510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K161375

B. Purpose for Submission:

To establish substantial equivalence to a predicate device and to obtain clearance for a new assay: the Alere i RSV Test.

C. Measurand:

RSV NS2 and nucleocapsid genes

D. Type of Test:

Qualitative isothermal nucleic acid amplification assay for the amplification and detection of specific RSV A and RSV B RNA sequences.

E. Applicant:

Alere Scarborough, Inc.

F. Proprietary and Established Names:

Alere™ i RSV
Alere™ i Instrument
Alere™ i RSV Control Swab 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 – Respiratory Virus Panel Nucleic Acid Assay System

OOI – Real Time Nucleic Acid Amplification System

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The Alere i RSV assay performed on the Alere i Instrument is a rapid, molecular, *in vitro* diagnostic test utilizing an isothermal nucleic acid amplification technology for the qualitative detection of respiratory syncytial virus (RSV) viral RNA in direct nasopharyngeal swabs and nasopharyngeal swabs eluted in viral transport media from patients with signs and symptoms of respiratory infection. It is intended for use as an aid in the diagnosis of RSV in children <18 years and adults >60 years in conjunction with clinical and epidemiological risk factors.

2. Indication(s) for use:

   Same as Intended Use.

3. Special conditions for use statement(s):

   For Prescription Use

4. Special instrument requirements:

   To be used only with the Alere i Instrument

I. Device Description:

The Alere i RSV assay consists of a single-use Sample Receiver containing the elution buffer, a single-use Test Base comprising two sealed reaction tubes, each containing a lyophilized pellet, disposable Transfer Cartridge for transfer of the eluted sample to the Test Base, the Alere i Instrument to process the sample and to record raw data, and embedded software to analyze and interpret the data. The test also includes Alere i RSV positive and negative external control swabs for quality control purposes. The controls are also available separately as an accessory item. The Alere Universal Printer and Barcode Scanner are also available as accessories.

To perform the assay, the Sample Receiver and the Test Base are inserted into the Alere i Instrument and the elution buffer is automatically heated by the instrument. The sample is added to the Sample Receiver and transferred via the Transfer Cartridge to the Test Base. Resuspending the lyophilized pellet contained within the Test Base initiates the target amplification. Heating, mixing, and detection by fluorescence are provided by the

instrument, with results automatically reported.

The test performs individual amplification reactions for RSV A and B within the Test Base by targeting regions within the RNA genomes of the two viruses. The amplification target for RSV A is the nonstructural protein gene 2 (NS2); the amplification target for RSV B is the nucleocapsid gene (N). However, separate results for RSV A and RSB are not reported. Alere i RSV technology is isothermal and does not require a heat denaturation step to generate a single strand target for amplification. Instead a thermostable, strand-displacing DNA polymerase, a thermostable nicking endonuclease, and two oligonucleotide templates (primers) are utilized. Reverse transcriptase is also performed in the reaction. The products of Alere i RSV amplification are two complementary oligonucleotides 30-40 nucleotides in length. A product of this size is of sufficient length to be highly unique amongst genomes. The exact length of the products depends on the specific placement of the templates relative to one another and relative to their complementary sequences within the target genome.

The Alere i RSV test uses fluorescently-labeled molecular beacon probes for sensitive and specific real-time detection. Results (positive, negative, or invalid) are displayed by the Alere i Instrument. Results are also stored in an on-board archive and are assigned to a sample ID that has been entered into the Alere i Instrument by the operator, and the date/time the test was performed. Data can be retrieved and downloaded by the operator at any time after testing. An external Alere Universal Printer can be attached via USB to the Alere i Instrument to print test results.

Interpretation of results:

Alere i RSV consists of three fluorescence channels, ROX for the RSV A and RSV B channels and FAM for the Internal Control (IC) channel. Their fluorescent processes physically occur in two separate tubes: ROX for RSV A in Tube 1 while ROX for RSV B and FAM for the Internal Control in Tube 2. The response curves are analyzed within a decision algorithm to form an assay result. Results are reported to the end user as RSV positive, negative or invalid.

Quality Control

Alere i RSV contains an internal control in the tube with the RSV B reagents. The control has been designed to monitor functionality of the amplification/detection process and reagents. This RNA oligonucleotide contains 5' and 3' ends that are complementary to the target template set's recognition regions but with a spacer region that differs from the target's spacer region. Detection of the IC occurs via a molecular beacon that specifically detects the amplified product generated from the IC RNA oligonucleotide. In positive samples where target amplification is strong, the internal control is ignored and the target amplification serves as the 'control' to confirm that the clinical sample was not inhibitory and that assay reagent performance was robust. At a low frequency, clinical samples can contain inhibitors that may generate invalid results.

Procedural Control Valid displayed on the instrument screen indicates that the assay reagents

maintained their functional integrity and the sample did not significantly inhibit assay performance.

