510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K181443

B. Purpose for Submission:

This is a new 510(k) submission for the determination of Substantial Equivalence for the Mesa Biotech Accula RSV Test. Mesa Biotech, Inc. has submited a combined 510(k) and CLIA waiver package for dual review.

C. Measurand:

RSV L Gene RNA

D. Type of Test:

RT-PCR amplification followed by hybridization and colorimetric visualization of amplified products on a test strip

E. Applicant:

Mesa Biotech, Inc.

F. Proprietary and Established Names:

Accula RSV Test

G. Regulatory Information:

1. Regulation section:

   21 CRF 866.3980, Respiratory viral panel multiplex nucleic acid assay

2. Classification:

   Class II

3. Product code:

   OCC – Respiratory Virus Panel Nucleic Acid Assay System

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The Accula RSV Test performed on the Accula Dock is a molecular *in vitro* diagnostic test utilizing polymerase chain reaction (PCR) and lateral flow technologies for the qualitative, visual detection of respiratory syncytial virus (RSV) viral RNA. The Accula RSV Test uses a nasal swab specimen collected from patients with signs and symptoms of respiratory infection. The Accula RSV assay is intended as an aid in the diagnosis of RSV infection in children and adults in conjunction with clinical and epidemiological risk factors.

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

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 Accula Dock Instrument

I. Device Description:

The Accula RSV Test is a semi-automated, colorimetric, multiplex reverse-transcription polymerase chain reaction (RT-PCR) nucleic acid amplification test to qualitatively detect RSV A and RSV B viral RNA from unprocessed nasal swabs that have not undergone prior nucleic acid extraction. The system integrates nucleic acid extraction, reverse transcription, nucleic acid amplification using a novel Mesa Biotech technology, and hybridization-based visual detection into a completely self-contained and semi-automated system. The Accula RSV system consists of a small reusable Dock to drive the automated testing process and a single-use disposable test cassette that contains all the reagents.

Upon insertion of a Test Cassette, the Dock will detect and identify the cassette type. After the user transfers a clinical sample into the cassette and closes the dock lid, the embedded firmware will control fluid flow of the sample into the various chambers of the cassette.

Amplicon detection requires the hybridization of two internal probes to generate a signal on the Accula RSV detection strip. Dyed polystyrene microspheres are conjugated to oligonucleotide probes to form an amplicon-microsphere complex by hybridization to an internal region of the amplicon. The complex migrates through the pores of the detection strip membrane and across capture zones which contain oligonucleotides complementary to an amplicon region distinct from the detection probe binding site. Hybridization of the amplicon-microsphere complex to a capture zone probe retards the flow of the specific amplicon. This allows for the generation of a visible signal in the form of a colored line at the capture zone.

Interpretation of results:

Results are interpreted visually by the operator after the test has completed. A colored line of any intensity at the “RSV” location indicates a positive result if the test is valid. A Negative Control line at the end of the test strip controls for non-specific binding or amplification and must be absent for a valid test. A positive control line at the beginning of the strip tests for amplification effectiveness and is necessary to interpret a test as “negative” for RSV.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Alere i RSV

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

   K161375

3. Comparison with predicate:

Table 1: Similarities Between Accula RSV and Predicate Device

Similarities		
Item	**Mesa Biotech Accula RSV Test**	**Alere i RSV Test**
Assay Targets	RSV virus	Same
Sample Type	Nasal Swab	Same
Assay Results	Qualitative	Same
Intended Users and Locations	Clinical Lab and Point of Care	Same
Nucleic Acid Purification	No	Same
Internal Control	Yes	Same
Positive and Negative Control Swabs	Yes	Same

