Package Insert

cobas® Influenza A/B & RSV

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

For In Vitro Diagnostic Use

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 & RSV assay. Follow all instructions to ensure correct assay performance.

NOTE: The **cobas®** Liat® System consists of the **cobas®** Liat® Analyzer together with a disposable assay tube that holds the reagents required to perform a test on the **cobas®** Liat® Analyzer. For the remainder of this Package Insert, the **cobas®** Liat® System may be referred to as the System and the **cobas®** Liat® Analyzer may be referred to as the Analyzer.

I. Intended Use

The **cobas**® Influenza A/B & RSV Nucleic acid test for use on the **cobas**® Liat® System (**cobas**® Influenza A/B & RSV) is an automated multiplex real-time RT-PCR assay for the rapid in vitro qualitative detection and discrimination of Influenza A virus, Influenza B virus and respiratory syncytial virus (RSV) 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, Influenza B, and RSV in humans and is not intended to detect Influenza C.

Negative results do not preclude Influenza virus or RSV 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 during the 2013-2014 and the 2014-2015 influenza seasons when Influenza A/H3 and A/H1N1 pandemic 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 and RSV are the leading causes of respiratory infections. 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.

RSV is a common virus that leads to mild, cold-like symptoms in adults, but can cause severe illness in infants, young children, the elderly, and adults with chronic heart or lung disease. Symptoms of RSV infections include nasal congestion, sore throat, cough, sputum production, difficulty breathing, and fever.

Signs and symptoms of Influenza and RSV infections overlap extensively with other infectious causes. Automated and rapid assays that identify patients infected with Influenza and RSV can aid in effective control, proper choice of treatment, and prevention of outbreaks.

III. Principle of the Procedure

The **cobas**® Liat® Influenza A/B & RSV assay is an automated in vitro diagnostic test for the qualitative detection of Influenza A, Influenza B, and RSV RNA in nasopharyngeal (NP) swab specimens. The sample-to-result time is ~20 minutes.

The test is performed on the Analyzer which automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in biological samples using real-time PCR assays. The assay targets a well-conserved region of the matrix gene of Influenza A (Inf A target), the non-structure protein gene of Influenza B (Inf B target), and the matrix gene of RSV (RSV target). An Internal Process Control (IPC) is also included. The IPC is present to control for adequate processing of the target virus through all steps of the assay process and to monitor the presence of inhibitors in the RT-PCR processes.

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 **cobas**® Influenza A/B & RSV assay tube that holds the nucleic acid purification and RT-PCR reagents, and hosts the sample preparation and RT-PCR processes.

IV. Reagents and Instruments

A. Materials Provided

The **cobas®** Influenza A/B & RSV assay tube kit (Cat # 08160104190) contains sufficient reagents to process 20 specimens or quality control samples. The kit contains 20 **cobas®** Influenza A/B & RSV assay tubes and 2 **cobas**® Liat® Transfer Pipette Packs (12 **cobas**® Liat® Transfer Pipettes /pack) (Cat #09329676001). A Package Insert Barcode Card (Cat # 07997060001) with 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
  - Thermo Fisher™ Scientific Remel™ M4RT® (Cat # R12565, R12566, R12567), Remel™ M4® (Cat # R12550), Remel™ M5® (Cat # R12555), or Remel™ M6® (Cat # R12563, R12568, R12569).
- Other Acceptable Collection Material:
  - Universal Transport Medium (UTM-RT®), without beads (Copan Cat # 3C047N)
  - Thermo Fisher™ Scientific Remel™ M4RT® (Cat # R12622, R12591)
  - Thomas Scientific MANTACC™ 0.9% Saline Solution, 3 mL in 10mL Tube, 50 Tubes per Pack (20A00K984), or equivalent
  - Pre-aliquotted 3 mL 0.9% physiological saline**
  - Sterile flexible flocked swab with a synthetic tip (e.g. Dacron, nylon, or rayon) can be used. DO NOT use cotton or calcium alginate swabs, or swabs with wood shafts.

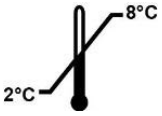
* BD Cat # 220531 is also described as BD universal viral transport (UVT) 3-mL collection kit with flexible flocked swab. This product may not be available in all countries.

** If the collection media and saline listed above are not available, CLIA certified moderate and high complexity laboratories only may prepare and package equivalent 3 mL of 0.9% physiological saline for use with **cobas**® Influenza A/B & RSV test.

- CONTROL **cobas**® Influenza A/B & RSV Quality Control Kit, Cat # 07402686190, containing:
  - **cobas**® Influenza A/B & RSV Positive Control (Positive Control), Cat # 07758537001
  - NEG BUF (Negative Control), Cat # 09587373001
  - Transfer Pipettes, 200 µL, Cat # 07898541001
  - Control Kit Barcode Card, Cat # 08165564001
    - Negative Control Barcode Label, Cat # 08165629001
    - Positive Control Barcode Label, Cat # 08165602001

Note: NEG BUF refers to Negative Buffer.

V. Storage and Handling



- Store the **cobas**® Influenza A/B & RSV assay tube and the **cobas**® Influenza A/B & RSV Control at 2-8ºC.
- The **cobas**® Liat® Transfer Pipette Pack may be stored at room temperature following first removal from the kit.
- Ensure clean gloves are used when removing transfer pipettes from the **cobas**® Liat® Transfer Pipette Pack.
- Reseal the **cobas**® Liat® Transfer Pipette Pack immediately after removing the necessary pipette(s).
- 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 & RSV 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.
- Only use collection kits, collection media or 0.9% physiological saline, and swab types listed in Section IV.
- Do not use a damaged **cobas®** Influenza A/B & RSV assay tube. Do not use a **cobas**® Influenza A/B & RSV 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 & RSV assay tube is used to process one test. Do not reuse a spent **cobas®** Influenza A/B & RSV assay tube. If a **cobas**® Influenza A/B & RSV 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 & RSV assay tubes during or after the run on the Analyzer.
- Dispose of a used **cobas®** Influenza A/B & RSV 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 **cobas**® Liat® System User Guide.
- Sample collection should be performed by specifically trained personnel using the recommended swab types. Specimen collection must be performed using the recommended swab types. Inadequate or inappropriate sample collection, storage, and transport may yield false test results. DO NOT use cotton or calcium alginate swabs, or swabs with wood shafts.
- Ensure there is no sign of leakage from the collection tube prior to running the test.
- Use only the transfer pipettes contained in the **cobas®** Influenza A/B & RSV assay kit and **cobas®** Influenza A/B & RSV Control kit. Use of alternative transfer pipettes may lead to invalid results.
- Change gloves before removing a transfer pipette from the **cobas**® Liat® Transfer Pipette Pack and after handling each sample or control to avoid contamination of reagents and pipettes.

VII. Specimen Collection, Handling, and Storage

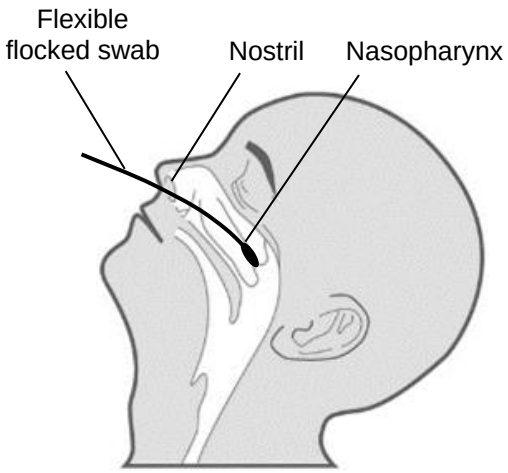
A. Nasopharyngeal Swab Collection

Materials:

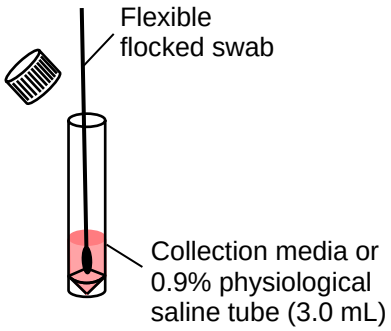
- Sterile flexible flocked swab with a synthetic tip (e.g. Dacron, nylon, or rayon). DO NOT use cotton or calcium alginate swabs, or swabs with wood shafts.
- Tube containing 3 mL of collection media or 0.9% physiological saline.

