510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K232377

B Applicant

Healgen Scientific LLC

C Proprietary and Established Names

Healgen Rapid COVID-19 Antigen Test

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QVF	Class II	21 CFR 866.3982 - Simple Point-Of-Care Device To Directly Detect SARS-Cov-2 Viral Targets From Clinical Specimens In Near-Patient Settings	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain 510(k) clearance for the Healgen Rapid COVID-19 Antigen Test.

B Measurand:

Nucleocapsid protein antigen from SARS-Coronavirus 2 (SARS-CoV-2)

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 Healgen Rapid COVID-19 Antigen Test is a lateral flow immunochromatographic assay intended for the rapid, qualitative detection of SARS-CoV-2 nucleocapsid protein antigens directly in anterior nasal swabs from individuals with signs and symptoms of upper respiratory infection within the first six (6) days of symptom onset. The test is intended for use as an aid in the diagnosis of SARS-CoV-2 infections (COVID-19) in symptomatic individuals when tested at least twice over three days with at least 48 hours between tests; or when tested once, and negative by the Healgen Rapid COVID-19 Antigen Test and followed with a molecular test.

The test does not differentiate between SARS-CoV and SARS-CoV-2.

A negative test result is presumptive, and it is recommended these results be confirmed by a molecular SARS-CoV-2 assay. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other patient management decisions.

Positive results do not rule out co-infection with other respiratory pathogens.

Performance characteristics for SARS-CoV-2 were established from May 2022 to July 2022 when SARS-CoV-2 Omicron was the predominant SARS-CoV-2 variant in circulation. When other SARS-CoV-2 virus variants are emerging, performance characteristics may vary.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

IVD - For In Vitro Diagnostic Use Only

D Special Instrument Requirements:

N/A

IV Device/System Characteristics:

A Device Description:

The Healgen Rapid COVID-19 Antigen Test is a lateral flow immunochromatographic membrane assay that uses monoclonal antibodies to detect nucleocapsid protein from SARS-CoV-2 virus in anterior nasal swab specimens. The test strip is composed of a sample pad, reagent pad, reaction membrane, and absorbing pad housed within a test cassette. The reagent pad contains a colloidal gold-conjugated monoclonal antibody, which recognizes and binds to the nucleocapsid protein of SARS-CoV-2; the reaction membrane in the test line (T) contains the second antibody that recognizes another epitope of the nucleocapsid protein. The whole strip is fixed inside a plastic cassette. **Figure 1** shows the test principle.

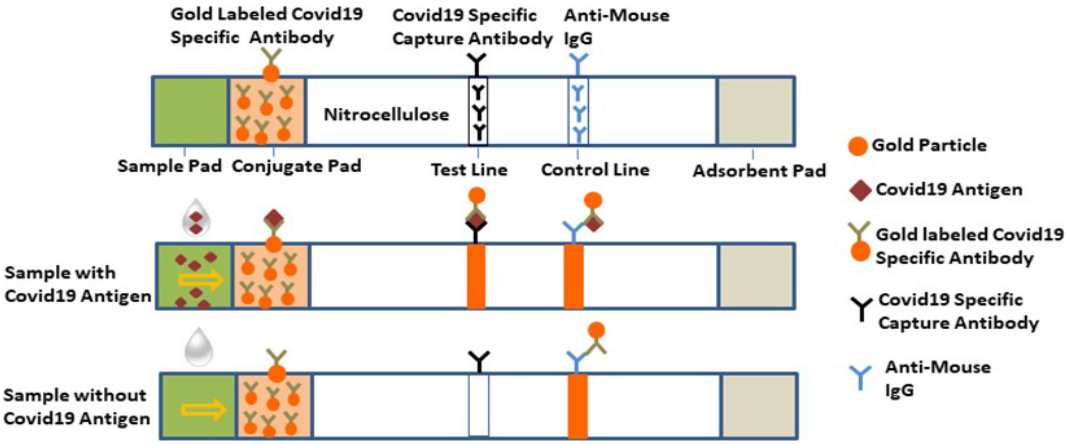

Figure 1. Diagram of Device Principle

External quality controls are required but not included with the test kit and are sold separately as the *COVID-19 Antigen Control Kit*. The control swabs should be processed according to the Instructions for Use (IFU) and are intended to be used as quality control samples to demonstrate that the test is performing and is being performed correctly. The Negative Control Swab is composed of negative control buffer dried onto a swab, with a blue shaft and containing 0.1% sodium azide preservative. The Positive Control Swab also contains SARS-CoV-2 recombinant antigen extract dried onto a swab at a concentration of 0.1 ng/mL, with a red shaft and containing 0.1% sodium azide preservative.

