510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K253318

B Applicant

Hangzhou Clongene Biotech Co., Ltd.

C Proprietary and Established Names

Clungene RSV Antigen Rapid Test

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
GQG	Class I	21 CFR 866.3480 - Respiratory Syncytial Virus Serological Reagents	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the Clungene RSV Antigen Rapid Test

B Measurand:

Nucleoprotein antigen from Respiratory Syncytial Virus (RSV)

C Type of Test:

Qualitative lateral flow immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Clungene RSV Antigen Rapid Test is a lateral flow immunoassay intended for the qualitative detection of respiratory syncytial virus (RSV) nucleoprotein antigen in

nasopharyngeal swab specimens from patients with signs and symptoms of respiratory infection. The test is intended for in vitro diagnostic use as an aid in the diagnosis of RSV infections. Negative results do not preclude RSV infection and should not be used as the sole basis for treatment or patient management decisions. A negative test is presumptive. It is recommended that negative test results be confirmed by viral cell culture or an FDA-cleared molecular assay.

C Special Conditions for Use Statement(s):

Rx - For prescription use only

IVD – For *in vitro* diagnostic use

D Special Instrument Requirements:

Not applicable

IV Device/System Characteristics:

A Device Description:

The Clungene RSV Antigen Rapid Test is a lateral flow immunoassay intended for the qualitative detection of respiratory syncytial virus (RSV) nucleoprotein antigen in human nasopharyngeal swab specimens from individuals with signs and symptoms of respiratory infection, suspected of RSV by their healthcare provider. The test is intended for *in vitro* diagnostic use as an aid in the diagnosis of RSV infection. The Clungene RSV Antigen Rapid Test is validated for use with direct specimens without transport media.

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

B Principle of Operation:

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.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Nano-Check RSV Test

B Predicate 510(k) Number(s):

K240280

C Comparison with Predicate(s):

Device & Predicate Device(s):	K240280	K253318
Device Trade Name	Nano-Check RSV Test	Clungene RSV Antigen Rapid Test
General Device Characteristic Similarities		
Intended Use/Indications For Use	The Nano-Check RSV Test is a rapid immunochromatographic assay for the qualitative detection of respiratory syncytial virus (RSV) nucleoprotein antigen in anterior nasal swab specimens from patients with signs and symptoms of respiratory infections. This test is intended for in vitro diagnostic use to aid in the diagnosis of RSV infections in infants and pediatric patients aged 6 months to 6 years old, and adults over 60 years of age. Negative results do not preclude RSV infection and should not be used as the sole basis for treatment or other management decisions. A negative test is presumptive. It	The Clungene RSV Antigen Rapid Test is a lateral flow immunoassay intended for the qualitative detection of respiratory syncytial virus (RSV) nucleoprotein antigen in nasopharyngeal swab specimens from patients with signs and symptoms of respiratory infection. The test is intended for in vitro diagnostic use as an aid in the diagnosis of RSV infections. Negative results do not preclude RSV infection and should not be used as the sole basis for treatment or patient management decisions. A negative test is presumptive. It is recommended that negative test results be confirmed by viral cell culture or an FDA-cleared molecular assay.

	is recommended that negative test results be confirmed by viral cell culture or an alternative method, such as an FDA-cleared molecular assay.	
Regulation Number	21 CFR 866.3480	Same
Regulatory Class	Class II	Same
Product Code	GQG	Same
Indication Type	Prescription Use only	Same
Technology	Immunochromatographic assay	Same
Test Result Type	Qualitative	Same
Test Target	Nucleoprotein of RSV	Same
Instrumentation	None	Same
Device Format	Cassette	Same
Reading time	15-20 minutes	Same
General Device Characteristic Differences		
Specimen Type	Anterior nasal swab	Nasopharyngeal swab

VI Standards/Guidance Documents Referenced:

Document	Title	Publisher	Applicable study
21 CFR 866.3480	Respiratory syncytial virus serological reagents	FDA	All
11135:2014	Sterilization of health care products - Ethylene oxide - Requirements for development, validation and routine control of a sterilization process for medical devices	ISO	Sterility
11737-2:2020	Sterilization of health care products - Microbiological methods	ISO	Sterility
10993-7	Biological Evaluation of Medical Devices – Part 7: Ethylene Oxide Sterilization Residuals	ISO	Sterility
10993-1 -11:2017	Biological Evaluation of Medical Devices – Acute systemic toxicity	ISO	Biocompatibility
10993-5: Third Edition 2009-06-01	Biological Evaluation of Medical Devices - Tests for in vitro cytotoxicity	ISO	Biocompatibility

Document	Title	Publisher	Applicable study
10993-10: 2021	Biological Evaluation of Medical Devices –Tests for irritation and skin sensitization	ISO	Biocompatibility
10993-23:2021	Biological Evaluation of Medical Devices – Intracutaneous reactivity	ISO	Biocompatibility
10993-11:2017 USP-NF (151) Pyrogen test	Biological Evaluation of Medical Devices – Pyrogen test	ISO	Biocompatibility

VII Performance Characteristics:

A Analytical Performance:

1. Precision/Reproducibility:

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 1 is presented for site-to-site reproducibility.

Table 1. 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 2. 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%)

2. Linearity:

Not applicable. This is a qualitative assay with binary, visually read results

3. Analytical Specificity/Interference:

a. Cross Reactivity and Microbial Interference:

Cross reactivity and microbial interference studies were conducted to determine potential assay interference from other respiratory pathogens/microbial flora that may be present in nasal samples. A comprehensive panel of viruses, bacteria, fungi, and PNF was evaluated.

For the cross-reactivity study, organisms were diluted in PNF and tested in triplicate per lot using three (3) lots of devices in the absence of RSV. No cross-reactivity was observed with the organisms tested (Table 3).

For the microbial interference study, organisms were diluted in PNF in the presence of low levels (2x LoD) of RSV-A and tested in triplicate (3) using three (3) lots of devices. No microbial interference was observed (Table 3).

Table 3. Cross Reactivity and Microbial Interference Study Results

Microorganism	Concentration	Test Results			
		Cross Reactivity		Microbial Interference	
		n/N[1]	Result Agreement (%)[2]	n/N[1]	Result Agreement (%)[2]
Adenovirus 1	1.6 X 10^6 $TCID_{50}$/mL	0/9	100%	9/9	100%
Adenovirus 2	1.1 X 10^7 $TCID_{50}$/mL	0/9	100%	9/9	100%
Adenovirus 3	8.9 X 10^8 $TCID_{50}$/mL	0/9	100%	9/9	100%
Adenovirus B7	8.9 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
SARS-CoV-2, USA-WA1/2020	3.8 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Human coronavirus (OC43)	1.6 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Human coronavirus (229E)	2.8 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Human coronavirus (NL63)	1.8 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Human herpesvirus	1.2 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Parainfluenza virus type 1	8.9 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Parainfluenza virus type 2	8.9 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Parainfluenza virus type 3	8.5 X 10^6 $TCID_{50}$/mL	0/9	100%	9/9	100%
Parainfluenza virus type 4	1.6 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Measles virus	2.1 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Mumps virus	1.2 X 10^6 $TCID_{50}$/mL	0/9	100%	9/9	100%
Rhinovirus	2.2 X 10^6 PFU/mL	0/9	100%	9/9	100%
Herpes simplex virus	1.4 X 10^6 $TCID_{50}$/mL	0/9	100%	9/9	100%
Varicella virus	2.9 X 10^6 $TCID_{50}$/mL	0/9	100%	9/9	100%
Epstein-Barr Virus	3.2 X 10^7 Copies/mL	0/9	100%	9/9	100%
Rotavirus A	5.0 X 10^6 $TCID_{50}$/mL	0/9	100%	9/9	100%
Enterovirus 68	1.5 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Enterovirus 71	8.9 X 10^6 $TCID_{50}$/mL	0/9	100%	9/9	100%