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 collection media or 0.9% physiological saline tube

B. Specimen Handling & Storage

- After the nasopharyngeal swab sample from the patient has been collected in collection media, specimens should be immediately added to the **cobas®** Influenza A/B & RSV assay tube and the **cobas**® Influenza A/B & RSV assay should be run on the Analyzer as soon as possible but no later than 4 hours after adding the sample to the **cobas**® Influenza A/B & RSV assay tube.
- If specimens cannot be immediately added to the assay tube for testing, nasopharyngeal swab specimens collected in collection media 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.
- Specimen collected in 0.9% physiological saline should be run as soon as possible on the Analyzer. Specimens collected in 0.9% physiological saline solution may be stored up to 4 hours at room temperature or up to 72 hours at 2-8°C if immediate testing is not possible.

VIII. Assay Procedure

Note:

- Consult the **cobas**® Liat® System User Guide on the detailed operations of the System.
  - To avoid error and sample cross contamination, change gloves before removing transfer pipettes from the **cobas**® **Liat**® Transfer Pipette Pack and work on one sample at a time. DO NOT add multiple samples into multiple **cobas**® Influenza A/B & RSV assay tubes at the same time.

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

Before using a new lot of **cobas**® Influenza A/B & RSV assay tubes, the Add Lot procedure must be performed on the Analyzer to validate the **cobas**® Influenza A/B & RSV 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 & RSV assay tube against the list of validated assay tube lots in step 1 below to check if the lot was previously added.

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

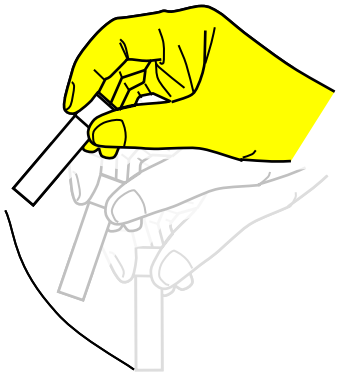
- Package Insert Barcode: On the Package Insert Barcode Card contained in this **cobas**® Influenza A/B & RSV assay tube pack. This barcode is lot-specific; match the lot number next to the barcode with the lot number on the **cobas**® Influenza A/B & RSV assay tubes.
- **cobas**® Influenza A/B & RSV assay tube Barcode: on the **cobas**® Influenza A/B & RSV assay tube sleeve.
- Negative Control Barcode: on the **cobas**® Influenza A/B & RSV Quality Control (QC) kit Barcode Card contained in the QC Kit. Match the lot number next to the barcode with the lot number on the Negative Buffer tube.
- Positive Control Barcode: on the QC 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 & RSV assay tube kit:
  - 〈 Package Insert Barcode Card
  - 〈 2 **cobas**® Influenza A/B & RSV assay tubes
  - 〈 2 transfer pipettes from the **cobas**® Liat® Transfer Pipette Pack
- From **cobas**® Influenza A/B & RSV Quality Control (QC) kit:
  - 〈 Negative Control: Negative Control Barcode, (see Control Kit Barcode Card), 1 Negative Buffer tube (used as the negative control sample)
  - 〈 Positive Control: Positive Control Barcode, (see Control Kit Barcode Card), 1 Positive Control tube, 1 Negative Buffer 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 & RSV assay tube pack
      〈 Negative Control Barcode on the Control Kit Barcode Card
      〈 1 Negative Buffer tube (used as the negative control sample)
      〈 1 **cobas®** Influenza A/B & RSV assay tube from this lot
      〈 1 transfer pipette from the **cobas®** Liat® Transfer Pipette Pack
   b. Select **Assay Menu** on the main menu of the 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 & RSV 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 tube ID*".
   f. Take a Negative Buffer tube from the QC Kit; this is used as the negative control sample. Hold the Negative Buffer 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 Negative Buffer has pooled at the bottom of the tube. If not, repeat the shake down procedure.



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

   g. Using the Negative Buffer as sample, run the assay following the Running **cobas®** Influenza A/B & RSV assay Procedure, steps B.2.b-h (Add Sample) and B.3 (Insert **cobas®** Influenza A/B & RSV assay tube).
   h. If *"Negative control result accepted."* is displayed at the end of the run, select **Confirm**. If the result is rejected, repeat the negative control run (step A.1)
   i. Select **Confirm**.
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, Influenza B, and RSV at the bottom of the tube
      〈 1 Negative Buffer tube, containing a unit dose of buffer to be mixed with the positive control
   b. Hold the Negative Buffer 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 liquid has pooled at the bottom of the tube. If not, repeat the shake down procedure.

c. Using the provided transfer pipette, transfer the liquid from the Negative Buffer 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 Negative Buffer. 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 Negative Buffer tube.

iii. Slowly release the bulb completely while keeping the pipette tip below the liquid surface. You will see the liquid rising into the pipette. After releasing the bulb completely, withdraw the pipette from the Negative Buffer 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 & RSV assay tube from this lot

〈 1 transfer pipette from the **cobas**® Liat® Transfer Pipette Pack

b. On the Analyzer, select **Scan** and scan the Positive Control Barcode on the Control Kit Barcode Card. The Analyzer will prompt "*Add positive control & scan tube ID*".

c. After the Positive Control tube from step A.2 has sat for 5 minutes, use the transfer pipette from the **cobas**® Liat® Transfer Pipette Pack 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 & RSV assay Procedure, steps B.2.b-h (Add Sample) and B.3 (Insert **cobas®** Influenza A/B & RSV assay tube).

e. If "*Positive control result accepted.*" is displayed at the end of the run, select **Confirm**, select **Back** 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 to transfer the lot information to the other Analyzers at your site. This allows the other Analyzers to use this **cobas®** Influenza A/B & RSV assay tube lot without performing Add Lot on each Analyzer. Consult the software specific User Guide for details of operation.

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

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

Materials:

- From **cobas®** Influenza A/B & RSV assay tube kit:
  - 〈 **cobas®** Influenza A/B & RSV assay tube from a lot that has been added to the Analyzer. See Section A for Add cobas® Influenza A/B & RSV tube lot instructions.
  - 〈 Transfer pipette from the **cobas®** Liat® Transfer Pipette Pack.

Procedure:

1. Scan Barcode

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

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

   b. Select **Scan** and scan the **cobas®** Influenza A/B & RSV 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.

   d. If patient verification is activated, the Analyzer will display the status of verification.

      i. If patient verification is successful, the Analyzer may prompt confirmation of entered information before proceeding with running the assay.

      ii. If patient verification fails, the Analyzer will display a notification that verification failed:

         〈 And may require acknowledgement before proceeding with running the assay or

         〈 You cannot proceed with running the assay. Contact your lab administrator if this occurs.

   e. The Analyzer will prompt “Add sample & rescan tube ID.”

2. Add Sample

   Change gloves before taking a transfer pipette out of the **cobas®** Liat® Transfer Pipette Pack to avoid contamination of the pipette pack. Use a transfer pipette from the **cobas®** Liat® Transfer Pipette Pack to load ~200 µL of the sample into the **cobas®** Influenza A/B & RSV 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 the pipette to be inserted into the sample tube. Avoid lifting the swab completely out of the sample tube.

b. 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 & RSV assay tube.

f. While watching through the viewing window in the sleeve, carefully insert the pipette into the **cobas**® Influenza A/B & RSV 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 & RSV assay tube. Avoid creating bubbles in the sample. Do not release the pipette bulb.

Note: Do not puncture the **cobas**® Liat® 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 & RSV 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 & RSV 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 & RSV 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 & RSV assay tube.