B Principle of Operation:

The Healgen Rapid COVID-19 Antigen Test uses immunochromatographic-based technology in a lateral flow design to detect nucleocapsid protein from SARS-CoV-2 virus. Anterior nasal swab specimens are collected by a healthcare professional (HCP). Following sample collection, the nasal swab is transferred to the prefilled extraction tube to lyse the sample and solubilize the viral nucleoproteins. Four (4) drops of the lysed sample is loaded onto the test cassette sample well, and the test result is read by the HCP after 15 minutes. If antigen is present, the colloidal gold monoclonal antibody conjugates bind to the nucleocapsid protein, and the antigen-antibody complex then migrate up along the reaction membrane, which will be captured by the second specific anti-SARS-2 antibody coated on the T line. The sample migrates along the test strip across two distinct areas: the test line and the internal procedural control line. If SARS-CoV-2 viral antigens are present, they will be captured by antibodies and bound to the test line of the test strip, and a colored line will be visible in the test window of the cassette. A red line will always appear in the control line (C), which is coated with secondary antibody recognizing the primary antibody in the migrating sample.

The Healgen Rapid COVID-19 Antigen Test gives three possible results: positive, negative or invalid. A positive result will present two colored lines beside the “C” and “T” regions of the test window and indicates that SARS-CoV-2 antigen was detected. A negative result will present only one colored line next to the “C” region of the test window and indicates that SARS-CoV-2 antigen was not detected. If no line is seen in the “C” region after 20 minutes, then the result is invalid and should be repeated with new test materials.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Sofia 2 SARS Antigen+ FIA, Sofia 2 SARS Antigen+ FIA Control Swab Set

B Predicate 510(k) Number(s):

DEN220039

C Comparison with Predicate(s):

Device & Predicate Device(s):	Device K232377	Predicate DEN220039
Device Trade Name	Healgen Rapid COVID-19 Antigen Test, COVID-19 Antigen Control Kit	Sofia 2 SARS Antigen+ FIA, Sofia 2 SARS Antigen+ FIA Control Swab Set
Intended Use/ Indications For Use	The Healgen Rapid COVID-19 Antigen Test is a lateral flow immunochromatographic assay intended for the rapid, qualitative detection of SARS-CoV-2 nucleocapsid protein antigens directly in anterior nasal swabs from individuals with signs and symptoms of upper respiratory infection within the first six (6) days of symptom onset. The test is intended for use as an aid in the diagnosis of SARS-CoV-2 infections (COVID-19) in symptomatic individuals when tested at least twice over three days with at least 48 hours between tests; or when tested once, and negative by the Healgen Rapid COVID-19 Antigen Test and followed with a molecular test. The test does not differentiate between SARS-CoV and SARS-CoV-2. A negative test result is presumptive, and it is recommended these results be confirmed by a molecular SARS-CoV-2 assay. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other patient management decisions. Positive results do not rule out co-infection with other respiratory	The Sofia 2 SARS Antigen+ FIA is a lateral flow immunofluorescent sandwich assay that is used with the Sofia 2 instrument for the rapid, qualitative detection of SARS-CoV-2 nucleocapsid protein antigens directly in anterior nasal swab specimens from individuals with signs and symptoms of upper respiratory infection (i.e., symptomatic) when testing is started within 6 days of symptom onset. The test is intended for use as an aid in the diagnosis of SARS-CoV-2 infections (COVID-19) in symptomatic individuals when tested at least twice over three days with at least 48 hours between tests. The test does not differentiate between SARS-CoV and SARS-CoV-2. A negative test result is presumptive, and it is recommended these results be confirmed by a molecular SARS-CoV-2 assay. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other patient management decisions. Positive results do not rule out co-infection with other respiratory pathogens. Performance characteristics for

