April 19, 2024

Healgen Scientific LLC
℅ Jinjie Hu
President and Principal Consultant
Axteria Biomed Consulting
8040 Cobble Creek Circle
Potomac, Maryland 20854

Re: K232377
Trade/Device Name: Healgen Rapid COVID-19 Antigen Test
Regulation Number: 21 CFR 866.3982
Regulation Name: Simple Point-Of-Care Device To Directly Detect SARS-Cov-2 Viral Targets From Clinical Specimens In Near-Patient Settings
Regulatory Class: Class II
Product Code: QVF
Dated: March 19, 2024
Received: March 19, 2024

Dear Jinjie Hu:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device"

(https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Silke Schlottmann -S

Digitally signed by Silke Schlottmann -S
Date: 2024.04.19 09:08:30 -04'00'

Silke Schlottmann, Ph.D.
Deputy Assistant Director
Bacteriology Respiratory and Medical Countermeasures Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

HEALGEN RAPID COVID-19 ANTIGEN TEST
INSTRUCTIONS FOR USE

REF **GCCOV-502a-NA**

IVD ℞ Only

INTENDED 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 specimens 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 either: 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.

SUMMARY

Coronaviruses belong to the β genus. Coronavirus disease 19 (COVID-19) is an acute respiratory infectious disease in patients infected by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The virus incubation period typically ranges from 1 to 14 days, and symptoms may appear within 2 to 14 days following exposure. Possible symptoms include fever or chills, fatigue, new loss of taste or smell, shortness of breath or difficulty breathing, cough, nasal congestion or runny nose, sore throat, myalgia, nausea or vomiting, headache, and diarrhea.

This test is for detection of SARS-CoV-2 nucleocapsid protein antigen to aid in the diagnosis of SARS-CoV-2 infection. Antigen is generally detectable in upper respiratory specimens during the acute phase of infection.

PRINCIPLE

The Healgen Rapid COVID-19 Antigen Test uses antibodies to detect nucleocapsid protein from SARS-CoV-2 in direct nasal (anterior nares) swabs. If SARS-CoV-2 nucleocapsid antigen is present in the sample a line will form at the test line at “T”, which means the test is positive. If SARS-CoV-2 nucleocapsid protein antigen is not in the sample a test line will not appear at “T” which means the test is negative. A control will always appear at “C” on a test that is functioning properly and when the test procedure has been followed.

MATERIALS SUPPLIED

20 Test Cassettes
20 Sterile Swabs
20 Pre-filled buffer tubes with flip-top
2 Tube Holders
1 Instructions for Use
1 Quick Reference Guide

MATERIALS REQUIRED BUT NOT PROVIDED

Timer
External Controls: COVID-19 Antigen Control Kit
(GCCOV(Ag)-PN10/GCCOV(Ag)-PN20)

WARNINGS AND PRECAUTIONS

1. For prescription use only.
2. For in vitro diagnostic use only.
3. Read all instructions carefully before performing the test. Failure to follow the instructions may result in inaccurate results.
4. Serial testing should be performed in symptomatic individuals with negative results at least twice over three days (with 48 hours between tests). You may need to purchase additional tests to perform this serial (repeat) testing or follow up testing with a molecular test.
5. Do not use the kit past its expiration date.
6. Do not use if any of the test kit contents or packaging is damaged.
7. Swabs, tubes, and test devices are for single use only. Do not re-use.
8. Do not interchange or mix components from different kit lots.
9. Testing should only be performed using the swabs provided within the kit. Do not touch the swab tip.
10. To obtain accurate results, do not use visually bloody or overly viscous samples.
11. Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents.
12. Wear appropriate personal protection equipment and gloves when running each test and handling patient specimens. Change gloves between handling of specimens.
13. Dispose of used contents as biohazardous wastes in accordance with federal, state, and local requirements.
14. Do not open the Test Cassette until you are ready to perform the test. Once opened, the test cassette should be used within one hour.
15. Do not read test results before 15 minutes or after 20 minutes. Results read before 15 minutes or after 20 minutes may lead to a false positive, false negative, or invalid results.
16. Do not use the kit to evaluate patient specimens if either the positive control swab or negative control swab fails to give the expected results.
17. Do not move test device during result development.

