510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

k113323

B. Purpose for Submission:

New device

C. Measurand:

Influenza A and Influenza B RNA from nasopharyngeal swabs

D. Type of Test:

Qualitative real-time reverse transcription-polymerase chain reaction (RT-PCR) for detection of Influenza A and B RNA from nasopharyngeal swab specimens.

E. Applicant:

QIAGEN GmbH

F. Proprietary and Established Names:

artus® Infl A/B RG RT-PCR Kit

G. Regulatory Information:

1. Regulation section:

   866.3980

2. Classification:

   Class II

3. Product codes:

   OCC, OOI, JJH

4. Panel:

   Microbiology

H. Intended Use:

1. Intended use(s):

The *artus*® Infl A/B RG RT-PCR Kit is a multiplex real time PCR *in vitro* diagnostic test for the qualitative detection and identification of Influenza A and Influenza B virus RNA in nasopharyngeal swab specimens using the Rotor-Gene® Q MDx instrument. The test is intended for use as an aid in the differential diagnosis of Influenza A and Influenza B viral infections in patients symptomatic for respiratory tract infection in conjunction with clinical and epidemiological risk factors. It is not intended to detect Influenza C virus.

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

Performance characteristics for Influenza A were established during the 2009/2010 and 2010/2011 flu seasons when Influenza A (H3N2) and Influenza A/2009 (H1N1) were the predominant Influenza A viruses in circulation. When other Influenza A viruses emerge, 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 departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

Not Applicable

4. Special instrument requirements:

Qiagen Rotor-Gene® Q MDx instrument with 72-well rotor

Qiagen EZ1 Advanced

I. Device Description:

The *artus* Infl A/B RG RT-PCR Kit contains reagents and instructions for the detection and differentiation of Influenza A and Influenza B viral RNA in nasopharyngeal swabs of symptomatic patients.

The assay utilizes the EZ1 DSP Virus Kit (QIAGEN) and the EZ1 Advanced XL instrument with the EZ1 Advanced XL DSP Virus Card v. 1.0 for viral nucleic acid extraction. The *artus* Infl A/B RG RT-PCR Kit in conjunction with the Rotor-Gene Q MDx instrument (with 72-well rotor) and the Influenza Assay Package v. 1.0.7 (QIAGEN) is used for amplification and detection.

Pathogen detection by the reverse transcription-polymerase chain reaction (RT-PCR) is based on the reverse transcription of the RNA into complementary DNA. In real-time PCR the amplified product is detected via fluorescent dyes. These are linked to oligonucleotides that bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run (i.e., in real time) allows the detection of the accumulating product without having to re-open the reaction tubes after the PCR run.

The *artus* Infl A/B RG RT-PCR procedure is briefly described below:

1. Sample collection: Collect nasopharyngeal swab specimens from symptomatic patients using a polyester, nylon, or rayon swab and place it into virus transport medium.

2. Nucleic acid extraction: Add the Influenza A/B internal control (IC) to the carrier RNA before starting the extraction procedure. Extract viral RNA using the EZ1 DSP Virus Kit in combination with the EZ1 Advanced XL instrument. The IC is added by the EZ1 Advanced to each sample during extraction.

3. Real-time RT-PCR: Add the extracted RNA and positive and negative control material to Influenza A/B Master mix. Perform real time RT-PCR using the Rotor-Gene Q MDx instrument.

4. Result interpretation: The Influenza Assay Package evaluates the results of the positive and negative controls to determine if the run is valid. If the run is valid, the internal control and target-specific results of each specimen are evaluated.

Interpretation of Control Results

The Rotor-Gene Q software determines whether control results are valid or invalid and therefore whether the run is valid or invalid.

Conditions required for a valid run

Name	Influenza A result	Influenza B result	Status
Inf A Co	Valid	–	Valid
Inf B Co	–	Valid	Valid
NTC	–	–	Valid

Invalid runs

If the Influenza A Control (Inf A Co), Influenza B Control (Inf B Co), or no template control (NTC) result is determined to be invalid, the software will provide a flag/warning message. If the run is invalid due to failure of the Influenza A Control, Influenza B Control, or no template control, all samples in that run must be retested using remaining purified nucleic acids.

Interpretation of Process Control Results

For each Process Control the Rotor-Gene Q software indicates the results for influenza A and influenza B (RNA detected or RNA not detected).

If the expected results for one or more of the Process Controls are not met the run is considered invalid and all the samples in that PCR run must be extracted again and retested.

Interpretation of Specimen Results

For each sample in a valid run, the Rotor-Gene Q software indicates the status of the analysis (valid or invalid) and the results for influenza A and influenza B (RNA detected or RNA not detected). A description of the sample results provided by the Rotor-Gene Q software is provided in the table below.

Sample results determined by the Rotor-Gene Q software

Name	Flag/ warning	Influenza A result	Influenza B result	Status	Interpretation of result
Sample ID	_	RNA detected	RNA not detected	Valid	Influenza A viral RNA detected
Sample ID	_	RNA not detected	RNA detected	Valid	Influenza B viral RNA detected
Sample ID	_	RNA not detected	RNA not detected	Valid	Influenza viral RNA not detected
Sample ID	_	RNA detected	RNA detected	Valid	Influenza A viral RNA and influenza B viral RNA detected*
Sample ID	SAMPLE IC FAIL	Invalid	Invalid	Invalid	Not determined: signal in the Control channel is out of specification range.
Sample ID	InfA_ SAMPLE_ EARLY_CT	Invalid	Invalid	Invalid	Not determined: Influenza A test channel failed.
Sample ID	INFA_ SAMPLE_ INVALID_ DATA	Invalid	Invalid	Invalid	Not determined: influenza A test channel failed, caused by double intersection.**
Sample ID	InfB_ SAMPLE_ EARLY_CT	Invalid	Invalid	Invalid	Not determined: influenza B test channel failed.
Sample ID	INFB_ SAMPLE_ INVALID_ DATA	Invalid	Invalid	Invalid	Not determined: influenza B test channel failed, caused by double intersection.**

*Dual infections with Influenza A and B are rare. For a sample with a dual infection test result, the sample needs to be retested

** Amplification curve crosses the threshold twice.

Retesting invalid samples

Samples with invalid results must be re-extracted and retested.

Materials Provided

The contents of the *artus* Infl A/B RG RT-PCR Kit are sufficient for 96 tests in two to four PCR runs on the Rotor-Gene Q MDx. The Rotor-Gene Q MDx rotor holds up to 72 reaction tubes.

Kit contents

artus Infl A/B RG RT-PCR Kit			(96)
Catalog no.			4524245
Number of reactions			96
Blue	Influenza A/B Master		8 x 174 µl
Yellow	Influenza Mg-Sol*	Mg-Sol**	600 µl
Red	Influenza A Control		200 µl
Brown	Influenza B Control		200 µl
Green	Influenza A/B IC†	IC**	2 x 1000 µl
White	Water (PCR grade)		1000 µl
	Instructions For Use (Handbook)		1

*Magnesium solution.

† Internal control.

** See page **Error! Bookmark not defined.** for symbols list with definitions

Materials Required but Not Provided

For RNA purification

Reagents

- EZ1 DSP Virus Kit (cat. no. 62724), version 4

Equipment

- EZ1 Advanced XL instrument (cat. no. 9001492)
- EZ1 Advanced XL DSP Virus Card v1.0 or higher, with firmware 1.0.1 and protocol “DSP Virus version 1.0” or higher (cat. no. 9018703)

For PCR

- Consumables
- Pipets (adjustable)
- Sterile, RNase-free pipet tips with filters
- Strip Tubes and Caps, 0.1 ml, for use with 72-well rotor (cat. no. 981103 or 981106)

Equipment

- Vortex mixer
- Laboratory timer
- Benchtop centrifuge with rotor for 2 ml reaction tubes
- Rotor-Gene Q MDx instrument with 72-well rotor (cat. no. 9002035)
- Rotor-Gene Q Software version 2.1.0 or higher
- Influenza Assay Package 1.0.7 or higher
- Cooling block (Loading Block 72 x 0.1 ml Tubes, cat. no. 9018901)

Primer / probe sets in the *artus* Infl A/B RG RT-PCR Kit

Component	Target	Target Gene	Probe Fluorophore	Detection Range (nm)
Influenza A/B Master	Influenza A Virus	Matrix	Fam	510 +/- 5
	Influenza B Virus	Matrix	IRD700	712 +/- 5
	Internal Control	Synthetic sequence	Texas Red	610 +/- 5