Device & Predicate Device(s):	Device K232377	Predicate DEN220039
	pathogens. Performance characteristics for SARS-CoV-2 were established from May 2022 to July 2022 when SARS-CoV-2 Omicron was the predominant SARS-CoV-2 variant in circulation. When other SARS-CoV-2 virus variants are emerging, performance characteristics may vary.	SARS-CoV-2 were established during the 2021-2022 SARSCoV-2 pandemic when SARS-CoV-2 Omicron was the predominant SARS-CoV-2 variant in circulation. When other SARS-CoV-2 virus variant are emerging, performance characteristics may vary. This test is intended for prescription use only and can be used in Point-of-Care settings.
General Device Characteristic Similarities		
Regulation	21 CFR 866.3982	Same
Target Analyte	Nucleocapsid protein antigen from SARS-CoV-2	Same
Specimen Type	Direct anterior nasal (nares) swabs	Same
Intended Use Population	Symptomatic individuals within 6 days of symptom onset	Same
Test Time	15 minutes after the extracted sample is added to the test cassette sample well	Same
Serial Testing	Yes	Same
Results Reported	Qualitative (positive, negative, invalid)	Same
Quality Controls	Built-in procedural control (control line region (C)) in the test strip and external quality controls (separately packaged kit).	Built-in procedural control in the test strip and external quality controls (included in kit).
General Device Characteristic Differences		
Instrumentation	None	Sofia 2 Instrument
Principle of Operation	Lateral flow immunochromatographic assay	Lateral flow immunofluorescence sandwich assay
Result Interpretation	Visually read - visual interpretation of the presence or absence of colored line(s) on the control and test line(s) of the test strip is used to determine Positive, Negative, or Invalid results.	Instrument read - the Sofia 2 instrument scans the test strip and measures the fluorescent signal by processing the results using method specific algorithms. Sofia 2 displays the test results (Positive, Negative, or Invalid) on the screen.
Storage	Room temperature or refrigerated (2 to 30°C, 36 to 86°F)	Room temperature, 59°F to 86°F (15°C to 30°C)

VI Standards/Guidance Documents Referenced:

No standard/guidance documents referenced.

VII Performance Characteristics (if/when applicable):

A. Analytical Performance:

1. Precision/Reproducibility:

A multisite precision study was performed at three external, CLIA-waived testing sites to evaluate reproducibility of the Healgen Rapid COVID-19 Antigen Test. Testing consisted of three replicates each of positive (prepared at 3x LoD), low positive (prepared at 1x LoD), and negative samples tested by three (3) untrained operators per site over 5 days, i.e., 3 replicates × 3 operators × 3 sites × 5 days = 135 replicates per concentration for a total of 405 total data points collected. Three (3) test lots were used in this study, and lot-to-lot variability was also assessed (see **Table 2** below).

Fifty (50) µL of the prepared sample were applied to kit swabs, shipped and stored frozen at -20°C until testing. The results were ≥ 90% agreement between expected and read result within run, by lot, by operator, by day, between sites and overall.

Table 1. Summary Results of Multisite Precision Study (Reproducibility)

Site	Negative		Weak Positive		Positive	
	Correct Reads/Total	NPA	Correct Read /Total	PPA	Correct Reads/Total	PPA
1	44/45	97.8%	45/45	100.0%	45/45	100.0%
2	45/45	100.0%	45/45	100.0%	45/45	100.0%
3	45/45	100.0%	45/45	100.0%	45/45	100.0%
Total	**134/135**	**99.2%**	**135/135**	**100.0%**	**135/135**	**100.0%**

Table 2. Summary Results of Lot-to Lot Precision Study

Lot	Negative		Weak Positive		Positive	
	Correct Reads/Total	NPA	Correct Read /Total	PPA	Correct Reads/Total	PPA
1	45/45	100.0%	45/45	100.0%	45/45	100.0%
2	44/45	97.8%	45/45	100.0%	45/45	100.0%
3	45/45	100.0%	45/45	100.0%	45/45	100.0%
Total	**134/135**	**99.2%**	**135/135**	**100.0%**	**135/135**	**100.0%**

2. Linearity:

Not Applicable; the Healgen Rapid COVID-19 Antigen Test device is a qualitative assay.

