CLIA Waiver by Application Approval Determination

Decision Summary

A. Document Number

CW200009

B. Parent Document Number

K201269

C. CLIA Waiver Type:

Dual 510(k) and CLIA Waiver by Application (Dual Submission)

D. Applicant

Mesa Biotech, Inc.

E. Proprietary and Established Names

Accula Strep A Test

F. Measurand (analyte)

Group A *Streptococcus* nucleic acid

G. Sample Type(s)

Throat swab specimens

H. Type of Test

Semi-automated PCR amplification followed by hybridization and colorimetric visualization of amplified products on a test strip for qualitative detection

I. Test System Description

1. Overview

The Accula Strep A Test is a semi-automated, colorimetric polymerase chain reaction (PCR) nucleic acid amplification test to qualitatively detect *Streptococcus pyogenes* (Group A β-hemolytic *Streptococcus*, Strep A) bacterial nucleic acid from unprocessed throat swabs that have not undergone prior nucleic acid extraction. The system integrates nucleic acid extraction, a novel Mesa Biotech PCR nucleic acid amplification technology,

and hybridization-based visual detection into a completely self-contained and automated system. The Accula Strep A 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 an Accula Strep 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 Strep A 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. The appearance of any shade of blue at the "Strep" position on the test strip indicates a valid result that is interpreted as positive for Strep A. A positive control ("C") line at the beginning of the strip tests for amplification effectiveness and is necessary to interpret a test as "negative" for Strep A. A negative control ("NC") line at the end of the test strip controls for non-specific binding or amplification and must be absent for a valid result.

2. Test System Components

The Accula Strep A Test kit contains the following test components:

- Collection Swabs (25): sterile swabs for throat sample collection
- Strep A Buffer (25): single-use vial of solution containing 2.5 mL of buffer with salts, dimethyl sulfoxide and < 0.01% sodium azide
- Transfer Pipette (25): single-use, fixed volume pipette used to transfer sample from the Strep A Buffer vial into the Test Cassette
- Accula Strep A Test Cassette (25): single-use, foil-pouched with desiccant and Test Cassette containing lyophilized reagents for target amplification and detection
- Strep A Positive Control Swab (1): contains inactivated Strep A bacteria dried onto a swab; positive for Strep A
- Strep A Negative Control Swab (1): contains buffer solution dried onto a swab; negative for Strep A

J. Demonstrating "Simple"

- The device is a fully automated instrument and a single-use cassette containing the assay reagents.

- The test uses unprocessed throat swab specimens.

- An untrained operator can conduct the test by performing five simple steps: 1) add swab specimen directly to a pre-aliquoted buffer in a test vial and rotate five times against the wall of the vial to elute sample, 2) insert Strep A cassette into Accula Dock, 3) transfer liquid sample to cassette with fixed volume pipette, 4) close lid on Accula Dock to initiate test run, and 5) read the results.

- 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.

- No technical or specialized operator training is required in order to use the test.

- Technical or specialized training is not required for troubleshooting or error code interpretation.

- The kit is packaged with a Quick Reference Guide (QRG) 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 QRG or Package Insert to determine test validity.

- There are no required electronic or mechanical maintenance tasks required.

- The test provides a direct readout of test results via the presence or absence of a colored line(s). No calculations, conversions, or calibrations are required. Results are interpreted as positive, negative, or invalid for Strep A by following the QRG or Package Insert.

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

1. Risk Analysis

   Risk analysis was performed by Mesa Biotech using the Failure Modes and Effects Analysis (FMEA) Method, according to ISO 14971, 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. Any errors that are not addressed by the quality control features of the Accula Dock and the Test Cassette were evaluated in Flex Studies. All risks of harm to the

patient or operator were mitigated to an acceptable level and were supported by flex studies and/or operator instructions.