J. Substantial Equivalence Information:

1. Predicate device name(s):
   ProFlu+ Assay

2. Predicate 510(k) number(s):
   k110968

3. Comparison with predicate:

Name	***artus* Infl A/B RG RT-PCR Kit**	**ProFlu+ Assay**
510(k) No.	k113323	k110968
Regulation	866.3980	866.3980
Product Code	OCC	OCC
Device Class	II	II
Similarities		

Intended Use	The *artus*® Infl A/B RG RT-PCR Kit is a multiplex real time PCR *in vitro* diagnostic test for the qualitative detection and identification of Influenza A and Influenza B virus RNA in nasopharyngeal swab specimens using the Rotor-Gene® Q MDx instrument. The test is intended for use as an aid in the differential diagnosis of Influenza A and Influenza B viral infections in patients symptomatic for respiratory tract infection in conjunction with clinical and epidemiological risk factors. It is not intended to detect Influenza C virus. Negative results do not preclude respiratory virus infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Performance characteristics for Influenza A were established during the 2009/2010 and 2010/2011 flu seasons when Influenza A (H3N2) and Influenza A/2009 (H1N1) were the predominant Influenza A viruses in circulation. When other Influenza A viruses emerge, 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 departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.	The ProFlu™ + Assay is a multiplex Real-Time PCR (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 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/Hl, 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 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.
Specimen Type	Nasopharyngeal swab	Nasopharyngeal swab
Assay Targets	Influenza A, Influenza B	Influenza A, Influenza B, RSV
Amplification and Detection Technology	Multiplex real-time PCR	Multiplex real-time PCR
Assay Controls	Influenza A Control, Influenza B Control, Influenza A/B Internal Control, each prepared from *in vitro* transcripts.	Influenza A RNA Control, Influenza B RNA Control, RSV A RNA Control, RSV B RNA Control and Internal RNA Control, each prepared from *in vitro* transcripts.
Influenza A Virus Target	Matrix gene	Matrix gene
Differences		
Influenza B Virus Target	Matrix	Non-structural NS1 and NS2
Nucleic Acid Extraction	Automated extraction using the EZ1 DSP Virus Kit with the EZ1 Advanced XL instrument	Automated extraction using the Roche MagNA Pure LC System wih the MagNA Pure Total Nucleic Acid Isolation Kit or the bioMerieux NucliSENS easyMAG System with the Automated Magnetic Extraction Reagents.
Amplification and Detection Instrument System	Rotor-Gene® Q MDx	Cepheid SmartCycler® II

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

Not Applicable

L. Test Principle:

The real-time PCR process simultaneously amplifies and detects nucleic acid targets in a single closed-tube reaction. Detection of Influenza RNA and Internal Control (IC) is based on three processes: nucleic acid isolation, reverse transcription, and real time PCR amplification/detection. Human respiratory specimens (nasopharyngeal swabs) from symptomatic patients are processed initially to isolate and purify viral nucleic acid from the cellular specimen matrix. After initial reverse transcription of RNA into complementary DNA (cDNA), amplification proceeds during which the probe anneals specifically to a region of the template between the forward and reverse primers. As primer extension and amplification occurs, the exonuclease activity of the Taq polymerase cleaves the probe separating the reporter dye away from the quencher. This generates an increase in fluorescent signal upon excitation from a light source of appropriate wavelength. With each cycle, additional reporter dye molecules are cleaved from their respective probes, yielding increased fluorescence signal. The amount of fluorescence at any given cycle is dependent on the amount of PCR product (amplicons) present at that time. Fluorescent intensity is monitored at each PCR cycle by fluorescent detection modules within the real-time instrument.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   The reproducibility of the artus Infl A/B RG RT-PCR test was evaluated using 3 investigational sites. A panel of 10 simulated specimens was provided for testing. Five of the specimens contained influenza A and the other 5 specimens contained influenza B. Each half of the panel included duplicate low-positive and moderate positive test samples. The test panel samples were prepared from whole virus. Influenza A test samples were made using Influenza A/California/7/09-like virus and the Influenza B test samples were made using Influenza B/Florida/4/2006-like virus. The virus stocks were diluted in influenza-negative clinical sample matrix that was comprised of pooled, residual, de-identified nasopharyngeal swab specimens. Low positive specimens where diluted to 2X the LoD, Moderate positives were made to 10X the LoD. Influenza A and Influenza B negative samples represent dilution levels that are 0.001x LoD.

   The 10-member panel plus 3 controls were tested by 2 different technologists each day for 6 days. The overall percent agreement for the *artus* Infl A/B RG RT-PCR test is summarized below.

Panel member	Agreement with expected result	Average C_T	CV%	95% confidence interval
Influenza A Neg	33/36 (91.7%)	29.76	2.2	78–97%
Influenza A Low Pos	72/72 (100%)	31.37	1.1	95–100%
Influenza A Mod Pos	72/72 (100%)	29.23	1.5	95–100%
Influenza B Neg	36/36 (100%)	29.78	2.1	90–100%
Influenza B Low Pos	72/72 (100%)	30.42	1.2	95–100%
Influenza B Mod Pos	72/72 (100%)	28.32	1.2	95–100%
Influenza A Control	36/36 (100%)	34.25	2.2	90–100%
Influenza B Control	36/36 (100%)	31.14	2.3	90–100%
Negative control	36/36 (100%)	27.42	1.8	90–100%

The average Ct for the negative samples is based on the IC.

b. Linearity/assay reportable range:

Not Applicable

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

<u>*Controls*</u>

Controls provided with the artus Infl A/B RG RT-PCR Kit:

<u>Internal Control</u>

The Influenza A/B Internal Control (IC) is a 1280 bp *in vitro* transcript of an artificial sequence with no homologies to influenza sequences. It is provided with the *artus* Infl A/B RG RT-PCR Kit. The IC is added to each sample by the EZ1 Advanced XL during the nucleic acid extraction process and to the control at PCR set-up by the user.

For the negative control (No Template) and the positive controls (Influenza A Control and Influenza B Control): The IC is added to the Influenza A/B Master mix aliquot that is prepared specifically for use with Controls. (Separate Influenza A/B Master mixes are prepared for Controls and specimens; the Master mix for samples does not contain IC because it is added to each sample prior to RNA extraction.)

In the No Template Control, a result for the IC that is outside of the acceptance ranges identifies a failure of the PCR while a result within the acceptance range indicates that the IC primers and probes are performing as expected. In influenza-negative samples, the Influenza A/B Internal Control identifies failure of the RNA extraction process or inhibition of the PCR reaction.

Positive Controls

Two positive controls, the Influenza A Control and the Influenza B Control, are provided with the *artus* Infl A/B RG RT-PCR Kit. The positive controls are added in place of extracted sample RNA to individual PCR reaction tubes containing Influenza A/B Master mix and Internal Control. The positive controls identify failures of the PCR caused by reagent problems, PCR set-up error, or failure of the Rotor-Gene Q MDx instrument. A result within the acceptance range for both controls indicates the assay is functioning as intended. Both controls must generate a valid result in order for the run to be reported as valid by the *artus* Influenza software. (The Internal Control Channel is not evaluated for the Positive Controls.)

The Influenza A Control is an *in vitro* transcript (IVT) corresponding to the amplicon generated with the Influenza A primers. The IVT is present at a concentration three times the limit of detection of the *artus* Infl A/B RG RT-PCR Kit.

The Influenza B Control is an IVT corresponding to the amplicon generated with the Influenza B primers. The IVT is present at a concentration five times the limit of detection of the *artus* Infl A/B RG RT-PCR Kit.

Negative (No Template) Control

PCR grade water is provided with the *artus* Infl A/B RG RT-PCR Kit and serves as the No Template control for the Influenza A/B Master. It is added in place of extracted sample RNA to an individual reaction tube containing the Influenza A/B Master mix with Internal Control. The No Template control result identifies possible sample-to-sample carryover or contamination of the master mix with target nucleic acid.

Controls not provided with the *artus* Infl A/B RG RT-PCR Kit:

Process Controls

The package insert for the *artus* Infl A/B RG RT-PCR Kit recommends to include influenza A positive, influenza B positive, and influenza negative Process Controls to be used starting from the extraction step. Since these controls are intended to mimic patient samples, characterized clinical samples can be used for this purpose.

Sample Stability

The stability of nasopharyngeal swab specimens, pre and post nucleic acid purification, was evaluated by testing simulated clinical samples stored at conditions reflecting the storage and handling instructions in the package insert of the artus Infl A/B RG RT-PCR Kit. The stability of nasopharyngeal swab specimens stored at -65 to -90°C (-70°C) was also demonstrated in the clinical study using prospectively collected specimens.

Simulated clinical samples were prepared by spiking cultured influenza virus strains A/California/7/09-like or B/Florida/4/2006-like into clinical sample matrix. Each virus strain was present at a concentration of three times the Limit of Detection (LoD) of the artus Infl A/B RG RT-PCR assay.