Table 2: Differences Between Accula RSV and Predicate Device

Differences		
Item	**Mesa Biotech Accula RSV Test**	**Alere i RSV Test**
Intended Use	The Accula RSV Test performed on the Accula Dock is a molecular *in vitro* diagnostic test utilizing polymerase chain reaction (PCR) and lateral flow technologies for the qualitative, visual detection of respiratory syncytial virus (RSV) viral RNA. The Accula RSV Test uses a nasal swab specimen collected from patients with signs and symptoms of respiratory infection. The Accula RSV assay is intended as an aid in the diagnosis of RSV infection in children and adults in conjunction with clinical and epidemiological risk factors. Negative results do not preclude RSV virus infection and should not be used as the sole basis for treatment or other patient management decisions.	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.
RSV Target	L viral polymerase gene	NS2 gene and nucleocapsid gene N
Assay Technology	PCR amplification and visual identification of amplified products by hybridization to a test strip	Isothermal nucleic acid amplification and detection of amplified products using molecular beacon probes
Detection	Dyed microparticle conjugates specifically detect amplified products	Fluorescently-labeled molecular beacons identify amplified RNA targets
Instrument	Amplification controlled by the Accula Dock	Amplification performed on the Alere i Instrument
Results Interpretation	Visual interpretation of colored lines on a test strip	Optical detection of fluorescence by the Alere i instrument

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

Not applicable

L. Test Principle:

Nucleic acid amplification plus hybridization to a membrane and chromatographic visual detection

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   The reproducibility of the Accula RSV Test was tested in a study using contrived nasal swabs at three CLIA-waived sites (sites 1 through 3) and one moderately complex site (site 4) based in the United States. The objective of this study was to test panels of contrived nasal swab samples with the Accula RSV Test to demonstrate reproducibility of the assay in the hands of multiple users at multiple sites over multiple non-consecutive days.

   Samples were provided to testing operators in panels of 3 samples (RSV Low Positive, Moderate Positive, and Negative). The targeted concentrations for the Moderate Positive samples were approximately 3X the respective limit of detection (LoD), the targeted concentrations for the Low Positive samples were approximately 1X the respective LoD, and the Negative samples contained no RSV. Samples were blinded and randomized. Each operator tested one panel per day, testing a maximum of three samples at a time. Each sample was tested in triplicate (from separate swabs) (2 operators x 1 run x 3 swabs x 5 non-consecutive days = 30 observations for each site per sample type). Results are reported for counts and percent agreement with expected results. Reproducibility was evaluated by site, by operator and by day.

Table 3: Site-to-Site Reproducibility: Percent Agreement and Total Counts

	Agreement By Site								Overall Percent Agreement and 95% CI	
	Site 1		Site 2		Site 3		Site 4			
	%	Count	%	Count	%	Count	%	Count		
LP[1] RSV	100%	30/30	100%	30/30	100%	30/30	100%	30/30	100% (120/120)	(96.9%, 100%)
MP[1] RSV	100%	30/30	100%	30/30	100%	31/31*	100%	30/30	100% (121/121)	(96.9%, 100%)
TN[1,2]	100%	30/30	100%	30/30	100%	29/29*	100%	30/30	100% (119/119)	(96.9%, 100%)

[1] LP = Low Positive, MP = Moderate Positive; TN = True Negative
[2] Percent agreement is for negative results
* One negative swab was mistakenly spiked with MP RSV

Agreement of actual results with expected results was 100%. There were no differences observed within run (replicates tested by one operator), between runs (five different days), between sites (four sites), or between operators (eight operators).

b. *Linearity/assay reportable range:*

Not Applicable; this is a qualitative assay.

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

Quality Control:

Each test cassette contains two internal process controls: an internal positive control and a negative control. The positive control is a non-infectious RNA molecule of the MS2 bacteriophage. The negative control is a non-RSV nucleic acid capture probe intended to monitor for non-specific binding. Each test kit also contains separate control swabs with inactivated RSV virus as well as a negative control swab to verify reagent integrity. The manufacturer recommends positive controls be run for each new lot or shipment of kits received, and for each new operator performing the test. Additional positive control swabs are available for purchase from the manufacturer.

Specimen Stability

Contrived samples of RSV A were tested in triplicate on the Accula RSV assay in an experiment to determine specimen stability. Samples were prepared near the empirical LoD in pooled negative matrix containing the Accula RSV nasal swab buffer solution and stored under various conditions. The contrived specimens were prepared by spiking cultured viruses onto rayon-tipped swabs, which were then dipped into pooled negative nasal swab matrix (PNNS); the material was pooled and aliquoted into appropriately sized tubes for storage. Test conditions and the results of the testing are summarized in the table below:

Table 4: Specimen Stability After Elution in Sample Buffer

Test Sample	Storage Condition	Duration	Pos. RSV / Expected	Pass/Fail
1	None	0	3/3	Pass
2	15-30°C	1 Hour	3/3	Pass
3	15-30°C	4 Hours	3/3	Pass
4	15-30°C	24 Hours	3/3	Pass
5	2-8°C	24 Hours	3/3	Pass
6	2-8°C	72 Hours	3/3	Pass
7	-20°C	72 Hours	3/3	Pass
8	-20°C	1 Week	3/3	Pass
9	-80°C	72 Hours	3/3	Pass
10	-80°C	2 Weeks	3/3	Pass
11	-20°C	1 Freeze/thaw	3/3	Pass

12	-20°C	2 Freeze/thaw	3/3	Pass
13	-20°C	3 Freeze/thaw	3/3	Pass

Mesa Biotech has indicated that specimens should be transferred to the nasal swab buffer sample tube immediately after collection and stored at 2-8°C for no longer than 24 hours. Swabs that have not been eluted into nasal swab buffer can be stored in the original swab packaging for up to two hours at room temperature. Tests supporting the storage of the un-eluted swab sample in the original packaging are described in the decision summary for CW180006.

<u>Shelf Life</u>

Accula RSV test kits are being tested for shelf life by storage at 30°C for different lengths of time. At each time interval (day 0, 2 weeks, 4 weeks, and at 3 month intervals beyond) stored test kits are used to test control swabs and simulated RSV samples prepared in clinical matrix. Contrived specimens are prepared in clinical matrix near the empirical LoD. Negative samples and control swabs are tested as one replicate. Acceptance criteria for shelf life studies are as follows: all positive samples and controls must yield a 100% positive rate and all negative samples and controls must yield a 100% negative rate. 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 demonstrates that the three lots tested are stable for the first three time points tested (up to and including 4 weeks). The firm will continue stability testing and the kit storage conditions for the Accula RSV test will be labeled as stable at 15-30°C for the latest time period for which acceptance criteria have been met in the ongoing stability study. Expiration dating will be extended as the study continues provided that correct results are obtained for all three test lots during the testing period.

d. *Detection limit:*

The objective of the Analytical Sensitivity Study was to identify the limit of detection (LoD) of the Accula RSV assay using characterized strains of RSV A and RSV B. Two RSV A strains (RSV A2 and RSV A Long) and two RSV B strains (RSV B1 and RSV B 18537) were tested in replicates of twenty (20) for each concentration. Clinical matrix was used as dilution material for all LoD determination/confirmation experiments.

To determine the limit of detection, a range-finding study was first conducted to establish the lower limit of analytical sensitivity using virus dilutions tested in replicates of n=5. Following identification of the lower end of the detectable concentrations in the range finding study, virus concentrations which produced at least 3/5 detectable results were tested in replicates of 20. If the dilution tested did not result in at least 19/20 "detected" results, a two-fold higher concentration was tested again with 20 replicates.The final concentrations whereby at least 95% of results (≥19/20) were positive for the target virus are shown in the table below:

Table 5: Accula RSV Limits of Detection

RSV Strain Tested	Concentration	Detected (Observed / Expected)
RSV A2	300 $TCID_{50}$/mL	20/20
RSV A Long	300 $TCID_{50}$/mL	20/20
RSV B1	10 $TCID_{50}$/mL	20/20
RSV B 18537	0.5 PFU/mL	19/20

e. *Analytical reactivity:*

Inclusivity verification was evaluated for the Accula RSV test using nine RSV strains. The chosen strains represented clinical RSV A and RSV B isolates that were obtainable for testing. Virus was diluted into a pooled clinical matrix to create contrived samples at an estimated 2X LoD concentration based on the empirical LoD studies. Each strain was tested in triplicate. The RSV strains, test concentrations, and results are shown below:

Table 6: Accula RSV Reactivity

Virus Tested	Subtype	Concentration Tested	RSV Positive Results / Expected
RSV 2012-10	RSV B	1 PFU/mL	3/3
RSV 2012-11	RSV B	1 PFU/mL	3/3
RSV A 12/2014	RSV A	600 $TCID_{50}$/mL	3/3
RSV A 3/2015	RSV A	600 $TCID_{50}$/mL	3/3
RSV B 12/2014	RSV B	20 $TCID_{50}$/mL	3/3
RSV 2006 Isolate	RSV A	600 $TCID_{50}$/mL	3/3
RSV CH93(18)-18	RSV B	20 $TCID_{50}$/mL	3/3
RSV 9320	RSV B	20 $TCID_{50}$/mL	3/3
RSV B 3/2015	RSV B	20 $TCID_{50}$/mL	3/3

f. *Analytical specificity:*

To determine the analytical specificity of the Accula RSV assay, 41 commensal and common pathogenic microorganisms (21 viruses, 19 bacteria, 1 fungus) including some that may be present in the nasal cavity or nasopharynx were tested. No cross-reactivity was observed for any of the microorganisms tested. The organisms tested and the results are shown in the table below:

Table 7: Accula RSV Specificity/Cross-Reactivity

Organism Tested	[1,2]Concentration Tested	RSV Positive Results / Tested
Adenovirus Type 1	5.10E+05	0/3
Adenovirus Type 7A	3.31E+04	0/3
Human Coronavirus 229E	1.10E+04	0/3
Human Coronavirus OC43	2.95E+05	0/3
Human CMV AD-169	1.10E+04	0/3
Human CMV Towne	1.90E+04	0/3
Human CMV Merlin	2.52E+04	0/3
Echovirus Type 11	2.95E+05	0/3
Human Enterovirus 71	1.04E+04	0/3
Human Metapneumovirus	1.01E+05	0/3
Measles virus	2.95E+05	0/3
Mumps virus	9.75E+04	0/3
*Parainfluenza 1	2.52E+04	0/3
Parainfluenza 2	1.10E+04	0/3
Parainfluenza 3	1.18E+04	0/3
Rhinovirus Type A1	3.31E+04	0/3
Rhinovirus Type A16	3.31E+04	0/3
Rhinovirus Type B14	3.02E+04	0/3
[3]Epstein-Barr virus	3.98E+07	0/3
Influenza A/CA/07/2009	2.15E+04	0/3
Influenza B/Mass/2/2012	5.00E+05	0/3
Candida albicans	9.80E+05	0/3
Bordetella pertussis	4.22E+06	0/3
Escherichia coli	1.92E+07	0/3
Haemophilus influenzae	1.20E+06	0/3
Klebsiella pneumoniae	4.15E+07	0/3
Lactobacillus sp.	3.00E+06	0/3
Legionella longbeachae	9.65E+06	0/3
Moraxella catarrhalis	1.99E+05	0/3
Mycobacterium tuberculosis	3.62E+06	0/3
Neisseria gonorrhoeae	6.30E+06	0/3
Neisseria meningitidis	1.28E+06	0/3
Neisseria subflava	7.30E+06	0/3
Proteus vulgaris	2.07E+07	0/3
Pseudomonas aeruginosa	6.05E+05	0/3
Staphylococcus aureus	6.95E+07	0/3
Staphylococcus epidermidis	3.24E+07	0/3
Streptococcus pneumonia	2.09E+06	0/3
Streptococcus pyogenes	2.72 E+07	0/3
Streptococcus salivarius	2.32E+06	0/3
[4]*Mycoplasma pneumoiae*	2.81E+05	0/3

* Replicate repeated due to an invalid result.

[1] Virus concentrations in $TCID_{50}$/mL
[2] Bacteria and *C. albicans* concentrations in CFU/mL
[3] EBV concentration in cp/mL
[4] *Mycoplasma pneumoniae* concentration in CCU/mL

g. *Interfering Substances:*

To assess substances with the potential to interfere with the performance of the Accula RSV test, two RSV strains or a negative control were tested in replicates of three (3) with each interfering substance at the "worst case" concentration. The RSV strains selected for testing were RSV A2 and RSV B1. Virus was serially diluted into a pooled clinical matrix to achieve a 1.5X LoD concentration.

Each RSV strain was tested with the "worst case" interferent concentration, representing the highest concentration likely to be found in a respiratory sample.