2. Fail-Safe and Failure Alert Mechanisms

a. Mechanical controls

Table 1. Check Methods and Respective Error Codes/Messages

Check	Description	Addressed in Flex Study
Altitude	After boot-up, the Dock senses the ambient air pressure, thereby determining approximate altitude. If the altitude is above 8000', an error is displayed, and the Dock is rebooted.	7
Ambient temperature	The Dock checks the ambient temperature. An error is displayed if ambient temperature is found to be out of the range of 15°C to 30°C.	7
Cassette Insertion	The Dock monitors electrical connections to determine when a cassette is inserted.	2
Cassette temperature	The Dock checks the cassette temperature, and displays an error if found to be out of range.	8
Sample	The Dock detects the addition and approximate volume of sample in the Lysis reaction chamber. If no sample or insufficient sample is detected, an error message is displayed. When the sample is detected, the Dock prompts the user to close the Dock Lid.	3
Cassette timeout	If a cassette is inserted into the Dock and no sample is inserted within 5 minutes, the Dock will invalidate the cassette, abort the assay, and display an error message	1

b. Built-in Procedural (Internal) Controls

Each test cassette contains two internal process controls: an internal positive control and internal negative control. The positive control is a segment of the *rbcL* plant gene. The negative control is a non-Strep A 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 Strep A Test kit contains two external control swabs: a Strep A positive swab that contains inactivated *S. pyogenes* and a Strep A negative swab that contains dried buffer solution without *S. pyogenes*. 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 Mesa Biotech.

3. Flex Studies

Operational limits of the device outside of recommended conditions were evaluated in the experiments listed below. Additional studies (Tilt and Displacement, Dock; Power Disturbances, Dock; Drop Test, Cassette) were previously submitted and reviewed (K171641/S001) and are still applicable.

1. Time between addition of sample and closing Dock lid
2. Time between removal of foil tab and addition of sample
3. Variable sample volumes added to cassette
4. Time between assay completion and results interpretation
5. Low Light Usage
6. Variable Elution Methods
7. Environmental Requirements, Dock
8. Environmental Requirements, Cassette

Descriptions of the flex studies are provided below. Unless indicated, each study tested negative samples (10 µL pooled, negative throat swab matrix prepared with Strep A buffer (PNTS) placed onto a swab) and Strep A positive samples (10 µL of PNTS spiked with *S. pyogenes* strain BAA-946 and placed onto a swab) at a level of 3x LoD (for moderate positive) and 1x LoD (for low positive). Each swab sample was prepared in duplicate and each eluted in PNTS. The duplicates were consolidated into a single sample and divided into nine aliquots for triplicate testing by three different operators for a total of nine Accula Strep A Tests (3 replicates x 3 operators) performed for each test condition at each Strep A concentration (3x LoD, 1x LoD or negative).

Flex Study 1: Time between addition of sample and closing Dock lid

The effect of extended time between addition of sample and Dock lid closing (i.e., initiation of test) was evaluated. Samples were added to the sample port and the Dock lid was closed after 0 minutes (no delay), 3 minutes, 5 minutes or 120 minutes to automatically start the test. No false positive or false negative results were observed for up to and including 3 minutes delayed lid closing. Invalid results were observed beginning at the five minutes delayed lid closing, which mitigates the risk of reporting an erroneous result. This study demonstrates that accurate test results can be obtained if the dock lid is closed within 3 minutes of adding the sample to the cassette.

Flex Study 2: Time between removal of foil tab and addition of sample

The effect of extended time between removing the foil port tab and adding the sample before closing the dock to initiate the test was evaluated. The foil port tab was removed, and the cassettes were placed in the Docks, but not fully seated since the Dock will time out after five minutes if it does not detect sample. After the appropriate time delay (i.e., 0 minutes, 30 minutes, 1 hour, 4 hours, 8 hours or 20 hours), the cassette was fully seated, sample added, and the Dock lid closed to begin the test. No false positive or false negative results were observed for up to and including eight hours delayed sample addition. High invalid rates (> 5%) were observed after a 20 hour delay, which mitigates the risk of reporting an erroneous result. This study demonstrates that accurate test results can be obtained if the sample is added within eight hours of removing the foil port tab.