The study evaluated routine storage conditions for pre-processed samples and purified nucleic acid samples. Additionally, the impact of leaving the purified nucleic acid on the EZ1 Advanced XL for up to three hours was evaluated. Although this is outside of routine storage conditions (short term at 2 – 8°C or long term storage at -65 to -90°C). Prolonged storage of purified samples on the EZ1 was also evaluated as it is reasonable to anticipate that a user will occasionally not immediately retrieve the purified nucleic acids at the conclusion of the EZ1 Advanced XL run.

For evaluation of pre-processed samples, 3 ml of each simulated sample was placed in an empty Copan vial along with a nasopharyngeal swab and stored at the designated temperature condition. Purified nucleic acid eluates (60µl) were stored in the elution vials provided with the EZ1 DSP Virus Kit. A freeze / thaw cycle is defined as the number of thaws minus one.

Storage of samples at 2 – 8°C for up to eight days.

For analysis of short term refrigerated storage of pre-processed samples, simulated samples were stored at -65°C to -90°C for four weeks and then stored for eight days at 2 - 8°C. During storage at 2 – 8°C, samples were tested after three days, five days and eight days. (The experimental design was chosen to conserve the negative clinical sample matrix by allowing evaluation of frozen and 4°C storage with the same samples. The preceding storage at -65°C to -90°C for four weeks and the resulting freeze thaw provides added assurance of sample stability at 2 – 8°C for up to eight days by exposing the

samples to additional storage conditions.)

Storage of samples for up to six weeks at -65°C to -90°C with one freeze / thaw at week four.

For analysis of -70°C storage and freeze-thaw stability, 3 ml of each simulated sample was prepared and one aliquot of 0.4 ml was tested at Test Time Pont 0. The remaining 2.6 ml was stored at -65°C to -90°C. After four weeks the sample was thawed (5 h at 2-8°C) and split into aliquots of 0.4 ml. One aliquot was processed and tested, the other aliquot was returned to the freezer. After two additional weeks the remaining aliquot was thawed, processed and tested. For each sample type, the observed result agreed with the expected result for all replicates at each time point. For the simulated negative sample, the IC of each replicate was within the acceptance range of Ct 26 – 36 at each time point.

Storage of purified RNA at 2 – 8°C

For analysis of short term stability, eluates of simulated samples were initially tested immediately after processing. The residual eluate was placed at 2-8°C and tested again after six hours and eight hours of storage. All measurements agreed with the expected results, demonstrating the stability of purified RNA stored at 2 – 8°C for up to eight hours.

Storage of purified RNA at -70°C

For analysis of long term and freeze/thaw stability of purified RNA, eluates of simulated samples were initially tested directly after processing. The residual eluate was then stored at -65°C to -90°C. After two weeks the eluate was thawed at 2 – 8°C for one hour and returned to storage at -65 to -90°C (without testing). At four weeks, the eluate was thawed again for five hours at 2-8°C and tested. The remaining eluate was returned to the freezer. At six weeks the eluate was thawed for the third time and tested. All measurements agreed with the expected results demonstrating the stability of purified RNA for two freeze-thaw cycles and six weeks storage at -65° to -90°C.

Storage of purified RNA on the EZ1 Advanced XL for up to three hours.

For analysis of short term stability of purified RNA on the EZ1 Advanced XL instrument, eluates of Simulated Samples were left on the instrument for three hours after the EZ1 Advanced XL run was finished. Testing was performed immediately after the EZ1 Advanced XL run was complete. The eluates were returned to the instrument platform and tested again at two hours and three hours. All measurements agreed with the expected results, demonstrating the stability of purified RNA that remains on the EZ1 Advanced XL instrument for up to three hours.

Stability of nasopharyngeal swab specimens and of purified RNA from those

specimens was demonstrated by testing simulated samples stored under defined conditions. Samples were prepared by diluting Influenza A strain A/California/7/09-like virus or Influenza B strain B/Florida/4/2006-like virus in clinical sample matrix to a concentration of three times the LoD. Samples were processed for nucleic acid purification using EZ1 DSP Virus Kit with the EZ1 Advanced XL instrument. Testing was performed with the artus Infl A/B RG RT-PCR Kit on the RGQ instrument. The observed results agreed with the expected result for each sample and each time point.

Stability of simulated nasopharyngeal swab specimens and purified RNA was demonstrated for each of the sample types and storage conditions shown in the table below:

Sample	**Tested Storage Condition**
Simulated NP Swab Sample	8 days at 2-8 °C
	6 weeks at -65 to -90°C (2 freeze thaws)
Purified RNA	8h at 2-8 °C
	6 weeks at -65 to -90 (3 freeze thaws)
	3h onboard EZ1 Advanced XL Instrument

d. Detection limit:

The limit of detection (LoD) of the *artus* Infl A/B RG RT-PCR Kit was determined and confirmed for six influenza A strains (two strains representing each of the influenza A subtypes of H1N1, H3N2, and 2009 H1N1) and two influenza B strains. Samples were prepared from re-cultured and re-titered virus diluted in nasopharyngeal clinical matrix. The LoD of each strain was initially determined by limited dilution testing of three replicates per dilution level. The result was confirmed by testing an additional 20 replicates at the LoD concentration. The LoD, defined as the level of virus that yields at least a 95% (19/20) detection rate with the *artus* Infl A/B RG RT-PCR Kit, ranged from 10e1.1 to 10e-0.1 $TCID_{50}$/ml. The strains included in the analysis and the confirmed LoD are summarized in the table below.

Influenza Strain	**LoD Concentration ($TCID_{50}$/mL)**
A/New Caledonia/20/1999 (H1N1)	10e0.5
A/Brisbane59/2007-like virus (H1N1)	10e1.1
A/Hong Kong/8/68(TC-adapted H3N2)	10e0.2
A/Wisconsin/67/2005 (H3N2)	10e0.4
A/California/7/09-like virus (2009 H1N1)	10e0.9
A/Hamburg/05/09 (2009 H1N1)	10e0.4
B Brisbane/20/2008-like virus	10e-0.1
B/Florida/4/2006-like virus	10e0.9

e. Analytical specificity:

Cross reactivity:

The analytical specificity of the *artus* Infl A/B RG RT-PCR Kit was evaluated by testing a panel of respiratory pathogens consisting of 31 virus strains (including seven influenza A and three influenza B strains) and 18 bacterial strains. The pathogens were tested at medically relevant levels. Human genomic DNA and RNA were also evaluated. Test samples were prepared by diluting the pathogen culture stock in Universal Transport Medium (UTM). Initial test concentrations of the potentially cross-reactive pathogens ranged from 10e3.2 to 10e6.1 TCID50/ml for viruses and from 10e2 to 10e6 cfu/ml for bacteria. Purified human genomic DNA (hDNA) and human genomic RNA (hRNA) were purchased and prepared at 0.1 ng/µl and 2 pg/µl, respectively. A volume of 10µl was added directly to the master mix.

The *artus* Infl A/B RG RT-PCR Kit did not cross-react with 10 influenza strains, 21 other respiratory viral pathogens, or 16 of 18 bacterial pathogens present at medically relevant levels. Two of the 18 bacterial pathogens, *Bordetella pertussis* and *Streptococcus pneumoniae*, generated invalid results at concentrations of 10e3 cfu/ml and 2 x 10e5 cfu/ml, respectively. The invalid results were generated by either absence of Ct values for the IC or Ct values for the IC that fell out of the acceptable range. Re-extraction and analysis of samples spiked with *Streptococcus pneumoniae* also generated invalid results, suggesting the presence of a potential inhibitor. Upon dilution of the eluates valid results were obtained. A spiked specimen was made with a concentration 1 log lower than the initial preparation. Similarly, a new spiked specimen containing *Bordetella pertussis* was also made to a final concentration of 1 log lower than the original sample, yielding valid results. There were no instances of cross-reactivity causing a false positive result for Influenza A or Influenza B. A limitation has been added to the package insert to indicate that the presence of *Bordetella pertussis* or *Streptococcus pneumoniae* can yield invalid results.

Cross-reactivity results for the *artus* Infl A/B RG RT-PCR Kit.