Alere i RSV External Controls are designed for use with Alere i RSV. The Positive Control swab is coated with inactivated RSV A and inactivated RSV B virus dried onto a swab. The Negative Control swab is coated with inactivated Streptococcus Group C dried onto a swab. External control swabs are provided with the Alere i RSV kit and are also available separately.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Quidel Molecular RSV + hMPV Assay

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

   K131813

3. Comparison with predicate:

	Alere i RSV	**Quidel Molecular RSV + hMPV**
510(k) Number	K161375	K131813
Assay Targets	RSV	RSV and hMPV
Product Code	OCC, OOI	OCC, OEM
RSV Target	NS2 gene and N gene	NS2 genes and L viral polymerase
Device Technology	Isothermal nucleic acid amplification	RT-PCR nucleic acid amplification
Results Interpretation	Automated	Same
Time to Result	<15 minutes	< 70 minutes
Specimen Types	Nasopharyngeal swab (NPS), NPS eluted in Viral Transport Media (VTM)	NPS and Nasal Swab
Instrument	Alere i Instrument	Cepheid SmartCycler II System, the Applied Biosystems 7500 Fast Dx RT-PCR Instrument, or the Life Technologies QuantStudio Dx RT-PCR Instrument
Intended Use	The Alere i RSV assay performed on the Alere i Instrument is a rapid, molecular, *in vitro*	The Quidel Molecular RSV + hMPV Assay is a multiplex Real-Time PCR (RT-PCR) assay for the qualitative detection and identification of

	diagnostic test utilizing an isothermal nucleic acid amplification technology for the qualitative detection of respiratory syncytial virus (RSV) viral RNA in direct nasopharyngeal swabs and nasopharyngeal swabs eluted in viral transport media from patients with signs and symptoms of respiratory infection. It is intended for use as an aid in the diagnosis of RSV in children <18 years and adults >60 years in conjunction with clinical and epidemiological risk factors.	respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) ribonucleic acid (RNA) extracted from nasal and nasopharyngeal swab specimens from patients with signs and symptoms of respiratory infection. This in vitro diagnostic test is intended to aid in the differential diagnosis of RSV and hMPV infection in humans in conjunction with clinical and epidemiological risk factors. This test is not intended to differentiate the two subtypes of RSV or the four genetic sub-lineages of hMPV. Negative results do not preclude RSV infection and/or hMPV infection and should not be used as the sole basis for diagnosis, 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. The use of additional laboratory testing and clinical presentation must be considered in order to obtain the final diagnosis of respiratory viral infection. The Quidel Molecular RSV + hMPV Assay can be performed using the Life Technologies QuantStudio Dx RT-PCR Instrument, the Applied Biosystems 7500 Fast Dx RT-PCR Instrument, or the Cepheid SmartCycler II System.

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

Not applicable.

L. Test Principle:

Isothermal nucleic acid amplification

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility Study

A reproducibility study of Alere i RSV was conducted by operators from three sites using panels of blind coded specimens containing negative, low positive (at the limit of detection), and moderate positive (above the limit of detection) RSV A and RSV B samples.

Participants tested multiple samples of each panel member on five different days. The percent agreement with expected results for the RSV A moderate positive and low positive samples were 100% (89/89) and 98.9% (89/90), respectfully. The percent agreement with expected result for the RSV B moderate positive and low positive samples were 98.9% (89/90) and 100% (90/90), respectfully. All of the true negative samples (90) generated negative test results. There were no significant differences observed within run (replicates tested by one operator), between run (five different days), between sites (three sites), or between operators (nine operators).
The Reproducibility Study site-to-site qualitative results (agreements with expected results) are presented in the table below.

Alere i RSV - Overall Reproducibility for All Three Sites for All Samples

	Site						Overall Percent Agreement and 95% CI	
	Site 1		Site 2		Site 3			
	% Agreement	Count	% Agreement	Count	% Agreement	Count		
LP[1] RSV A	96.7%	29/30	100%	30/30	100%	30/30	98.9% (89/90)	(94.0%, 99.8%)
MP[1] RSV A	100%	29/29	100%	30/30	100%	30/30	100% (89/89)	(95.9%, 100%)
LP[1] RSV B	100%	30/30	100%	30/30	100%	30/30	100% (90/90)	(95.9%, 100%)
MP[1] RSV B	100%	30/30	96.7%	29/30	100%	30/30	98.9% (89/90)	(94.0%, 99.8%)
TN[1,2]	100%	30/30	100%	30/30	100%	30/30	100% (90/90)	(95.9%, 100%)

[1] LP = Low Positive, MP = Moderate Positive; TN = True Negative
[2] Percent agreement correlates to the percent of negative results