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

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

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

c. Immediately insert the **cobas**® Influenza A/B & RSV assay tube into the Analyzer until the assay tube clicks into place. The **cobas**® Influenza A/B & RSV 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 & RSV assay tube barcode (step 3a) and insert the **cobas**® Influenza A/B & RSV assay tube again. Once the **cobas**® Influenza A/B & RSV 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, "Remove tube slowly and carefully." and opens the assay tube entry door automatically.

a. Lift the **cobas**® Influenza A/B & RSV assay tube out of the Analyzer and dispose of the used assay tube according to your institution's safety guidelines for hazardous material.

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, Influenza B, and RSV, or “Assay Invalid.”

The Analyzer automatically interprets the results from measured fluorescent signals. Embedded calculation algorithms determine the PCR cycle threshold (Ct) and evaluate the Ct 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”.

Like the viral targets, the IPC target is also evaluated in every assay run. In the case that Influenza A, Influenza B, and RSV 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”. In some cases, high concentration of one or more target viruses may inhibit the amplification of IPC or other targets. IPC is not taken into consideration when a target virus is detected.

The following table 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	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	Presence or absence of Influenza B cannot be determined. Repeat assay with same sample or, if possible, new sample.
RSV	RSV Not Detected	Negative test for RSV (no RSV RNA detected)
	RSV Detected	Positive test for RSV (RSV RNA present)
	RSV Indeterminate	Presence or absence of RSV cannot be determined. Repeat assay with same sample or, if possible, new sample.
Assay Invalid		Presence or absence of Influenza A, Influenza B, and RSV 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 Code	Sample and External Controls	Negative Control (Add Lot)	Positive Control (Add Lot)
g0*	IPC Not Detected or Indeterminate. Repeat Run	IPC Not Detected or Indeterminate. Repeat Run	IPC Not Detected or Indeterminate. Repeat Run
g1			
g2			
g3			
g4			
x4	At least one or more Target Indeterminate. Repeat Run	N/A	N/A
FP	N/A	At least one Target is Detected or Indeterminate. Repeat Run	N/A
b0	N/A	N/A	At least one Target is Not Detected or Indeterminate. Repeat Run
b1			
b2			
b3			
b4			
a0	N/A	N/A	At least one Target is Not Detected or Indeterminate. Repeat Run
a1			
a2			
a3			
a4			
r0	N/A	N/A	At least one Target is Not Detected or Indeterminate. Repeat Run
r1			
r2			
r3			
r4			

Note*: Failure code g0 does 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 assay 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.

Dual infections of Influenza A and Influenza B are rare. If the test result is “Influenza A Detected” and “Influenza B Detected”, the assay should be repeated with the same patient specimen, or if possible, with a newly collected specimen. Specimens that have repeat “Influenza A Detected” and “Influenza B Detected” results should be sent to a laboratory for confirmatory testing.

E. Quality Control **CONTROL**

Internal Process Control (IPC): is an encapsulated RNA that is included in each **cobas®** Influenza A/B & RSV 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.

External Controls: provide additional quality control to monitor the integrity of reagents, the functionality of the Analyzer and to ensure that the test procedure is followed correctly. External Controls are run during the Add **cobas®** Influenza A/B & RSV 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:

- From the **cobas®** Influenza A/B & RSV Assay Kit:1 **cobas®** Influenza A/B & RSV assay tube, 1 transfer pipette from the **cobas**® Liat® Transfer Pipette Pack.
- From QC Kit: 1 Negative Buffer tube (used as the negative control sample), and Negative Control Barcode on the Control Kit Barcode Card.

Procedure:

The Negative Buffer is used as the sample for the Negative Control run.

1. Take a Negative Buffer tube from the QC Kit.
2. Hold the Negative Buffer 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 Negative Buffer has pooled at the bottom of the tube. If not, repeat the shake down procedure.
3. Using the Negative Buffer as sample, run the assay following the Running **cobas®** Influenza A/B & RSV assay procedure steps B.2.b-h (Add Sample) and B.3 (Insert **cobas**® Influenza A/B & RSV 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,” “Influenza B Not Detected,” and “RSV Not Detected” for the negative control to pass.

Positive Control

Materials:

〈 From the **cobas®** Influenza A/B & RSV Assay Kit: 1 **cobas®** Influenza A/B & RSV assay tube, 1 transfer pipette from the **cobas**® Liat® Transfer Pipette Pack.

〈 From QC Kit: 1 Positive Control tube, 1 Negative Buffer 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, Influenza B, and RSV. Follow the directions below to dissolve the positive control in Negative Buffer and run it on the **cobas®** Influenza A/B & RSV assay.

1. Follow step A.2. of the Add **cobas®** Influenza A/B & RSV 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 & RSV 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 & RSV assay Procedure steps B.2.b-h (Add Sample) and B.3 (Insert **cobas**® Influenza A/B & RSV 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,” “Influenza B Detected,” and “RSV Detected” for the positive control to pass.

IX. Limitations

- The performance of the **cobas®** Influenza A/B & RSV assay was evaluated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- Users in a point of care environment should not prepare (formulate, measure, aliquot) 0.9% physiological saline. CLIA certified moderate and high complexity laboratories may prepare and package equivalent 3 mL of 0.9% physiological saline for use with **cobas**® Influenza A/B & RSV test, but performance with these alternative solutions has not been established. When using 0.9% physiological saline solution, ensure that the collection tube is an appropriate height for the swab such that the score mark on the swab is not higher than the height of the tube. The **cobas**® Influenza A/B & RSV assay has not been reviewed by FDA for use with 0.9% physiological saline.
- As with other tests, negative results do not preclude Influenza A, Influenza B, or RSV infection and should not be used as the sole basis for treatment or other patient management decisions. Results from the **cobas®** Influenza A/B & RSV 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.
- False negative results may occur if one or more target viruses inhibits amplification of other targets.
- If the virus mutates in the target regions, Influenza viruses A or B or RSV may not be detected or may be detected less predictably.
- This test has not been evaluated for patients without signs and symptoms of Influenza and RSV 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.

I. Expected Values

The rate of positivity found in influenza testing is dependent on many factors including the method of specimen collection, the test method used, geographic location, and the disease prevalence in specific localities. In the **cobas**® Influenza A/B & RSV assay prospective clinical study, a total of 313 specimens were determined to be evaluable during the 2013-2014 influenza season from January 2014 to May 2014. The number and percentage of influenza A, influenza B and RSV positive cases per specified age group, as determined by the **cobas**® Influenza A/B & RSV assay, are presented in the tables below:

Age Group	Number of Nasopharyngeal Swab Specimens	Number of Influenza A Positives	Influenza A Positivity Rate
≤ 5 years	135	5	3.7%
6 to 21 years	124	10	8.1%
22 to 59 years	45	3	6.7%
≥ 60 years	9	1	11.1%
Total	313	19	6.1%

Age Group	Number of Nasopharyngeal Swab Specimens	Number of Influenza B Positives	Influenza B Positivity Rate
≤ 5 years	135	4	3.0%
6 to 21 years	124	11	8.9%
22 to 59 years	45	5	11.1%
≥ 60 years	9	0	0.0%
Total	313	20	6.4%

Age Group	Number of Nasopharyngeal Swab Specimens	Number of RSV Positives	RSV Positivity Rate
≤ 5 years	135	8	5.9%
6 to 21 years	124	2	1.6%
22 to 59 years	45	3	6.7%
≥ 60 years	9	0	0.0%
Total	313	13	4.2%

A total of 1048 specimens were determined to be evaluable during the 2014-2015 influenza season from October 2014 to April 2015. The number and percentage of influenza A, influenza B and RSV positive cases per specified age group, as determined by the **cobas®** Influenza A/B & RSV assay, are presented in the tables below:

Age Group	Number of Nasopharyngeal Swab Specimens	Number of Influenza A Positives	Influenza A Positivity Rate
≤ 5 years	185	21	11.4%
6 to 21 years	284	78	27.5%
22 to 59 years	460	76	16.5%
≥ 60 years	119	29	24.4%
Total	1048	204	19.5%

Age Group	Number of Nasopharyngeal Swab Specimens	Number of Influenza B Positives	Influenza B Positivity Rate
≤ 5 years	185	1	0.5%
6 to 21 years	284	7	2.5%
22 to 59 years	460	18	3.9%
≥ 60 years	119	3	2.5%
Total	1048	29	2.8%

Age Group	Number of Nasopharyngeal Swab Specimens	Number of RSV Positives	RSV Positivity Rate
≤ 5 years	185	54	29.2%
6 to 21 years	284	17	6.0%
22 to 59 years	460	23	5.0%
≥ 60 years	119	6	5.0%
Total	1048	100	9.5%

II. Performance Characteristics

A. Clinical Studies

The **cobas®** Influenza A/B & RSV assay was evaluated at 12 CLIA waived healthcare facilities. Prospective nasopharyngeal swab (NPS) specimens were collected from patients with signs and symptoms of respiratory infection in the US during the 2013-2014 and 2014-2015 flu seasons, and were tested prospectively at the study sites. Additionally, retrospective NPS specimens were obtained from 2 reference laboratories and were distributed to and tested at 3 of the 12 sites. The retrospective specimens were worked into the daily workload of those sites for testing.