Pathogen	Test Concentration		Positive Replicates/ Total Replicates	
			InfA	InfB
Influenza virus strains				
A/California/7/09-like virus	10e5.0	$TCID_{50}$/ml	3/3	0/3
A/Hong Kong/8/68 (TC-adapted)	10e5.0	$TCID_{50}$/ml	3/3	0/3
A/PR/8/34	10e5.0	$TCID_{50}$/ml	3/3	0/3

A2/Wisconsin/67/2005	10e5.0	$TCID_{50}$/ml	3/3	0/3
A/Solomon Islands/3/2006 (H1N1)-like virus	10e5.0	$TCID_{50}$/ml	3/3	0/3
A/Duck/Potsdam2243/84	10e5.0	$TCID_{50}$/ml	3/3	0/3
A/Brisbane59/2007 (H1N1)-like virus	10e6.1	$TCID_{50}$/ml	3/3	0/3
B/Brisbane/60/2008-like virus	10e5.0	$TCID_{50}$/ml	0/3	3/3
B/Florida/4/2006-like virus	10e5.0	$TCID_{50}$/ml	0/3	3/3
B/Malaysia/2506/2004	10e5.0	$TCID_{50}$/ml	0/3	3/3
Other respiratory virus strains				
RSVA VR-26	10e5.0	$TCID_{50}$/ml	0/3	0/3
RSVB VR-1400	10e4.45	$TCID_{50}$/ml	0/3	0/3
PIV1 VR-94	10e3.45	$TCID_{50}$/ml	0/3	0/3
PIV2 VR-92	10e5.0	$TCID_{50}$/ml	0/3	0/3
PIV3 VR-93	10e5.0	$TCID_{50}$/ml	0/3	0/3
PIV4a VR-1378	10e3.95	$TCID_{50}$/ml	0/3	0/3
ADVE 4 VR-1572	10e5.0	$TCID_{50}$/ml	0/3	0/3
ADVB 3 VR-3	10e5.0	$TCID_{50}$/ml	0/3	0/3
ADVC 5 VR-5	10e5.0	$TCID_{50}$/ml	0/3	0/3
Echovirus 11 VR-41	10e5.0	$TCID_{50}$/ml	0/3	0/3
Rhinovirus 1a VR-1559	10e5.0	$TCID_{50}$/ml	0/3	0/3
Rhinovirus 39 VR-340	10e5.0	$TCID_{50}$/ml	0/3	0/3
Coxsackie B1 VR-28	10e5.0	$TCID_{50}$/ml	0/3	0/3
229E VR-740	10e4.2	$TCID_{50}$/ml	0/3	0/3
OC43 VR-1558	10e4.45	$TCID_{50}$/ml	0/3	0/3
CMV VR-538	10e4.95	$TCID_{50}$/ml	0/3	0/3
HSV VR-260	10e5.0	$TCID_{50}$/ml	0/3	0/3
Varicella-zoster virus VR-1367	10e3.95	$TCID_{50}$/ml	0/3	0/3
EBV VR-603	10e3.0	cfu/ml *	0/3	0/3
Measles VR-24	10e3.2	$TCID_{50}$/ml	0/3	0/3
Mumps virus VR-106	10e5.0	$TCID_{50}$/ml	0/3	0/3
Bacterial strains				
Bordetella pertussis	10e3.0	cfu/ml	3 Invalid/3	3 Invalid/3
	10e2.0	cfu/ml	0/3	0/3
Chlamydophila pneumoniae	10e5.0	$TCID_{50}$/ml	0/3	0/3

Corynebacterium sp.	10e3.0	cfu/ml	0/3	0/3
Escherichia coli	10e6.0	cfu/ml	0/3	0/3
Haemophilus influenzae	10e6.0	cfu/ml	0/3	0/3
Lactobacillus sp.	10e3.0	cfu/ml	0/3	0/3
Legionella spp	9 x 10e5.0	bacteria/ml	0/3	0/3
Moraxella catarrhalis	10e3.0	cfu/ml	0/3	0/3
Mycobacterium tuberculosis avirulent	10e2.0	cfu/ml	0/3	0/3
Mycoplasma pneumoniae	10e3.0	cfu/ml	0/3	0/3
Neisseria ssp. #14685	10e2.0	cfu/ml	0/3	0/3
Neisseria meningitidis	10e6.0	bacteria/ml	0/3	0/3
Pseudomonas aeruginosa	10e6.0	cfu/ml	0/3	0/3
Staphylococcus aureus Protein A producer	10e6.0	bacteria/ml	0/3	0/3
Staphylococcus epidermidis	10e6.0	bacteria/ml	0/3	0/3
Streptococcus pneumoniae	2 x 10e5.0	cfu/ml	3 Invalid/3	3 Invalid/3
	2 x 10e4	cfu/ml	0/3	0/3
Streptococcus pyogenes	10e3.0	cfu/ml	0/3	0/3
Streptococcus salivarius	10e6.0	cfu/ml	0/3	0/3
Human genomic DNA / RNA				
Human genomic DNA	0.1 ng/µl		0/3	0/3
Human lung RNA	2 pg/µl		0/3	0/3

*Epstein Barr virus was purchased from ATCC. A titer was not provided. The guaranteed minimum concentration was stated to be 10e4 cfu/ml.

Invalid results originally obtained with *Bordetella pertussis* and *Streptococcus pneumoniae* were resolved by diluting the test samples 10-fold and re-analyzing. A summary of the triplicate measurements are presented below.

Cross-reactivity results for Bordetella pertussis

Test Concentration	Inf A Ct* (green channel)	Inf B Ct* (crimson channel)	IC Ct* (orange channel)	Comment
10e3 cfu/ml	n.d.	n.d.	42.19	Invalid
	n.d.	n.d.	n.d.	Invalid
	n.d.	n.d.	n.d.	Invalid
10e2 cfu/ml	n.d.	n.d.	33.41	Valid
	n.d.	n.d.	34.28	Valid
	n.d.	n.d.	33.14	Valid

* n.d. Not Detected

Cross-reactivity results for Streptococcus pneumoniae

Test Concentration	Inf A Ct* (green channel)	Inf B Ct* (crimson channel)	IC Ct* (orange channel)	Comment

2 X 10e5 cfu/ml	n.d.	n.d.	37.79	Invalid
	n.d.	n.d.	36.89	Invalid
	n.d.	n.d.	37.87	Invalid
2 X 10e4 cfu/ml	n.d.	n.d.	33.67	Valid
	n.d.	n.d.	33.61	Valid
	n.d.	n.d.	33.47	Valid

* n.d. Not Detected

Inclusivity:

The analytical reactivity of the *artus* Infl A/B RG RT-PCR Kit was demonstrated by testing 18 strains of influenza A, including four strains originally identified in non-human species, and six strains of influenza B at concentrations near the limit of detection (LoD) of the test. Samples were prepared from whole virus diluted in clinical sample matrix and were selected to represent temporal and geographic diversity. The initial test concentration of each virus strain was the highest LoD concentration previously determined for the respective type or subtype. Viral stocks were obtained from ATCC or Novartis. For each strain, three aliquots at the initial test concentration were processed for nucleic acid extraction and purification using the EZ1 Advanced XL. The resulting three eluates for each strain were tested with the *artus* Infl A/B RG RT-PCR Kit on the RGQ. Reactivity was considered to be successfully demonstrated if three of three eluates generated a positive result in the appropriate target-specific channel. For strains generating fewer than three (of three) positive results, three aliquots of a higher concentration were processed and tested. This process was repeated until a virus concentration generating three of three positive results was obtained. Results from the analysis are summarized below.

Influenza A Virus Strain	**Subtype**	**Concentration**	**Number Positive for Influenza A / Number Tested**
A/Virginia/ATCC2/2009	2009 H1N1	1×10^{1} $TCID_{50}$/mL	1/3
		1×10^{2} $TCID_{50}$/mL	3/3
A/PR/8/34	H1N1	1×10^{1} $TCID_{50}$/mL	3/3
A/FM/1/47	H1N1	1×10^{1} $CEID_{50}$/mL	3/3
A/Solomon Islands/3/2006 (H1N1)-like virus	H1N1	1×10^{1} $TCID_{50}$/mL	3/3
A/Mal/302/54	H1N1	1×10^{1} $CEID_{50}$/mL	3/3
A/New Jersey/8/76	H1N1	1×10^{1} $CEID_{50}$/mL	3/3