STORAGE

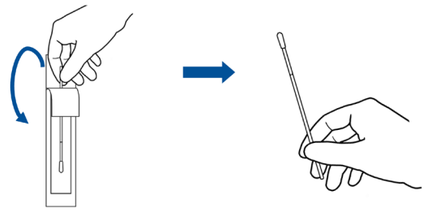
1. The kit can be stored at room temperature or refrigerated (2-30°C/36-86°F) in the original sealed pouch.
2. Do not freeze any of the test kit components.
3. Keep away from direct sunlight.
4. Do not use the test device and reagents after the expiration date.
5. Unused test devices that have been outside of the sealed pouch for more than one hour should be discarded.
6. Close the kit box and secure contents when not in use.

SPECIMEN COLLECTION

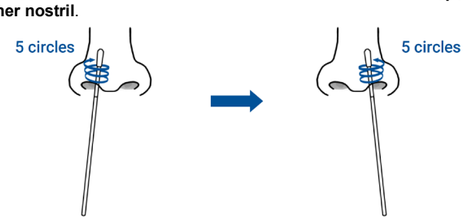
Acceptable specimen type for testing is direct anterior nasal swab specimen. Inadequate specimen collection, improper specimen handling and/or transport may yield false results.

Samples should be tested as soon as possible after collection. Based on data generated with the Healgen Rapid COVID-19 Antigen Test, nasal swabs are stable for up to 4 hours at room temperature but should be stored in a sterile container if not tested immediately.

1. Open the swab pouch by peeling the cover back. Hold the **plastic stick end** of the swab and remove from pouch. **Do not touch the swab tip.**



2. To collect the anterior nasal swab sample, tilt the patient’s head back 70 degrees and insert the swab tip into nostril, at about **1/2 to 3/4 inches deep**. For children, young children, swab should not be inserted more than 1/2 inch. Rotate the swab along the inside walls of nostril in a complete circle at least **5 times**. Remove swab from nostril and use the same swab to **repeat in the other nostril**.

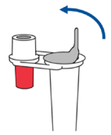


TEST PROCEDURE

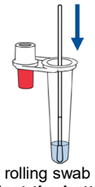
Check expiration on each individual test package or outer box before using.

The test should be performed at room temperature (15-30°C). Allow the test materials to reach room temperature prior to use. Do not open the Test Cassette until you are ready to use.

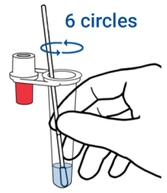
1. While maintaining tube upright, remove seal from tube. Avoid spilling liquid.



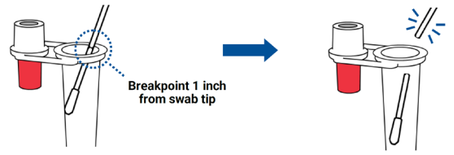
2. Insert swab tip in tube opening.



3. Grab swab shaft and mix well by rolling swab in a circular motion **at least 6 times**. **Press the swab tip against the bottom and sides of tube while rolling swab**.

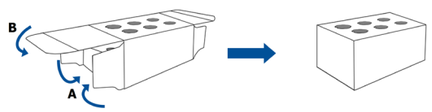


4. While holding the tube in your hand, place the swab’s tip near the top of the tube. Gently bend the swab until it snaps at the breakpoint.

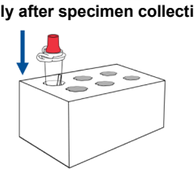


5. Hold tube upright. Close the flip cap of the tube tightly to ensure there is a tight fit so it does not leak. **Do not remove red cap or swab tip from tube**.

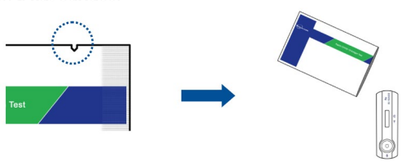
6. Fold tube holder by folding vertical flaps toward center (**A**). Hold vertical flaps in place and hold horizontal flap down and inward (**B**).



7. Allow the swab to sit in buffer tube for **at least 1 minute, but no longer than 30 minutes immediately after specimen collection.**

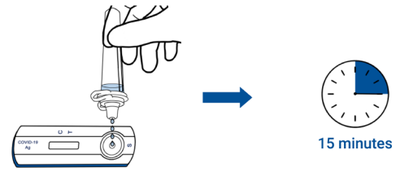


8. Open the test cassette pouch by tearing the area circled below. Place the test cassette on a **flat surface**.



9. **Gently remove red cap from tip**. Ensure tip remains secure within tube opening.

10. Invert the tube and gently squeeze, from the middle of the tube, to add **4 drops** of solution into the sample well, labeled as “S” on the test cassette. **Start timer for 15 minutes**.