Each patient's specimen was tested by the **cobas®** Influenza A/B & RSV and an FDA-cleared laboratory-based multiplexed real-time reverse transcriptase PCR (RT-PCR) test (comparator test). The **cobas®** Influenza A/B & RSV assay results were compared against the results from the comparator test. A total of 1,350 prospective NPS specimens and 292 retrospective NPS specimens were included in the performance analysis.

For prospective specimens, a total of 1,421 subjects were enrolled in this study. Of these, 41 specimens did not meet eligibility criteria. Additionally, 17 and 13 specimens were excluded due to invalid results from the Analyzer and the comparator tests, respectively. As such, a total of 1,350 prospective nasopharyngeal swab (NPS) specimens were included in the performance analysis. Compared to the comparator test, the **cobas®** Influenza A/B & RSV assay demonstrated positive agreement of 98.3%, 95.2% and 97.0% for Inf A, Inf B and RSV, respectively; and negative agreement of 96.0%, 99.4% and 98.7% for Inf A, Inf B, and RSV, respectively.

Prospective NPS Specimens

Inf A		Comparator Test		
		Positive	Negative	Total
Liat	Positive	172	47[a]	219
	Negative	3	1128	1131
	Total	175	1175	1350

	%	95% CI
Positive Agreement	98.3%	(95.1% - 99.4%)
Negative Agreement	96.0%	(94.7% - 97.0%)

[a] Forty-one **cobas®** Influenza A/B & RSV positive, lab-based RT-PCR negative specimens were tested by PCR/sequencing. Of these, 18 were positive and 23 were negative by PCR/sequencing.

Inf B		Comparator Test		
		Positive	Negative	Total
Liat	Positive	40	8[a]	48
	Negative	2	1300	1302
	Total	42	1308	1350

	%	95% CI
Positive Agreement	95.2%	(84.2% - 98.7%)
Negative Agreement	99.4%	(98.8% - 99.7%)

[a] Six **cobas®** Influenza A/B & RSV positive, lab-based RT-PCR negative specimens were tested by PCR/sequencing. were positive and 1 was negative by PCR/sequencing.

RSV		Comparator Test		
		Positive	Negative	Total
Liat	Positive	96	16[a]	112
	Negative	3	1235	1238
	Total	99	1251	1350

	%	95% CI
Positive Agreement	97.0%	(91.5% - 99.0%)
Negative Agreement	98.7%	(97.9% - 99.2%)

[a] Fifteen **cobas®** Influenza A/B & RSV positive, lab-based RT-PCR negative specimens were tested by PCR/sequencing. Of these, 3 were positive and 12 were negative by PCR/sequencing.

For retrospective specimens, a total of 300 specimens were tested at clinical sites. Of these, 5 and 3 specimens were excluded due to invalid results from the System and the comparator tests, respectively. As such, a total of 292 retrospective nasopharyngeal swab (NPS) specimens were included in the performance analysis. Compared to the comparator test, the **cobas®** Influenza A/B & RSV assay demonstrated positive agreement of 98.7%, 99.0% and 98.8% for Inf A, Inf B and RSV, respectively; and negative agreement of 99.1%, 99.5% and 96.6% for Inf A, Inf B, and RSV, respectively.

Retrospective NPS Specimens

Inf A		Comparator Test		
		Positive	Negative	Total
Liat	Positive	76	2[a]	78
	Negative	1	213	214
	Total	77	215	292

	%	95% CI
Positive Agreement	98.7%	(93.0% - 99.8%)
Negative Agreement	99.1%	(96.7% - 99.7%)

[a] One **cobas®** Influenza A/B & RSV positive, lab-based RT-PCR negative specimens were tested by PCR/sequencing. The 1 sample was negative by PCR/sequencing.

Inf B		Comparator Test		
		Positive	Negative	Total
Liat	Positive	97	1	98
	Negative	1	193	194
	Total	98	194	292

	%	95% CI
Positive Agreement	99.0%	(94.4% - 99.8%)
Negative Agreement	99.5%	(97.1% - 99.9%)

RSV		Comparator Test		
		Positive	Negative	Total
Liat	Positive	83	7[a]	90
	Negative	1	201	202
	Total	84	208	292

	%	95% CI
Positive Agreement	98.8%	(93.6% - 99.8%)
Negative Agreement	96.6%	(93.2% - 98.4%)

[a] Six **cobas®** Influenza A/B & RSV positive, lab-based RT-PCR negative specimens were tested by PCR/sequencing. Of these, all 6 were positive by PCR/sequencing.

During the clinical study testing of prospective and retrospective specimens, the **cobas®** Influenza A/B & RSV assay initial invalid rate was 1.8% (29/1,656 specimens, 95% CI: 1.2% - 2.5%). Of these 29 specimens with initial invalid results, 5 specimens had 2 invalid or aborted runs, 16 specimens had 1 invalid run and were not repeated due to unavailability of residual samples, and 8 specimens had an initial invalid run and a repeat test per product instructions for use yielded a valid result.

B. Reproducibility

Reproducibility study assesses the total variability of the **cobas®** Influenza A/B & RSV assay across operators, study sites, testing days, Analyzers, and **cobas®** Influenza A/B & RSV assay tube lots. The **cobas®** Influenza A/B & RSV assay was evaluated at 3 sites. Two operators at each of the 3 sites tested a 10 member reproducibility panel in triplicate on 5 different days, for a total of ~900 runs (10 panel members · 3 replicates · 2 operators · 5 days · 3 sites). Nine Analyzers and 3 **cobas®** Influenza A/B & RSV assay tube lots were used. The reproducibility panel comprises a high negative, a low positive, and a moderate positive for each of Influenza A, Influenza B and RSV, in addition to a negative sample. For a given virus, the expected result for the true negative and the high negative panel member is "Not Detected," while the expected result for the low positive and moderate positive panel member is "Detected." Percent agreement with expected result, mean Ct, and Ct %CV for each site are shown in the tables below.