A/NWS/33	H1N1	1×10^{1} $CEID_{50}$/mL	1/3
		1×10^{2} $CEID_{50}$/mL	3/3
A1/Denver/1/57	H1N1	1×10^{1} $CEID_{50}$/mL	1/3
		1×10^{2} $CEID_{50}$/mL	2/3
		1×10^{3} $CEID_{50}$/mL	3/3
A/Weiss/43	H1N1	1×10^{1} $CEID_{50}$/mL	0/3
		1×10^{3} $CEID_{50}$/mL	2/3
		1×10^{4} $CEID_{50}$/mL	3/3
A/Victoria x187 (TF11016B1)	H3N2	1×10^{1} $TCID_{50}$/mL	3/3
A2/Aichi2/68	H3N2	1×10^{0} $CEID_{50}$/mL	2/3 ‡
		1×10^{1} $CEID_{50}$/mL	3/3
A/Victoria/3/75	H3N2	1×10^{0} $CEID_{50}$/mL	1/3
		1×10^{1} $CEID_{50}$/mL	3/3
A/Alice	H3N2	1×10^{0} EID_{50}/mL	1/3
		1×10^{1} EID_{50}/mL	2/3
		1×10^{2} EID_{50}/mL	3/3
A/MRC2	H3N2	1×10^{0} $CEID_{50}$/mL	0/3 †
		1×10^{1} $CEID_{50}$/mL	2/3
		1×10^{2} $CEID_{50}$/mL	3/3
(H9N2-like)	H9N2	1×10^{0} $TCID_{50}$/mL	3/3
A/Duck/Potsdam2243/84 (H5N6-like)	H5N6	1×10^{0} $TCID_{50}$/mL	3/3
A/Swine/Iowa/15/30	H1N1	1×10^{0} $CEID_{50}$/mL	0/3
		1×10^{2} $CEID_{50}$/mL	3/3
A/Equine/2/Miami/63 (H3N8-like)	H3N8	1×10^{0} $CEID_{50}$/mL	0/3
		1×10^{2} $CEID_{50}$/mL	0/3

		1×10^{4} $CEID_{50}$/mL	3/3

‡ For A2/Aichi2/68, one replicate at 1×10^{0} $CEID_{50}$/mL was initially invalid. The sample was re-processed for RNA purification and gave a valid result of negative for Inf A on re-test.

† For A/MRC2, one replicate at 1×10^{0} $CEID_{50}$/ml was invalid. The sample was not re-tested because the other two replicates were negative, requiring testing of this

Influenza B Virus Strain	Concentration	Number Positive for Influenza B / Number Tested
B/Lee/40	1×10^{1} $TCID_{50}$/mL	3/3
B/Allen/45	1×10^{1} $CEID_{50}$/mL	3/3
B/Taiwan/2/62	1×10^{1} $CEID_{50}$/mL	3/3
B/Hong Kong/5/72	1×10^{1} $CEID_{50}$/mL	3/3
B/Maryland/1/59	1×10^{1} $CEID_{50}$/mL	3/3
B/Malaysia/2506/2004	1×10^{1} $TCID_{50}$/mL	3/3

Interfering Substances

The potential for blood or medications that might be present in a nasopharyngeal swab specimen to interfere with the detection of low levels of influenza A or influenza B by the *artus* Infl A/B RG RT-PCR test was evaluated. A total of 24 substances representing the active ingredient in over-the-counter or prescription medications were tested against two influenza strains: A/California/7/09-like virus or B/Florida/4/2006-like virus. For each test sample, three aliquots were processed for RNA purification using the EZ1 DSP Virus Kit with the EZ1 Advanced XL instrument. The resulting three eluates were tested with the *artus* Infl A/B RG RT-PCR Kit in separate runs on one Rotor-Gene Q MDx (RGQ).

If one or more of the three replicates was negative for the influenza A or influenza B reference strain, testing was repeated with a lower concentration of the interfering substance until the reference strain was successfully detected in three of three replicates. The active ingredients from 24 over-the-counter or prescription medications and whole blood were evaluated for interference with the *artus* Infl A/B RG RT-PCR test. Samples containing the influenza strain A/California/7/09-like virus or B/Florida/4/2006-like virus at the LoD concentration of 10e1 TCID50/ml and the potential interferent were processed

for RNA purification in triplicate and tested. The reference influenza strains were successfully detected in all samples except for one aliquot containing the reference influenza B strain and Mupirocin at 10 mg/ml. The reference influenza B strain was successfully detected in three of three aliquots with Mupirocin at 2 mg/ml. (Mupirocin is an antibiotic ointment used to treat skin infections. The product tested contains Mupirocin at 20mg / g of ointment.)

Interfering Substance			**Reference Strain Influenza A**		**Reference Strain Influenza B**	
Active Ingredient	**Source**	**Concen-tration**	**Sample ID**	**Inf A Ct (green channel)**	**Sample ID**	**Inf B Ct (crimson channel)**
Human Blood †	Human Blood	5% v/v	155001	33.45	155085	30.99
			155002	33.61	155086	31.01
			155003	33.18	155087	31.13
Zanamivir	Relenza	3 mg/ml	155004	32.5	155088	32.12
			155005	32.69	155089	31.59
			155006	32.13	155090	31.11
Oseltamivir	Tamiflu	15 mg/ml	155007	34.45	155091	33.52
			155008	34.08	155092	33.44
			155009	33.67	155093	32.81
NaCl with preservatives	Olynth Salin	10% v/v (0.9 µg/ml)[#]	155010	32.9	155094	31.66
			155011	32.37	155095	31.25
			155012	33.02	155096	31.41
Phenyl-ephrine	Visadron	10% v/v (125 µg/ml)	155013	32.41	155097	31.26
			155014	32.54	155098	31.12
			155015	32.69	155099	31.51
Oxy-metazoline	Wick Sinex Schnupfen	10% v/v (500 µg/ml)	155016	32.62	155100	31.84
			155017	32.4	155101	31.56
			155018	32.42	155102	31.29
Budesonide	Pulmicort 1.0 mg/2ml	40ug/ml	155019	32.4	155103	31.35
			155020	32.5	155104	31.93
			155021	32.26	155105	31.05
Fluticasone propionate	Flutide Nasal Punpspray	2.5% v/v	155022	33.22	155106	31.29
			155023	32.64	155107	30.42
			155024	32.45	155108	30.82
Luffa opperculata	Luffa opperculata	4.5 mg/ml	155025	32.41	155109	31.18
			155026	32.19	155110	31.01
			155027	32.66	155111	31.63
Sulfur	Sulfur	4.5 mg/ml	155028	32.35	155112	31.21
			155029	32.16	155113	31.49
			155030	32.08	155114	31.24
Galphimia	Galphimia	4.5	155031	36.99	155115	32.77

Interfering Substance			Reference Strain Influenza A		Reference Strain Influenza B	
Active Ingredient	**Source**	**Concen-tration**	**Sample ID**	**Inf A Ct (green channel)**	**Sample ID**	**Inf B Ct (crimson channel)**
glauca	glauca	mg/ml	155032	36.88	155116	32.34
			155033	35.91	155117	32.68
Histaminum hydro-Chloricum	Histaminu m hydro-Chloricum	4.5 mg/ml	155034	32.6	155118	31.58
			155035	32.97	155119	31.43
			155036	32.73	155120	31.28
Beclometh-asone dipropionate	Beclomet Nasal Aqua (nasal spray)	61.73 µg/ml	155037	33.17	155121	30.43
			155038	33.09	155122	30.77
			155039	33.71	155123	31.11
Flunisolide	Syntaris	25 µg/ml	155040	32.55	155124	31.42
			155041	32.05	155125	31.04
			155042	32.37	155126	31.43
Triamcinolone acetonide	Triamcinol one acetonide	27.5 µg/ml	155043	32.56	155127	31.85
			155044	32.06	155128	31.54
			155045	32.41	155129	31.48
Guaifenesin ¥	Fagusan	1.33 mg/ml	155046	32.52	155130	31.24
			155047	32.32	155131	31.53
			155048	32.35	155132	31.9
Diphenhy-dramine hydrochloride	Diphenhy-dramine hydro-chloride	0.5 mg/l	155049	32.61	155133	31.31
			155050	32.43	155134	31.81
			155051	32.71	155135	31.32
Dextro-methorphan hydrobromide	Hustenstill er Ratiopharm	1 mg/ml	155052	32.48	155136	31.08
			155053	32.88	155137	31.14
			155054	32.46	155138	31.17
Pseudo-ephedrine hydrochloride	RhinoPRO NT	20 µg/ml	155055	32.95	155139	31.53
			155056	33.11	155140	31.25
			155057	32.78	155141	31.77
Benzocoaine	Anaesthesin	1.44 mg/ml	155061	37.35	155142	34.48
			155062	35.51	155143	33.92
			155063	n.d.	155144	33.01
			155173 *	33.17	n/a	n/a
Menthol	Menthol	5 mg/ml	155064	32.7	155145	31.2
			155065	32.99	155146	31.82
			155066	32.98	155147	31.76
Tobramycin	Tobramaxin	0.3 mg/ml	155067	32.51	155148	31.34
			155068	32.64	155149	31.2
			155069	32.46	155150	31.6

Interfering Substance			Reference Strain Influenza A		Reference Strain Influenza B	
Active Ingredient	**Source**	**Concen-tration**	**Sample ID**	**Inf A Ct (green channel)**	**Sample ID**	**Inf B Ct (crimson channel)**
Mupirocin	Infectop-yoderm	10 mg/ml	155070	34.16	155151	36.32
			155071	34.33	155152	n.d.**
			155072	34.15	155153	33.28
		2 mg/ml	n/a	n/a	155177	31.5
			n/a	n/a	155178	32.48
			n/a	n/a	155179	31.71
Amoxicillin	Amoxihexal	1 mg/ml	155073	32.3	155154	31.51
			155074	32.45	155155	31.28
			155075	32.65	155156	31.1
Dexamethason	Dexa-Ratiopharm	1.53 µmol/L	155076	32.66	155157	31.66
			155077	32.46	155158	31.24
			155078	32.66	155159	31.25
FluMist Influenza Vaccine §	FluMist Influenza Vaccine	$10^{6.5-7.5}$ FFU (fluor-escent focus units)	60050	20.45	60050	17.04
			60050.1	20.41	60050.1	17.28
			60050.2	20.41	60050.2	17.18
No Interfering Substance	n/a	n/a	155082	32.67	155160	30.77
			155083	32.39	155161	31.01
			155084	32.54	155162	30.76

† The human blood sample was stored frozen before testing.

