DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
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Silver Spring, MD 20993-0002

July 25, 2016

IQuum, Inc.
David W. Gates, Ph.D.
Senior Director, Regulatory Affairs
4300 Hacienda Drive
Pleasanton, CA 94588

Re: K153544
Trade/Device Name: cobas® Influenza A/B & RSV Nucleic Acid Test for Use on the cobas® Liat System (cobas® Liat Influenza A/B & RSV);
cobas® Influenza A/B & RSV Quality Control Kit
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory Viral Panel Multiplex Nucleic Acid Assay
Regulatory Class: II
Product Code: OCC, OZE, OOI, JJX
Dated: May 31, 2016
Received: June 1, 2016

Dear Dr. Gates:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the

electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Steven R. Gitterman -S

for Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K153544

Device Name

1) cobas® Influenza A/B & RSV Nucleic Acid Test for Use on the cobas® Liat System (cobas® Liat Influenza A/B & RSV); 2) cobas® Influenza A/B & RSV Quality Control Kit

Indications for Use *(Describe)*

1) The cobas® Influenza A/B & RSV Nucleic Acid Test for Use on the cobas® Liat System (cobas® Liat 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 diagnosis and differentiation 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 BSL 3+ facility is available to receive and culture specimens.

2) The cobas® Influenza A/B & RSV Quality Control Kit contains External Controls for use with the cobas® Liat Influenza A/B & RSV assay. External Controls are run during the Add cobas® Liat Influenza A/B & RSV Tube Lot procedure. Additional External Controls should be tested in accordance with local, state, federal and/or accrediting organization requirements as applicable.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(K) SUMMARY

Summary Date: June 16, 2016

A. 510(k) Number: K153544

B. Purpose for Submission:

The purpose of this submission is to request 510k clearance of the cobas® Influenza A/B & RSV Nucleic Acid Test for Use on the cobas® Liat System (cobas® Liat Influenza A/B & RSV) and the cobas® Influenza A/B & RSV Quality Control Kit.

C. Measurand:

The cobas® Influenza A/B & RSV Nucleic Acid Test for Use on the cobas® Liat System is a rapid, automated *in vitro* diagnostic test for the qualitative detection of Influenza A, Influenza B and RSV RNA from nasopharyngeal swab (NPS) specimens from patients with signs and symptoms of respiratory infection.

D. Type of Test:

Multiplex nucleic acid assay for the qualitative detection of Influenza A, Influenza B and RSV RNA from NPS specimens, including nucleic acid isolation and multiplex real-time RT-PCR amplification using the cobas® Liat System.

E. Applicant:

IQuum, Inc. (a wholly-owned subsidiary of Roche Molecular Systems Inc.)
700 Nickerson Road
Marlborough, MA 01752
Tel: 508-229-3200
Fax: 508-970-0119

Contact name: Lingjun Chen
Title: Vice President, Point-of-care Operational Development
Tel: 508-229-3203
Email: lingjun.chen@roche.com

F. Proprietary and Established Names:

1) cobas® Influenza A/B & RSV Nucleic Acid Test for Use on the cobas® Liat System (cobas® Liat Influenza A/B & RSV)
2) cobas® Influenza A/B & RSV Quality Control Kit

G. Regulatory Information:

1. Regulation section:

21 CFR 866.3980, Respiratory Viral Panel Multiplex Nucleic Acid Assay

2. Classification:

Class II

3. Product code:

OCC, OZE, OOI, JJX

4. Panel:

Microbiology (83)

H. Intended Use:

1. Intended use(s):

   1) The cobas® Influenza A/B & RSV Nucleic Acid Test for Use on the cobas® Liat System (cobas® Liat 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 diagnosis and differentiation 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.