Alere i RSV - Reproducibility by Operator for All Sample Types

	Site 1			Site 2			Site 3			
Sample/ Operator	**X***	**Y***	**Z***	**X***	**Y***	**Z***	**X***	**Y***	**Z***	**Total**
RSV A MP[1]	10/10	9/9	10/10	10/10	10/10	10/10	10/10	10/10	10/10	89/89
RSV A LP[1]	10/10	10/10	9/10	10/10	10/10	10/10	10/10	10/10	10/10	89/90
RSV B MP[1]	10/10	10/10	10/10	9/10	10/10	10/10	10/10	10/10	10/10	89/90
RSV B LP[1]	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	90/90
TN[1,2]	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	90/90
% Agreement	100 %	100 %	98.0 %	98.0 %	100 %	100 %	100 %	100 %	100 %	447/449

[1] LP = Low Positive, MP = Moderate Positive; TN = True Negative
[2] Percent/fraction agreement correlates to the percent/fraction of negative results.
* Individual Operator results are shown as number of samples detected per total number tested.

b. Linearity/assay reportable range:

Not applicable; this is a qualitative assay.

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

Specimen Stability

To provide data supporting the specimen storage recommendations stated in the product package insert, an analytical study was carried out to evaluate specimen stability.

Contrived positive nasal swab samples were prepared using the RSV A (Strain A2) and RSV B (Strain 9320) dilutions in pooled negative clinical matrix with targeted concentrations near the respective limit of detection (LOD) levels. Mock negative nasal swab samples were prepared using pooled clinical matrix. Swabs were prepared by coating 10 µL of the virus dilution or clinical matrix onto the swab. Swab coating was done by lightly scratching the swab surface with the pipette tip as the dilution was pipetted to ensure liquid was absorbed into the swab tip. All prepared swabs were tested at N=10 at each of the following three time points/conditions: For Direct Swabs, Time 0, 3 hours at 30 °C, 22 hours at 2-8 °C. Swabs tested after 22 hours at 2-8 °C were obtained from the group of swabs stored for 3 hours at 30 °C for a total of 25 hours elapsed before testing. For swabs eluted in VTM, 200 µL was tested according to the VTM assay procedure at the following time points: 0, 9 hours at 30 °C, 16 hours at 2-8 °C, 48 hours at 2-8 °C. Positive samples were considered stable as long as they tested positive in Alere i RSV. Negative samples were considered stable as long as they generated negative results in the test. Results of the Direct Swab and VTM stability studies are shown in the tables below.

Alere i RSV Swab Direct Sample Storage

Sample Type	Time 0		3 Hours at 30 °C		22 Hours at 2-8 °C	
	RSV A	RSV B	RSV A	RSV B	RSV A	RSV B
Negative Matrix	0/10	0/10	0/10	0/10	0/10	0/10
RSV A	10/10	0/10	10/10	0/10	10/10	0/10
RSV B	0/10	10/10	0/10	10/10	0/10	10/10

Alere i RSV Swab Eluted in VTM Sample Storage

Sample Type	Time 0		9 Hours at 30 °C		16 Hours at 2-8 °C		48 Hours at 2-8 °C	
	RSV A	RSV B	RSV A	RSV B	RSV A	RSV B	RSV A	RSV B
Negative Matrix	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
RSV A	10/10	1/10	10/10	0/10	10/10	0/10	10/10	0/10
RSV B	0/10	10/10	0/10	10/10	0/10	10/10	0/10	10/10

Swab direct method samples generated expected results for each sample type at each storage time point.

VTM method samples generated expected results for each sample type at each storage time point except at Time 0 for the clinical matrix diluent which generated one RSV false positive result. This sample was not retested and all later testing generated expected results. One RSV A VTM sample at Time 0 generated a positive signal in the RSV B channel but does not affect the final answer provided by the Alere i RSV test.

The Product Insert states that NPS samples can be stored at room temperature for up to 2 hours. If the swab will be held longer than two hours, it must be refrigerated at 2-8°C and tested within 24 hours from the time of sample collection. The Product Insert also states that eluted swab samples (VTM) can be held at 15-30°C for up to 8 hours or at 2-8°C for 24 hours prior to testing with Alere i RSV.

Shelf Life

Alere performed a product stability study to generate stability data to support the expiry assigned to Alere i RSV test kits and control swabs. The test components were tested at specified time points using the RSV A & B Positive QC LOD Controls, and RSV Positive Control Swabs as the positive samples. The QC LOD Controls are approximately 3X the LOD when 50µl of a control is added to the sample receiver. Elution Buffer, UTM, Negative Controls Swabs, and presumed negative nasal swabs were tested as negative samples. The table below details the sample type and number of replicates tested at each time point for each condition.