Saline solution: a commonly used term for a sterile solution of sodium chloride (NaCl) of 0.90% w/v, about 300mOsm/L or 9.0g per liter. Therefore it can be assumed that a concentration of 0.9g/L was tested.

¥ Guaifenesin serves the same function as mucin. The source material, Fagusan, is an alternative to Mucinex.

* Sample 155173 is a repeat (including RNA purification and testing) of the originally invalid result from sample 155063. The original result was invalid because the IC Ct was outside of the acceptance range required for samples negative for Inf A and Inf B.

** Sample 155152 was valid and negative for Inf B. A new sample was prepared with a five fold lower concentration of Mupirocin. The new sample was re-extracted in triplicate and tested as sample IDs 155177 – 155179. The influenza B reference strain was successfully detected in three of three replicates with the lower concentration of Mupirocin.

§ The FluMist vaccine was tested without Influenza A and Influenza B reference strains. Testing was performed as part of the Cross-Reactivity analytical verification study (DHF-114-VER-006). Positive results for influenza A and B were expected with the *artus* Infl A/B RG RT-PCR Kit because the vaccine contains RNA from Influenza A strains A/California/7/2009 (H1N1) and A/Perth/16/2009(H3N2) and from Influenza B strain B/Brisbane/60/2008.

f. Carry over/Contamination

The potential for carryover or cross-contamination to occur during nucleic acid purification, using the EZ1 Advanced XL instrument with the EZ1 DSP Virus Kit, or during amplification / detection using the *artus* Infl A/B RG RT-PCR Kit with the Rotor-Gene Q MDX was evaluated by processing and testing samples with a high concentration of Influenza A in alternating sequence with samples containing the same analyte at a concentration that tested positive approximately 10% of the time (high negative). The *artus* Infl A/B RG RT-PCR test (including extraction using the EZ1 DSP Virus Kit with the EZ1 Advanced XL) showed no evidence of carryover or cross-contamination when 5 runs of a panel of 6 members of mock samples containing influenza A at a concentration just below the limit of detection of the assay were extracted and tested in alternating order with a panel of 6 members of mock samples of the same strain present at a high concentration. Analysis of the negative rate of the high negative samples is not affected by the presence of high positive samples and supporting the conclusion that carryover and cross-contamination do not occur during RNA purification with the EZ1 DSP Virus Kit on the EZ1 Advanced XL instrument or during testing with the *artus* Infl A/B RG RT-PCR Kit on the RotoGene Q MDx.

g. LoB Study:

The Limit of Blank (LoB) was established by testing sixty influenza-negative nasopharyngeal swab specimens with the *artus* Infl A/B RG RT-PCR Kit. The robustness of the Internal Control (IC) signal was evaluated by assessing the IC signals in the same set of samples.

Sixty residual, de-identified nasopharyngeal swab specimens collected in UTM were tested in the study. The specimens were negative for Influenza A and Influenza B by culture. Each of the sixty specimens was processed for RNA purification and tested once. Specimens that generate a Ct value in the Infl A or Infl B channel of the RGQ within 45 cycles are determined to be positive for Influenza A or Influenza B RNA, respectively. For the Limit of Blank study, the number of PCR cycles was changed from the usual 45 cycles to 50 cycles to ensure that influenza-negative specimens do not generate a Ct close to (but later than) the assay cutoff of 45 cycles. The use of a pre-launch version of the RGQ software allows the PCR cycling profile to be manually applied. RNA purification was performed with one lot of EZ1 DSP Virus Kits

on three EZ1 Advanced XL instruments. Testing included three lots of the *artus* Infl A/B RG RT-PCR Kit, three RGQ instruments and three operators.

Sixty influenza-negative (by culture) NPS specimens were processed for RNA purification and tested with the *artus* Infl A/B RG RT PCR Kit. The Influenza A/B Internal Control demonstrated robust performance in the *artus* Infl A/B RG RT PCR test. None of the 60 specimens generated a Ct for Influenza A or Influenza B through 50 PCR cycles, indicating that the LoB for the assay is greater than 50 Ct. The Internal Control Ct of each of the 60 specimens was within the acceptance range of 26 – 36 Ct. The average Ct for the Internal Control was 31.17 with a CV of 1.6%. These results support the assay cutoff of less than 45 Ct for discriminating positive from negative specimens.

2. Comparison studies:

   a. Method comparison with predicate device:

   Not Applicable

   b. Matrix comparison:

   The impact of freezing on the performance characteristics of the *artus* Infl A/B RG RT-PCR Assay was assessed using prospectively collected nasopharyngeal swab specimens. All prospectively collected specimens were originally characterized when the UTM was fresh (i.e., portions of UTM were subjected to viral culture and nucleic acid extraction prior to freezing). An aliquot from all prospectively collected nasopharyngeal swab specimens were stored at -70°C or lower for at least 21 days before retesting with the *artus* Infl A/B RG RT-PCR Assay. Nucleic acid was extracted from a 400 µL portion of frozen UTM using the EZ1 Advanced XL/DSP Virus system for testing by the *artus* Infl A/BRT-PCR Kit; the eluate was either tested immediately or stored at −70°C or below for later testing. The aliquot used for this study was the primary backup specimen for the prospective analysis in the event that a specimen failed or was lost. A total of 244 specimens were evaluable when comparing the investigational *artus* method to viral culture, or the *artus* Infl A/B RG RT-PCR result from specimens tested prior to freezing. The overall positive agreement of testing frozen UTM with the *artus* Infl A/B RG RT-PCR test relative to fresh UTM was 100% for Influenza A and 98.2% for Influenza B. The overall negative agreement for both Influenza A and Influenza B was 100%. The results are summarized below in a 2x2 comparison of fresh vs. frozen NP swab specimens in UTM.

Influenza A Test Results from Frozen UTM Relative to Fresh UTM among Prospectively Collected NP Swab Specimens

Influenza A								
		Fresh UTM – *artus* Influenza A			Performance			
					Calculated Result		95% Confidence Interval	
		Positive	**Negative**	***Total***	**Metric**	**%**	**Lower CI**	**Upper CI**
Frozen UTM *artus* Infl A	**Positive**	27	0	27	*Positive Agreement*	100	0.88	1
	Negative	0	217	217	*Negative Agreement*	100	0.98	1
	Total	27	217	244				

Influenza B Test Results from Frozen UTM Relative to Fresh UTM among Prospectively Collected NP Swab Specimens

Influenza B								
		Fresh UTM – *artus* Influenza B			Performance			
					Calculated Result		95% Confidence Interval	
		Positive	**Negative**	***Total***	**Metric**	**%**	**Lower CI**	**Upper CI**
Frozen UTM *artus* Infl B	**Positive**	54	0	54	*Positive Agreement*	98.2	0.90	1
	Negative	1	189	190	*Negative Agreement*	100	0.98	1
	Total	55	189	244				

3. Clinical studies:

To assess performance of the *artus* Infl A/B RG RT-PCR Kit a multi-center study was performed. Three independent clinical trial testing sites, separated geographically within the United States, were used.

The study was conducted in three parts. The first part included testing fresh prospectively collected nasopharyngeal swab specimens from individuals with a respiratory tract infection wherein said infection was suspected of being caused by a respiratory virus. Only one specimen was collected per subject; specimens were subjected to viral culture, and nucleic acids were extracted from these specimens within 36 hours of collection for investigational *artus* testing. The second part of the trial involved testing (at two clinical sites, Site 1 and Site 2) of prospectively collected and archived clinical samples over two influenza seasons from the Site 2. The third part of the trial involved multisite testing of retrospectively collected specimens acquired from the CDC Influenza Banking Program. In total, the results of 928 specimens were evaluated in this clinical evaluation. Each part of the study is discussed in detail below.