   2) The cobas® Influenza A/B & RSV Quality Control Kit contains External Controls for use with the cobas® Liat Influenza A/B & RSV assay. External Controls are run during the

Add cobas® Liat Influenza A/B & RSV Tube Lot procedure. Additional External Controls should be tested in accordance with local, state, federal and/or accrediting organization requirements as applicable.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Requires the cobas® Liat System

I. Device Description:

The cobas® Liat Influenza A/B & RSV Nucleic Acid Test for Use on the cobas® Liat System ("cobas® Liat Influenza A/B & RSV assay") is a rapid, automated *in vitro* diagnostic test for the qualitative detection of influenza A, influenza B, and RSV RNA in nasopharyngeal swab (NPS) specimens eluted in viral transport media.

The assay targets a well-conserved region of the matrix gene of influenza A (Inf A target), the non-structural 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 viruses and to monitor the presence of inhibitors in the sample preparation and RT-PCR.

The assay utilizes a single-use disposable cobas® Liat Tube that holds the sample purification and PCR reagents, and hosts the sample preparation and PCR processes. The cobas® Liat Tube uses a flexible tube as a sample vessel. It contains all required unit dose reagents pre-packed in tube segments, separated by peelable seals, in the order of reagent use.

The cobas® Liat System automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in biological samples. The cobas® Liat System performs all assay steps from clinical sample and reports assay result automatically. During the testing process, multiple sample processing actuators of the cobas® Liat System compress the cobas® Liat Tube to selectively release reagents from tube segments, move the sample from one segment to another, and control reaction volume, temperature, and time to conduct sample preparation, nucleic acid extraction, target enrichment, inhibitor removal, nucleic acid elution and real-time PCR. An embedded microprocessor controls and coordinates the actions of these sample processors to perform all required assay processes within the closed cobas® Liat Tube.

Positive and negative controls are provided in the cobas® Influenza A/B & RSV Quality Control Kit. The positive control comprises inactivated Influenza A, Influenza B and RSV virus in a dried format. The negative control comprises Universal Transport Media (UTM).

To perform the cobas® Liat Influenza A/B & RSV assay, an operator first collects a nasopharyngeal swab and places the swab into UTM. The operator transfers the sample into cobas® Liat Influenza A/B & RSV assay tube using a transfer pipette, and scans the tube barcode to identify the test and the sample barcode to code the sample ID with the assay run on the

cobas® Liat System. The cobas® Liat Tube is then inserted into the cobas® Liat System. The system performs all the test steps and outputs interpreted results (e.g. Influenza A Detected, Influenza B Not Detected, RSV Not Detected) in ~20 minutes. A report of the interpreted results can be viewed on the cobas® Liat System's LCD screen, and printed directly through a USB or network connected printer. No reagent preparation or additional steps are required other than adding the sample to the cobas® Liat Tube. Because all the reagents are contained within the cobas® Liat Tube and no sample or reagent needs to be removed from the tube, cross-contamination between samples is minimized.

The results are interpreted by the cobas® Liat System software from measured fluorescent signals and real time curve recognition algorithm. All possible final test results are described below.

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.
RSV	RSV Not Detected	Negative test for RSV (no RSV RNA detected)
	RSV Detected	Positive test for RSV (RSV RNA present)
	RSV Indeterminate. Repeat Assay.	Presence or absence of RSV cannot be determined. Repeat assay with same sample or, if possible, new sample.
Assay Invalid. Repeat Assay		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		

If the test result is "Indeterminate" or "Invalid", the assay should be repeated with the same patient specimen, or if possible, with a newly collected specimen. Specimens that have repeat "Indeterminate" or "Invalid" results should be sent to a laboratory for confirmatory testing.