MERS-CoV	8.0 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Influenza A (H1N1, Brisbane/59/07)	3.6 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Influenza A (H1N1, California/7/09)	8.0 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Influenza A (H3N2 Brisbane/10/07)	5.3 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Influenza A (H3N2, Victoria/361/11)	3.8 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Influenza A (H3N2, HongKong/8/68)	1.3 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Influenza A (H3N2, Wisconsin/67/05	1.1 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Influenza B (Yamagata/16/88)	1.3 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Influenza B (Yamagata, 504/00	6.3 X 10^5 $TCID_{50}$/mL	0/9	100%	9/9	100%
Influenza B (Victoria, Malaysia/2506/04)	1.9 X 10^6 $TCID_{50}$/mL	0/9	100%	9/9	100%
Influenza B (Victoria, 2/87)	7.6×10^5 TCID50/mL	0/9	100%	9/9	100%
Influenza B (Victoria, /78/2015)	2.8×10^6 TCID50/mL	0/9	100%	9/9	100%
Human Metapneumovirus	6.3×10^5 TCID50/mL	0/9	100%	9/9	100%
Coxsackievirus	1.6×10^6 TCID50/mL	0/9	100%	9/9	100%
Bordetella pertussis	8.7 X 10^7 CFU/ml	0/9	100%	9/9	100%
Chlamydophila pneumoniae	9.1 X 10^6 CFU/ml	0/9	100%	9/9	100%
Mycoplasma pneumonieae[3]	4.2 X 10^5 CFU/ml	0/9	100%	9/9	100%
Pseudomonas aeruginosa	2.8 X 10^8 CFU/ml	0/9	100%	9/9	100%
Staphylococcus aureus	9.5 X 10^8 CFU/ml	0/9	100%	9/9	100%
Haemophilus influenzae	8.0 X 10^7 CFU/ml	0/9	100%	9/9	100%
Streptococcus pneumoniae	1.0 X 10^7 CFU/ml	0/9	100%	9/9	100%
Streptococcus pyogenes	1.3 X 10^8 CFU/ml	0/9	100%	9/9	100%
Streptococcus salivarius	1.3 X 10^7 CFU/ml	0/9	100%	9/9	100%

Streptococcus mutans	1.2 X 10^6 CFU/ml	0/9	100%	9/9	100%
*Legionell*a *pneumophila*	1.6 X 10^6 CFU/ml	0/9	100%	9/9	100%
Klebsiella pneumoniae	5.8 X 10^7 CFU/ml	0/9	100%	9/9	100%
Staphylococcus epidermidis	1.2×10^9 CFU/mL	0/9	100%	9/9	100%
Mycobacterium tuberculosis	1.2×10^7 CFU/mL	0/9	100%	9/9	100%
Candida albicans	3.6×10^6 CFU/mL	0/9	100%	9/9	100%
Corynebacterium diphtheriae	1.4×10^6 CFU/mL	0/9	100%	9/9	100%
Moraxella catarrhalis	2.2×10^8 CFU/mL	0/9	100%	9/9	100%
Lactobacillus sp.	5.0×10^7 CFU/mL	0/9	100%	9/9	100%
Neisseria meningitidis	8.8×10^7 CFU/mL	0/9	100%	9/9	100%
Escherichia coli	2.6 X 10^7 CFU/ml	0/9	100%	9/9	100%
Pooled human nasal wash	N/A	0/9	100%	9/9	100%

[1] # of positive results/# of replicates.
[2] Agreement with the expected result.
[3] Tested at lower than recommended concentration.

b. <u>Endogenous/Exogenous Substances Interference:</u>

The Clungene RSV Antigen Rapid Test was evaluated for performance in the presence of a panel of common interfering endogenous and exogenous substances. Potentially interfering substances were prepared in PNF to the recommended concentration. Virus negative PNF specimens were evaluated in triplicate/lot using three (3) lots of devices to confirm that the potentially interfering substances were not cross-reactive with the test. Positive samples were also prepared in PNF at 2x LoD (RSV-A) and were evaluated in the presence of interfering substances in triplicate/lot using three (3) lots of devices to confirm that these substances do not interfere with detection of RSV-A. No interference was observed (Table 4).