Flex Study 3: Variable sample volumes added to cassette

The effect of insufficient or excessive sample addition on Accula Strep A Test performance was evaluated. While the Accula Strep A Test comes with a pre-calibrated pipette volume of 60 µL, the Dock firmware has a feature to estimate the amount of sample added. If the sample volume is too low, the Dock reports an error message and the test is aborted; this feature was disabled to evaluate the effect on variable sample volumes. Immediately after the foil port tab was removed, the appropriate sample volume (i.e., 25 µL, 40 µL, 50 µL, 60 µL (control), 75 µL, 100 µL and 125 µL) was added and the Dock lid closed to begin the test. No false positive or false negative results were observed for sample volumes between 50 µL and 100 µL. For sample volumes outside of this range, high invalid rates (> 5%) were observed, which mitigates the risk of reporting an erroneous result. For each volume, no false positive or false negative results were observed. This study demonstrates that accurate test results can be obtained if sample volumes are between 50 µL and 100 µL.

Flex Study 4: Time between assay completion and results interpretation

The effect of delayed result interpretation was evaluated. After the testing was completed, cassettes were immediately interpreted and scanned. Results were then interpreted at the following time delays after test completion: 1 hour, 3 hours, 5 hours, 24 hours, 48 hours and 1 week. No false positive or false negative results were observed for up to and including one week delayed interpretation. This study demonstrates that test results can be accurately interpreted up to 1 week after the test is completed.

Flex Study 5: Low Light Usage

The effect of result interpretation under lowlighting conditions was evaluated. After the testing was completed, results were interpreted under the following light conditions: “normal” laboratory lighting (~46 FC or 500 LUX), low lighting (home or warehouse, ~14 FC or 150 LUX), and very low lighting (twilight, ~5 FC or 54 LUX). No false positive or false negative results were observed under any light condition tested. This

study demonstrates that test results can be accurately interpreted under various light conditions.

Flex Study 6: Variable Elution Methods

The effect of variability in sample elution on Accula Strep A Test performance was evaluated. Elution of contrived swab samples were performed with the following methods: press against the wall and rotating 10 times, press against the wall and rotate 5 times (control), press against the wall and rotate two times, rotate 10 times without pressing swab against the wall, and dipping the swab without rotation and without pressing against the wall. Elution samples were prepared in duplicate and consolidated into a single sample. Samples were divided into nine aliquots for triplicate testing by three different operators for a total of nine Accula Strep A Tests (3 replicates x 3 operators). No false positive or false negative results were observed with any elution method tested. This study demonstrates that accurate test results can be obtained regardless of if the swab is pressed against the wall of the vial as well as with increased or decreased swab rotations.

Flex Study 7: Environmental Requirements, Dock

The specified operating conditions (temperature, 15° – 30°C; relative humidity, 20% - 80%; altitude, up to 8,000 feet) for the Accula Strep A Test were evaluated at extreme ranges. The Dock firmware has QC features to measure the temperature and ambient air pressure (to determine the approximate altitude). If testing is attempted outside of the specified temperature range or altitude, an error message is reported; these features were disabled for the evaluation. Strep A positive samples (n=3 per condition) and negative samples (n=2 per condition) were run using 5 Docks under the following six conditions: at the specified altitude (up to 8,000 feet) at 13°C / 20% RH, 13°C / 80% RH, 32°C / 20% RH, or 32°C / 80% RH as well as at an elevated altitude (10,000 feet) at ambient relative humidity at 13°C and 32°C. (Testing in the environmental chamber set to 13°C / 20% RH was never able to reach a relative humidity level below 27%.) No false positive or false negative results were observed with any condition tested. This study demonstrates that accurate test results can be obtained when the Accula Strep A Test is operated at the extreme range of the specified temperature, relative humidity and altitude.

Flex Study 8: Environmental Requirements, Cassette

Storage of Accula Strep A Test cassettes outside of the specified operating conditions (temperature, 15° – 30°C; relative humidity, 20% - 80%) was evaluated at extreme ranges. Cassettes with the foil port tab in place were stored outside the pouch at 13° or 32°C at a high relative humidity (80%) for 45 minutes, 60 minutes, or 75 minutes prior to testing. Cassettes with the foil port tab removed were stored outside the pouch at 13° or 32°C at a high relative humidity (80%) for 5 minutes or 7 minutes prior to testing. Strep A positive samples (n=3 per condition) and negative samples (n=2 per condition) were tested with cassettes after storage. No false positive or false negative results were observed under any condition tested. This study demonstrated that the Accula Strep A

Test cassette is functional outside the pouch with the foil port tab in place at temperature from 13° - 32°C and at a relative humidity of 80% for up to 75 minutes. This study also demonstrated that the Accula Strep A Test cassette is functional outside the pouch with the foil port tab removed at temperature from 13° - 32°C and at a relative humidity of 80% for up to 7 minutes.