3. Analytical Specificity/Interference:

a. Cross-Reactivity and Microbial Interference (Analytical Specificity)

The purpose of the cross-reactivity and microbial interference studies is to evaluate potential cross-reactants and microbial interferents for their impact on the Healgen Rapid COVID-19 Antigen Test performance. For this testing, live, commercial sources of the organisms were obtained, excluding MERS which used inactivated virus. Three replicates each of microorganism prepared in negative clinical matrix were tested in the absence and presence of 2x LoD inactivated SARS-CoV-2 virus (USA-WA1/2020) on 3 test lots. Fifty (50) µL of the prepared sample were applied to the kit swabs and eluted into the extraction buffer tube per the IFU. No cross-reactivity or microbial interference was observed with these organisms at the tested concentration.

Table 3. Cross-reactivity and Microbial Interference Study Results

Virus/Microorganism	Concentration	Cross-Reactivity (% Negative Agreement)	Interference (% Positive Agreement)
Human coronavirus 229E	8.00×10^5 $TCID_{50}$/mL	100 (3/3)	100 (3/3)
Human coronavirus OC43	7.00×10^6 $TCID_{50}$/mL	100 (3/3)	100 (3/3)
Human coronavirus NL63	2.93×10^4 $TCID_{50}$/mL	100 (3/3)	100 (3/3)
MERS-coronavirus	7.00×10^5 $TCID_{50}$/mL	100 (3/3)	100 (3/3)
Adenovirus 10	2.39×10^6 $TCID_{50}$/mL	100 (3/3)	100 (3/3)
Adenovirus 21	1.14×10^6 $TCID_{50}$/mL	100 (3/3)	100 (3/3)
Human metapneumovirus	3.95×10^5 $TCID_{50}$/mL	100 (3/3)	100 (3/3)
Parainfluenza virus Type 1	2.23×10^6 $TCID_{50}$/mL	100 (3/3)	100 (3/3)
Parainfluenza virus Type 2	2.23×10^5 $TCID_{50}$/mL	100 (3/3)	100 (3/3)
Parainfluenza virus Type 3	4.00×10^6 $TCID_{50}$/mL	100 (3/3)	100 (3/3)
Parainfluenza virus Type 4a	7.00×10^4 $TCID_{50}$/mL	100 (3/3)	100 (3/3)
Influenza A (H1N1)	4.00×10^8 $CEID_{50}$/mL	100 (3/3)	100 (3/3)
Influenza A (H3N2)	7.00×10^5 $TCID_{50}$/mL	100 (3/3)	100 (3/3)
Influenza B	7.00×10^5 $TCID_{50}$/mL	100 (3/3)	100 (3/3)
Enterovirus 68	2.23×10^6 $TCID_{50}$/mL	100 (3/3)	100 (3/3)
Enterovirus 71	4.00×10^7 $TCID_{50}$/mL	100 (3/3)	100 (3/3)
Respiratory syncytial virus	2.23×10^6 $TCID_{50}$/mL	100 (3/3)	100 (3/3)
Rhinovirus 60	8.00×10^5 $TCID_{50}$/mL	100 (3/3)	100 (3/3)
Haemophilus influenzae	1.74×10^8 CFU/mL	100 (3/3)	100 (3/3)
Streptococcus pneumoniae	3.35×10^8 CFU/mL	100 (3/3)	100 (3/3)
Streptococcus pyogenes	5.98×10^8 CFU/mL	100 (3/3)	100 (3/3)
Candida albicans	1.19×10^8 CFU/mL	100 (3/3)	100 (3/3)
Bordetella pertussis	4.90×10^9 CFU/mL	100 (3/3)	100 (3/3)
Mycoplasma pneumoniae	6.75×10^7 CCU/mL	100 (3/3)	100 (3/3)
Chlamydia pneumoniae	4.25×10^7 CFU/mL	100 (3/3)	100 (3/3)
Legionella pneumophila	9.20×10^9 CFU/mL	100 (3/3)	100 (3/3)
Staphylococcus aureus	5.00×10^6 CFU/mL	100 (3/3)	100 (3/3)
Staphylococcus epidermidis	1.75×10^8 CFU/mL	100 (3/3)	100 (3/3)
Pooled human nasal wash	NA	100 (3/3)	100 (3/3)

In silico evaluation of SARS-CoV-1 coronavirus, Human coronavirus HKU1, *Mycobacterium tuberculosis*, and *Pneumocystis jirovecii* was also conducted. The N protein sequence (GenBank ID: UUL70282.1) derived from an Omicron variant of SARS-CoV-2, belonging to the sublineages BA.1, BA.1.1, BA.2 (Accession Number OP160218) was used for the analysis.