Table 4. Interfering Substances Study Results

Potentially Interfering Substances	**Concentration Tested**	**Test Results**			
		No analyte		**With Analyte**	
		n/N[1]	**Result Agreement (%)[2]**	**n/N[1]**	**Result Agreement (%)[2]**
Purified Mucin Powder	2.5 mg/mL	0/9	100%	9/9	100%
Whole Blood	4%	0/9	100%	9/9	100%
Phenylephrine HCl	15% v/v	0/9	100%	9/9	100%
Oxymetazoline HCl	15% v/v	0/9	100%	9/9	100%
Sodium Chloride with Preservatives	15% v/v	0/9	100%	9/9	100%

Beclomethasone	0.5 mg/mL	0/9	100%	9/9	100%
Budesonide Nasal Spray	15% v/v	0/9	100%	9/9	100%
Mometasone Furoate	0.5 mg/mL	0/9	100%	9/9	100%
Dexamethasone	5 mg/mL	0/9	100%	9/9	100%
Flunisolide	5 mg/mL	0/9	100%	9/9	100%
Triamcinolone Acetonide	5 mg/mL	0/9	100%	9/9	100%
Fluticasone Propionate	5 mg/mL	0/9	100%	9/9	100%
Histamine diHydrochloride	10 mg/mL	0/9	100%	9/9	100%
Benzocaine	3 mg/mL	0/9	100%	9/9	100%
Menthol	3 mg/mL	0/9	100%	9/9	100%
Zanamivir	5 mg/mL	0/9	100%	9/9	100%
Ribavirin	5 mg/mL	0/9	100%	9/9	100%
Oseltamivir Phosphate	5 mg/mL	0/9	100%	9/9	100%
Peramivir	100 µg/mL	0/9	100%	9/9	100%
Mupirocin	10 mg/mL	0/9	100%	9/9	100%
Tobramycin	5 µg/mL	0/9	100%	9/9	100%
D-Biotin	3500 ng/mL	0/9	100%	9/9	100%
Remdesivir	0.2 mg/mL	0/9	100%	9/9	100%
Molnupiravir	2.5 mg/mL	0/9	100%	9/9	100%
Paracetamol	50 µg/mL	0/9	100%	9/9	100%
Euphorbium Nasal Spray	15% v/v	0/9	100%	9/9	100%
Sinna Nasal Spray	15% v/v	0/9	100%	9/9	100%
Quantum HEALTH TheraZinc	15% v/v	0/9	100%	9/9	100%
Leukocytes	5×10^6 cells/mL	0/9	100%	9/9	100%

[1] # of positive results/# of replicates.
[2] Agreement with the expected result.

4. Detection Limit and Assay Reportable Range:

a. Limit of Detection (LoD):

An LoD study was conducted to determine the lowest detectable concentration of two (2) strains of RSV-A (2006 isolate and ATCC-2012-10), and two (2) strains of RSV-B (ATCC-2012-11 and BWV/14617/85) at which at least 95% of all true positive replicates return a positive result. Testing was conducted on three (3) lots of test devices.

A preliminary LoD was first determined by testing serial 10-fold dilutions of virus stocks diluted in PNF in three (3) replicates per device lot for a total of 9 replicates per dilution. A 50 µL sample of each virus diluted in PNF was pipetted onto the dry swab. The swab was then tested per the IFU. The preliminary LoD of each virus was confirmed by testing an additional twenty samples/lot for each viral stock at the preliminary LoD concentration. If the preliminary LoD yielded 100%-positive test results/lot from each 1:10 dilution, it was further evaluated using a 2-fold dilution series, in 20 replicates/lot for each level, to refine the LoD.

As per the acceptance criteria for confirmation of the LoD, at least 95% of the replicates (≥ 19/20/lot) should be positive to be considered as the confirmed LoD. The confirmed LoDs observed were identical for the three (3) lots of devices tested for each virus strain.

Table 5. Limit of Detection- Clungene RSV Antigen Rapid Test

Virus strains	LoD	#Positive/ #Total	% Positive
RSV-A (2006 isolate)	1.5 x 10^2 $TCID_{50}$/mL	60/60	100.0%
RSV-A (ATCC-2012-10)	6.4 x10^3 $TCID_{50}$/mL	60/60	100.0%
RSV-B (ATCC-2012-11)	1.9 x 10^2 $TCID_{50}$/mL	60/60	100.0%
RSV-B (B WV/14617/85)	2.2 x 10^3 $TCID_{50}$/mL	60/60	100.0%

b. High Dose Hook Effect:

A high-dose hook effect study was conducted to evaluate whether high concentrations of target analyte could yield false negative results. Each sample was prepared beginning at a high "stock" viral concentration and subsequently diluted in 1:10 serial dilutions down to 1x LoD. Following preparation, 50 µL of each sample was applied to swabs, which were then processed according to the Instructions for Use (IFU). Three (3) lots of devices were tested, and all samples were tested in triplicate per lot. The results demonstrated no evidence of a high-dose hook effect across the concentrations evaluated in this study.