L. Demonstrating "Insignificant Risk of an Erroneous Result" –Accuracy

1. Study Design:

The objective of the clinical study was to evaluate the performance of the Accula Strep A 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 Strep A Test were evaluated in a prospective, multi-center study conducted at nine point of care (POC; e.g., physician office laboratory, urgent care, outpatient clinics) investigational sites representative of a CLIA-waived environment in the United States between Mary 2019 and January 2020.

b. Operators:

Twenty-four operators representative of intended CLIA waived users across the nine clinical testing sites participated in the study. The participants consisted of administrative personnel, medical assistants, nurses, and other patient care providers. The test operators who participated in the study were untrained in the use of any Accula 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 QRG. 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 with signs and symptoms of pharyngitis and completed informed consent prior to sample collection. Patient demographics included male and female patients for all ages.

e. Samples:

Two throat swab specimens were collected from each participant: one Copan FLOQswab for immediate use with the Accula Strep A Test and Copan eSwab Liquid Amies Collection System for comparator testing.

f. Comparator Method:

Blood Agar culture and an FDA-cleared molecular assay were used for concurrent comparator testing at one reference laboratory.

g. Exclusions:

Table 2 summarizes the number of evaluable results included in the Accula Strep A performance analysis. Throat swabs were collected from 669 participants; however, only 663 Accula Strep A Test assays were performed due to exclusion of six samples with protocol deviations or incidents. An additional four samples with unresolved invalid/error Accula Strep A Test results were excluded from analysis. When using the Blood Agar culture comparator, an additional five results were excluded from the analysis due to protocol deviations or incidents, for a total of 654 Accula Strep A Test and Blood Agar culture results available for analysis. When using the molecular assay comparator, an additional 11 results were excluded from the analysis (six results excluded due to protocol deviations or incidents, and five results excluded due to invalid results), for a total of 648 Accula Strep A Test and molecular assay comparator results available for analysis.

Table 2. Summary of Evaluable Results for Accula Strep A Test Performance Analysis

Exclusions	**Accula Strep A Test**	**Blood Agar culture**	**Molecular Comparator**
Protocol Deviations/Incidents	6	5	6
Unresolved Invalid/Error Result	4	-	5
Total Excluded Results	10	5	11
Total Evaluable Results	**659**	**654[1]**	**648[1]**

[1]Total number of Accula Strep A Test and comparator results included in performance analysis.

2. Test Performance:

a. Method Comparison:

All samples with valid Accula Strep A Test and comparator results (**Table 2**) are included in the performance analysis. All samples with discrepant results when compared to either culture or the molecular comparator assay as well as 10% of samples with non-discrepant results were also tested with a second FDA-cleared molecular assay.

Sensitivity and specificity for the Accula Strep A Test when compared to Blood Agar culture are shown in **Table 3**.

Table 3. Accula Strep A Test Clinical Performance vs. Blood Agar Culture

		Blood Agar Culture		
		Positive	Negative	Total
Accula Strep A Test	Positive	126	13[2]	139
	Negative	5[1]	510	515
	Total	131	523	654
	Sensitivity: 126/131 = 96.2% (95% CI: 91.4-98.4%) Specificity: 510/523 = 97.5% (95% CI: 95.8-98.5%) Positive Predictive Value: 126/139 = 90.6% (95% CI: 84.7-94.5%) Negative Predictive Value: 510/515 = 99.0% (95% CI: 97.7-99.6%)			

[1]Strep A was not detected in 2/5 False Negative specimens using an FDA-cleared assay for discordant analysis.
[2]Strep A was detected in 7/13 False Positive specimens using an FDA-cleared assay for discordant analysis.

Positive and negative percent agreement for the Accula Strep A Test when compared to an FDA-cleared molecular assay are shown in **Table 4**.