There was no significant sequence homology with *M. tuberculosis* and *P. jirovecii* genomes, suggesting cross-reactivity would not occur; however, cross-reactivity cannot be ruled out. Homologous N protein sequences were identified in SARS-coronavirus, Human coronavirus HKU1, and MERS coronavirus. Human coronavirus HKU1 shares 41.18% to 49.00% homology or identity with the SARS-CoV-2 Omicron N protein sequence, and MERS coronavirus shares 52.87% to 54.04% identity. Homology is relatively low; however, cross-reactivity cannot be ruled out. The N protein sequence of SARS coronavirus shares 79.01% to 97.61% sequence identity with that of SARS CoV-2 Omicron variant indicating that cross-reactivity is likely. Therefore, the intended use states that it cannot distinguish SARS-CoV-2 and SARS-CoV.

b. Endogenous/Exogenous Interference

An endogenous/exogenous substances interference study was conducted in which samples of 2x LoD inactivated SARS-CoV-2 virus (USA-WA1/2020) were prepared in negative clinical matrix and tested in the presence of potentially interfering substances on 3 test lots, to evaluate endogenous/exogenous interference on the Healgen Rapid COVID-19 Antigen Test. Fifty (50) µL of the prepared sample were applied to the kit swabs and eluted into the extraction buffer tube per the IFU. No interference was observed with the tested interfering substances at the tested concentration.

Table 4. Interfering Substances Study Results

Substance	Active Ingredient	Concentration	% Positive Agreement	% Negative Agreement
Whole Blood		4%	100 (3/3)	100 (3/3)
Human Leukocytes		1×10^7 cells/mL	100 (3/3)	100 (3/3)
Mucin		0.5%	100 (3/3)	100 (3/3)
Chloroseptic	Menthol, Benzocaine	3 mg/mL	100 (3/3)	100 (3/3)
NasoGEL (NeilMed)	Sodium Chloride, Sodium Bicarbonate, Sodium Hyaluronate	5%	100 (3/3)	100 (3/3)
CVS Nasal Drops	Phenylephrine	15%	100 (3/3)	100 (3/3)
Afrin Naal Spray	Oxymetazoline	15%	100 (3/3)	100 (3/3)
CVS Nasal Spray	Cromolyn	15%	100 (3/3)	100 (3/3)
ZICAM Cold Remedy + Multi-Symptom Relief	Galphimia glauca, luffa operculata, sabadilla	5%	100 (3/3)	100 (3/3)
Homeopathic (Alkalol)	Galphima glauca, Luffa operculata, Sabadila 6X	15%	100 (3/3)	100 (3/3)
Sore throat phenol spray	Phenol	15%	100 (3/3)	100 (3/3)
Hand soap		1%	100 (3/3)	100 (3/3)
Hand sanitizer		1%	100 (3/3)	100 (3/3)
Tobramycin	Tobramycin	4 µg/mL	100 (3/3)	100 (3/3)
Mupirocin	Mupirocin	10 mg/mL	100 (3/3)	100 (3/3)
Fluticasone Propionate	Glucocorticoid	15%	100 (3/3)	100 (3/3)
Tamiflu	Oseltamivir Phosphate	5 mg/mL	100 (3/3)	100 (3/3)

c. High-dose Hook Effect Study

The purpose of the hook effect study is to evaluate if a hook effect occurs when high levels of the target analyte are present in the test sample. For this, 1:1, 1:10 and 1:100 dilutions of heat inactivated SARS-CoV-2 virus (USA-WA1/2020) were prepared in negative clinical matrix and tested on the Healgen Rapid COVID-19 Antigen Test. Fifty (50) µL of the

prepared sample were applied to the kit swabs and eluted into the extraction buffer tube per the IFU. The highest concentration tested was 5.75×10^6 $TCID_{50}$/mL, and no high-dose hook effect was observed.

