainfluenza Type 1	2.8×10^3 $TCID_{50}$/mL	+	–	–	+
Human Parainfluenza Type 2	1.4×10^5 $TCID_{50}$/mL	+	–	–	+
Human Parainfluenza Type 3	1.6×10^5 $TCID_{50}$/mL	+	–	–	+
Measles	7.9×10^4 $TCID_{50}$/mL	+	–	–	+
Human Metapneumovirus	7×10^3 $TCID_{50}$/mL	+	–	–	+
Mumps virus	7.9×10^4 $TCID_{50}$/mL	+	–	–	+
Respiratory syncytial virus type B	1.4×10^4 $TCID_{50}$/mL	+	–	–	+
Rhinovirus Type 1A	1.6×10^5 $TCID_{50}$/mL	+	–	–	+
Cytomegalovirus	4.5×10^4 $TCID_{50}$/mL	+	–	–	+
Epstein Barr virus	1.9×10^5 copies/mL	+	–	–	+
Bordetella pertussis	1.8×10^5 CFU/mL	+	–	–	+
Chlamydia pneumoniae	8×10^4 $TCID_{50}$/mL	+	–	–	+
Corynebacterium sp.	5.0×10^6 CFU/mL	+	–	–	+
Escherichia coli	6.6×10^6 CFU/mL	+	–	–	+
Haemophilus influenzae	3×10^6 CFU/mL	+	–	–	+
Lactobacillus sp.	1.6×10^6 CFU/mL	+	–	–	+
Legionella pneumophila	7×10^6 CFU/mL	+	–	–	+
Moraxella catarrhalis	5.8×10^6 CFU/mL	+	–	–	+
Neisseria meningitidis	3.2×10^6 CFU/mL	+	–	–	+
Neisseria sp.	1.8×10^6 CFU/mL	+	–	–	+
Pseudomonas aeruginosa	1.6×10^6 CFU/mL	+	–	–	+
Staphylococcus aureus	4.5×10^6 CFU/mL	+	–	–	+
Staphylococcus epidermidis	6×10^6 CFU/mL	+	–	–	+
Streptococcus pneumoniae	1.9×10^6 CFU/mL	+	–	–	+
Streptococcus pyogenes	3.7×10^6 CFU/mL	+	–	–	+
Streptococcus salivarius	4.3×10^6 CFU/mL	+	–	–	+

G. Interfering Substances

The **cobas®** Influenza A/B assay was evaluated with potentially interfering substances that may be encountered in respiratory specimens. Medically and/or physiologically relevant concentrations of potential interferents were tested with 2 Influenza A strains and 2 Influenza B strains at 3x LOD (10^{-1}–10^{-2} $TCID_{50}$/mL). Results showed that substances tested did not interfere in the detection of Influenza A and B strains.

Potential Interferent	Active Ingredient	Concentration
Mucin: bovine submaxillary gland, type I-S	Purified mucin protein	0.1 mg/mL and 25 mg/mL
Blood	-	5% (v/v)
Nasal spray – Afrin	Oxymetazoline	5% (v/v)
Nasal corticosteroids – Veramyst	Fluticasone	5% (v/v)
Nasal gel – Zicam	Galphimia glauca, Histaminum hydrochloricum, Luffa operculata, Sulphur	5% (v/v)
Throat lozenges, oral anesthetic and analgesic – Cepacol	Benzocaine, Menthol	5 mg/mL
Antibiotic, nasal ointment – Bactroban	Mupirocin	5 mg/mL
Antiviral drug – Relenza	Zanamivir	5 mg/mL
Antiviral drug – Tamiflu	Oseltamivir	7.5 mg/mL
Antimicrobial, systemic	Tobramycin	4 µg/mL

H. Performance using Fresh vs. Frozen Samples

The **cobas®** Influenza A/B assay was tested by comparing its performance using fresh and frozen specimens. One Influenza A strain and one Influenza B strain were individually spiked into NPS matrix at different viral loads, including levels near LOD and levels reflecting the clinical range. For each strain, 60 samples were tested immediately while another 60 samples were frozen at -80°C for 7 days, thawed and then tested. Fresh and frozen samples demonstrated 100% detection across all levels of viral load, demonstrating that the **cobas®** Influenza A/B assay had equivalent performance for fresh and frozen samples.