If an assay is aborted due to run error, or if an assay is aborted by user, the test should be repeated with the same sample or, if possible, a new sample. Roche Service Representative should be contacted 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.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Hologic Prodesse ProFlu+™

Roche cobas® Influenza A/B Nucleic Acid Test for Use on the cobas® Liat System

2. Predicate 510(k) number(s):

K073029, K081030, K092500, K110968, K132129

K111387

3. Comparison with predicate:

Item Name	Device: cobas® Liat Influenza A/B & RSV	Predicate: cobas® Liat Influenza A/B	Predicate: ProFlu+
Intended Use	The cobas® Influenza A/B & RSV Nucleic Acid Test for Use on the cobas® Liat System 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 diagnosis and differentiation 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	The cobas® Influenza A/B Nucleic Acid Test for Use on the cobas® Liat System 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	The ProFlu+ Assay is a multiplex Real Time RT-PCR *in vitro* diagnostic test for the rapid and qualitative detection and discrimination of Influenza A Virus, Influenza B Virus, and Respiratory Syncytial Virus (RSV) nucleic acids isolated and purified from nasopharyngeal (NP) swab specimens obtained from symptomatic patients. This test is intended for use to aid in the differential diagnosis of Influenza A, Influenza B and RSV viral infections in humans and is not intended to detect Influenza C. Negative results do not preclude Influenza or RSV virus infection and should not be used as the sole basis for treatment or other 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. The use of additional laboratory testing and clinical presentation must be considered in order to obtain the final diagnosis of respiratory viral

Item Name	Device: cobas® Liat Influenza A/B & RSV	Predicate: cobas® Liat Influenza A/B	Predicate: ProFlu+
	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.	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.	infection. Performance characteristics for Influenza A Virus were established when Influenza A/H3 and A/H1 were the predominant Influenza A viruses in circulation (2006-2007 respiratory season). Performance characteristics for Influenza A were confirmed when Influenza A/H1, Influenza A/H3, and Influenza A/2009 H1N1 were the predominant Influenza A viruses in circulation (2008 and 2009). When other Influenza A viruses are emerging, performance characteristics may vary. If infections 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 BSL 3+ facility is available to receive and culture specimens.

Item Name	Device: cobas® Liat Influenza A/B & RSV	Predicate: cobas® Liat Influenza A/B	Predicate: ProFlu+
Regulation	21 CFR 866.3980	(same)	(same)
Product Code	OCC	(same)	(same)
Assay Target	Influenza A Influenza B RSV	Influenza A Influenza B	Influenza A Influenza B RSV
Sample Type	Nasopharyngeal Swab	(same)	(same)
Internal Control	Yes for sample preparation and RT-PCR performance using encapsulated RNA	(same)	Yes for RT-PCR performance only using *in vitro* transcribed RNA. Extraction control is recommended but not provided.
Influenza A Viral Target	Well conserved region of the matrix gene	(same)	(same)
Influenza B Viral Target	Well conserved region of the non-structural protein (NSP) gene	(same)	(same)
RSV Viral Target	Well conserved region of the matrix (M) gene	N/A	Polymerase gene
Extraction Method	Integrated silica-magnetic bead-based nucleic acid extraction	(same)	Silica-magnetic bead-based nucleic acid extraction using Roche MagNA Pure LC System, or bioMérieux NucliSENS easyMag
Assay Method	RT-PCR for detecting the presence / absence of viral RNA in clinical specimens	(same)	(same)
Detection Technique	Multiplex assay using different reporter dyes for each target	(same)	(same)

Item Name	Device: cobas® Liat Influenza A/B & RSV	Predicate: cobas® Liat Influenza A/B	Predicate: ProFlu+
Assay Result	Qualitative	(same)	(same)
Assay Instrument	cobas® Liat System	(same)	Roche MagNA Pure LC System, or bioMérieux NucliSENS easyMag Cepheid SmartCycler II Real Time Instrument Laboratory equipment, e.g. pipettors, centrifuge, vortex, cold block, biosafety cabinet
Self Contained System Assay	Yes, integrated PC, software, and touch-screen display	(same)	No, different instruments, and external PC computers required
All Assay Reagents Contained in Disposable	Yes, no manual reagent addition required	(same)	No, multiple manual reagent handling and preparation steps required
Sample Volume Detection	Yes, automatically checks that input sample volume exceeds lower limit	(same)	No
Automated Assay	Yes, sample preparation, amplification, detection, and result interpretation	(same)	No, multiple manual steps required
Error Diagnostic System	Yes, monitors and records system parameters for error recovery or assay abort if unrecoverable	(same)	No
PCR Curve Pattern Recognition	Yes, ensures abnormal PCR curves are called “Invalid” or “Indeterminate”	(same)	No