Table 5. High-Dose Hook Effect Study Results

Stock Dilution	Concentration	% Expected Agreement
1:1	5.75×10^6 $TCID_{50}$/mL	100 (3/3)
1:10	1.15×10^6 $TCID_{50}$/mL	100 (3/3)
1:100	1.15×10^5 $TCID_{50}$/mL	100 (3/3)
Negative	0	100 (3/3)

4. Assay Reportable Range:

Not Applicable; the Healgen Rapid COVID-19 Antigen Test is a qualitative assay.

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

a. Internal Control

The Healgen Rapid COVID-19 Antigen Test has an internal, built-in procedural control. When sample is added and migrates up the test strip, a red line will appear in the control line region (C), which is coated with secondary antibody recognizing the primary antibody in the migrating sample. The control line should always appear in a test that functions accurately when the test procedure is followed.

b. External Controls

The Healgen Rapid COVID-19 Antigen Test is required to be run with external quality controls as specified under the CLIA regulations. External quality controls are sold separately as the *COVID-19 Antigen Control Kit*. The Negative Control Swab is composed of negative control buffer dried onto a swab, and the Positive Control Swab also contains SARS-CoV-2 recombinant antigen dried onto a swab at a concentration of 0.1 ng/mL. Lot-to-lot reproducibility of the external positive and negative control swabs was evaluated with three (3) external control lots as follows: 4 replicates per lot of positive or negative external control swabs were tested on 3 different test kit lots for a total of 36 positive or negative external control results. All results were as expected.

Table 6. Summary Results of External Controls Validation

Control	Control Lot #	Test Lot #	n	# of Neg	# of Pos	% Agreement
Negative Control Swab	1	1	4	4	0	100
		2	4	4	0	100
		3	4	4	0	100
	2	1	4	4	0	100
		2	4	4	0	100
		3	4	4	0	100
	3	1	4	4	0	100
		2	4	4	0	100
		3	4	4	0	100

Control	Control Lot #	Test Lot #	n	# of Neg	# of Pos	% Agreement
Positive Control Swab	1	1	4	0	4	100
		2	4	0	4	100
		3	4	0	4	100
	2	1	4	0	4	100
		2	4	0	4	100
		3	4	0	4	100
	3	1	4	0	4	100
		2	4	0	4	100
		3	4	0	4	100

c. Reagent Stability/Shelf-Life

Healgen Rapid COVID-19 Antigen Test: A real-time reagent stability study was conducted to support a shelf-life of 18 months. Three lots of the Healgen Rapid COVID-19 Antigen Test were stored at 2 to 8°C and 30 ± 3°C. At each timepoint test devices were visually inspected and tested with 5 replicates of a low positive sample (prepared at 2x LoD) and one replicate each of a high negative (prepared at 0.1x LoD) and a negative sample. Testing was performed at 0, 3, 6, 9, 12, 15, 18 and 20 months. All samples at all timepoints gave the expected results, supporting a shelf-life of the test device for 18 months under the intended storage condition.

COVID-19 Antigen Control Kit: Three lots of the COVID-19 Antigen Control Kit were stored at 2 ± 3°C and 30 ± 3°C and then each lot was tested in duplicate on 2 test kit lots at Time 0 and every 3 months to 27 months. All samples at all timepoints and storage conditions gave the expected results, supporting a shelf-life of the control kit for 24 months under the intended storage condition.

d. Specimen Stability

A specimen stability study in which 3 replicates each of low positive (prepared at 2x LoD) and negative samples (NCM) were prepared and tested on 3 test lots. Fifty (50) µL of the prepared sample were applied to kit swab and incubated at room temperature (RT). Swabs were tested at 0, 2, 4 and 6 hours. All samples gave the expected result, demonstrating that swab specimens are stable up to 4 hours after collection when stored at RT.

