CLIA Waiver by Application

Approval Determination Decision Summary

A. Document Number:

CW170007

B. Parent Document:

K171641

C. Purpose for Submission:

This submission was a Dual 510(k) and CLIA Waiver by Application (Dual Submission) tracked as K171641 and CW170007. CW170007 was submitted to request CLIA Waived categorization of the Accula Flu A/Flu B Assay.

D. Measurand (Analyte):

Influenza A PB2 Gene RNA
Influenza B M Gene RNA

E. Sample Type(s):

Direct Nasal Swab Specimens

F. Type of Test:

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

G. Applicant:

Mesa Biotech, Inc.

H. Proprietary and Established Names:

Accula Flu A/Flu B Assay

I. Test System Description:

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

Results are interpreted visually by the operator after the test has completed. A colored line of any intensity at the “Flu A” and/or “Flu B” location indicates a positive result for that influenza virus type, 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 control line at the beginning of the strip displays amplification effectiveness and is necessary to interpret a test as “negative” for influenza A and influenza B.

J. Demonstrating “Simple”:

The Accula Flu A/Flu B Assay 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 there is no reagent preparation 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 complete 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 Influenza A and Influenza B.

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; 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 resultswere 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 previously used cassette.
   - 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 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 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-influenza 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 Flu A/Flu B test kit contains two external positive control swabs (one Influenza A and one Influenza B swab). The positive control swabs contain either inactivated Influenza A or inactivated Influenza B dried onto the swab head and are recommended for testing when receiving a new lot of reagents or when a new operator uses the test. Both 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 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. Delay between sample collection and elution in the provided buffer.
6. Operator uses method(s) other than the SOP for eluting the swab.
7. Test is performed at extremes of temperature and humidity.
8. Test is performed with a dropped or damaged cassette.
9. Test is performed and results are read under sub-optimal lighting.
10. Test is performed with instrument seated at an angle or instrument is moved while a test is in progress.
11. Instrument is plugged and un-plugged repeatedly prior to beginning a test.

Detailed descriptions of the flex studies are presented below. For flex studies 1 through 6, 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 both moderate (3X LOD) and low (1X LOD) positive Influenza A and Influenza B concentrations, or negative matrix. All samples were tested in triplicate for a total of 27 Accula Flu A/Flu B tests performed per test condition and per target (3 replicates x 3 concentration levels x 3 operators = 27 tests). Strains used for testing were A/California/07/2009 or B/Massachusetts/02/2012.

For flex studies 7 through 11, 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 Dock was closed and the test begun after the appropriate time delay condition. The timer was started immediately to accurately add sample at the later timepoints. Time points tested were zero minutes delay (SOP), 5 minutes, 10 minutes, 15 minutes, and 120 minutes.Correct results for all 27 tests were obtained for all time points up to and including 10 minutes delayed lid closing for both Influenza A and Influenza B samples. Invalid and false 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, or 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 sample. Immediately after placing the docks (unseated), all foil tabs were removed. For time zero, the cassette was properly seated, and sample was added immediately (< 10 seconds). For later timepoints, the sample was added and hte Dock was closed and the test begun after the appropriate time delay condition. The timer was started immediately to accurately add sample at the later timepoints. Time points tested were zero minutes delay (SOP), 5 minutes, 10 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, and 8 hours. Correct results for all 27 tests were obtained for all time points up to and including 4 hours of delayed sample addition for both Influenza A and Influenza B samples. Invalid and false results were observed beginning at the 6 hour time point. This study demonstrates that the test is very robust with respect to delays in addition of the sample 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 amount was pipetted into the sample port. 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), 75µl, and 100µl. Correct results for all 27

tests were obtained for volumes of 50µl, and 60µl (SOP). Invalid results were observed above 60µl of sample and below 40µl. False results were observed at the 100µl level. The results of this study demonstrated that invalid results are obtained if volumes above or below the pre-calibrated pipette volume of 60µl is used for sample addition to the cassette; and incorrect results are possible if the volume greatly exceeds the pre-established volume.