Table 6. High-Dose Hook Effect Study Results

RSV Strain	Concentration (TCID50/mL)	#Positive/ #Total	% Positive
RSV-A (2006 Isolate)	1.5×10^6 (10000×LoD)	9/9	100.0%
	1.5×10^5 (1000×LoD)	9/9	100.0%
	1.5×10^4 (100×LoD)	9/9	100.0%
	1.5×10^3 (10×LoD)	9/9	100.0%
	1.5×10^2 (1×LoD)	9/9	100.0%
RSV-A (ATCC-2012- 10)	6.4×10^6 (1000×LoD)	9/9	100.0%
	6.4×10^5 (100×LoD)	9/9	100.0%
	6.4×10^4 (10×LoD)	9/9	100.0%
	6.4×10^3 (1×LoD)	9/9	100.0%
RSV-B (ATCC-2012- 11)	3.8×10^4 (200×LoD)	9/9	100.0%
	1.9×10^4 (100×LoD)	9/9	100.0%
	1.9×10^3 (10×LoD)	9/9	100.0%
	1.9×10^2 (1×LoD)	9/9	100.0%
RSV-B (B WV/14617/85)	8.9×10^5 (400×LoD)	9/9	100.0%
	2.2×10^5 (100×LoD)	9/9	100.0%
	2.2×10^4 (100×LoD)	9/9	100.0%
	2.2×10^3 (100×LoD)	9/9	100.0%

c. Inclusivity:

Analytical reactivity testing for the Clungene RSV Antigen Rapid Test was conducted to ensure that the device can adequately detect a variety of strains for RSV viruses. A selection of temporally, geographically, and genetically diverse RSV-A and RSV-B strains were tested for inclusivity. A series of ten-fold dilutions of each virus strain was spiked into PNF and tested to determine an approximate LoD of the test for each virus. The lowest concentration with 100% positive replicates was identified and additional 2-fold dilutions below that approximate LoD were tested to demonstrate inclusivity. Three (3) lots of devices were tested, and all samples were tested in triplicate per lot. Based on the dilution series, the minimum detectable concentration was defined as the lowest concentration for which all nine (9) replicates (3 replicates/lot X 3 lots) were detected. Results are summarized below and demonstrate that the test tests can detect the analytes across a range of viral strains.

Table 7. Inclusivity Results

Strain	Lowest Concentration with 100% Detection
RSV-A (A2)	1.6×10^3 $TCID_{50}$/mL
RSV-A(Long)	8.0×10^2 PFU/mL
RSV-A(1/2015 Isolate #1)	6.3×10^1 $TCID_{50}$/mL
RSV-A (2/2015 Isolate 2)	1.17×10^1 $TCID_{50}$/mL
RSV-B(CH93(18)-18)	3.15×10^2 $TCID_{50}$/mL
RSV-A (A2023/06 12NSMM)	7.0×10^2 $TCID_{50}$/mL
RSV-B (B1)	7.0×10^2 $TCID_{50}$/mL
RSV-B (18537)	1.24×10^3 $TCID_{50}$/mL
RSV-B (9320)	1.4×10^3 $TCID_{50}$/mL

d. Assay Reportable Range:

Not applicable; the device is a binary qualitative assay that is visually read.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a. Controls:

i. *Internal Controls*:

The Clungene RSV Antigen Rapid Test contains a built-in internal procedural control. The appearance of the control line 'C'-Line on the test strip ensures that sufficient flow of the sample occurred and that the reagents are functioning properly during the assay. If no visible signal appears on the control line, the test result is invalid, and the operator is instructed in the labelling to test the sample again with another test device.

ii. External Controls:

The Clungene RSV Antigen Rapid Test contains one positive external control swab and one negative external control swab that allows for monitoring of the performance of the assay. The positive control swab contains non-infectious recombinant RSV nucleocapsid protein, and the negative control swab non-infectious recombinant Group C Streptococci protein dried on the swab tip.