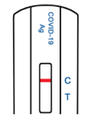
11. **After 15 minutes** read the test results visually in the result window, labeled as “C” and “T” on the test cassette. **Do not read result before 15 minutes or after 20 minutes.**

INTERPRETATION OF RESULTS

POSITIVE

If the Control (C) line and the Test (T) line are visible, the test is positive. Any visible faint red or pink test (T) line with a visible control (C) line should be read as positive. Repeat testing is not needed for individuals with a positive result.

NEGATIVE



If the Control (C) line is visible, but the Test (T) line is not visible, the test is negative. A negative test result indicates that the virus that causes COVID-19 was not detected in the sample.

NOTE: Negative results are presumptive and may be confirmed with a molecular assay, if necessary, for patient management. Individuals with symptoms of COVID-19 and initial negative results should be tested again after 48 hours or followed up with a molecular test.

INVALID

If a control (C) line is not visible, the test is not valid. Invalid tests should be repeated with a new test.

LIMITATIONS

1. This test is only for the detection of proteins from SARS-CoV-2, not for any other viruses or pathogens.
2. The test is intended to only be used with direct anterior nasal swabs and is not validated for use with swabs in viral transport media.
3. This test is only for use with individuals who show symptoms of upper respiratory infection within 6 days of symptom onset.
4. Serial testing should be performed in individuals with negative results at least twice over three days (with 48 hours between tests) for symptomatic individuals.
5. This test is not for use in at-home test settings.
6. This test is read visually and has not been validated for use by those with impaired vision or color-impaired vision.
7. This test should not be used beyond the expiration date listed on the packaging. Use of expired tests can lead to incorrect results.
8. Accurate results are dependent on adequate specimen collection, transport, storage, and processing. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
9. Test results should be interpreted in conjunction with other clinical and laboratory information available to the healthcare provider. Results from the

device should be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.

10. A negative test result does not preclude the possibility of infection with other bacteria or viruses.
11. A negative test result may occur if the level of antigen in the sample is below the detection limit of the test.
12. All COVID-19 antigen test negative results are presumptive and confirmation with a molecular assay may be necessary.
13. There is a higher chance of false negative results with antigen tests than with laboratory-based molecular tests due to the sensitivity of the test technology. This means that there is a higher chance this test will give a false negative result in an individual with COVID-19 as compared to a molecular test, especially in samples with low viral load.
14. Positive test results do not rule out co-infections with other non-SARS viral or bacterial pathogens.
15. Positive test results do not differentiate between SARS-CoV and SARS-CoV-2.
16. False positive test results are more likely when prevalence of upper respiratory infection is low in the community.
17. This test detects both viable (live) and non-viable SARS-CoV-2 virus. Test performance depends on the amount of virus (antigens) in the sample and may or may not correlate with viral culture results performed on the same sample. A false-negative test result may occur if the level of viral antigen in a sample is below the detection limit of the test or if the sample was collected or transported improperly.
18. There is a risk of erroneous results (i.e., false negatives) due to the presence of novel, emerging respiratory viral variants (e.g., specific strains or isolates).
19. The performance of this test was established based on the evaluation of a limited number of clinical specimens collected between May 2022 and July 2022. The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
20. Based on sequence analysis, a potential for cross-reactivity between the SARS-CoV-2 test and HKU1 exists. Wet testing for HKU1 coronavirus was not conducted and therefore, cross-reactivity between SARS-CoV-2 and HKU1 coronavirus cannot be ruled out. measures)

PERFORMANCE CHARACTERISTICS

Clinical Performance

Clinical performance of the Healgen Rapid COVID-19 Antigen Test was established with 806 anterior nasal swab (ANS) samples prospectively collected from symptomatic subjects between May 2022 and July 2022 at six clinical point of care sites. Samples (ANS for the investigational device and nasopharyngeal (NP) swab for the comparator) were collected from sequentially enrolled subjects presenting with symptoms of upper respiratory infection within 6 days of onset of symptoms. Results obtained with the Healgen Rapid COVID-19 Antigen Test were compared to a composite comparator method of two FDA cleared, highly sensitive RT-PCR assays. A third FDA cleared RT-PCR comparator assay was performed on comparator samples with discordant results between the first and second RT-PCR tests. The final result for the comparator was determined using a 2 out of 3 rules. Testing was performed by operators who had no prior experience in the laboratory and were representative of the intended users. Operators used only the QRI to conduct testing without comprehensive training provided.

Of the 806 subjects, 164 were confirmed positive by the comparator and 642 were confirmed negative. The positive percent agreement (PPA) was 85.4% and the negative percent agreement (NPA) was 99.7% (see tables below).