Alere i RSV Kit Stability Test Conditions

Condition / Time Point	Sample Type	Replicates	Total Tests
Time 0 All conditions	RSV A and B LOD Presumed Neg. Swab Elution Buffer VTM Pos. Control Swabs Neg. Control Swabs	105 105 50 50 25 20 20	375
28-32°C: Months 1 and 3	RSV A LOD RSV B LOD Elution Buffer	10 10 10	30
45°C: Months 1,2,3 and 4	RSV A LOD RSV B LOD Elution Buffer Pos. Control Swabs	10 10 10 5	35

To perform testing of RSV A and B QC LOD controls, 50µL of the control material was added to the Sample Receiver and mixed by pipetting up and down and swirling the pipette tip for 10 seconds. To perform testing of the Elution Buffer, no samples was added to the Sample Receiver. The buffer was transferred to the Test Base using the Transfer Cartridge when prompted by the instrument. For testing VTM samples, 200µL of VTM was added to the Sample Receiver and mixed by pipetting up and down and swirling the pipette tip for 10 seconds. Tests were considered stable as long as they continued to produce positive results for positive samples and negative results for negative samples.

Data generated so far supports the following conditions for Alere i RSV kit storage conditions:

Alere i RSV Kit Stability Results

Lot	Months of Stability at Various Temperatures	
	28-32°C	45°C
Transfer Lot 1	3 Months	3 Months
Transfer Lot 2	3 Months	3 Months
Transfer Lot 3	3 Months	2 Months
Transfer Lot 4	1 Month	N/A

Swab Type Compatibility Study

Alere performed a swab type validation study to establish which swabs are acceptable for use with the Alere i RSV assay. Swab samples were prepared using RSV A and RSV B strains diluted to approximately 3 times the limit of detection. Swabs were prepared by coating 10µL of the RSV A virus dilution, RSV B virus dilution or clinical matrix diluent on the swab. Ten samples of each swab type were prepared for each sample type (RSV A,

RSV B, or clinical matrix). Swab coating was done by lightly scratching the swab surface with the pipette tip as the solution was pipetted to ensure liquid was absorbed into the swab tip. All swabs were tested within 10 minutes of coating.

Swabs tested in this study include: Puritan Small Tapered Foam Tipped Swab; Puritan PurFlock Ultra Flocked Swab – Mini-tip; Puritan HydraFlock Flocked Swab – Mini-tip; Puritan HydraFlock Micro Ultra Fine Tip Flocked Swab; Copan Mini-tip Nylon Flocked Swab; Copan Ultra Mini-tip (Pediatric) Nylon Flocked Swab; Copan Ultra-Thin Mini-tip Nylon Flocked Swab; and BD CultureSwab Liquid Stuart with Foam Reservoir.

The BD CultureSwab Liquid Stuart Collection and Transport System includes a foam media reservoir soaked with Liquid Stuart's transport media. The inoculated swab was inserted into the tube containing the Liquid Stuart's Media soaked foam reservoir so that the swab head made contact with the sponge. Swabs were tested immediately after contacting the media soaked reservoir.

All of the swabs, except the Puritan PurFlock Ultra Flocked Swab – Mini-tip generated the expected results for all ten replicates for each sample type. A precaution has been added to the package insert warning the Puritan PurFlock Ultra Flocked Swab – Mini-tip is not cleared for use with this assay. All other commonly used flocked swabs appear to be acceptable.

Viral Transport Media Validation Study

Thirteen common transport media were tested in a VTM validation study to determine if the particular media type is acceptable for use with VTM specimens in the Alere i RSV assay. Presumed negative nasal swabs were eluted in 3 mL of each VTM type. Swabs were eluted by swirling and mixing the swab for 10 seconds. For each VTM type, n=10 clinical matrix diluent (negative sample), RSV A and RSV B samples were tested at a volume of 200 µL on the Alere i RSV test according to the VTM assay procedure. Dilutions of RSV A and RSV B were prepared at a concentration approximately 3 times the VTM limit of detection level in each VTM type clinical matrix diluent. A VTM was considered acceptable if positive RSV samples generated positive RSV results on the Alere i RSV assay and negative samples generated negative results. A summary of the testing is shown in the table below.