Item Name	Device: cobas® Liat Influenza A/B & RSV	Predicate: cobas® Liat Influenza A/B	Predicate: ProFlu+
Automated Result Interpretation	Yes, results reported as "Detected" or "Not Detected" for each virus	(same)	No, manual result interpretation
User	Hospital nurse and CLIA moderate complexity laboratory technologist. Nurses and medical assistants at emergency rooms, urgent care clinics, and outpatient clinics, including physician's offices.	(same)	High complexity laboratory technologist
Test Availability	Random access, on-demand test	(same)	Batch processing
Time-to-result	~20 minutes	(same)	≥4 hours

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

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. Docket number FDA-2008-D-0095.

L. Test Principle:

The cobas® Liat Influenza A/B & RSV assay uses established nucleic acid test chemistry and assay protocol for viral RNA detection. The sample preparation methodology is based on chaotropic agent-based lysis and magnetic particle based nucleic acid purification. First, the NPS sample is diluted and mixed with an internal process control (IPC) comprising an encapsulated RNA. Chaotropic and proteolytic reagent then disrupts the three dimensional structure in macromolecules such as proteins and nucleic acids in the sample, and denatures them. Second, nucleic acids are isolated from lysates through binding to the surface of silica magnetic beads in the presence of a chaotropic salt, which removes water from hydrated molecules in solution. Third, the beads are separated from the lysates using a magnetic field, and the lysate removed. Fourth, the beads with captured nucleic acids are washed to remove possible inhibitors in the sample. Finally, the captured nucleic acids are eluted under low-salt conditions into a small volume of elution buffer.

Target amplification and detection uses TaqMan-probe based real-time polymerase chain reaction (RT-PCR). The Inf A primer and probe set is designed to specifically detect the matrix RNA from Influenza A virus. The Inf B primer and probe set is designed to specifically detect the non-structural protein (NSP) RNA from Influenza B viruses. The RSV primer and probe set is designed to specifically detect the matrix RNA from RSV. An IPC primer and probe set is also included to amplify the target region of the encapsulated RNA internal control.

Eluted viral RNA is first transcribed into cDNA using reverse transcriptase. This cDNA then undergoes a polymerase chain reaction (PCR) where the reaction mixture is repeatedly heated to denature the nucleic acid, and cooled to allow annealing of primers and extension of annealed primers by DNA polymerase to logarithmically amplify the specific region of the cDNA. Dual-labeled fluorogenic hydrolysis (TaqMan) probes anneal to the specific target sequences located between the binding regions of forward and reverse primers. During the extension phase of the PCR cycle, the 5′ nuclease activity of the polymerase degrades the probes, causing the reporter dyes to separate from the quenchers, thus generating fluorescent signals. Fluorescence intensities are monitored at each PCR cycle. When the fluorescence intensities exceed pre-determined thresholds, cycle threshold (Ct) values are returned for the specific analyte corresponding to the fluorescence channel.

All these sample preparation and real-time PCR amplification processes are conducted in a closed cobas® Liat Tube in ~20 minutes.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility study assesses the total variability of the cobas® Liat Influenza A/B & RSV assay across operators, study sites, testing days, cobas® Liat Analyzers, and cobas® Liat Influenza A/B & RSV Assay Tube lots. The cobas® Liat Influenza A/B & RSV assay was evaluated at 3 sites. Two (2) 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 (9) cobas® Liat Analyzer and 3 cobas® Liat Influenza A/B & RSV Assay Tube lots were used. The reproducibility panel comprises a high negative (C_5), a low positive (C_{95}), and a medium positive (C_{100}) 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**	**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 (C5)	29 / 30	37.0	-	30 / 30	-	-	29 / 30	35.7	-	88 / 90 (97.8%)	92.3% - 99.4%
Flu A Low Positive (C95)	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 (C100)	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 (C5)	30 / 30	-	-	31 / 31	-	-	30 / 30	-	-	91 / 91 (100.0%)	96.0% - 100.0%
Flu B Low Positive (C95)	30 / 30	-	-	30 / 30	-	-	29 / 29[†]	-	-	89 / 89 (100.0%)	95.9% - 100.0%
Flu B Moderate Positive (C100)	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
RSV High Negative (C5)	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
RSV Low Positive (C95)	30 / 30	-	-	31 / 31	-	-	30 / 30	-	-	91 / 91 (100.0%)	96.0% - 100.0%
RSV Moderate Positive (C100)	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%