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), 30 minutes, 1 hour, and 15 hours. Correct results for all 27 tests were obtained for the SOP condition, the 30 minute timepoint, and the one hour time point for both Influenza A and Influenza B samples. This study demonstrates that accurate test results can be obtained if the operator delays eluting the sample from the swab for up to one hour.

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. Correct results for all 27 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. False results were obtained if the swab was rotated without pressing against the wall, and if the swab was only dipped into the vial with no pressing and no rotating. 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 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.). For each environmental condition combination, a set of five swab samples (two negative and

three flu A/B dual 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: Test is performed with a dropped or damaged cassette

For this study, 97 test cassettes were dropped onto tile flooring at six orientations from three heights: 3 ft., 4 ft., and 5 ft. After dropping the test cassettes, the damaged cassettes were each run with a sample of either Pooled Negative Nasal Sample, weak positive sample at 1X LoD Flu A/Flu B, or moderately positive sample at 3X LoD Flu A/Flu B. Passing results required that the operator was able to run the dropped cassette on the dock assembly and that the operator observed the correct result. Three cassettes were damaged beyond their ability to run a test. Sixteen cassettes were partially opened by the impact and could not run without snapping the case back into place. After snapping back into place, all 16 cassettes were successfully run and produced the expected results. Five cassettes contained damaged dessicant tablets due to the drop from 5 feet. Four out of five produced the expected results, the fifth produced an invalid result. Of the remaining undamaged cassettes, four produced invalid results which is consistent with the initial invalid rate of the Accula Flu A/Flu B test. There were no cases of a false result after using a dropped cassette.

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

Testing was performed under three quantified lighting conditions: 500 LUX (normal laboratory lighting), 150 LUX (low-level lighting), and 54 LUX (very low-level lighting). with samples including Pooled Negative Nasal Sample, weak positive at 1X LoD Flu A/Flu B, and moderate positive at 3X LoD Flu A/Flu B. 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 Study 10: Test is performed with instrument seated at an angle or instrument is moved while a test is in progress

In this study, tests were executed with the Dock placed on inclines ranging from 10° to 16°. Additionally, mid-test translation of the Accula Dock was performed to determine effects on the assay. At 16° of incline, all 5 Docks tested produced an error code, preventing execution of the Flu A/Flu B test. The incline was reduced by 2° increments until the Dock allowed execution of the test without error codes. The largest allowable tilt angles ranged between 10° and 14°. In all cases, Flu A/Flu B tests executed on Docks at the maximum allowed incline produced correct results, when tested with Nasal Swab Buffer, or with Flu A/Flu B swabs eluted into Nasal Swab Buffer. No invalid or incorrect results were encountered during tilt testing.

In testing lateral movement during the assay, tests were started with Flu A/Flu B Test Cassettes using samples of either Nasal Swab Buffer, or with Flu A/Flu B swabs eluted into Nasal Swab Buffer. At three key stages of the test (corresponding to microfluidic transfer of the sample within the cassette), the Docks (5 total) were moved laterally a distance of approximately 1 ft., at two discrete speeds of approximately 1 ft./sec and 0.3 ft./sec. The Accula test performance was found to be unaffected by translational disturbance generating correct results in each case. This study demonstrates the test is unaffected by operating on an uneven surface, or if the dock is moved while a test is in progress.

Flex Study 11: Instrument is plugged and un-plugged repeatedly prior to beginning a test

In this study, five separate Accula docks were repeatedly un-plugged ten times prior to initiating a test. Flu A/Flu B Test Cassettes were then subsequently run on the Docks using samples of either Nasal Swab Buffer or with Flu A/Flu B swabs eluted into Nasal Swab Buffer. In all cases, the Docks correctly executed the tests and produced correct results.

The results of the flex studies demonstrated that the test is robust to procedural variation and insensitive to the majority of environmental stresses. Based on the results of the flex studies, no additional cautions or limitations were needed for the device labeling.