Alere i RSV VTM Validation Study Results

VTM Type	Sample Type	Replicates	RSV A Results*	RSV B Results*
Liquid Amies Transport Media	Matrix Diluent	N=10	-	-
	RSV A Dilution	N=10	+	-
	RSV B Dilution	N=10	-	+
UTM	Matrix Diluent	N=10	-	-
	RSV A Dilution	N=10	+	-
	RSV B Dilution	N=10	-	+
M4	Matrix Diluent	N=10	-	-
	RSV A Dilution	N=10	+	-
	RSV B Dilution	N=10	+4/ -6	+
M4-RT	Matrix Diluent	N=10	-	-
	RSV A Dilution	N=10	+	-
	RSV B Dilution	N=10	-	+
M5	Matrix Diluent	N=10	-	-
	RSV A Dilution	N=10	+	-
	RSV B Dilution	N=10	-	+
M6	Matrix Diluent	N=10	-	-
	RSV A Dilution	N=10	+	-
	RSV B Dilution	N=10	-	+
DMEM	Matrix Diluent	N=10	-	-
	RSV A Dilution	N=10	+	-
	RSV B Dilution	N=10	-	+
Starplex Multitrans Media	Matrix Diluent	N=10	-	-
	RSV A Dilution	N=10	+	-
	RSV B Dilution	N=10	-	+
Tryptose Phosphate Broth	Matrix Diluent	N=10	-	-
	RSV A Dilution	N=10	+	-
	RSV B Dilution	N=10	-	+
0.9% Saline Solution	Matrix Diluent	N=10	-	-
	RSV A Dilution	N=10	+	-
	RSV B Dilution	N=10	-	+
Phosphate Buffered Broth	Matrix Diluent	N=10	-	-
	RSV A Dilution	N=10	+	-
	RSV B Dilution	N=10	-	+
2.5% Veal Infusion Broth	Matrix Diluent	N=10	-	-
	RSV A Dilution	N=11	+10 / 1 invalid	-10 / 1 invalid
	RSV B Dilution	N=10	-	+
Vircell Transport Media	Matrix Diluent	N=10	-	-
	RSV A Dilution	N=10	+	-
	RSV B Dilution	N=10	-	+

* Unless otherwise indicated, + or – represents 10/10 concurrence with the expected result.

Four RSV B samples generated positive results in the RSV A channel in the M4 media. The positive signal in the RSV A channel does not change the outcome of the Alere i RSV assay result and all ten replicates for the RSV B eluted in M4 media generated positive signals in the RSV B channel.

d. Detection limit:

The objective of the Analytical Sensitivity Study was to identify the limit of detection (LOD) of the Alere i RSV using characterized strains of RSV A and RSV B. For the purposes of the study, the LOD level was defined as those concentration of RSV A and RSV B that produced positive Alere i RSV test results approximately 95% of the time when tested in multiple replicates.

The Swab Direct Method samples were tested on the Alere i RSV test according to the swab direct assay procedure. VTM samples were each eluted into a separate vial containing 3 mL of UTM. The two swab eluates were combined and mixed thoroughly by gentle vortex. Each swab eluate was tested on the Alere i RSV test according to the VTM assay procedure.

For both methods, if 100% positive results were obtained with the starting dilution, a series of 2-fold dilutions beginning with the dilution that produced a positive result was prepared. Two-fold dilutions were tested in triplicate until a negative result was obtained. Then 20 replicates of the lowest 2-fold dilution that produced a positive result were tested. If 95% positive results were obtained (19/20 positive) that dilution level was reported as the LOD level.

If testing of 2-fold dilutions did not generate a 95% positive results, dilutions that were half-way between the lowest 100% positive 2-fold dilution and the 2-fold dilution below it were tested until a dilution testing 95% positive was determined.

LOD dilutions were stored at 2-8°C until conclusion of testing each day. These dilutions were used to determine concentration in targets/mL via qPCR. The empirically determined LOD for each specimen type and each RSV strain is shown in the table below along with the corresponding genome equivalents/mL.

Alere i RSV Limits of Detection

Specimen Type	Strain	$TCID_{50}$/mL	$TCID_{50}$/test*	Targets/mL	Targets/test*
Swab Direct	RSV A	5.82×10^{2}	5.82	7.80×10^{4}	7.8×10^{2}
	RSV B	6×10^{1}	6.0×10^{-1}	5.43×10^{3}	5.43×10^{1}
VTM	RSV A	9.15×10^{3}	6.10	1.06×10^{6}	7.07×10^{2}
	RSV B	9.64×10^{2}	6.4×10^{-1}	1.48×10^{5}	9.87×10^{1}

* Per test amount is the total amount added to the Alere i system (into 2.5 mL buffer)

e. Analytical reactivity:

Various RSV A and RSV B strains were obtained from their respective vendors and provided with a vendor designated titer in PFU/mL (RSV B1 did not have a titer and is presented as dilution factors). Samples were prepared using several concentrations of each of the quantified viable RSV A and B strains diluted in clinical matrix diluent. A 1:100 dilution of each stock of virus was prepared for the starting concentration. All strains were diluted per the dilution scheme described below until the lowest dilution at which 3/3 replicates were positive was identified.

Dilutions of each virus were prepared in clinical matrix diluent and 10µL of each dilution was dispensed on to sterile foam swabs. All viral dilutions were run in triplicate on the Alere i RSV according to the swab direct assay procedure. The lowest level of each strain that generated positive results on all replicates was identified as the lowest level detected by Alere i RSV. If the initial testing concentration was positive, the strain was diluted 10-fold and tested (n=3) until a negative result was obtained.

When a negative result was obtained, two-fold dilutions were tested, starting at the lowest positive dilution and tested in triplicate. A dilution level was considered positive if all three replicates generated a positive result. At least one out of three negative results was considered a negative result in the study.

