CLIA Waiver by Application Approval Determination
Decision Summary

I. Document Number

CW260006

II. Parent Document Number

K253318

III. CLIA Waiver Type

CLIA Waiver by Application

IV. Applicant

Hangzhou Clongene Biotech Co., Ltd.

V. Proprietary and Established Names

Clungene RSV Antigen Rapid Test

VI. Measurand (analyte)

Nucleoprotein antigen from Respiratory Syncytial Virus (RSV)

VII. Sample Type(s)

Nasopharyngeal swab specimens

VIII. Type of Test

Qualitative lateral flow immunoassay

IX. Test System Description

A Overview

The Clungene RSV Antigen Rapid Test is a lateral flow immunoassay that operates based on the double antibody sandwich technique principle. Nasopharyngeal swab specimens from individuals are processed using extraction buffer in a buffer tube, and the extracted sample is then added to the specimen well. When present in the sample, respiratory syncytial virus (RSV) nucleoprotein antigen reacts with anti-RSV antigen antibody that has been conjugated with colored micro particles, forming an antigen-antibody labeled complex. This complex migrates across the membrane through capillary action until it reaches the test line, where it

is captured by pre-coated anti-RSV antigen antibody. If the specimen contains RSV nucleoprotein antigen, a colored line will appear in the test line (T) region, indicating a positive result, while the absence of RSV nucleoprotein antigen will result in no colored line appearing in this region, indicating a negative result. As a procedural control, a colored line will always appear at the control line (C) region, confirming that the proper volume of specimen has been added, and that membrane wicking has occurred.

The test result should be read at 15-20 minutes after adding the extracted sample to the specimen well. One line in the control line region (C), and another line in the test line region (T) indicates a positive result, regardless of color intensity. When only one line is present in the control region (C) and no line is present in the test line region (T), results are negative result. If the control line(C) fails to appear, the result is invalid, and the operator should review the procedure and repeat the test with a specimen and new test cassette.

B Test System Components

Materials Provided

The Clungene RSV Antigen Rapid Test contains reagents sufficient to run 25 tests and includes:

- 25 Test cassettes: each cassette with desiccant in individual foil pouch.
- 25 Buffer tubes: tube sealed with foil film containing 0.3 mL of extraction buffer.
- 25 Sterilized swabs: single use swab for specimen collection.
- 1 Positive Control Swab*: non-infectious recombinant RSV nucleocapsid protein is dried on the swab tip
- 1 Negative Control Swab*: non-infectious recombinant Group C Streptococci protein is dried on the swab tip
- 1 Instructions for Use
- 1 Quick Reference Card
- 1 Workstation

***** Contact manufacturer (1-800-319-8684; Mon-Fri: 9AM-5PM EST) to request additional positive or negative control swabs.

Materials Required but not Provided

- Timer
- Required personal protective equipment

X. Specific Contents for CLIA Waiver

A Demonstrating “Simple”:

The Clungene RSV Antigen Rapid Test is designed to be simple and easy to use incorporating the following key features:

1. The test is self-contained.
2. The test uses an unprocessed nasopharyngeal swab specimen directly.

3. The test needs only basic, non-technique-dependent specimen manipulation and reagent handling. An untrained operator can conduct the test by performing a few simple steps: .

   -Collect sample using the provided sterile swabs.

   -Place the swab in the buffer tube with extraction buffer; mix by rotating.

   -Dispense 3 drops from the Reagent tube onto the test device.

   -Wait 15-20 minutes.

   -Visually inspect the test (T) and control (C) lines on the test device to read the result.
4. The supplied extraction diluents are premeasured and provided in single-use vials.
5. No pipetting is required. Dropper tips for the extraction diluent are provided for adding sample dropwise to the test cassette.
6. The test does not require any operator intervention during the analysis step.
7. The test does not require technical or specialized training for troubleshooting or interpretation of multiple or complex error codes.
8. The test does not require any electronic or mechanical maintenance beyond simple tasks.
9. The test produces results that do not require operator calibration, interpretation, or calculation.
10. The test produces easily determinable results, such as positive, negative or invalid.
11. The kit is packaged with a Quick Reference Card (QRC) and Instruction for Use (IFU) that outlines the test process in easy to follow steps with illustrations.

B Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-Safe Mechanisms

1. Risk Analysis:

   Risk analysis was performed by the firm using the Failure Modes and Effects Analysis (FMEA) Method in accordance with ISO 14971. The FMEAs included identification and addressing of potential risks or error sources, analyzing potential causes, effects and the existing measures or mitigation factors related to the Clungene RSV Antigen Rapid Test. The elements considered included operator error, environmental factors, specimen and reagent handling, storage, device calibration, and external controls.

   Potential sources of errors that could adversely affect system performance were identified and mitigated first through system design verification and validation studies and then through additional precautions and warnings in the labeling. The identified risks which could result in erroneous test results were evaluated in flex studies that stressed the functional limits of the test system (see item 3 below).

2. Fail-Safe and Failure Alert Mechanisms:

   The Clungene RSV Antigen Rapid Test was designed to include numerous features and fail-safe mechanisms built into the system to prevent erroneous results.

   a) Design Features

   i. Each test cassette is packaged in a foil pouch with desiccant to maintain the integrity of the test device and reagents.

   ii. The foil pouch is printed with the assay name, lot number, and expiration date to ensure clarity and appropriate use.

iii. The kit provides swab and extraction reagent to ensure efficient nasal specimen collection and antigen extraction.

iv. Swab collected nasal sample is directly added into the tube with sample processing solution, and the extracted antigen is then applied directly to the test cassette, eliminating additional specimen-transfer steps.

v. The tube with sample processing solution is sealed with foil printed with the reagent name, lot number and expiration date to ensure clarity and appropriate use.

vi. The leak-proof cap of the tube with sample processing solution helps prevent sample loss and contamination.

vii. Each test cassette features distinct position marks within the results window to facilitate clear and accurate result interpretation. The control line is denoted as "C", the RSV test line is denoted as "T".

viii. Each positive and negative external control swabs provided with the device are clearly marked on the labeling with control swab name, lot number, and expiration date to ensure clarity and appropriate use.

b) <u>Fail-safe Features</u>

i. Internal Quality Control

The presence of a colored line at the control region (C) in the result window acts as an internal procedural control to ensure adequate migration has occurred and the functional integrity of the test cassette has been maintained. If the control line does not develop at 15 minutes, the test result is considered invalid and a new test should be performed.

ii. External Quality Control

External quality controls are used to demonstrate that the reagents and assay procedure perform properly. Each Clungene RSV Antigen Rapid Test contains one external positive control swab and one negative control swab. The package insert specifies that any additional external controls can be requested from the manufacturer.

The positive control swab and one negative control swab are recommended for testing when receiving a new lot of reagents/shipment or when a new operator uses the test. End users are expected to adhere to all applicable local, state, and federal regulations governing quality control procedures. The control swabs should be processed and tested in accordance with the assay's Instructions for Use, following the same procedure as for patient samples and are ready for use. Each control swab should produce the expected positive or negative results to verify the test performance. If external controls do not perform as expected, users are instructed to not use the test results and to repeat the tests or to contact the manufacturer.

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

The flex study was a non-blinded robustness evaluation of the Clungene RSV Antigen Rapid Test, designed to assess test performance under possible real-world deviations from standard operating conditions. Testing was conducted across three (3) lots of the

Clungene RSV Antigen Rapid Test and three (3) lots of control swabs, with each lot tested by a separate operator.

Contrived positive samples were prepared by diluting RSV-A (ATCC-2012-10) to 2× LoD in a pooled human nasal fluid matrix confirmed RSV-negative by RT-PCR. Negative samples consisted of the same matrix without virus. A total of 18 conditions were evaluated, spanning pre-analytical variables (e.g., swab insertion depth, buffer volume, nasal discharge), procedural variations (e.g., sample application angle, swab rotation time, dropper tip handling), environmental factors (e.g., light source, temperature storage, non-level surface), and physical disturbances (e.g., drop, shock, displacement, vibration). Results were read at the IFU-specified timepoint unless reading time itself was the variable under evaluation. The summary of results are presented in Table 1 below.