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 Flu A/Flu B Assay 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 Flu A/Flu B Assay were evaluated in a multi-site prospective study during the 2016-2017 influenza season in the U.S. Sixteen 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:

There were a total of 23 operators representative of intended CLIA waived users across the sixteen clinical testing sites. 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 Flu A/Flu B Assay 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.

e. Samples:

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

f. Comparative Method:

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

g. Exclusions:

A total of 1331 nasal swab specimens were enrolled in the study. Of those, 73 specimens were unevaluable: 4 did not meet eligibility criteria, 53 samples were rejected due to protocol deviations, and 16 samples returned invalid results after repeat testing. A total of 1258 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 Flu A/Flu B assay were retested per the product instructions. The initial invalid rate was 9.1% (116/1272) (95% CI: 7.6% to 10.8%). After repeat testing the invalid rate was 1.1% (14/1272) (95% CI: 0.7% to 1.8%). There were two clinical specimens (2/1258; or 0.16%) positive for Influenza A and Influenza B (double positives) observed on the Accula Flu A/Flu B test during the clinical study. The two specimens were also double positive according to the comparator method.

Sensitivity and Specificity for the Accula Flu A/Flu B assay are shown below:

Table 1 – Influenza A Performance on the Accula Flu A/Flu B Versus Molecular Comparator

Accula Flu A/Flu B	Comparator		
	Positive	**Negative**	**Total**
Positive	289	60[a]	349
Negative	9[b]	900	909
Total	298	960	1258
Sensitivity: 97% (95% CI: 94.4-98.4%)			
Specificity: 94% (95% CI: 92.0-95.1%)			

[a] Flu A was detected in 47/60 false positive specimens using an alternative FDA-cleared molecular influenza assay.
[b] Flu A was not detected in 3/9 false negative specimens using an alternative FDA-cleared molecular influenza assay.

Table 2 - Influenza B Performance on the Accula Flu A/Flu B Versus Molecular Comparator

Accula Flu A/Flu B	Comparator		
	Positive	**Negative**	**Total**
Positive	126	14[a]	140
Negative	8[b]	1110	1118
Total	134	1124	1258
Sensitivity: 94% (95% CI: 88.7-97.0%)			
Specificity: 99% (95% CI: 97.9-99.3%)			

[a] Flu B was detected in 9/14 false positive specimens using an alternative FDA-cleared molecular influenza assay.
[b] Flu B was not detected in 5/8 false negative specimens using an alternative FDA-cleared molecular influenza assay.

The study results demonstrated that users untrained in the test procedure of the Accula Flu A/Flu B assay were able to perform the test and interpret the results with high accuracy. Performance was comparable across study sites and operators.

b. Performance with Analyte Concentrations Near the Cutoff:

A study was conducted to evaluate the performance of Accula Flu A/Flu B with weakly reactive samples when testing was performed by untrained users. Randomized blind-coded panels, containing negative or low positive (C_{95}) Influenza A or Influenza B specimens, were tested with the Accula Flu A/Flu B at three CLIA waived sites (60 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 Flu A/Flu B with samples near the assay cutoff are summarized in the table below:

Table 3 - Detection of Influenza A and Influenza B with Samples Near the Cutoff

Sample Type	Site 1 Detection	Site 2 Detection	Site 3 Detection	Overall Detection	Overall 95% CI
Flu A Low Positive	95.0% (19/20)	100.0% (20/20)	95.0% (19/20)	96.7% (58/60)	89-99%
Flu B Low Positive	95% (19/20)	100.0% (20/20)	95% (19/20)	96.7% (58/60)	89-99%
True Negative	0% (0/19)*	0% (0/20)	0% (0/20)	0% (0/59)	0-6%

* One sample produced repeated invalid results and was excluded from analysis.

The study results demonstrated that users untrained in the test procedure of the Accula Flu A/Flu B assay 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-three 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 includes the Assay Package Insert, Dock Manual, and Quick Reference Instructions written in simple language and containing graphics to facilitate comprehension of the instructions.

N. Conclusion:

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