Part 1

A total of 272 subjects were enrolled in the fresh prospective clinical study during the 2010/2011 flu season. In order to be eligible for the study, subjects must have signed an informed consent and must have had symptoms of an acute respiratory tract infection for less than five days. Considering these criteria, a total of 18 subjects were considered ineligible due to unconfirmed consent (i.e. improper consenting etc.), or subjects were excluded due to (a) the duration of the subjects symptoms exceeding five days, or (b) the subject's symptoms were not properly documented. Consequently, there were 254 subjects that met the study eligibility criteria from three sites.

The demographics for the prospective study are as follows:

Age Group (Years)	Site 1		Site 2		Site 3		All Sites	
	N	%	N	%	N	%	N	%
<5	0	0	0	0	90	45.0	90	35.4
≥5 and ≤21	3	11.1	1	3.7	102	51.0	106	41.7
≥22 and ≤59	23	85.2	22	81.5	6	3.0	51	20.1
>60	1	3.7	3	11.1	2	1.0	6	2.4
Total	27	100	27	100	200	100	254	100

All Sites Combined – Influenza A								
					Performance			
		Culture/DFA Influenza A			**Calculated Result**		**95% Confidence Interval**	
		Positive	**Negative**	***Total***	**Metric**	**%**	**Lower CI**	**Upper CI**
***artus* Infl A/B RG RT-PCR Test**	**Positive**	15	12	27	*Sensitivity*	100	0.80	1
	Negative	0	227	227	*Specificity*	95.0	0.91	0.97
	Total	15	239	254				

Results at Each Site – Influenza A								
Site 1								
					Performance			
		Culture/DFA Influenza A			**Calculated Result**		**95% Confidence Interval**	
		Positive	**Negative**	***Total***	**Metric**	**%**	**Lower CI**	**Upper CI**
***artus* Infl A/B RG RT-PCR Test**	**Positive**	2	2	4	*Sensitivity*	100	0.34	1
	Negative	0	23	23	*Specificity*	92.0	0.75	0.98
	Total	2	25	27				

Site 2								
					Performance			
		Culture/DFA Influenza A			Calculated Result		95% Confidence Interval	
		Positive	Negative	*Total*	Metric	%	Lower CI	Upper CI
***artus* Infl A/B RG RT-PCR Test**	**Positive**	4	3	7	*Sensitivity*	100	0.51	1
	Negative	0	20	20	*Specificity*	86.9	0.68	0.95
	Total	4	23	27				

Site 3								
					Performance			
		Culture/DFA Influenza A			Calculated Result		95% Confidence Interval	
		Positive	Negative	*Total*	Metric	%	Lower CI	Upper CI
***artus* Infl A/B RG RT-PCR Test**	**Positive**	9	7	16	*Sensitivity*	100	0.70	1
	Negative	0	184	184	*Specificity*	96.3	0.93	0.98
	Total	9	191	200				

All Sites Combined – Influenza B								
					Performance			
		Culture/DFA Influenza B			Calculated Result		95% Confidence Interval	
		Positive	Negative	*Total*	Metric	%	Lower CI	Upper CI
***artus* Infl A/B RG RT-PCR Test**	**Positive**	45	11	56	*Sensitivity*	100	0.92	1
	Negative	0	198	198	*Specificity*	94.7	0.91	0.97
	Total	45	209	254				

Results at Each Site – Influenza B								
Site 1								
					Performance			
		Culture/DFA Influenza B			Calculated Result		95% Confidence Interval	
		Positive	Negative	*Total*	Metric	%	Lower CI	Upper CI
***artus* Infl A/B RG RT-PCR Test**	**Positive**	1	1	2	*Sensitivity*	100	0.21	1
	Negative	0	25	25	*Specificity*	96.2	0.81	0.99
	Total	1	26	27				

Site 2								
					Performance			
		Culture/DFA Influenza B			Calculated Result		95% Confidence Interval	

		Positive	Negative	*Total*	Metric	%	Lower CI	Upper CI
***artus* Infl A/B RG RT-PCR Test**	**Positive**	2	0	2	*Sensitivity*	100	0.34	1
	Negative	0	25	25	*Specificity*	100	0.87	1
	Total	2	25	27				

Site 3

					Performance			
		Culture/DFA Influenza B			Calculated Result		95% Confidence Interval	
		Positive	**Negative**	***Total***	**Metric**	**%**	**Lower CI**	**Upper CI**
***artus* Infl A/B RG RT-PCR Test**	**Positive**	42	10	52	*Sensitivity*	100	0.92	1
	Negative	0	148	148	*Specificity*	93.7	0.89	0.96
	Total	42	158	200				

The overall sensitivity of the *artus* Infl A/B RG RT-PCR test relative to viral culture was 100% for both Influenza A and Influenza B. The overall specificity of the investigational *artus* test for Influenza A and Influenza B was 95.0% and 94.7%, respectively. There were a total of 23 discordant results in the fresh prospective clinical evaluation. All discordant results were positive by the *artus* Infl A/B test, but negative by standard viral culture. Twelve of these were Influenza A positive, while 11 were Influenza B positive. The Ct-values of the 12 discordant Influenza A specimens are compared with the Ct-values of the 15 concordant positive specimens in as well as the Ct-values of the 11 discordant Influenza B specimens are compared with the Ct-values of the 45 concordant positive specimens were compared. The distribution of Ct-values between the concordant positive specimens and the discordant specimens formed two distinct populations for both Influenza A and Influenza B. The comparison is presented below.

Analysis of the *artus* Infl A/B RG RT-PCR Test Ct-Values among Culture Positive and Culture Negative Specimens for Flu A and Flu B Positive Specimens

	Influenza A Ct-Values			Influenza B Ct-Values		
	Culture Positive	**Culture Negative**	**All *artus* A/B Positive**	**Culture Positive**	**Culture Negative**	**All *artus* A/B Positive**
N	15	12	27	45	11	56
Mean	22.99	32.44	27.19	21.24	31.57	23.27
St. Dev.	2.70	4.01	5.80	3.32	4.48	5.44
Lower CI*	21.49	29.88	24.89	20.24	28.56	21.81
Upper CI*	24.48	34.99	29.48	22.24	34.58	24.73
Min	18.46	29.15	18.46	15.65	24.55	15.65
Max	26.80	42.74	42.74	28.05	37.03	37.03
Range	8.34	13.59	24.28	12.40	12.48	21.38
Median	23.27	31.09	26.80	20.85	33.19	22.31

Bi-directional sequencing was performed on 24 of the 27 Influenza A positive specimens, and all 56 Influenza B positive specimens. Three Influenza A positive specimens from Site 2 were not tested with bi-directional sequencing and all three were culture negative.

Of the remaining 24 Influenza A positive specimens, only two specimens could not be confirmed as Influenza A; both specimens were culture negative. Both specimens had high Ct-values relative to other discordant culture negative specimens.

All 56 Influenza B positive specimens were submitted for bi-directional sequencing; a total of 49 were confirmed as Influenza B.

Part 2

In the second part of the clinical evaluation prospectively collected and archived clinical specimens from Site 2 were used. The specimens enrolled in the study spanned two influenza seasons (between 8/24/2009-1/13/2010 and 1/14/2011-5/10/2011) and represented an all-comers study. They were included in the study based on the inclusion criteria of the study protocol. Samples from this study were analyzed at two sites. A total of 198 specimens were shipped to Site 1 for *artus* Infl A/B testing, while 265 were tested at Site 2 with the *artus* Infl A/B test. The reference method used for this study was one of two FDA cleared high performance molecular tests for influenza. The following tables represent the results of performance evaluation through comparison to each FDA cleared molecular device individually at each site, at all sites combined, and finally at all sites combined

The demographics for the prospectively collected and archived study are as follows:

Age Group (Years)	Site 1		SITE 2		All Sites	
	N	%	N	%	N	%
<5	5	2.5	2	0.8	7	1.5
≥5 and ≤21	27	13.7	16	6.0	43	9.3
≥22 and ≤59	138	70.1	189	71.3	327	70.8
>60	24	12.2	56	21.1	80	17.3
Total	197	100	265	100	462	100

Comparison of *artus* Infl A/B RG RT-PCR Test Results in the Site 2 Prospectively collected and Archived Specimen Study Relative to an FDA Cleared Molecular Reference Result for Influenza A