I. CLIA Waiver Study

The accuracy of the **cobas®** Influenza A/B assay was evaluated at 12 CLIA waived intended use sites. A total of 33 untrained operators representative of CLIA waived site intended operators participated in the study. Prospective specimens were collected from patients with signs and symptoms of Influenza in the US during the 2008-2009, 2013-2014 and 2014-2015 flu seasons. The initial test invalid rate was 1.6%.

The **cobas®** Liat® assay results were compared against that from an FDA-cleared lab-based real-time reverse transcriptase PCR (RT-PCR) test in 842 prospective specimens and 300 retrospective specimens. The study demonstrated assay positive agreement of 97.7% and 98.6% for Influenza A and Influenza B, respectively; and negative agreement of 99.2% and 99.4% for Influenza A and Influenza B, respectively.

Clinical Performance of cobas® Liat® assay vs. FDA cleared lab-based RT-PCR Assay

Influenza A		Lab-based RT-PCR		
		Positive	Negative	Total
cobas® Liat®	Positive	173	8[a]	181
	Negative	4[b]	956	960
	Total	177	964	1142[c]

	%	95% CI
Positive Agreement	97.7%	(94.3% - 99.1%)
Negative Agreement	99.2%	(98.4% - 99.6%)

[a] Of 8 **cobas®** Liat® positive, lab-based RT-PCR negative specimens, 3 were positive and 5 were negative by PCR/sequencing. 1 was positive and 7 were negative by Culture.

[b] Of 4 **cobas®** Liat® negative, lab-based RT-PCR positive specimens, 3 were positive and 1 was negative by PCR/sequencing. 1 was positive, 1 was negative and 2 were not tested by Culture.

[c] 1 specimen was indeterminate for Inf A by the lab-based RT-PCR due to late Ct. This sample was positive for Influenza A by **cobas®** Liat® and PCR/sequencing.

Influenza B		Lab-based RT-PCR		
		Positive	Negative	Total
cobas® Liat®	Positive	142	6[a]	148
	Negative	2[b]	992	994
	Total	144	998	1142

	%	95% CI
Positive Agreement	98.6%	(95.1% - 99.6%)
Negative Agreement	99.4%	(98.7% - 99.7%)

[a] Of 6 **cobas®** Liat® positive, lab-based RT-PCR negative specimens, 4 were positive and 2 were negative by PCR/sequencing. All 6 were negative by Culture.

[b] Of 2 **cobas®** Liat® negative, lab-based RT-PCR positive specimens, 1 was positive and 1 was negative by PCR/sequencing. 1 was negative and 1 was not tested by Culture.

The **cobas®** Liat® assay results were also compared against those from viral culture in 784 prospective specimens. The study demonstrated assay sensitivity was 97.5% and 96.9% for Influenza A and Influenza B, respectively; specificity was 97.9% for both Influenza A and Influenza B.

Clinical Performance of cobas® Liat® assay vs. Culture

Influenza A		Culture		
		Positive	Negative	Total
cobas® Liat®	Positive	77	15[a]	92
	Negative	2[b]	690	692
	Total	79	705	784

	%	95% CI
Sensitivity	97.5%	(91.2% - 99.3%)
Specificity	97.9%	(96.5% - 98.7%)

[a] Of 15 **cobas®** Liat® positive, Culture negative specimens, 9 were positive and 6 were negative by PCR/sequencing. 7 were positive, 1 was indeterminate due to high Ct, and 7 were negative by lab-based RT-PCR.

[b] 2 **cobas®** Liat® negative, Culture positive specimens were positive by PCR/sequencing. 1 was positive, and 1 was negative by lab-based RT-PCR.

Influenza B		Culture		
		Positive	Negative	Total
cobas® Liat®	Positive	31	16[a]	47
	Negative	1[b]	736	737
	Total	32	752	784

	%	95% CI
Sensitivity	96.9%	(84.3% - 99.4%)
Specificity	97.9%	(96.6% - 98.7%)

[a] Of 16 **cobas®** Liat® positive, Culture negative specimens, 14 were positive and 2 were negative by PCR/sequencing. 10 were positive, and 6 were negative by lab-based RT-PCR.