Influenza A Reproducibility

	Site 1			Site 2			Site 3			Total	
Sample	**Agreement w/ expected result**	**Avg Ct**	**Ct %CV**	**Agreement w/ expected result**	**Ct Avg**	**Ct %CV**	**Agreement w/ expected result**	**Ct Avg**	**Ct %CV**	**Agreement w/ expected result**	**95% CI**
Negative	30 / 30	-	-	31 / 31	-	-	30 / 30	-	-	91 / 91 (100.0%)	96.0% - 100.0%
Flu A High Negative*	29 / 30	37.0	-	30 / 30	-	-	29 / 30	35.7	-	88 / 90 (97.8%)	92.3% - 99.4%
Flu A Low Positive*	30 / 30	32.7	2.9%	30 / 30	32.1	1.6%	30 / 30	32.3	1.6%	90 / 90 (100.0%)	95.9% - 100.0%
Flu A Moderate Positive*	30 / 30	30.4	1.0%	30 / 30	30.0	1.2%	30 / 30	30.1	0.9%	90 / 90 (100.0%)	95.9% - 100.0%
Flu B High Negative*	30 / 30	-	-	31 / 31	-	-	30 / 30	-	-	91 / 91 (100.0%)	96.0% - 100.0%
Flu B Low Positive*	30 / 30	-	-	30 / 30	-	-	29 / 29†	-	-	89 / 89 (100.0%)	95.9% - 100.0%
Flu B Moderate Positive*	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
RSV High Negative*	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
RSV Low Positive*	30 / 30	-	-	31 / 31	-	-	30 / 30	-	-	91 / 91 (100.0%)	96.0% - 100.0%
RSV Moderate Positive*	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Total Agreement	299 / 300 (99.7%)			303 / 303 (100.0%)			298 / 299 (99.7%)			900 / 902 (99.8%)	99.2% - 99.9%

† One of 30 Flu B Low Positive replicates yielded an "Assay Invalid. Repeat Assay" result, and was not repeated.
* 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**	**Ct Avg**	**Ct %CV**	**Agreement w/ expected result**	**Ct Avg**	**Ct %CV**	**Agreement w/ expected result**	**Ct Avg**	**Ct %CV**	**Agreement w/ expected result**	**95% CI**
Negative	30 / 30	-	-	31 / 31	-	-	30 / 30	-	-	91 / 91 (100.0%)	96.0% - 100.0%
Flu A High Negative*	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Flu A Low Positive*	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Flu A Moderate Positive*	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Flu B High Negative*	29 / 30	35.1	-	31 / 31	-	-	30 / 30	-	-	90 / 91 (98.9%)	94.0% - 99.8%
Flu B Low Positive*	30 / 30	31.9	1.8%	30 / 30	31.6	1.4%	29 / 29†	31.6	1.5%	89 / 89 (100.0%)	95.9% - 100.0%
Flu B Moderate Positive*	30 / 30	30.8	1.3%	30 / 30	30.4	1.4%	30 / 30	30.5	1.3%	90 / 90 (100.0%)	95.9% - 100.0%
RSV High Negative*	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
RSV Low Positive*	30 / 30	-	-	31 / 31	-	-	30 / 30	-	-	91 / 91 (100.0%)	96.0% - 100.0%
RSV Moderate Positive*	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Total Agreement	299 / 300 (99.7%)			303 / 303 (100.0%)			299 / 299 (100.0%)			901 / 902 (99.9%)	99.4% - 100.0%

† One of 30 Flu B Low Positive replicates yielded an “Assay Invalid. Repeat Assay” result, and was not repeated.
* 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

RSV Reproducibility

	Site 1			Site 2			Site 3			Total	
Sample	**Agreement w/ expected result**	**Ct Avg**	**Ct %CV**	**Agreement w/ expected result**	**Ct Avg**	**Ct %CV**	**Agreement w/ expected result**	**Ct Avg**	**Ct %CV**	**Agreement w/ expected result**	**95% CI**
Negative	30 / 30	-	-	31 / 31	-	-	30 / 30	-	-	91 / 91 (100.0%)	96.0% - 100.0%
Flu A High Negative*	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Flu A Low Positive*	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Flu A Moderate Positive*	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Flu B High Negative*	30 / 30	-	-	31 / 31	-	-	30 / 30	-	-	91 / 91 (100.0%)	96.0% - 100.0%
Flu B Low Positive*	30 / 30	-	-	30 / 30	-	-	29 / 29†	-	-	89 / 89 (100.0%)	95.9% - 100.0%
Flu B Moderate Positive*	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
RSV High Negative*	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
RSV Low Positive*	29 / 30	33.0	3.7%	31 / 31	32.8	3.4%	30 / 30	32.8	2.7%	90 / 91 (98.9%)	94.0% - 99.8%
RSV Moderate Positive*	30 / 30	30.6	2.9%	30 / 30	30.9	1.6%	30 / 30	30.5	2.5%	90 / 90 (100.0%)	95.9% - 100.0%
Total Agreement	299 / 300 (99.7%)			303 / 303 (100.0%)			299 / 299 (100.0%)			901 / 902 (99.9%)	99.4% - 100.0%

† One of 30 Flu B Low Positive replicates yielded an “Assay Invalid. Repeat Assay” result, and was not repeated.
* 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 & RSV assay was evaluated using 3 strains of Influenza A, 2 strains of Influenza B, and 2 strains of RSV. The LOD was determined by limiting dilution studies using these titered viruses. The viruses were spiked into negative nasopharyngeal swab (NPS) in UTM sample matrix, and then tested using the **cobas®** Influenza A/B & RSV assay. The LOD was determined as the lowest virus concentration that was detected ≥95% of the time (i.e. concentration at which at least 19 out of 20 replicates tested positive). The LOD was 2×10^{-3} - 2×10^{-2} $TCID_{50}$/mL for Influenza A strains, 2×10^{-3} - 4×10^{-3} $TCID_{50}$/mL for Influenza B strains, and 4×10^{-1} $TCID_{50}$/mL for RSV strains.

Virus Strain	LOD ($TCID_{50}$/mL)
A/Brisbane/10/07	2.0×10^{-2}
A/Brisbane/59/07	2.0×10^{-3}
A/NY/01/2009	2.0×10^{-2}
B/Florida/04/06	2.0×10^{-3}
B/Malaysia/2506/04	4.0×10^{-3}
RSV A	4.0×10^{-1}
RSV B	4.0×10^{-1}

D. Analytical Reactivity

The reactivity study evaluates the ability to detect Influenza and RSV strains representing temporal and geographical diversity. The **cobas®** Influenza A/B & RSV assay was evaluated with 28 Influenza A, 15 Influenza B, and 7 RSV strains. Influenza A strains included 14 Influenza A/H1 strains (including 3 H1N1 pdm09 strains), 12 Influenza A/H3 strains (including 1 H3N2v strain), 1 Influenza A/H7N9 strain, and 1 Influenza A/H5N1 reassortant strain. Influenza B strains included that from both the Victoria lineage and Yamagata lineage. RSV strains included both RSV Type A and Type B strains. The **cobas®** Influenza A/B & RSV assay detected all strains at the concentrations tested.

Virus Strain	Type / Subtype	Test Concentration	Inf A Result	Inf B Result	RSV Result
A/Aichi/2/68	Influenza A/H3N2	1.0×10^{2} $CEID_{50}$/mL	+	–	–
A/Alice	Influenza A/H3N2	5.0×10^{1} $CEID_{50}$/mL	+	–	–
A/Anhui/1/2013	Influenza A/H7N9 (Eurasian lineage)	1.0×10^{3} $TCID_{50}$/mL	+	–	–
A/Brisbane/10/07	Influenza A/H3N2	2.0×10^{-2} TCID50/mL	+	–	–
A/Brisbane/59/07	Influenza A/H1N1	2.0×10^{-3} TCID50/mL	+	–	–
A/Cambodia/X0810301/2013(H5N1)-PR8-IDCDC-RG34B	Influenza A/H5N1 reassortant	2.5×10^{1} $CEID_{50}$/mL	+	–	–
A/Denver/1/57	Influenza A/H1N1	1.0×10^{2} $CEID_{50}$/mL	+	–	–
A/FM/1/47	Influenza A/H1N1	1.0×10^{2} $CEID_{50}$/mL	+	–	–
A/H3/Perth/16/09	Influenza A/H3N2	2.5×10^{-1} $TCID_{50}$/mL	+	–	–
A/Hong Kong/8/68	Influenza A/H3N2	1.0×10^{2} $TCID_{50}$/mL	+	–	–
A/Indiana/8/2011	Influenza A/H3N2v	5.0×10^{-1} $TCID_{50}$/mL	+	–	–
A/Mal/302/54	Influenza A/H1N1	4.0×10^{2} $CEID_{50}$/mL	+	–	–
A/MRC2	Influenza A/H3	1.0×10^{2} $CEID_{50}$/mL	+	–	–
A/New Caledonia/20/99	Influenza A/H1N1	1.0×10^{2} $TCID_{50}$/mL	+	–	–
A/New Jersey/8/76	Influenza A/H1N1	1.0×10^{1} $CEID_{50}$/mL	+	–	–
A/NY/01/2009	Influenza A/H1N1 pdm09	2.0×10^{-2} TCID50/mL	+	–	–
A/NY/02/2009	Influenza A/H1N1 pdm09	2.5×10^{-2} $TCID_{50}$/mL	+	–	–
A/NY/03/2009	Influenza A/H1N1 pdm09	2.0×10^{-1} $TCID_{50}$/mL	+	–	–
A/Port Chalmers/1/73	Influenza A/H3N2	1.0×10^{2} $CEID_{50}$/mL	+	–	–
A/PR/8/34	Influenza A/H1N1	5.0×10^{0} $TCID_{50}$/mL	+	–	–
A/Solomon Island/3/2006	Influenza A/H1N1	5.0×10^{-2} $TCID_{50}$/mL	+	–	–
A/Swine/1976/31	Influenza A/H1N1	1.0×10^{1} $CEID_{50}$/mL	+	–	–
A/Swine/Iowa/15/30	Influenza A/H1N1	1.0×10^{2} $CEID_{50}$/mL	+	–	–