The lowest detectable dilutions were stored frozen at -80 °C until conclusion of testing each day. This dilution was used to determine concentration in targets/mL via validated qPCR. A summary of the results of the reactivity testing is presented in the table below.

Alere i RSV Analytical Reactivity Results

Strain	Subtype	Concentration (PFU/mL or Genome Equivalents)		Alere i RSV Result (n=3)
		PFU/mL	Genome Equivalents/mL	
RSV A Long	A	9.38×10^{-3}	1.75×10^{3}	Positive
RSV B1	B	1:20,000[a]	2.37×10^{3}	Positive
RSV B 18537	B	1.00×10^{-1}	1.37×10^{3}	Positive

[a] RSV B1 PFU/mL was not available from the vendor and is presented as a dilution factor.

f. Analytical specificity:

To determine the analytical specificity of Alere i RSV, 40 commensal and pathogenic microorganisms (21 bacteria, 18 viruses, and 1 yeast) that may be present in the nasal cavity or nasopharynx were tested. All of the following microorganisms were

negative when tested at concentrations ranging from 10^3 to 10^{10} cells/mL or CFU/mL (bacteria), 10^4 to 10^8 $TCID_{50}$/mL (viruses), and 10^8 cells/mL (yeast). Some cross-reactivity was observed for *E. coli* at concentrations greater than 2.75 x10^9, *Moraxella catarrhalis* at concentrations greater than 1.50 x10^9, and *Proteus vulgaris* at concentrations greater than 4.69 x 10^8 and has been noted in the package insert.

Alere i RSV Analytical Specificity

Bacteria	**Viruses**	**Yeast**
Bordetella pertussis	Adenovirus Type 1	*Candida albicans*
Corynebacterium diphtheriae	Adenovirus Type 7	
Escherichia coli	Enterovirus/Coxsackievirus B4	
Haemophilus influenzae	Enterovirus Type 70	
Klebsiella pneumoniae	Epstein Barr Virus	
Lactobacillus plantarum	Human Coronavirus 229E	
Legionella pneumophila	Human Coronavirus OC43	
Moraxella/Branhamella catarrhalis	Human Cytomegalovirus	
Mycobacterium tuberculosis	Human Echovirus 7 (Wallace)	
Mycoplasma pneumoniae	Human Metapneumovirus	
Neisseria gonorrhoeae	Influenza A	
Neisseria meningitidis	Influenza B	
Neisseria sicca	Measles (Edmonston)	
Neisseria subflava	Mumps (Enders)	
Proteus vulgaris	Parainfluenza 1	
Pseudomonas aeruginosa	Parainfluenza 2	
Staphylococcus aureus	Parainfluenza 3	
Staphylococcus epidermidis	Rhinovirus type 1A	
Streptococcus, GroupA		
Streptococcus pneumonia		
Streptococcus salivarius		

In addition, *in silico* analysis was performed to determine whether there is any significant overlap between Alere i RSV target nucleic acid sequence and the genomes of the following upper respiratory tract microorganism. None of the organisms maintained genomic sequence that was significantly similar to the Alere i RSV target sequences.

Alere i RSV Specificity (*in silico*)

Viruses	**Bacteria**
Adenovirus 2	*Bordetella bronchiseptica*
Adenovirus 3	*Chlamydia pneumonia*
Adenovirus 4	*Chlamydia trachomatis*
Adenovirus 5	*Neisseria mucosa*
Adenovirus 11	*Proteus mirabilis*
Adenovirus 14	

Adenovirus 31	
Coronavirus NL63	
Coxsackievirus B35	
Echovirus 6	
Echovirus 9	
Echovirus 11	
Enterovirus 71	

g. Potentially Interfering Substances:

An analytical study was performed to assess the potential interference effects of 17 substances naturally present in respiratory specimens or that may be artificially introduced into the nasal cavity/nasopharynx.

Alere i RSV Interfering Substances Tested

Substance	Concentration
Mucin	0.0625%
Whole Blood	1%
NeoSynephrine Cold and Sinus Extra Strength Spray	20%
Afrin PumpMist Original	20%
Ocean Saline	20%
Chloroseptic Max	20%
Zicam Allergy Relief	20%
Beclomethasone	0.068 mg/mL
Budesonide	0.051 mg/mL
Dexamethasone	0.48 mg/mL
Flunisolide	0.04 mg/mL
Fluticasone propionate	0.04 mg/mL
Mometasone furoate	0.04 mg/mL
Mupircoin	4.3 mg/mL
Tobramycin	1.44 mg/mL
Triamcinolone	0.04 mg/mL
Zanamivir (Relenza)	0.284 mg/mL

Swab samples were prepared using RSV A and RSV B strains diluted to approximately three times the VTM limit of detection in clinical matrix diluent. Swabs were prepared by coating 10µL of the virus dilution onto the swab. Swab coating was done by lightly scratching the swab surface with the pipette tip as the solution was pipetted to ensure liquid was absorbed into the swab tip.