Table 7. Results of Sample Stability Study

	% Agreement (Correct Read /Total)			
Sample	**0 hours**	**2 hours**	**4 hours**	**6 hours**
Low positive (2x LoD)	100 (9/9)	100 (9/9)	100 (9/9)	100 (9/9)
Negative (NCM)	100 (9/9)	100 (9/9)	100 (9/9)	100 /9)

6. Detection Limit:

a. Limit of Detection (Analytical Sensitivity)

For the limit of detection study, heat-inactivated SARS-CoV-2 virus, isolate 2019-nCoV/USA-WA1/2020, was prepared in negative clinical matrix (NCM) composed of leftover, RT-PCR negative clinical swab specimens collected from healthy volunteers, eluted

in 0.5 to 1mL saline or PBS, and pooled together. Testing was performed on 3 different test kit lots. A preliminary LoD was first established in a range-finding study using 3 replicates per concentration per lot of the Healgen Rapid COVID-19 Antigen Test. Thereafter, the LoD was confirmed using 20 replicates per lot of 1:1,000, 1:2,000, and 1:4,000 serial dilution preparations. For both studies, fifty (50) µL of the prepared sample were applied to kit swab and eluted into the extraction buffer tube prior to testing with the Healgen Rapid COVID-19 Antigen Test device per the IFU. A 98.3% (58/60) positive concurrence rate was observed at 1:2,000 sample, and the LoD was determined as 5.75×10^3 $TCID_{50}$/mL.

Table 8. Summary Results of Limit of Detection Study

			Range Finding		Confirmatory	
Dilution	**Analyte Concentration ($TCID_{50}$/mL)**	**Analyte per Swab ($TCID_{50}$/swab)**	**# Positive**	**% Positive**	**# Positive**	**% Positive**
1:500	2.3×10^4	1.15×10^3	9/9	100.0%		
1:1000	1.15×10^4	5.75×10^2	9/9	100.0%		
1:2000	**5.75×10^3**	**2.875×10^2**	**9/9**	**100.0%**		
1:4000	2.875×10^3	1.438×10^2	4/9	44.4%		
1:1000	1.15×10^4	5.75×10^2			60/60	100.0%
1:2000	**5.75×10^3**	**2.875×10^2**			**58/60**	**96.7%**
1:4000	2.875×10^3	1.438×10^2			51/60	85.0%

b. WHO International Standard LoD

A study was performed to determine the LoD of the Healgen Rapid COVID-19 Antigen Test device with the WHO International Standard for SARS-CoV-2 Antigen (NIBSC 21/368) in real clinical matrix. The results are shown below for range finding and confirmatory LoD studies, and the LoD for the WHO International Standard was determined to be 250 IU.

Table 9. Results of LoD Study with WHO SARS-CoV-2 Standard

Dilution	**Analyte Concentration (IU)**	**Analyte per Swab (IU/swab)**	**# Positive**	**% Positive**	**# Positive**	**% Positive**
1:20	1000	50	3/3	100.0%		
1:40	500	25	3/3	100.0%		
1:80	**250**	**12.5**	**3/3**	**100.0%**		
1:160	125	6.25	2/3	66.6%		
1:40	500	25			20/20	100.0%
1:80	**250**	**12.5**			**20/20**	**100.0%**
1:160	125	6.25			13/20	65.0%

c. Inclusivity (Analytical Reactivity)

For the inclusivity study, commercially obtained sources of alpha (B.1.1.7), delta (B.1.617.2), omicron (B.1.1.529), beta (B.1.351), gamma (P1) and kappa (B.1.617.1) SARS-CoV-2 virus were tested to evaluate the ability of the Healgen Rapid COVID-19 Antigen Test device to detect a variety of SARS-CoV-2 variants. The study utilized a dilution series for each lineage with testing of each dilution in triplicate on 3 test lots. The lowest concentration that gave 100% positive results for each of the variants using the Healgen Rapid COVID-19 Antigen Test is provided below.

Table 10. Summary Results of Inclusivity Testing

SARS-CoV-2 Variant	Lowest Concentration with 9/9 Positive Results ($TCID_{50}$/mL)
B.1.1.7 (Alpha)	1.00×10^2
B.1.351 (Beta)	3.83×10^2
B.1.617.2 (Delta)	1.10×10^2
P1 (Gamma)	6.30×10^2
B.1.617.1 (Kappa)	1.90×10^2
B.1.1.529 (Omicron)	2.51×10^2

7. Assay Cut-Off:

   Not Applicable; the Healgen Rapid COVID-19 Antigen Test device gives a non-numerical, visually read qualitative result and does not use an instrument.