Table 1. Summary Results of Flex Studies

Conditions Tested	**Variations Tested under each Condition**	**# of positive results/#of replicates tested**	
		Positive samples*	**Negative samples***
Reading Time	5 min	0/9	0/9
	10 min	6/9	0/9
	12–30 min (3 timepoints)	27/27	0/27
Light Source	All 7 sources (flashlight, incandescent, halogen, fluorescent, LED, sunny, cloudy)	63/63	0/63
Sample Volume	1 drop (~35 µL)	All 9 invalid results	All 9 invalid results
	2–6 drops (5 volumes)	45/45	0/45
Non-Level Surface	4 tilt angles (15°, 30°, 60°, 90°)	36/36	0/36
Displacement	3 distances (1 m, 3 m, 5 m)	27/27	0/27
Drop Height	3 heights (0.6 m, 1.2 m, 1.8 m)	27/27	0/27
Shock	3 repeats	27/27	0/27
Storage at −20°C	9 timepoints (0, 1, 2, 3, 5, 7, 9, 12, and 15 days)	81/81	0/81
Storage at 45°C	9 timepoints ((0, 1, 2, 3, 5, 7, 9, 12, and 15 days)	81/81	0/81
Nasal Discharge on Swab	Heavy nasal discharge	12/12	0/12
Swab Insertion Depth	Swab touches tube bottom	9/9	0/9
	Swab in buffer, not touching bottom	9/9	0/9
	Swab not in buffer	0/9	0/9
Swab Rotation Time	3 durations (10 s, 20 s, 40 s)	27/27	0/27*
Swab Tip Squeezing	4 squeeze counts (0, 2, 5, 10)	36/36	0/36
Buffer Volume	0.1 mL (33%)	6/9 were invalids and remaining 3 yielded	4/9 were invalids and remaining 5 yielded

		negative results	negative results
	0.2–0.4 mL (3 volumes)	9/9	0/9
Dropper Tip Cycles	6 cycles (0–5)	54/54	0/54
Sample Application Angle	3 angles (vertical, 60°, 30°)	27/27	0/27
Extracted Sample Stability (swab in buffer)	3 timepoints (0, 30, 60 min)	27/27	0/27
Extracted Sample Stability (swab removed)	3 timepoints (0, 30, 60 min)	27/27	0/27
Shaking of Test Device	2 timepoints (0 min, 5 min)	18/18	0/18
Vibration Speed	4 speeds (0, 250, 500, 750 rpm)	36/36	0/36

*(n/N per variation) X # (of variations tested)

C Demonstrating "Insignificant Risk of an Erroneous Result" - Accuracy

1. Comparison study:

A prospective clinical study was conducted to evaluate the performance of the Clungene RSV Antigen Rapid Test using nasopharyngeal (NP) swab specimens. A total of seven hundred and twenty-two (722) NP swab specimens were prospectively collected from patients with signs and symptoms consistent with respiratory tract infection from five (5) clinical sites between November 2024 and April 2025. Two NP swabs were collected sequentially from each subject by the study operator/s. The sample for the RT-PCR was collected first from one nostril and placed into transport media for comparator testing. The second swab for the RSV Antigen Rapid Test was collected from the other nostril and tested immediately with the Clungene RSV Antigen Rapid Test at the site. Results from the candidate device were compared to NP swab specimen results when tested with an FDA cleared RT-PCR assay for RSV to demonstrate performance.

Patient Demographics

Demographic information was collected for all seven hundred and twenty-two (722) patients included in the study.