A volume of 50µL of each interfering substance was dispensed into sterile tubes. Blank swabs or RSV A or RSV B inoculated swabs were placed into each tube in triplicate. Swabs were stirred to absorb all of the liquid into the swab head. Blank swabs containing only the potentially interfering substance were tested to determine if

the substance causes false positive or invalid results in the assay. None of the substances were found to affect test performance at the concentrations tested. False negative results were observed when mucin was present at a concentration of 0.125% w/v or greater and has been noted in the package insert Limitations section.

h. Microbial Interference:

Alere i RSV testing was performed in the presence of non-RSV respiratory pathogens. Vendor provided stocks of RSV A and B strains were diluted in UTM to approximately three times the limit of detection. Contrived RSV A and B positive swab specimens were prepared by coating 10 microliters of virus dilution onto each swab. Fifty microliters of each of the four potentially interfering viruses were dispensed into sterile tubes.

One of the RSV A or RSV B inoculated swabs was placed into each tube (n=3 RSV A and n=3 RSV B swabs total for each non-RSV virus). Swabs were stirred to absorb all of the liquid into the swab head. Swabs were tested immediately as a swab direct sample according to the Alere i RSV test procedure. A table of the potentially interfering viruses and the concentrations tested is shown in the table below.

List of Potentially Interfering Organisms and Concentrations Tested

Potential Interfering Organism	Concentration ($TCID_{50}$/ml)
Adenovirus Type 1	1.58×10^7
Rhinovirus Type 1A	1.58×10^7
Influenza A	5.00×10^7
Influenza B	1.00×10^8

All co-infection swabs produced the expected outcome (RSV A or RSV B signal detected) for all three replicates tested.

i. Carry-over:

To test for the possibility of cross-contamination between runs, a single dilution containing both RSV A and RSV B was prepared in clinical matrix diluent using RSV A and RSV B diluted to approximately 30 times each strain's limit of detection level. Swab direct samples were prepared by coating 10µL of the virus dilution onto the swab. Swab coating was done by lightly scratching the swab surface with the pipette tip as the solution was pipetted to ensure liquid was absorbed into the swab tip. VTM method samples were tested on the Alere i RSV test according to the VTM assay procedure.

Positive and negative swab samples were tested per the product instructions. Testing between positive and negative samples alternated for a total of 15 rounds (15 positives and 15 negatives) on one Alere i instrument. All positive and negative daily controls generated the expected results on each day of testing. A summary of the results of the Direct Swab and VTM testing is shown in the table below.

Alere i RSV Carryover Testing Results

Sample Type	Number Tested	Alere i RSV Result (Positive Samples)		Alere i RSV Result (Negative Samples)	
		RSV A Result	RSV B Result	RSV A Result	RSV B Result
Swab Direct	15	+	+	-	-
VTM	15	+	+	-	-

All positive samples generated RSV A positive and RSV B positive signals. All negative samples generated RSV A negative and RSV B negative signals.

j. Assay cut-off:

Alere i RSV consists of three fluorescence channels, ROX for the RSV A and RSV B channels and FAM for the Internal Control (IC) channel. Their fluorescent processes physically occur in two separate tubes: ROX for RSV A in Tube 1 while ROX for RSV B and FAM for the Internal Control in Tube 2. The response curves are analyzed within a decision algorithm to form an assay result. For each response curve, the gradient values within the threshold window are compared to the gradient threshold and the results of this comparison ultimately determine the channel result, "Asserted", "Not Asserted", or "Indeterminate". The gradient threshold (cut-off) in the algorithm was derived through the RSV rehydration study and analysis of retrospective culture of unknown clinical samples. The gradients of positive and negative samples were analyzed. These thresholds were subsequently verified during the clinical study performed during the 2015-2016 respiratory season.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable. Performance of Alere i RSV was evaluated against the reference method in a prospective clinical study.

b. Matrix comparison:

Not applicable.

3. Clinical Studies:

A multi-center study was conducted at nine study sites located throughout the U.S. during the 2015-2016 respiratory season. Alere i RSV was used to evaluate fresh, prospectively collected nasopharyngeal swab specimens from children <18 years and adults ≥60 years with suspected respiratory infection and meeting the inclusion/exclusion criteria. Samples were tested with Alere i RSV within two hours of sample collection. At all sites, one swab specimen was tested directly on Alere i RSV, according to product instructions for testing swabs. The other swab was swirled in VTM for 10-20 seconds, the shaft cut-off,

and a sample of the VTM eluate tested with Alere i RSV. The remaining VTM containing the residual swab was stored refrigerated and shipped to the central or local laboratory for reference testing.

