CLIA Waiver by Application

Approval Determination Decision Summary

A. Document Number:

CW180006

B. Parent Document:

K181443

C. Purpose for Submission:

This submission was a Dual 510(k) and CLIA Waiver by Application (Dual Submission) tracked as K181443 and CW180006. CW180006 was submitted to request CLIA waived categorization for the Accula RSV Test performed on the Accula Dock.

D. Measurand (Analyte):

RSV L Viral Polymerase Gene RNA

E. Sample Type(s):

Direct Nasal Swab Specimens

F. Type of Test:

Semi-automated qualitative nucleic acid amplification assay for the amplification and detection of specific RSV A and RSV B RNA sequences.

G. Applicant:

Mesa Biotech, Inc.

H. Proprietary and Established Names:

Accula RSV Test

I. Test System 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.

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. Demonstrating “Simple”:

The Accula RSV Test is a “self-contained” test that uses unprocessed nasal swab specimens that are added directly to a pre-aliquoted buffer in a test vial following collection from the patient. The test system requires only non-technique-dependent reagent manipulation; the elution buffer is pre-loaded within each test vial and no reagent preparation is required of the user.

The test does not require any operator intervention during the analysis step. After adding the sample to the buffer test vial, the eluted sample is transferred to the cassette via a fixed volume transfer pipette. Heating, mixing, and target separation/detection are performed by the instrument which informs the user when the test is completed and the results are ready to be read.

No technical or specialized operator training is required in order to use the test. The kit is packaged with a Quick Reference Instructions (QRI) guide that outlines the test process in easy to follow steps with illustrations. Invalid results and error messages are clearly displayed on the instrument screen if the test encounters a problem prior to completion. Control lines on the nitrocellulose membrane can be compared to results interpretation pictures in the QRI or Package Insert to determine test validity.

The test provides a direct readout of test results. No calculations, conversions, or calibrations are required. Results are reported as positive, negative, or invalid for RSV.

The instrument requires no electronic or mechanical maintenance. There are no serviceable parts and the instrument is to be returned to Mesa Biotech for repair.

K. Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-safe Mechanisms:

1. Risk Assessment:

Risk analysis was performed by the firm using the Failure Modes and Effects Analysis (FMEA) Method to assess risks associated with human and environmental factors; the detailed analysis was included in the submission. Potential sources of errors that could adversely affect system performance were identified and mitigated first through system design and then through additional cautions in the labeling. The identified risks which could result in erroneous test results were evaluated in flex studies which stressed the functional limits of the test.

2. Fail-safe and Failure Alert Mechanisms

a. Lockout features

- The cassette is keyed and shaped only to fit into the proper location on the dock and only in the proper orientation.
- The dock displays an error code for 'no sample detected' if the cassette is inserted into the dock and the lid closed before adding a sample.
- The dock prevents re-use of a cassette by sealing the cassette closed after closing of the dock lid. The dock can detect a used cassette and displays an error message that a new cassette is required. The dock firmware will invalidate a cassette that it has determined to be previously used.
- If a cassette is removed before the test has completed the dock will display an error code. Test validity is further supported by the positive and negative control lines on the cassette should the operator attempt to read the result.
- The dock will not initiate a test if the dock lid is not fully closed. Dock will display message "sample detected close lid". If excessive time passes, the dock will invalidate the cassette.
- If the lid is opened during a test but the cassette is not disturbed the dock will provide an audible sound and display a message to close the lid.
- If a cassette is inserted into the dock and a sample is not added within five minutes, the dock will invalidate the cassette and display an error message.
- If power is lost during a test run, the test is cancelled and the dock displays an error code. The dock instructs the operator that the test cassette is used and needs to be replaced.

- The dock will abort an assay in progress if at any point during the run time the dock is tilted more than 15 degrees for longer than 1.5 seconds.

- The dock has a lockout function if an operator attempts to use the dock outside of the specified temperature range or above an altitude of 8000 feet.

b. Built-in Procedural 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 target intended to check for non-specific binding. These process controls are used to help the user determine the validity of the test result when reading the test strip.

c. External Controls

Each Accula RSV Test kit contains two external control swabs (one RSV positive and one RSV negative swab). Control testing is recommended when receiving a new lot of reagents or when a new operator uses the test. Controls can also be used to conform with local, state, or federal regulations; or to conform to the lab's quality control procedures. Control swabs are tested following the same procedure used for patient samples and are ready for use. Additional control swabs can be ordered from the company.