Table 4. Accula Strep A Test Clinical Performance vs. FDA-cleared Molecular Assay

		FDA-cleared Assay		
		Positive	Negative	Total
Accula Strep A Test	Positive	137	1[2]	138
	Negative	9[1]	501	510
	Total	146	502	648
	Positive Percent Agreement: 137/146 = 93.8% (95% CI: 88.7-96.7%) Negative Percent Agreement: 501/502 = 99.8% (95% CI: 98.9-100%) Positive Predictive Value: 137/138 = 99.3% (95% CI: 96.0-99.9%) Negative Predictive Value: 501/513 = 98.2% (95% CI: 96.7-99.1%)			

[1]Strep A was not detected in 5/9 False Negative specimens using an FDA-cleared assay for discordant analysis.
[2]Strep A was detected in 0/1 False Positive specimens using an FDA-cleared assay for discordant analysis.

During the prospective clinical study, a total of 56 samples did not produce valid results with the Accula Strep A during the initial test (56/663 = 8.4%). Of the 56 samples, 39 samples (39/663 = 5.8%) gave invalid results due to failed internal controls and 17 samples (17/663 = 2.6%) did not generate a result due to system errors caused by the Dock (e.g., cassette failure or misuse, inappropriate sample volume, or amplification error). After repeat testing of the 56 samples, per the Accula Strep A product instructions, seven results (5 invalids and 2 system errors) remained unresolved (7/719 = 1.0%). The seven samples were tested for a third time; three provided valid results while four remained unresolved (4/726 = 0.6%).

b. Performance with Analyte Concentrations Near the Cutoff:

A study was conducted to evaluate the performance of the Accula Strep A Test with weakly reactive samples when testing was performed by untrained users. A panel of contrived throat swabs were tested at three CLIA-waived sites by two untrained operators at each site. The nine-member panel consisted of triplicate swab preparations spiked with varying Strep A concentrations: low positive (~1x LoD), moderate positive (~2x LoD), and negative (no Strep A). Each operator tested the coded, randomized panel on 5 non-consecutive days over a period of two weeks (3 sites x 2 operators x 3 replicate swabs x 5 days = 90 results/panel member).

Results are reported as percent agreement: observed result/expected result x 100. Agreement by site is summarized in **Table 5**.

Table 5. Performance of Samples Near the Cutoff[1]

Sample Type	Observed / Expected (%)				Overall 95% CI
	Site 1	Site 2	Site 3	Overall	
Low Positive	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	89/90 (98.9%)	94.0-99.8%
Moderate Positive	29/30 (96.7%)	30/30 (100%)	28/29 (96.6%)	87/89 (97.8%)	92.2-99.4%
Negative	30/30 (100%)	30/30 (100%)	28/30 (93.3%)	88/90 (97.8%)	92.3-99.4%

[1]Twenty (6.9%) of the 270 samples tested did not yield a valid result after initial testing (14 invalid results and 6 system errors). After retesting, one invalid result remained unresolved (1/290 = 0.3%).

The study results demonstrate that untrained users were able to perform the test correctly and the test provided the expected result >95% of the time for samples near the cutoff.

c. Operator Questionnaire Results:

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

M. Labeling for Waived Devices

The labeling consists of:

1. Accula Strep A Test Package Insert
2. Accula Strep A Test Quick Reference Guide (QRG)
3. Accula Dock Manual

The following elements are present in the labeling:

- The QRG is written at no higher than a 7th grade reading level. It contains illustrations of the system components and procedure steps.
- The package insert and the QRG identify the test as CLIA-waived.
- The package insert contains a statement that a Certificate of Waiver is required to perform the test in a waived setting; information on how operators can obtain a certificate is also provided.
- Per 42 CFR 493.15(e)(1), the package insert contains a statement that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test.
- Instructions for quality control (QC) are integrated with procedural instructions for performing the test in both the package insert and the QRG.
- Appropriate cautions have been added to the Package Insert and QRG to ensure safe use of the product.
- The results of a Clinical Study that support the determination of eligibility for CLIA Waiver are included in the Package Insert.

N. Benefit-Risk Considerations

The Accula Strep A Test when compared to culture showed high sensitivity (96.2%) and specificity (97.5%). While acceptable (>95%), this level of sensitivity warrants the requirement for culture confirmation of negative test results. This requirement is sufficient to mitigate the risks associated with a false-negative result. The rapid results associated with high specificity offers a benefit to patients in management of a positive Strep A result.

O. Conclusion:

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