A total of 531 nasopharyngeal swab specimens were enrolled in the study. Of those, 25 specimens did not meet eligibility criteria. A total of 506 nasopharyngeal swab specimens were considered evaluable. Of the 506 Subjects that met inclusion/exclusion criteria, five samples returned invalid results after repeat testing via the reference method. Additionally, four direct swab specimens returned invalid results on the Alere i RSV after repeat testing leaving 497 Direct Swab and 501 VTM specimens evaluable for the purpose of data analysis. Patient age and gender distribution for the evaluable specimens is presented in the table below.

Clinical Study Participant Demographics

Age Group (Years)	Female	Male
<1	56	66
1-5	114	129
6-10	27	31
11-18	19	22
≥60	20	22
Total	236	270

During the prospective clinical study, the initial invalid rate for direct nasopharyngeal swab samples (before repeat testing per the product instructions) was 4.1% (21/506) (95% CI: 2.7% to 6.3%). After repeat testing per the product instructions, the invalid rate was 0.8% (4/506) (95% CI: 0.3% to 2.0%).

The initial invalid rate for nasopharyngeal swabs eluted in viral transport media was 2.2% (11/506) (95% CI: 1.2% to 3.9%). After repeat testing per the product instructions, the invalid rate was 0% (0/506) (95% CI: 0.0% to 0.8%).

Compared to an FDA-cleared molecular assay, the performance of the Alere i RSV for direct NPS and NPS swabs eluted in VTM is presented below, respectively.

Alere i RSV Direct Nasopharyngeal Swab Performance Compared to FDA-cleared Molecular Comparator

Alere i RSV	Comparator Method		
	Positive	Negative	Total
Positive	137	7[a]	144
Negative	2	351	353
Total	139	358	497
Sensitivity: 137/139 98.6% (95%CI: 94.9-99.6%)			
Specificity 351/358 98.0% (95%CI: 96.0-99.1%)			

[a]RSV nucleic acid was detected in 6/7 false positive specimens using an FDA-cleared molecular test

Alere i RSV Nasopharyngeal Swab Eluted in VTM Performance Compared to FDA-cleared Molecular Comparator

Alere i RSV	Comparator Method		
	Positive	Negative	Total
Positive	138	8[a]	146
Negative	2	353	355
Total	140	361	501
Sensitivity: 138/140 98.6% (95%CI: 94.9-99.6%)			
Specificity 353/361 97.8% (95%CI: 95.7-98.9%)			

[a]RSV nucleic acid was detected in 6/8 false positive specimens using an FDA-cleared molecular test

4. Clinical cut-off**:**

   Not applicable.

5. Expected values/Reference range:

   In the Alere i RSV prospective clinical study (described in the "Clinical Studies" section above), a total of 506 direct NPS specimens were evaluable by the Alere i RSV assay. The number and percentage of RSV positive cases per specified age group, as determined by the Alere i RSV assay, are presented in the table below:

Alere i RSV Expected Values

Age Group (Years)	Number NPS Swabs Tested	Number of RSV Positives	% RSV Positivity
<1	122	58	48%
1-5	243	82	34%
6-10	58	0	0%
11-18	41	1	2%
≥60	42	5	12%
Combined	506	146	29%

N. Instrument Name:

Alere i Instrument

O. System Descriptions:

1. Modes of Operation:

   The Alere i Instrument is a portable bench-top unit designed to perform in laboratory and point-of care environments. Alere i RSV components are color-coded to match the corresponding holder on the instrument and designed to only fit in the corresponding holder in one direction. Once properly placed into the instrument, the Alere i Instrument uses image analysis to automatically detect the presence of the Test Base and to check the test type and expiry date on the barcode present on the Test Base. This triggers the test sequence. Heating, mixing, and detection by fluorescence are provided by the instrument. The Alere i Instrument is equipped with two optical fluorescence modules, FAM and ROX. Each optical module consists of a LED excitation source and photodiode receiver configured to the fluorescent channel. Fluorescence signals are optically filtered by a confocal lens and mirror arrangement and processed by software running on an on-board computer. The results are calculated automatically and presented on the instrument graphical user interface and available for export in JSON format or printing.

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ___X_____ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No ___X_____

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ___X_____ or No _____

3. Specimen Identification:

   Specimen ID is entered using on screen keyboard or barcode scanner.

4. Specimen Sampling and Handling:

   Not applicable. The specimens are manually inserted in the Sample Receiver in the instrument.

5. Calibration:

   The Alere i Instrument is factory calibrated and does not require any further calibration and verification at user site. However, if the instrument was transported or moved, a performance check using Alere i Positive and Negative Controls is recommended to ensure proper functionality by the manufacture.

6. Quality Control:

   Quality control is addressed for each specific FDA-cleared assay to be run on the instrument (separately cleared).

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable.

Q. Proposed Labeling:

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

R. Conclusion:

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