Lot-to-lot precision of the external positive and negative control swabs was evaluated by testing ten (10) replicates each of three (3) lots of the external controls. For each external control lot, all positive and negative controls produced 100% agreement with the expected results, as summarized below.

Table 8. Summary of External Controls Precision Study Results

External Control	Control Lot No.	Test Result/Total Tests		% Agreement to Expected Result
		# Negative	# Positive	
External Positive Control	1	0/10	10/10	100%
	2	0/10	10/10	100%
	3	0/10	10/10	100%
External Negative Control	1	10/10	0/10	100%
	2	10/10	0/10	100%
	3	10/10	0/10	100%

b. Stability:

i. Specimen Stability:

A specimen stability study was conducted to evaluate the stability the specimen on swabs provided with the test kit (i.e., Copan Flexible FLOQSwab) and when tested under various conditions. Two test samples were prepared: negative samples (pooled negative nasal fluid, PNF) and contrived positive samples (prepared by spiking RSV-A at 2x LoD into PNF) and tested using three (3) device lots.

Fifty (50) µL of either three (3) positive or three (3) negative samples per lot were applied to dry Copan Flexible FLOQSwabs. Each swab was placed into a sterile, empty tube and stored under temperature conditions for specified durations as described below:

- ***Refrigerated (2-8 ºC):*** Samples were stored for 0, 6, 12, 24, and 48 hours.
- ***Ambient conditions (Room temperature, and 35 ºC):*** Samples were stored separately at room temperature, and 35 ºC for 0, 1, 2, 3, and 4 hours.
- ***Frozen (below -20 ºC):*** Samples were stored at -20 ºC for 0, 1, 3, and 5 days.
- ***Freeze-thaw stability:*** Samples were stored at -20 ºC for 24 hours, then transferred to room temperature for thawing, constituting one freeze-thaw cycle. Repeated to constitute to 2 freeze-thaw cycles. After each freeze-thaw cycle, tests were performed in triplicate using three device lots by three operators.

Subsequently, all exposed sample swabs were tested at each storage condition and timepoint and the results were compared with time 0. All negative samples produced negative results at all conditions and timepoints tested. All 2x LoD positive samples yielded expected positive results at all conditions and timepoints tested.

ii. Real Time Stability:

A real-time stability study was conducted to evaluate stability and determine the shelf-life of the unopened kit. To validate the shelf-life at 2-30°C, three (3) unopened Clungene RSV Antigen Rapid Test lots with three (3) unopened lots of external controls were stored at 4°C and 30°C. At defined intervals, an assessment of each lot was conducted with the following panel of test samples: negative clinical matrix and contrived positive samples prepared at 1x LoD of RSV-A virus.

Fifty (50) µL of each sample were applied to the swab and tested according to the IFU. Five replicates of each sample were tested per lot for each time point.

Baseline testing was performed within one month of each manufactured lot. Subsequent time testing was conducted every month for up to 3 months and then at 6, 12, 18, 21, 24, 25, 26, and 27 months. At the time of clearance, all study data have met the protocol defined acceptance criteria, and support storage of the test kits at 2-30°C for up to 26 months.

6. Assay Cut-Off:

Not applicable as this is a qualitative visually read assay without numeric data.

B Comparison Studies:

1. Method Comparison with Predicate Device:

See section C (clinical studies) below.

2. Matrix Comparison:

The Clungene RSV Antigen Rapid Test is intended for use with direct nasopharyngeal swab specimens. As the intended sample type is evaluated in the clinical study, a matrix comparison study is not applicable.

C Clinical Studies:

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 9. 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 10. 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%)		

1. Clinical Sensitivity:

   Please refer to Section VI.C (Clinical Studies) above for the clinical validation.

2. Clinical Specificity:

   Please refer to Section VI.C (Clinical Studies) above for the clinical validation.

3. Clinical Cut-Off:

   A clinical cut-off study is not applicable, there is no clinical cut-off related to the presence of RSV in patient samples.

D Expected Values/Reference Range:

A patient sample is expected to be negative for RSV.

VIII Proposed Labeling:

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

IX Conclusion:

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