A/Texas/50/2012	Influenza A/H3N2	1.0×10^{-1} $TCID_{50}$/mL	+	–	–
A/Victoria/3/75	Influenza A/H3N2	1.0×10^{2} $CEID_{50}$/mL	+	–	–
A/Victoria/361/2011	Influenza A/H3N2	2.0×10^{-2} $TCID_{50}$/mL	+	–	–
A/Weiss/43	Influenza A/H1N1	1.0×10^{3} $TCID_{50}$/mL	+	–	–
A/Wisconsin/67/05	Influenza A/H3N2	5.0×10^{-1} $TCID_{50}$/mL	+	–	–
B/Allen/45	Influenza B	5.0×10^{-1} $TCID_{50}$/mL	–	+	–
B/Brisbane/60/2008	Influenza B (Victoria lineage)	1.0×10^{-2} $TCID_{50}$/mL	–	+	–
B/Florida/04/06	Influenza B (Yamagata lineage)	2.0×10^{-3} TCID50/mL	–	+	–
B/Florida/07/04	Influenza B (Yamagata lineage)	5.0×10^{-2} $TCID_{50}$/mL	–	+	–
B/GL/1739/54	Influenza B	2.0×10^{0} $TCID_{50}$/mL	–	+	–
B/HongKong/5/72	Influenza B	2.5×10^{-1} $TCID_{50}$/mL	–	+	–
B/Lee/40	Influenza B	2.5×10^{-1} $TCID_{50}$/mL	–	+	–
B/Malaysia/2506/04	Influenza B (Victoria lineage)	4.0×10^{-3} TCID50/mL	–	+	–
B/Maryland/1/59	Influenza B	2.0×10^{-2} $TCID_{50}$/mL	–	+	–
B/Mass/3/66	Influenza B	1.0×10^{1} $TCID_{50}$/mL	–	+	–
B/Massachusetts/2/2012	Influenza B (Yamagata lineage)	5.0×10^{-3} $TCID_{50}$/mL	–	+	–
B/Nevada/03/2011	Influenza B (Victoria lineage)	2.5×10^{-1} $CEID_{50}$/mL	–	+	–
B/Taiwan/2/62	Influenza B	2.0×10^{-1} $TCID_{50}$/mL	–	+	–
B/Texas/6/2011	Influenza B (Yamagata lineage)	1.0×10^{-1} $TCID_{50}$/mL	–	+	–
B/Wisconsin/1/2010	Influenza B (Yamagata lineage)	5.0×10^{-1} $TCID_{50}$/mL	–	+	–
RSV A 2006 isolate	RSV A	4.0×10^{-1} $TCID_{50}$/mL	–	–	+
RSV A Long	RSV A	1.0×10^{2} $TCID_{50}$/mL	–	–	+
RSV A2	RSV A	1.0×10^{0} $TCID_{50}$/mL	–	–	+
RSV B 9320	RSV B	1.0×10^{0} $TCID_{50}$/mL	–	–	+
RSV B Ch93(18)-18	RSV B	4.0×10^{-1} $TCID_{50}$/mL	–	–	+
RSV B Wash/18537	RSV B	1.0×10^{0} $TCID_{50}$/mL	–	–	+
RSV B WV/14617/85	RSV B	1.0×10^{-1} $TCID_{50}$/mL	–	–	+

E. Cross Reactivity

Cross reactivity study evaluates potential cross-reactivity with non-influenza and non-RSV microorganisms that may be present in nasopharyngeal swab samples. The **cobas**® Influenza A/B & RSV assay was evaluated against a panel comprising human genomic DNA and 35 microorganisms. Bacteria and *Candida albicans* were tested at ≥ 10^6 CFU/mL. Viruses were tested at ≥ 10^5 $TCID_{50}$/mL, or the highest available concentration. The **cobas**® Influenza A/B & RSV assay showed no cross reactivity for the human genomic DNA or the microorganisms at the concentrations tested.

Microorganism	Test Concentration	Inf A Result	Inf B Result	RSV Result
Adenovirus Type 1	9.0×10^5 $TCID_{50}$/mL	–	–	–
Adenovirus Type 7	1.4×10^5 $TCID_{50}$/mL	–	–	–
Cytomegalovirus	4.5×10^4 $TCID_{50}$/mL	–	–	–
Epstein Barr Virus	2.5×10^5 $TCID_{50}$/mL	–	–	–
Herpes Simplex Virus	1.4×10^5 $TCID_{50}$/mL	–	–	–
Human Coronavirus 229E	8.0×10^3 $TCID_{50}$/mL	–	–	–
Human Coronavirus OC43	8.0×10^4 $TCID_{50}$/mL	–	–	–
Human Enterovirus 68	1.0×10^5 $TCID_{50}$/mL	–	–	–
Human Metapneumovirus	7.0×10^3 $TCID_{50}$/mL	–	–	–
Human Parainfluenza Type 1	3.7×10^5 $TCID_{50}$/mL	–	–	–
Human Parainfluenza Type 2	7.5×10^5 $TCID_{50}$/mL	–	–	–
Human Parainfluenza Type 3	4.5×10^5 $TCID_{50}$/mL	–	–	–
Human Rhinovirus Type 1A	8.0×10^5 $TCID_{50}$/mL	–	–	–
Measles	8.0×10^4 $TCID_{50}$/mL	–	–	–
Mumps Virus	8.0×10^4 $TCID_{50}$/mL	–	–	–
Varicella-Zoster Virus	4.4×10^3 $TCID_{50}$/mL	–	–	–
Bordetella pertussis	2.2×10^6 CFU/mL	–	–	–
Candida albicans	4.2×10^6 CFU/mL	–	–	–
Chlamydia pneumoniae	8.0×10^4 $TCID_{50}$/mL	–	–	–
Corynebacterium sp	3.6×10^6 CFU/mL	–	–	–
Escherichia coli	1.9×10^6 CFU/mL	–	–	–
Haemophilus influenzae	2.3×10^6 CFU/mL	–	–	–
Lactobacillus sp	1.9×10^6 CFU/mL	–	–	–
Legionella pneumophila	6.7×10^6 CFU/mL	–	–	–
Moraxella catarrhalis	2.5×10^6 CFU/mL	–	–	–
Mycobacterium tuberculosis	2.8×10^6 copies/mL†	–	–	–
Mycoplasma pneumoniae	2.9×10^6 copies/mL†	–	–	–
Neisseria elongate	2.0×10^6 CFU/mL	–	–	–
Neisseria meningitidis	2.2×10^6 CFU/mL	–	–	–
Pseudomonas aeruginosa	2.3×10^6 CFU/mL	–	–	–
Staphylococcus aureus	2.4×10^6 CFU/mL	–	–	–
Staphylococcus epidermidis	1.9×10^6 CFU/mL	–	–	–
Streptococcus pneumoniae	1.8×10^6 CFU/mL	–	–	–
Streptococcus pyogenes	2.5×10^6 CFU/mL	–	–	–
Streptococcus salivarius	4.3×10^6 CFU/mL	–	–	–
Human genomic DNA	1.0×10^4 copies/mL	–	–	–

† Testing was performed with genomic DNA due to difficulties in propagation of these bacteria.