Table 8: Interfering Substances Tested

Substance	Concentration
Mucin	5mg/mL
Whole Blood	1% (v/v)
Phenylephrine Nasal Spray	Neat
Oxymetazoline Nasal Spray	Neat
Ocean Saline Nasal Spray	Neat
Chloraseptic Max	Neat
Nasacort	Neat
Zicam Allergy Relief	Neat
Cepacol (Benzocaine)	3 mg/mL
Beclomethasone	1.6 mg/mL
Budesonide	3.2 mg/mL
Dexamethasone	30 mg/mL
Flunisolide	1.6 mg/mL
Fluticasone Propionate	0.25 mg/mL
Mometasone furoate	1 mg/mL
Triamcinolone	0.05 mg/mL
Zanamivir (Relenza)	50 mg/mL
Tobramycin	75 mg/mL
Mupircoin	20 mg/mL

No interference was observed for any of the substances tested above at the concentrations listed. No false positive results were observed for negative (control) samples.

h. *Assay cut-off:*

Not applicable; the detection signal is read visually.

2. Comparison studies:

   a. *Method comparison with predicate device:*

      Not applicable. Performance of the Accula RSV Test was evaluated against the comparator method in a prospective clinical study.

   b. *Matrix comparison:*

      Not applicable.

3. Clinical studies:

Clinical performance was evaluated in a multi-site study in the U.S. during the 2017-2018 RSV season. Accula RSV was used to evaluate fresh, prospectively collected nasal swab specimens from children and adults with suspected respiratory infection and meeting the inclusion/exclusion criteria. Samples were tested with the Accula RSV within one hour of sample collection. At all sites, one swab specimen was tested directly on the Accula RSV according to product instructions. The other swab was eluted in UTM and shipped to a reference laboratory for testing with a high performance, FDA-cleared molecular RSV assay. Elution and shipping of the reference swab sample was performed according to the kit instructions. Discrepant results were tested using alternative FDA-cleared molecular assays at the reference laboratory.

A total of 749 subjects were enrolled in the study with 55 specimens found to be unevaluable. Of those 55 specimens that were unevaluable, 39 samples were rejected due to protocol deviations, and 16 samples returned invalid results after repeat testing. A total of 694 nasal swab specimens were considered evaluable for the purpose of data analysis in the accuracy study. Patient age and gender distribution for the evaluable specimens is presented in the table below:

Table 9: Prospective Clinical Study Age and Gender Distribution

Age Group (Years)	Female	Male	Total
<5	164	207	371
6-18	67	67	134
19-59	78	31	109
≥60	54	26	80
Total	363	331	694

During the prospective clinical study, the initial invalid rate was 4.2% (30/710) (95% CI: 3.0% to 6.0%). After repeat testing (according to the product instructions) the invalid rate was 2.3% (16/710) (95% CI: 1.4% to 3.6%)). Clinical performance of the Accula RSV assay compared to an FDA-cleared molecular assay for nasal swab samples is presented below.

Table 10: Accula RSV Performance Compared to FDA-cleared Molecular Comparator.

Accula RSV	Comparator		
	Positive	Negative	Total
Positive	129	24[a]	153
Negative	14[b]	527	541
Total	143	551	694
Sensitivity:	90.2% (95% CI: 84.2-94.1%)		
Specificity:	95.6% (95% CI: 93.6-97.1%)		

[a] RSV was detected in 22/24 false positive specimens and 7/24 false positive specimens using two alternative FDA-cleared molecular RSV assays.
[b] RSV was not detected in 12/14 false negative specimens and 13/14 false negative specimens using two alternative FDA-cleared molecular RSV assays.

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

In the Accula RSV clinical study (described in the "Clinical Studies" section above), a total of 694 nasal swab specimens were evaluable by the Accula RSV assay. The number and percentage of RSV positive cases per specified age group, as determined by the Accula RSV assay, are presented in the tables below:

Table 12: RSV Expected Values

Age Group (Years)	Number of Specimens	Number of RSV Positives	RSV Positivity Rate
<5	371	134	36.1%
6-18	134	9	6.7%
≥19	189	10	5.3%
Total	694	153	22.0%

N. Instrument Name:

Accula Dock

O. System Descriptions:

1. Modes of Operation:

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

Yes ________ or No ___X____

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 by hand directly onto the test cassette.

4. Specimen Sampling and Handling:

   Not applicable. The specimens are manually inserted into the test cassette in the instrument.

5. Calibration:

   The Accula Dock is factory calibrated and does not require any further calibration at the user site.

6. Quality Control:

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

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

N/A

Q. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

R. Conclusion:

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