Table 2. Patient Demographics

Characteristics of the study population		**N=722**	**Percent (%)**
Sex	Male	321	44.5%
	Female	401	55.5%
Age	<6	146	20.2%
	6-21	232	32.1%
	21-59	239	33.1%
	≥60	105	14.5%
Race	Asian	134	18.6%
	Black or African American	92	12.7%

	White or Caucasian	362	50.1%
	Native Hawaiian or Other Pacific Islander	2	0.3%
	Other (Mixed race)	10	1.3%
	American Indian or Alaskan native	1	0.1%
	Prefer not to say	121	16.8%

Table 3. RSV Performance of the Clungene RSV Antigen Rapid Test with NP Swab Specimens Compared to RT-PCR

Clungene RSV Antigen Rapid Test	**Molecular Comparator**		
	Positive	**Negative**	**Total**
Positive	98	5	103
Negative	13	606	619
Total	111	611	722
Positive Percent Agreement (PPA)	88.3% (95% CI: 81.0%-93.0%)		
Negative Percent Agreement (NPA)	99.2% (95% CI: 98.1%-99.6%)		

2. Device Performance with Analyte Concentrations Near the Cutoff:

a. Multi-Lot, Multi-Site Precision and Reproducibility Study:

A multi-site reproducibility study was conducted to assess the performance consistency of the candidate device across different testing environments and operators. The study used a contrived sample panel consisting of a true negative sample (no analyte), a high negative sample at 0.05x LoD, a low positive sample at 1x LoD, and a moderate positive sample at 3x LoD. The evaluation was performed by two (2) untrained operators at each of three (3) testing sites over a five (5)-day period, providing an assessment of device's precision and reproducibility under varied operational conditions and critical concentration thresholds.

Contrived swab samples were prepared by spiking an RSV-A strain into pooled negative nasal fluid (PNF), with each diluted sample (50 µL) applied directly onto the sample collection swab head, while true negative swab samples consisted of 50 µL of negative PNF applied to the swab head. The contrived sample swabs were randomized and blinded to operators across all three (3) sites, with 150 replicates tested per concentration level (3 sites x 2 operators/site x 5 days x 5 replicates per day) yielding 450 total results.

All outcomes met the predefined acceptance criteria with no significant differences observed by operators, by days, and testing sites, confirming acceptable device performance reproducibility across the multi-site evaluation. The data in the Table 4 is presented for site-to-site reproducibility.

Table 4. Summary of Multi-Site Reproducibility Study Results

Sample	**# of positive result/# of total tested (% positive rate)**			**Total sample count (% positive rate)**
	Site 1	**Site 2**	**Site 3**	

True negative	0/150 (0.0%)	0/150 (0.0%)	0/150 (0.0%)	0/450 (0.0%)
High negative (0.05x LoD)	0/150 (0.0%)	0/150 (0.0%)	0/150 (0.0%)	0/450 (0.0%)
Low positive (1x LoD)	150/150 (100.0%)	150/150 (100.0%)	150/150 (100.0%)	450/450 (100.0%)
Moderate positive (3x LoD)	150/150 (100.0%)	150/150 (100.0%)	150/150 (100.0%)	450/450 (100.0%)

b. Multi-Lot, Single-Site Precision Study:

To supplement the initial five-day study, a second evaluation was conducted to determine the lot-to-lot precision of the Clungene RSV Antigen Rapid Test over 12 non-consecutive days. This single-site study utilized three (3) distinct test kit lots and three (3) concentrations of RSV-A and a negative sample. Consistent with the first study, RSV-A (same strain/lot) was spiked into pooled negative nasal fluid (PNF).

Samples were blinded and randomized before allotting them to the operators. 50µL of each sample was applied to dry nasal swabs and processed per the IFU of the candidate device. All panel members were tested in duplicate with 3 device lots per day and the study was conducted for 12 days (i.e., 1 site x 3 lots x 2 replicates per day x 12 days). A total of 72 results were obtained per concentration. All replicates prepared at each concentration, demonstrated 100% agreement with the expected results. The results from precision study are summarized below.