[†] 1 of 30 Flu B Low Positive (C95) replicates yielded an “Assay Invalid. Repeat Assay.” result, and was not repeated.

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 (C5)	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Flu A Low Positive (C95)	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Flu A Moderate Positive (C100)	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Flu B High Negative (C5)	29 / 30	35.1	-	31 / 31	-	-	30 / 30	-	-	90 / 91 (98.9%)	94.0% - 99.8%
Flu B Low Positive (C95)	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 (C100)	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 (C5)	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
RSV Low Positive (C95)	30 / 30	-	-	31 / 31	-	-	30 / 30	-	-	91 / 91 (100.0%)	96.0% - 100.0%
RSV Moderate Positive (C100)	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%

[†] 1 of 30 Flu B Low Positive (C95) replicates yielded an “Assay Invalid. Repeat Assay.” result, and was not repeated.

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 (C5)	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Flu A Low Positive (C95)	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Flu A Moderate Positive (C100)	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
Flu B High Negative (C5)	30 / 30	-	-	31 / 31	-	-	30 / 30	-	-	91 / 91 (100.0%)	96.0% - 100.0%
Flu B Low Positive (C95)	30 / 30	-	-	30 / 30	-	-	29 / 29[†]	-	-	89 / 89 (100.0%)	95.9% - 100.0%
Flu B Moderate Positive (C100)	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
RSV High Negative (C5)	30 / 30	-	-	30 / 30	-	-	30 / 30	-	-	90 / 90 (100.0%)	95.9% - 100.0%
RSV Low Positive (C95)	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 (C100)	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%

[†] 1 of 30 Flu B Low Positive (C95) replicates yielded an “Assay Invalid. Repeat Assay.” result, and was not repeated.

b. Linearity/assay reportable range:

Not Applicable

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

The cobas® Liat Influenza A/B & RSV assay has 3 controls: (1) internal process control, (2) positive control and (3) negative control.

Internal Process Control

The internal process control (IPC) comprises an encapsulated RNA that is pre-packed in each cobas® Liat Tube. When conducting an assay, it is first mixed with sample and then goes through all the test processes to monitor both the sample preparation and RT-PCR performance. The IPC RNA is detected in a separate channel by IPC specific primers and probe. If IPC Ct does not meet the acceptance criteria and Inf A, Inf B, and RSV are not detected, the assay run is called "Invalid" to avoid false negative results due to excessive sample inhibition or system operation outside the normal range.

Positive Control

Positive control is provided in the cobas® Influenza A/B & RSV Assay Quality Control Kit. The positive control comprises a pooled sample of inactivated Influenza A, Influenza B and RSV in a dried format. The positive control is designed to be detected at near LOD Ct using the cobas® Liat Influenza A/B & RSV assay.

To use the positive control, an operator transfers a unit dose of UTM from the Dilution UTM tube into the Positive Control tube using an included transfer pipette to dissolve and mix the dried positive control. The entire mixture is then transfer into the cobas® Liat Tube, and the cobas® Liat Tube is run on a cobas® Liat System according to the Instructions for Use.