3. <u>Flex Studies:</u>

Operational limits of the device identified in the Risk Analysis were tested in the following series of experiments:

1. Dock lid not closed immediately after adding a sample.
2. Sample not added to cassette immediately after removing foil tab.
3. Variable sample volumes added to cassette.
4. Operator delays reading the cassette after test is complete.
5. Test is performed and results are read under sub-optimal lighting.
6. Delay between sample collection and elution in the provided buffer.
7. Operator uses method(s) other than the SOP for eluting the swab.
8. Test is performed at extremes of temperature and humidity.
9. Exposure of the cassettes to temperature and humidity outside of the recommended ranges

Detailed descriptions of the flex studies are presented below. For flex studies 1 through 5, the experiments were set up as follows: sets of negative and positive swab samples were created by placing 10µl of diluted virus or nasal swab buffer onto a swab tip. Panels consisted of RSV positive samples at moderate (3X LOD) and low (1X LOD) concentrations, or negative matrix. Each sample was tested in triplicate by three different operators for a total of nine Accula RSV Tests performed per test condition for each RSV concentration (3X, 1X, or negative). The RSV strain used for flex testing was RSV A2.

For flex studies 6 through 9, experimental conditions varied and are described individually.

Flex Study 1: Dock lid not closed immediately after adding a sample

To perform this study, the sample port tab was removed and the cassette was placed in the Dock. Immediately (< 10 seconds) after the sample port tab had been removed, sample was pipetted into the sample port. The timer was started immediately to accurately measure the time of Dock lid closure. The Dock lid was closed and the test begun after the appropriate time delay condition. Time points tested were zero minutes delay (SOP), 5 minutes, 10 minutes, 15 minutes, and 120 minutes. Correct results for all 9 tests (3 replicates x 3 operators) were obtained for all time points up to and including 10 minutes delayed lid closing for the negative, low, and moderate concentration RSV samples. Invalid results were observed beginning at the 15-minute time point. This study demonstrates that accurate test results can be obtained if the dock lid is not closed immediately and is closed within 10 minutes of adding the sample to the cassette.

Flex Study 2: Sample not added to cassette immediately after removing foil tab

To perform this study, all sample port tabs were removed and the cassettes were placed in the Docks but were not fully seated; the Dock will time out after 5 minutes if it does not detect a sample. Therefore, the lockout mechanism was temporarily defeated to perform the testing. Immediately after placing the docks (unseated), all foil tabs were removed. The timer was started immediately to accurately add the sample at the later timepoints. For time zero, the cassette was properly seated, and sample was added immediately (< 10 seconds). For later timepoints, the sample was added after the appropriate time delay condition, the Dock was closed, and the test was then started. Time points tested were zero minutes delay (SOP), 30 minutes, 1 hour, 2 hours, and 4 hours. Correct results for all 9 tests (3 replicates x 3 operators) were obtained for the SOP time point. A single invalid result was observed for the 30-minute time point (1/27 invalid). Multiple invalid and incorrect results were observed beginning at the 1-hour time point. This study demonstrates that the test is robust with respect to delays up to 30 minutes in sample addition to the test cassette.

Flex Study 3: Variable sample volumes added to cassette

To perform this study, the sample port tab was removed and the cassette was placed in the Dock. Immediately (< 10 seconds) after the sample port tab had been removed, a variable sample volume was pipetted into the sample port. The Accula Dock has a feature that estimates the volume of sample added to the cassette and if the volume is too low, the test is aborted. For the purposes of this test, that feature was temporarily disabled. The Dock was closed and the test begun after the appropriate time delay condition. Variable sample volumes tested in this study were 25µl, 40µl, 50µl, 60µl (SOP), 70µl, 75µl, and 100µl. Correct results for all 9 tests (3 replicates x 3 operators) were obtained for volumes of 50µl, 60µl (SOP), and 70µl. Invalid results were observed below a sample volume of 50µl and above a sample volume of 70µl. No incorrect results were observed

during this study. The results of this study demonstrated that invalid results may be obtained if volumes above or below the pre-calibrated pipette volume of 60µl are used for sample addition to the cassette.

Flex Study 4: Operator delays reading the cassette after test is complete

In this study, samples were tested according to the package insert but results were read off the cassette at varying times following test completion. Time points tested were zero minutes delay (SOP), 1 hour, 3 hours, 5 hours, 24 hours, 48 hours, and 1 week. Correct results for all tests were obtained for all time points up to and including 1 week after the test was completed. This study demonstrates that the test can provide a correct and valid result interpretation beyond the recommended read time.