Table 5. Summary of Precision Study Results

Sample	**# of positive result/# of total tested (% positive rate)**			**Total sample count (% positive rate)**
	Lot 1	**Lot 2**	**Lot 3**	
True negative	0/24 (0.0%)	0/24 (0.0%)	0/24 (0.0%)	0/72 (0.0%)
High negative (0.05x LoD)	0/24 (0.0%)	0/24 (0.0%)	0/24 (0.0%)	0/72 (0.0%)
Low positive (1x LoD)	24/24 (100.0%)	24/24 (100.0%)	24/24 (100.0%)	72/72 (100.0%)
Moderate positive (3x LoD)	24/24 (100.0%)	24/24 (100.0%)	24/24 (100.0%)	72/72 (100.0%)

3. Operator questionnaire:

A total of twenty eight (28) untrained operators participated in a operator questionnaire assessment to evaluate comprehension of the product labeling, test procedure, and result interpretation. Each participant was provided with the Quick Reference Instructions (QRI) and test materials and instructed to perform the test without prior training. The questionnaire included 10 questions covering key aspects of test use, including comprehension of overall instructions, ease of following the sample collection, test procedure and result interpretation, and appropriate response to invalid results. Overall,

results were acceptable and support the use of this device in a CLIA-waived environment by untrained operators.

D Labeling for Waived Devices

The labeling submitted for the Clungene RSV Antigen Rapid Test consists of:

1. QRC
2. IFU
3. Package Labeling – kit box labels

The following elements are appropriately present:

- The QRC and the IFU identify the test as CLIA waived.
- The IFU contains a statement that a Certificate of Waiver is required to perform the test in a waived setting.
- A statement clearly states the specimen type.
- A statement that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test.
- The IFU contains a statement that any modification to the test or the manufacturer's instructions will result in the test being classified as high complexity. Step-by-step instructions for all control procedures, including frequencies and action to be taken if control results are out of range or invalid, or if other failure alert or fail-safe mechanisms are activated.
- The IFU and QRC provide instructions for conducting quality control procedures.
- A warning addressing color blindness when waived tests use color-coded reagents and/or endpoints.
- Telephone number to contact manufacturer for technical assistance or troubleshooting the test system which directs the user to call for assistance when the device or the control materials do not work as specified by the manufacturer.
- The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

XI. Benefit-Risk Considerations

After implementation of all risk control measures identified through the Design, Process, and Usability FMEA, the overall residual risks are acceptable and have been reduced to as low as reasonably practicable (ALARP) in accordance with ISO 14971. All hazards have been adequately mitigated through a combination of design features, fail-safe mechanisms, manufacturing controls, analytical verification, usability validation, and detailed labeling instructions. Importantly, no new risks were introduced as a result of implementing these risk controls, and additional controls did not create any unintended hazardous situations. Therefore, no further formal risk–benefit analysis is required.

Analytical performance studies demonstrated that the Clungene RSV Antigen Rapid Test reliably detects RSV antigen and distinguishes it from other common respiratory pathogens. Clinical validation performed in CLIA-waived environments showed that user-related errors were infrequent, and when they occurred, they did not lead to false positive results. The most

meaningful potential residual risk— misinterpretation of weak or faint test lines by lay users—primarily results in false negative outcomes. This risk is substantially reduced through:

- clearly illustrated QRC
- simplified workflow design
- intuitive labeling of the test cassette
- explicit interpretation examples including faint positives, and
- robust warnings and limitations within the IFU

Furthermore, internal fail-safe mechanisms (e.g., mandatory control line, bias toward invalid results under stress conditions) ensure that device malfunction or major procedural errors result in INVALID outcomes rather than false negatives or false positives, supporting FDA's requirement for insignificant risk of erroneous result in CLIA-waived settings.

Overall, the benefits of using the Clungene RSV Antigen Rapid Test in the intended population and CLIA-waived settings significantly outweigh the residual risks. When used according to the IFU, the device provides timely and clinically meaningful information that aids early detection of RSV infection, while maintaining a strong safety profile and a low likelihood of erroneous results.

XII. Conclusion

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