[b] 1 **cobas®** Liat® negative, Culture positive specimens was positive by PCR/sequencing and was negative by lab-based RT-PCR.

A Device Performance with Analyte Concentrations Near Cutoff study was performed to assess the capability of CLIA waived site intended operators to test true negative, weak negative and weak positive samples and obtain accurate results. Influenza A and Influenza B weak positive and weak negative concentrations were determined by dilution studies performed by professional operators using the FDA-cleared lab-based RT-PCR test. The weak negative sample is defined as the target concentration at which the professional operators of the lab-based RT-PCR test obtained negative results 95-99% of the time. The weak positive sample is defined as the target concentration at which the professional operators of the lab-based RT-PCR test obtained positive results 95-99% of the time.

At least sixty (60) weak positive and 60 weak negative samples for each of Influenza A and Influenza B, along with 60 true negative samples, were distributed equally among 3 CLIA waived sites and tested by 6 intended operators using the **cobas®** Liat® assay.

The **cobas®** Liat® assay yielded "Influenza A Detected" and "Influenza B Detected" result for 100% of Influenza A weak positive, and Influenza B weak positive samples, respectively. The **cobas®** Liat® assay further yielded "Influenza A Not Detected, Influenza B Not Detected" result for 100% of true negative samples. For Influenza A and Influenza B weak negative samples, the **cobas®** Liat® assay yielded "Influenza A Not Detected" and "Influenza B Not Detected" result in 83.3% and 16.4% of samples, respectively. The results of the study are presented below.

Sample Level	Untrained Intended Operators using the **cobas®** Liat® assay							
	Inf A Detected	Inf A Not Detected	Inf B Detected	Inf B Not Detected	Inf A Agreement	95%CI	Inf B Agreement	95% CI
True Negative	-	60 / 60	-	60 / 60	100%	94%-100%	100%	94%-100%
Inf A Weak Neg.*	10 / 60	50 / 60	-	60 / 60	83%	72%-91%	100%	94%-100%
Inf A Weak Pos.*	61 / 61	-	-	61 / 61	100%	94%-100%	100%	94%-100%
Inf B Weak Neg.	-	61 / 61	51 / 61	10 / 61	100%	94%-100%	16%	9% - 28%
Inf B Weak Pos.	-	60 / 60	60 / 60	-	100%	94%-100%	100%	94%-100%

* Guidance for Industry and Food and Drug Administration Staff: Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of In Vitro Diagnostic Devices January 30, 2008.

The expected result for the Inf A Weak Positive sample was "Influenza A Detected, Influenza B Not Detected", The expected result for the Inf B Weak Positive sample was "Influenza A Not Detected, Influenza B Detected", The expected result for Inf A Weak Negative, Inf B Weak Negative, and True Negative sample was "Influenza A Not Detected, Influenza B Not Detected".

Using risk analysis as a guide, analytical flex studies were conducted. The studies demonstrated that the test is insensitive to stresses of environmental conditions and potential user errors.

XI. Table of Symbols

Symbol	Used For	Symbol	Used For
	Consult instructions for use		Serial number
	*In Vitro* Diagnostic Medical Device		Use-by date
	Temperature Limit		Control
	Biological Risks		Negative Control
	Do not reuse		Positive Control
	Contains sufficient for <*n*> tests		Authorized Representative in the European community
	Catalogue number		Distributed by
	Batch code		Manufacturer
	This product fulfills the requirements of Directive 98/79/EC on *in vitro* diagnostic medical devices.		

XII. Manufacturer

Technical Support

If you have any questions or problems, please contact your Roche Service representative.

Test system problems may also be reported to the FDA through the MedWatch medical products reporting program (phone: 1-800-FDA-1088; fax: 1-800-FDA-0178; http://www.fda.gov/ medwatch).

Trademarks and Patents

See http://www.roche-diagnostics.us/patents

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