The positive control is required to be run during the "Add Liat Tube Lot" process, in which the cobas® Liat tube lot and end user site procedures are checked at the end user site. Additional positive control runs may be performed by the end-user to confirm the performance of a cobas® Liat System and a cobas® Liat Tube lot through detection of Influenza A, Influenza B and RSV targets, or as required by the end user's quality control standards.

Negative Control

Negative control is provided in the cobas® Influenza A/B & RSV Assay Quality Control Kit. The negative control comprises UTM. The solution is provided in unit dose quantity and labeled as Dilution UTM.

To use the negative control, an operator transfers the entire contents of the Dilution UTM tube into the cobas® Liat Tube using a transfer pipette and runs the assay following the Instructions for Use.

The negative control is required to be run during the "Add Liat Tube Lot" process, in which potential contamination and end user site procedures are checked at the end user site. Additional negative control runs may be performed by the end-user to check if there is contamination resulting in a false positive result, or as required by the end user's quality control standards.

d. Detection limit:

The Limit of Detection (LOD) of the cobas® Liat 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® Liat 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}

e. Analytical specificity (reactivity):

The reactivity study evaluates the ability to detect Influenza and RSV strains representing temporal and geographical diversity. The cobas® Liat 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 strains, 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® Liat 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	+	–	–

Virus Strain	Type / Subtype	Test Concentration	Inf A Result	Inf B Result	RSV Result
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	5.0×10^{-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	1.0×10^{0} $TCID_{50}$/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	–	–	+

f. Analytical specificity (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® Liat Influenza A/B & RSV assay was evaluated against a panel comprising human genomic DNA and 35 microorganisms. Bacteria and *Candida albicans* were tested at $\geq 10^6$ CFU/mL. Viruses were tested at $\geq 10^5$ $TCID_{50}$/mL, or the highest available concentration. The cobas® Liat 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†	–	–	–

Microorganism	Test Concentration	Inf A Result	Inf B Result	RSV Result
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.

g. 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® Liat 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 $\geq 10^6$ CFU/mL, and viruses were tested at $\geq 10^5$ $TCID_{50}$/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[†]	+	+	+

Microorganism	Test Concentration	1 Flu A, 1 Flu B &1 RSV strain at 3x LOD		
		Inf A Result	Inf B Result	RSV Result
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.

h. Interfering substances

The cobas® Liat 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

i. Carry-over/Cross-contamination:

A study was conducted to demonstrate that the single-use, self-contained cobas® Liat assay tube is not susceptible to carry-over contamination when alternating high positive and negative samples are tested. High positive samples comprised an influenza A virus spiked into negative nasopharyngeal swab in UTM matrix at a concentration greater than that expected in 95% or more of specimens of diseased patients in the intended use population. Negative samples comprised negative nasopharyngeal swab matrix. Twenty (20) high positive and 20 negative samples were tested on each of 2 cobas® Liat Systems (i.e. 40 high positive and 40 negative samples in total). High positive and negative samples were run alternating analyzer-to-analyzer and run-to-run. All 40 high positive samples tested were correctly reported as “Influenza A Detected”. All 40 negative samples tested were correctly reported as “Influenza A Not Detected”. There was no carry-over or cross contamination observed during this study.

j. Performance using Fresh vs. Frozen Samples:

The cobas® Liat Influenza A/B & RSV assay was tested by comparing its performance using fresh and frozen specimens. One (1) influenza A, 1 influenza B, and 1 RSV strain was individually spiked into nasopharyngeal swab in UTM matrix at different viral loads, including levels near the LOD and levels reflecting the clinical range. For each strain, at least 60 samples were tested immediately while at least another 60 samples were frozen at -80°C for 7 days, thawed and then tested. Ten (10) negative samples were also tested fresh and after being frozen at -80°C for 7 days. Fresh and frozen samples demonstrated 100% agreement with the expected result, demonstrating that the cobas® Liat Influenza A/B & RSV assay had equivalent performance for fresh and frozen samples.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

The cobas® Liat Influenza A/B & RSV assay was evaluated at 12 CLIA waived healthcare facilities. 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 two 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