B. Comparison Studies:

1. Method Comparison with Predicate Device:

   Please refer to **Section VII.C** (Clinical Studies) below for the clinical validation.

2. Matrix Comparison:

   Not Applicable; the Healgen Rapid COVID-19 Antigen Test device is only intended for use with direct, anterior nasal swabs, and no other sample type is claimed or presented.

C. Clinical Studies:

The clinical performance of the Healgen Rapid COVID-19 Antigen Test was evaluated in a multi-center, prospective study conducted from May 2022 to July 2022 using 32 untrained operators at six different CLIA-waived sites.

Testing is described as being performed in a simulated home environment. Enrolled subjects were aged 2 years or older symptomatic subjects within six (6) days post symptom onset (DPSO) and exhibiting symptoms at the time of collection. Symptomatic individuals were defined as exhibiting symptoms of fever or 2 or more of the following: chills, cough, shortness of breath/difficulty breathing, fatigue, muscle/body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, and diarrhea. A total of 806 evaluable subjects were collected and tested. Patient demographics and specimen positivity are summarized in the following tables.

Table 11. Overview of Patient Demographics

Age Group	Total #	Total Positive	Prevalence (%)
2 to 13 years	75	13	17.3
14 to 24 years	118	19	16.1
25 to 64 years	514	110	21.4
≥ 65 years	99	22	22.2

Table 12. Overview of Specimen Positivity Based on DPSO

DPSO	# Samples	# Positive Samples	% Positive
0	26	3	11.5
1	92	16	17.4
2	213	46	21.6
3	219	39	17.8
4	150	29	19.3
5	75	19	25.3
6	31	12	38.7

Two swabs were collected from each subject, one anterior nares (AN) swab was tested on the candidate device, and one nasopharyngeal (NP) swab was collected into 3 mL of viral transport media (VTM), stored at 2-8°C and tested within 120 hours using a composite comparator method (or frozen at -70°C for long-term storage). For comparator testing, samples were tested on two highly sensitive RT-PCR comparator assays; testing on a third comparator would then be performed if discordant results were observed between the first two tests. The comparator result for those samples was determined based on a 2 out of 3 rule. Results obtained on the candidate device were compared to the composite comparator method to determine a clinical sensitivity/positive percent agreement (PPA) of 85.4% and a specificity/negative percent agreement (NPA) of 99.7%.

Table 13. Summary of Clinical Performance

Candidate device	Composite Comparator Method		
	Detected	Not Detected	Total
Reactive	140	2	142
Non-reactive	24	640	664
Total	164	642	806
Positive Percent Agreement: 85.4% (95% CI: 79.1 to 90.0%)			
Negative Percent Agreement: 99.7% (95% CI: 98.9 to 99.9%)			

Table 14. Summary of Clinical Performance by DPSO

DPSO	Number of Samples	Candidate Positive	Comparator Positive	PPA (%)
Day 0	26	3	3	100.0
Day 1	92	14	16	87.5
Day 2	213	36	46	78.3
Day 3	219	31	39	79.5
Day 4	150	27	29	93.1
Day 5	75	17	19	89.5
Day 6	31	12	12	100

3. Clinical Sensitivity:

   Please refer to **Section VII.C** (Clinical Studies) above for the clinical validation, regarding the test sensitivity/PPA. The PPA for the test is 85.4% (140/164; 95% CI: 79.1% - 90.0%).

4. Clinical Specificity:

Please refer to **Section VII.C** (Clinical Studies) above for the clinical validation, regarding the test specificity/NPA. The NPA for the test is 99.7% (640/642; 95% CI: 98.9% - 99.9%).

D. Clinical Cut-Off:

Not Applicable; there is no clinical cutoff for the presence of SARS-CoV-2 in patient samples.

E. Expected Values/Reference Range:

Not Applicable; a patient sample is expected to test negative for SARS-CoV-2.

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.