F. Interfering Microorganisms

Interfering microorganism study evaluates whether non-influenza and non-RSV microorganisms that may be present in nasopharyngeal swab samples can interfere in the detection of Influenza A, Influenza B or RSV by the **cobas**® Influenza A/B & RSV assay. The panel comprising human genomic DNA and 35 microorganisms tested in the cross-reactivity study was tested for potential interference. Bacteria and *Candida albicans* were tested at ≥ 10^6 CFU/mL and viruses were tested at ≥10^5 $TCID_{50}$/mL or the highest available concentration, in the presence of 1 Influenza A strain, 1 Influenza B strain and 1 RSV strain at 3x LOD concentration in negative NPS in UTM matrix. Results show that the presence of human genomic DNA or the microorganisms at the concentrations tested did not interfere with the detection of Influenza A, Influenza B, or RSV.

Microorganism	Test Concentration	1 Flu A, 1 Flu B &1 RSV strain at 3x LOD		
		Inf A Result	Inf B Result	RSV Result
Adenovirus Type 1	9.0×10^5 $TCID_{50}$/mL	+	+	+
Adenovirus Type 7	1.4×10^5 $TCID_{50}$/mL	+	+	+
Cytomegalovirus	4.5×10^4 $TCID_{50}$/mL	+	+	+
Epstein Barr Virus	2.5×10^5 $TCID_{50}$/mL	+	+	+
Herpes Simplex Virus	1.4×10^5 $TCID_{50}$/mL	+	+	+
Human Coronavirus 229E	8.0×10^3 $TCID_{50}$/mL	+	+	+
Human Coronavirus OC43	8.0×10^4 $TCID_{50}$/mL	+	+	+
Human Enterovirus 68	1.0×10^5 $TCID_{50}$/mL	+	+	+
Human Metapneumovirus	7.0×10^3 $TCID_{50}$/mL	+	+	+
Human Parainfluenza Type 1	3.7×10^5 $TCID_{50}$/mL	+	+	+
Human Parainfluenza Type 2	7.5×10^5 $TCID_{50}$/mL	+	+	+
Human Parainfluenza Type 3	4.5×10^5 $TCID_{50}$/mL	+	+	+
Human Rhinovirus Type 1A	8.0×10^5 $TCID_{50}$/mL	+	+	+
Measles	8.0×10^4 $TCID_{50}$/mL	+	+	+
Mumps Virus	8.0×10^4 $TCID_{50}$/mL	+	+	+
Varicella-Zoster Virus	4.4×10^3 $TCID_{50}$/mL	+	+	+
Bordetella pertussis	2.2×10^6 CFU/mL	+	+	+
Candida albicans	4.2×10^6 CFU/mL	+	+	+
Chlamydia pneumoniae	8.0×10^4 $TCID_{50}$/mL	+	+	+
Corynebacterium sp	3.6×10^6 CFU/mL	+	+	+
Escherichia coli	1.9×10^6 CFU/mL	+	+	+
Haemophilus influenzae	2.3×10^6 CFU/mL	+	+	+
Lactobacillus sp	1.9×10^6 CFU/mL	+	+	+
Legionella pneumophila	6.7×10^6 CFU/mL	+	+	+
Moraxella catarrhalis	2.5×10^6 CFU/mL	+	+	+
Mycobacterium tuberculosis	2.8×10^6 copies/mL†	+	+	+
Mycoplasma pneumoniae	2.9×10^6 copies/mL†	+	+	+
Neisseria elongata	2.0×10^6 CFU/mL	+	+	+

Neisseria meningitidis	2.2×10^6 CFU/mL	+	+	+
Pseudomonas aeruginosa	2.3×10^6 CFU/mL	+	+	+
Staphylococcus aureus	2.4×10^6 CFU/mL	+	+	+
Staphylococcus epidermidis	1.9×10^6 CFU/mL	+	+	+
Streptococcus pneumoniae	1.8×10^6 CFU/mL	+	+	+
Streptococcus pyogenes	2.5×10^6 CFU/mL	+	+	+
Streptococcus salivarius	4.3×10^6 CFU/mL	+	+	+
Human Genomic DNA	1.0×10^4 copies/mL	+	+	+

† Testing was performed with genomic DNA due to difficulties in propagation of these bacteria.

G. Interfering Substances

The **cobas®** Influenza A/B & RSV 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, 2 Influenza B strains, and 2 RSV strains at 3x LOD. Results showed that substances at the concentrations tested did not interfere in the detection of Influenza A, Influenza B, and RSV.

Potential Interferent	Active Ingredient	Concentration
Mucin: bovine submaxillary gland, type I-S	Purified mucin protein	5 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. CLIA Waiver Study

As described in Section XI, the accuracy of the **cobas®** Influenza A/B & RSV assay was evaluated at 12 CLIA waived intended use sites. A total of 38 untrained operators representative of intended use operators at CLIA waived sites participated in the study. Prospective nasopharyngeal swab (NPS) specimens were collected from patients with signs and symptoms of respiratory infection in the US during the 2013-2014 and 2014-2015 flu seasons, and were tested prospectively at the study sites. Retrospective NPS specimens were obtained from 2 reference laboratories and were distributed to and tested at 3 of the 12 CLIA waived intended use sites. The retrospective specimens were worked into the daily workload of those sites for testing.

The **cobas®** Influenza A/B & RSV assay results were compared against that from an FDA-cleared laboratory-based multiplexed real-time reverse transcriptase PCR (RT-PCR) test (comparator test). A total of 1642 NPS specimens (1,350 prospective specimens and 292 retrospective specimens) were included in the performance analysis. The study demonstrated positive agreement of 98.4%, 97.9%, and 97.8% for Influenza A, Influenza B and RSV, respectively; and negative agreement of 96.5%, 99.4%, and 98.4% for Influenza A, Influenza B, and RSV, respectively.

Inf A		Comparator Test		
		Positive	Negative	Total
Liat	Positive	248	49[a]	297
	Negative	4	1341	1345
	Total	252	1390	1642

	%	95% CI
Positive Agreement	98.4%	(96.0% - 99.4%)
Negative Agreement	96.5%	(95.4% - 97.3%)

[a] Forty-two **cobas®** Influenza A/B & RSV positive, lab-based RT-PCR negative specimens were tested by PCR/sequencing. Of these, 18 were positive and 24 were negative by PCR/sequencing.

Inf B		Comparator Test		
		Positive	Negative	Total
Liat	Positive	137	9[a]	146
	Negative	3	1493	1496
	Total	140	1502	1642

	%	95% CI
Positive Agreement	97.9%	(93.9% - 99.3%)
Negative Agreement	99.4%	(98.9% - 99.7%)

[a] Six **cobas®** Influenza A/B & RSV positive, lab-based RT-PCR negative specimens were tested by PCR/sequencing. Of these, 5 were positive and 1 was negative by PCR/sequencing.

RSV		Comparator Test		
		Positive	Negative	Total
Liat	Positive	179	23[a]	202
	Negative	4	1436	1440
	Total	183	1459	1642

	%	95% CI
Positive Agreement	97.8%	(94.5% - 99.1%)
Negative Agreement	98.4%	(97.6% - 98.9%)

[a] Twenty-one **cobas®** Influenza A/B & RSV positive, lab-based RT-PCR negative specimens were tested by PCR/sequencing. Of these, 9 were positive and 12 were negative by PCR/sequencing.

Additionally, 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, Influenza B, and RSV weak positive and weak negative concentrations were determined by dilution studies performed by professional operators using an FDA-cleared laboratory-based RT-PCR test. The weak negative sample is defined as the target concentration at which the professional operators of the laboratory-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 laboratory-based RT-PCR test obtained positive results 95-99% of the time. For a given target virus, the expected result for the true negative and the weak negative samples is “Not Detected,” while the expected result for the weak positive samples is “Detected.”