All Sites Combined – Influenza A								
					Performance			
		FDA Cleared Molecular (1) Influenza A			Calculated Result		95% Confidence Interval	
		Positive	Negative	*Total*	Metric	%	Lower CI	Upper CI
***artus* Infl A/B RG RT-PCR Test**	**Positive**	73	9	82	*Positive Agreement*	98.6	0.93	1
	Negative	1	162	163	*Negative Agreement*	94.7	0.90	0.97
	Total	74	171	245				

					Performance			
		FDA Cleared Molecular (2) Influenza A			Calculated Result		95% Confidence Interval	
		Positive	Negative	*Total*	Metric	%	Lower CI	Upper CI
***artus* Infl A/B RG RT-PCR Test**	**Positive**	21	10	31	*Positive Agreement*	95.5	0.78	0.99
	Negative	1	185	186	*Negative Agreement*	94.9	0.91	0.97
	Total	22	195	217				

Results at Each Site – Influenza A								
Site 1								
					Performance			
		FDA Cleared Molecular (1) Influenza A			Calculated Result		95% Confidence Interval	
		Positive	Negative	*Total*	Metric	%	Lower CI	Upper CI
***artus* Infl A/B RG RT-PCR Test**	**Positive**	30	0	30	*Positive Agreement*	100.0	0.89	1
	Negative	0	4	4	*Negative Agreement*	100.0	0.51	1
	Total	30	4	34				

					Performance			
		FDA Cleared Molecular (2) Influenza A			Calculated Result		95% Confidence Interval	
		Positive	Negative	*Total*	Metric	%	Lower CI	Upper CI
***artus* Infl A/B RG RT-PCR Test**	**Positive**	20	10	30	*Positive Agreement*	95.2	0.77	0.99
	Negative	1	132	133	*Negative Agreement*	93.0	0.88	0.96
	Total	21	142	163				

Results at Each Site – Influenza A					
Site 2					
					Performance

		FDA Cleared Molecular (1) Influenza A			Calculated Result		95% Confidence Interval	
		Positive	**Negative**	***Total***	**Metric**	**%**	**Lower CI**	**Upper CI**
***artus* Infl A/B RG RT-PCR Test**	**Positive**	43	9	52	*Positive Agreement*	97.7	0.88	1
	Negative	1	158	159	*Negative Agreement*	94.6	0.90	0.97
	Total	44	167	211				

					Performance			
		FDA Cleared Molecular (2) Influenza A			Calculated Result		95% Confidence Interval	
		Positive	**Negative**	***Total***	**Metric**	**%**	**Lower CI**	**Upper CI**
***artus* Infl A/B RG RT-PCR Test**	**Positive**	1	0	1	*Positive Agreement*	100.0	0.21	1
	Negative	0	53	53	*Negative Agreement*	100.0	0.93	1
	Total	1	53	54				

Comparison of *artus* Infl A/B RG RT-PCR Test Results in the Site 2 Prospectively collected and Archived Specimen Study Relative to the Combined Reference Results for Influenza A

All Sites Combined – Influenza A								
					Performance			
		Combined FDA Cleared Molecular Influenza A			Calculated Result		95% Confidence Interval	
		Positive	**Negative**	***Total***	**Metric**	**%**	**Lower CI**	**Upper CI**
***artus* Infl A/B RG RT-PCR Test**	**Positive**	94	19	113	*Positive Agreement*	97.9	0.93	0.99
	Negative	2	347	349	*Negative Agreement*	94.8	0.92	0.97
	Total	96	366	462				

Comparison of *artus* Infl A/B RG RT-PCR Test Results in the SITE 2 Prospectively collected and Archived Specimen Study Relative to an FDA Cleared Molecular Reference Result for Influenza B

All Sites Combined – Influenza B								
					Performance			
		FDA Cleared Molecular (1) Influenza B			Calculated Result		95% Confidence Interval	
		Positive	Negative	*Total*	Metric	%	Lower CI	Upper CI
***artus* Infl A/B RG RT-PCR Test**	**Positive**	13	2	15	*Positive Agreement*	100.0	0.77	1
	Negative	0	230	230	*Negative Agreement*	99.1	0.97	1
	Total	13	232	245				

					Performance			
		FDA Cleared Molecular (2) Influenza B			Calculated Result		95% Confidence Interval	
		Positive	Negative	*Total*	Metric	%	Lower CI	Upper CI
***artus* Infl A/B RG RT-PCR Test**	**Positive**	1	0	1	*Positive Agreement*	100.0	0.20	1
	Negative	0	216	216	*Negative Agreement*	100.0	0.98	1
	Total	1	216	217				

Results at Each Site – Influenza B								
Site 1								
					Performance			
		FDA Cleared Molecular (1) Influenza B			Calculated Result		95% Confidence Interval	
		Positive	Negative	*Total*	Metric	%	Lower CI	Upper CI
***artus* Infl A/B RG RT-PCR Test**	**Positive**	0	0	0	*Positive Agreement*	-	-	-
	Negative	0	34	34	*Negative Agreement*	100.0	0.90	1
	Total	0	34	34				

					Performance			
		FDA Cleared Molecular (2) Influenza B			Calculated Result		95% Confidence Interval	
		Positive	Negative	*Total*	Metric	%	Lower CI	Upper CI
***artus* Infl A/B RG RT-PCR Test**	**Positive**	0	0	0	*Positive Agreement*	-	-	-
	Negative	0	163	163	*Negative Agreement*	100.0	0.98	1
	Total	0	163	163				

Results at Each Site – Influenza B								
Site 2								
					Performance			

		FDA Cleared Molecular (1) Influenza B			Calculated Result		95% Confidence Interval	
		Positive	**Negative**	***Total***	**Metric**	**%**	**Lower CI**	**Upper CI**
***artus* Infl A/B RG RT-PCR Test**	**Positive**	13	2	15	*Positive Agreement*	100	0.77	1
	Negative	0	196	196	*Negative Agreement*	99.0	0.96	1
	Total	13	198	211				

					Performance			
		FDA Cleared Molecular (2) Influenza B			Calculated Result		95% Confidence Interval	
		Positive	**Negative**	***Total***	**Metric**	**%**	**Lower CI**	**Upper CI**
***artus* Infl A/B RG RT-PCR Test**	**Positive**	1	0	1	*Positive Agreement*	100.0	0.21	1
	Negative	0	53	53	*Negative Agreement*	100.0	0.93	1
	Total	1	53	54				

Comparison of *artus* Infl A/B RG RT-PCR Test Results in the Prospectively collected and Archived Specimen Study Relative to the Combined Reference Results for Influenza B

All Sites Combined – Influenza B								
					Performance			
		Reference Result Influenza B			Calculated Result		95% Confidence Interval	
		Positive	**Negative**	***Total***	**Metric**	**%**	**Lower CI**	**Upper CI**
***artus* Infl A/B RG RT-PCR Test**	**Positive**	14	2	16	*Positive Agreement*	100	0.79	1
	Negative	0	446	446	*Negative Agreement*	99.6	0.98	1
	Total	14	448	462				

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:

   Influenza viruses often change from one season to the next, or even within a season, due to antigenic drift. Distribution of virus types and subtypes can also vary by geographic region. According to reports from the Centers for Disease Control and Prevention (CDC), during the 2010–11 flu season in the United States, the most commonly reported virus was influenza A (H3N2) although high

levels of 2009 influenza A (H1N1) and influenza B viruses circulated as well. Of 246,128 specimens tested for influenza viruses, 54,226 (22%) were positive. Of the positive specimens, 40,282 (74%) were influenza A viruses, and 13,944 (26%) were influenza B viruses. By comparison, in the southeast region of the United States, 10,026 specimens were positive for influenza, of which 6,071 (61%) were influenza A viruses and 3,955 (39%) were influenza B viruses.

In the prospective clinical study for the *artus* Infl A/B RG RT-PCR Kit, nasopharyngeal swab specimens were prospectively collected from 254 patients with symptoms of respiratory tract infection from February to April 2011 and analyzed fresh. Of the 254 specimens, 60 (23.6%) were positive. Of the positive specimens, 15 (25%) were positive for influenza A and 45 (75%) were positive for influenza B. There was no Influenza A and B dual positive specimen detected by the *artus* Infl A/B RG RT-PCR Kit or the reference method during the prospective clinical study.

In addition, a total of 462 prospectively collected archived specimens from patients with symptoms of respiratory tract infection from August 2009 to May 2011 were tested. Of the 462 specimens, 110 (23.8%) were positive. Of the positive specimens, 96 (87.3%) were positive for influenza A and 14 (12.7%) were positive for influenza B. There was no Influenza A and B dual positive specimen detected by the *artus* Infl A/B RG RT-PCR Kit or the reference methods during the clinical study.

N. Proposed Labeling:

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

O. Conclusion:

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