Flex Study 5: Delay between sample collection and elution in the provided buffer

For this study, samples were tested according to the package insert with one variable: the time between sample collection (sample spike onto swabs) and elution; the control condition was immediate elution and testing (SOP). The following wait times were tested between spike of sample onto swab and elution: 0 minutes (SOP), 1 hour, 2 hours, 8 hours, 15 hours, and 24 hours. Correct results for all tests were obtained for all time points up to and including the 24-hour time point. This study demonstrates that accurate test results can be obtained if the operator delays eluting the sample from the swab for up to 24 hours.

Flex Study 6: Operator uses method(s) other than the SOP for eluting the swab

For this study, samples were tested according to the package insert with one variable: the method for eluting the swab into the buffer vial was varied. The control condition was to rotate the swab back and forth 5 times while rolling against the side of the vial. Variables tested in the elution method included: pressing against the vial and rotating 10 times, pressing against the wall and rotating 5 times (SOP), pressing against the wall and rotating two times, rotate 10 times without pressing against the wall, dipping the swab with no rotation and no pressing against the wall. The elution liquid from each condition was split into nine aliquots and distributed between three operators (n=3 per operator) for testing. Correct results for all tests were obtained for the SOP condition, and all methods that involved pressing the swab against the side of the vial regardless of number of rotations. Correct results were obtained for all samples rotated 10 times without pressing against the wall. False results were obtained if the swab was only dipped into the vial with no pressing and no rotating (i.e.; the most challenging test condition). This study demonstrates that accurate test results can be obtained if the operator does not rotate the swab the full 5 times while pressing against the vial wall, or if the operator rotates the swab at least 10 times but fails to press the swab against the wall during the elution step.

Flex Study 7: Test is performed at extremes of temperature and humidity

In this study, the system was operated in a combination of temperatures (13°C and 32°C), relative humidity (20% and 80%), and simulated altitudes (sea level and 8000 ft.). The Accula Dock firmware contains both temperature and pressure lockout features that were

temporarily disabled in order to perform these tests. For each environmental condition combination, a set of five swab samples (two negative and three RSV positive samples) was run using 5 individual cassettes on 5 docks. Testing was performed according to the package insert. All five test cassettes produced the expected results in all the conditions tested. This study demonstrated that the test operates correctly in a range of temperatures, relative humidity, and altitudes.

Flex Study 8: Cassette is stored improperly prior to testing (Temperature and RH)

For this study, test cassettes were stored under conditions of high or low temperature outside of the recommended temperature range (i.e.; 13°C or 32°C) and at high RH (80%) for 45 minutes, 60 minutes, or 75 minutes prior to being used for the Accula RSV Test. Nasal swab buffer (n=2) and RSV positive control swabs (n=3) were tested for each condition. Correct results were obtained for all samples and for all conditions tested. This study demonstrated that the Accula RSV Test cassette is functional after exposure to temperatures from 13°C - 32°C and at a relative humidity of 80%, for up to 75 minutes.

Flex Study 9: Test is performed under sub-optimal lighting conditions

In this study, testing was performed under three quantified lighting conditions using a calibrated light meter: ~46 Foot Candles (FC; normal laboratory lighting), ~14 FC (low-level lighting), and ~5 FC (very low-level lighting). Five samples were tested each by three different operators. Samples consisted of RSV negative, RSV low positive (1X LOD), and RSV moderate positive (3X LOD). Test results were interpreted by three independent operators at low light conditions, then re-interpreted under "Normal Laboratory Lighting" and compared for accuracy. The test operators were able to correctly interpret the results of the tests at each lighting level. This study demonstrates the test can be correctly performed and interpreted in various lighting conditions.

Flex studies evaluating the robustness of the Accula dock have been reviewed previously under CW170007 for the Accula FluA/Flu B assay. These studies included the Accula Dock tilt and displacement test, power fluctuations and disturbances, and the dropped cassette test.