At least 60 weak positive and 60 weak negative samples for each of Influenza A, Influenza B and RSV, along with 90 true negative samples, were distributed equally among 3 CLIA waived sites and tested by intended operators using the **cobas®** Influenza A/B & RSV assay. The **cobas®** Influenza

A/B & RSV assay yielded the expected results for 100% of Influenza A, Influenza B and RSV weak positive samples and true negative samples. For Influenza A, Influenza B and RSV weak negative samples, the **cobas®** Influenza A/B & RSV assay yielded the expected results in 27%, 0% and 3% of samples, respectively. Given that the weak negative samples indeed contain the target viruses, the higher than the expected positive detection rates by the **cobas®** Influenza A/B & RSV assay in the weak negative samples seem to suggest that the **cobas®** Influenza A/B & RSV assay has higher analytical sensitivity than the FDA-cleared laboratory-based RT-PCR test that was used in the characterization of the samples in this study.

Overall, the results demonstrate that CLIA waived site intended operators were able to test true negative, weak negative and weak positive samples using the **cobas®** Influenza A/B & RSV assay and obtain accurate results.

Sample Level	Untrained Intended Operators using the **cobas®** Influenza A/B & RSV assay								
	Inf A Detected	Inf A Not Detected	Inf B Detected	Inf B Not Detected	RSV Detected	RSV Not Detected	Inf A Agreement (95% CI)	Inf B Agreement (95% CI)	RSV Agreement (95% CI)
True Negative	-	91 / 91	-	91 / 91	-	91 / 91	100% (96%-100%)	100% (96%-100%)	100% (96%-100%)
Inf A Weak Negative*	44 / 60	16 / 60	-	60 / 60	-	60 / 60	27% (17%-39%)	100% (94%-100%)	100% (94%-100%)
Inf A Weak Positive*	60 / 60	-	-	60 / 60	-	60 / 60	100% (94%-100%)	100% (94%-100%)	100% (94%-100%)
Inf B Weak Negative*	-	61 / 61	61 / 61	-	-	61 / 61	100% (94%-100%)	0% (0%-6%)	100% (94%-100%)
Inf B Weak Positive*	-	61 / 61	61 / 61	-	-	61 / 61	100% (94%-100%)	100% (94%-100%)	100% (94%-100%)
RSV Weak Negative*	-	60 / 60	-	60 / 60	58 / 60	2 / 60	100% (94%-100%)	100% (94%-100%)	3% (1%-11%)
RSV Weak Positive*	-	60 / 60	-	60 / 60	60 / 60	-	100% (94%-100%)	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.

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.

I. Matrix equivalency – UTM and 0.9% physiological saline

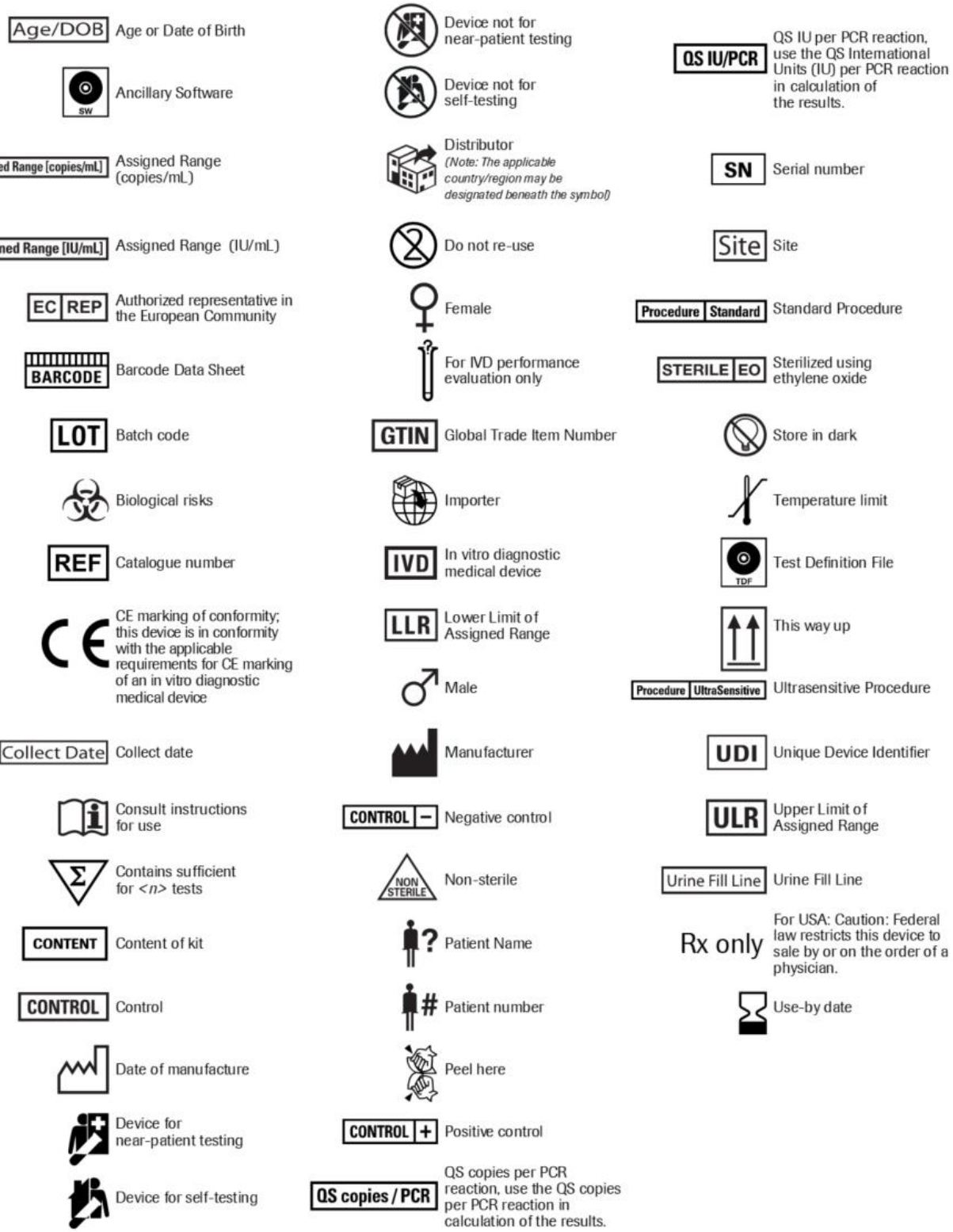
Equivalence between UTM and 0.9% physiological saline was evaluated by spiking cultured viruses (Influenza A, Influenza B and RSV A) at 3x LoD into paired clinical negative nasopharyngeal swab specimens collected in UTM and 0.9% physiological saline using the **cobas®** Influenza A/B & RSV Nucleic acid test for use on **cobas®** Liat® System. For each collection medium, 20 individual contrived low positive samples and 10 negative individual specimens were tested. All low positive

paired samples were positive in both sample matrices. All negative paired samples were negative in both sample matrices.

Specimen	Media	N	Influenza A	Influenza B	RSV A
			% Positive	% Positive	% Positive
Positive	0.9% Physiological Saline	20	100	100	100
	UTM	20	100	100	100
Negative	0.9% Physiological Saline	10	0	0	0
	UTM	10	0	0	0

The **cobas®** Influenza A/B & RSV assay has not been reviewed by FDA for use with 0.9% physiological saline.

III. Table of Symbols



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Document revision

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Doc Rev. 3.0 05/2024	Added information regarding description and use of the **cobas®** Liat® Transfer Pipette Packs (12 pipettes/pack, Cat #09329676001). Removed software version 3.2 references. Moved Rx only text from front page to above legal manufacturer. Updated Trademarks and patents section, including the link. Updated the harmonized symbol page. Please contact your local Roche Representative if you have any questions.