Each patient's specimen was tested by the cobas® Liat Influenza A/B & RSV and an FDA-cleared laboratory-based multiplexed real-time reverse transcriptase PCR (RT-PCR) test (comparator test). The cobas® Liat Influenza A/B & RSV assay results were compared against that 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 cobas® Liat and the comparator tests, respectively. As such, a total of 1,350 prospective NPS specimens were included in the performance analysis. Compared to the comparator test, the cobas® Liat 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] 41 Liat positive, comparator 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] 6 Liat positive, comparator 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	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] 15 Liat positive, comparator 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 cobas® Liat and the comparator tests, respectively. As such, a total of 292 retrospective NPS specimens were included in the performance analysis. Compared to the comparator test, the cobas® Liat 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] 1 Liat positive, comparator negative specimen was tested by PCR/sequencing. This 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] 6 Liat positive, comparator negative specimens were tested by PCR/sequencing. Of these, all 6 were positive by PCR/sequencing.

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

In the cobas® Liat 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® Liat Influenza A/B & RSV assay, are presented in the tables below:

Influenza A Positives by the cobas® Liat Influenza A/B & RSV Assay per Patient Age Group - 2013 to 2014 Influenza Season

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%

Influenza B Positives by cobas® Liat Influenza A/B & RSV Assay per Patient Age Group - 2013 to 2014 Influenza Season

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%

Table 24: RSV Positives by cobas® Liat Influenza A/B & RSV Assay per Patient Age Group - 2013 to 2014 Influenza Season

Age Group	Number of Nasopharyngeal Swab Specimens	Number of Influenza B Positives	Influenza B 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® Liat Influenza A/B & RSV assay, are presented in the tables below:

Influenza A Positives by the cobas® Liat Influenza A/B & RSV Assay per Patient Age Group - 2014 to 2015 Influenza Season

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%

Influenza B Positives by cobas® Liat Influenza A/B & RSV Assay per Patient Age Group - 2014 to 2015 Influenza Season

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%

RSV Positives by cobas® Liat Influenza A/B & RSV Assay per Patient Age Group - 2014 to 2015 Influenza Season

Age Group	Number of Nasopharyngeal Swab Specimens	Number of Influenza B Positives	Influenza B 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%

N. Instrument Name:

cobas® Liat System

O. System Descriptions:

1. Modes of Operation:

The cobas® Liat System functions as a point-of-care platform with sample-to-answer capabilities in which all sample processing steps as well as detection are carried out using a single-use, disposable cobas® Liat Tube.

2. Software:

Applicant's Hazard Analysis and software development processes for this line of product types has been provided:

Yes _X_ or No _____

3. Specimen Identification:

A sample barcode is scanned or a sample ID entered into the cobas® Liat System during the assay run process. After scanning the sample barcode, the corresponding sample is loaded directly into a cobas® Liat Tube using a transfer pipette. After the tube is capped, the tube barcodes is scanned by the cobas® Liat System and the tube is inserted into the system to start the test.

4. Specimen Sampling and Handling:

Specimen sampling and handling during the assay is controlled automatically using multiple sample processing modules contained within the cobas® Liat System. The sample processing modules are composed of two assemblies, a moving side assembly comprised of multiple sample processing plungers and clamps and a fixed side assembly. When performing an assay, a cobas® Liat Tube is inserted into the tube slot of a cobas® Liat System. The plungers and clamps selectively compress the cobas® Liat Tube segments against the fixed side assembly to release reagents from the segments, move the sample from one segment to another, and control reaction conditions.

5. Calibration:

Not required. The cobas® Liat Tube is single use and part of a closed system.

6. Quality Control:

An internal process control used in conjunction with procedural checks monitors instrument functionality, performance, fluidics, and result determination based on a pre-defined decision algorithm.

P. Conclusion:

The submitted information in this premarket notification is complete and demonstrates that the cobas® Liat Influenza A/B & RSV assay is substantially equivalent to the predicate devices.