L. Demonstrating "Insignificant Risk of an Erroneous Result" - Accuracy:

1. Study Design:

The objective of the study was to evaluate the performance of the Accula RSV Test in the hands of the intended users when performed in a CLIA waived setting.

a. Study Sites and Duration:

Clinical performance characteristics of the Accula RSV Test were evaluated in a multi-site prospective study during the 2017-2018 cold and influenza season in the U.S. Ten sites throughout the U.S. participated in the method comparison portion of

the clinical study. The sites consisted of primary and urgent care clinics, pediatric offices, and family practice offices. All the sites qualified as representative of CLIA waived intended use sites for this device.

b. Operators:

A total of 25 operators representative of intended CLIA waived users across the ten clinical testing sites participated in the study. The participants consisted of administrative personnel, medical assistants, nurses, research/study coordinators, administrative managers, and other patient care providers. The test operators who participated in the study were untrained in the use of the Accula RSV Test and none were trained laboratory technicians. Upon completion of the study, the operators at each site were asked to complete an Operator Questionnaire that asked them to rate the ease of use of the test procedure.

c. Instructions for Use:

The operators were given the product instructions and the Quick Reference Guide. No other materials or instructions were provided and the operators received no training in the use of the test.

d. Subjects (Patients):

To be enrolled in the study, subjects had to be patients presenting at the participating study sites with symptoms of respiratory disease and complete informed consent prior to sample collection. Patient demographics included male and female patients of all ages.

e. Samples:

Two nasal swabs were collected from each subject using standard collection methods. One swab was eluted in UTM and sent to the reference laboratory for comparator testing according to the shipping instructions for the comparator test. The other sample was immediately eluted into the supplied Accula RSV nasal swab buffer and tested with the Accula RSV Test within one hour of collection.

f. Comparative Method:

An FDA-cleared molecular RSV assay was used as the comparator method for demonstrating the performance accuracy in support of the CLIA waiver.

g. Exclusions:

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.

2. Test Performance:

a. Method Comparison:

Samples with invalid results by the Accula RSV Test were retested per the product instructions. The initial invalid rate was 4.2% (30/710) (95% CI: 3.0% to 6.0%). After repeat testing the invalid rate was 2.3% (16/710) (95% CI: 1.4% to 3.6%).

Sensitivity and Specificity for the Accula RSV Test are shown below:

Table 1 – RSV Performance on the Accula RSV Test Versus 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 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.

b. Performance with Analyte Concentrations Near the Cutoff:

A study was conducted to evaluate the performance of Accula RSV Test with weakly reactive samples when testing was performed by untrained users. Randomized blind-coded panels, containing negative or low positive (C_{95}) RSV were tested with the Accula RSV Test at three sites representative of CLIA waived sites (90 tests in total per site). Nine untrained users (3 operators per site) at the CLIA waived sites participated in the study. The panel testing was conducted over 14 days at each site, and the testing was integrated into the users' daily work flow. The results of testing of the Accula RSV with samples near the assay cutoff are summarized in the table below:

Table 2 - Detection of RSV with Samples Near the Cutoff

Sample Type	**Site 1 Detection**	**Site 2 Detection**	**Site 3 Detection**	**Overall Detection**	**Overall 95% CI**
RSV Low Positive	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90)	96-100%
True Negative	0% (0/30)	0% (0/30)	0% (0/30)	0% (0/90)	0-4%

The study results demonstrated that users untrained in the test procedure of the Accula RSV Test were able to perform the test correctly and the test provided the expected result for samples near the cut-off.

c. Operator Questionnaire Results:

Twenty-two of the operators completed the Operator Questionnaire and the results do not raise any concerns about the ability of untrained users to perform the test at intended use sites.

M. Proposed Labeling:

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

The labeling relevant to the test includes a Package Insert, Instrument Manual, and a Quick Reference Guide. The Quick Reference Guide is written in simple language and contains graphics to facilitate comprehension of the instructions. The Instrument Manual has been previously reviewed by FDA as a part of K171641.

N. Conclusion:

1. Benefit/Risk Justification:

   The Sensitivity of the Accula RSV Test (90.2%) is below the recommendations outlined in the FDA guidance document, "Guidance for Industry and FDA Staff: Recommendations for Clinical Laboratory Improvements Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of *In Vitro* Diagnostic Devices" (95% Sensitivity is recommended). However, the Accula RSV Test performance exceeds that of all other CLIA-waived RSV tests that utilize visually-read lateral flow technology. Thus, granting of CLIA waiver for the Accula RSV test will allow healthcare providers and patients access to a RSV lateral flow test with improved performance characteristics and will be a benefit to public health.

2. Waiver Decision:

   The submitted information in this CLIA waiver application supports a CLIA Waiver approval decision.