Table 1. Healgen Rapid COVID-19 Antigen Test Results versus RT-PCR Composite Comparator for Symptomatic Patients

Healgen Rapid COVID-19 Antigen Test	Composite Comparator		Total
	Positive	Negative	
Positive	140	2	142
Negative	24	640	664
Total	**164**	**642**	**806**

Positive Percent Agreement (PPA) = (140/164) = 85.4% (95% CI: 79.1% - 90%)
Negative Percent Agreement (NPA) = (640/642) = 99.7% (95% CI: 98.9% - 99.9%)

Table 2. Patient Demographics

Subject Age Group	Number of samples tested	Antigen Positives	Composite Comparator Positives	% Positive (by Comparator)
2-13 years of age	75	11	13	17.3%
14-24 years of age	118	17	19	16.1%
25-64 years of age	514	91	110	21.4%
≥ 65 years of age	99	21	22	22.2%
Total	**806**	**140**	**164**	**20.3%**

Table 3. Clinical Performance in Subjects on Different Symptomatic Days

Days Post COVID-19 Symptoms	Number of samples tested	Antigen Positives	Composite Comparator Positives	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	100.0%
Total	**806**	**140**	**164**	**85.4%**

Analytical Sensitivity: Limit of Detection (LoD)

LoD studies determine the lowest detectable concentration of SARS-CoV-2 at which approximately 95% of all (true positive) replicates test positive. Heat inactivated SARS- CoV-2 virus, isolate 2019-nCoV/USA-WA1/2020, was spiked into negative clinical matrix composed of pooled nasopharyngeal swab specimens in PBS/saline and serially diluted. A preliminary LoD test was performed by spiking 50 µL of each diluted sample onto the sample collection swab head in triplicate. The confirmatory LoD test was performed at the selected preliminary LoD concentration and at concentrations above and below the preliminary LoD with an additional 20 replicates on the Healgen Rapid COVID-19 Antigen Test. The Limit of Detection is 5.75 x 10^3 $TCID_{50}$/mL (2.875 x 10^2 $TCID_{50}$/swab).

Furthermore, the LoD was established using the 1st WHO International Standard for SARS-CoV-2 Antigen (NIBSC 21/368) in real clinical matrix of pooled swab specimens. Initially, a preliminary LoD was performed in range finding studies, and a confirmatory LoD test was then conducted to confirm the preliminary LoD concentration and additional dilutions bracketing the preliminary concentration. It was determined that the LoD of the 1st WHO International Standard for SARS-CoV-2 Antigen (NIBSC 21/368) for the Healgen Rapid COVID-19 Antigen Test was determined to be 250 IU/mL (12.5 IU/swab).

Inclusivity

Inclusivity testing was conducted with the currently available commercial stock strains of SARS-CoV-2 Alpha, Beta, Gamma, Delta, Kappa, and Omicron variants.

Table 4. 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

Analytical Specificity: Cross Reactivity (Exclusivity) and Microbial Interference

Various microorganisms were evaluated for cross-reactivity and microbial interference by wet testing with the Healgen Rapid COVID-19 Antigen Test. Human coronavirus HKU1 was not tested for cross-reactivity due to a lack of availability. The samples were tested in triplicate, and no cross-reactivity and no microbial interference were observed. Microbial concentrations and results are outlined in **Table 5.**

Table 5. Results of Microorganisms Tested for Cross-Reactivity and Microbial Interference

Microorganism	Concentration Tested	Cross-Reactivity	Interference
Human coronavirus 229E	8.00 × 10^5 $TCID_{50}$/mL	No	No
Human coronavirus OC43	7.00 x 10^6 $TCID_{50}$/mL	No	No
Human coronavirus NL63	2.93 x 10^4 $TCID_{50}$/mL	No	No
MERS-coronavirus	7.0 × 10^5 $TCID_{50}$/mL	No	No
Adenovirus 21	2.39 x 10^6 $TCID_{50}$/mL	No	No
Adenovirus 10	1.14 x 10^6 $TCID_{50}$/mL	No	No
Human Metapneumovirus	3.95 x 10^5 $TCID_{50}$/mL	No	No
Parainfluenza virus Type 1	2.23 x 10^6 $TCID_{50}$/mL	No	No
Parainfluenza virus Type 2	2.23 x 10^5 $TCID_{50}$/mL	No	No
Parainfluenza virus Type 3	4.00 x 10^6 $TCID_{50}$/mL	No	No
Parainfluenza virus Type 4a	4.00 x 10^3 $TCID_{50}$/mL	No	No
Influenza virus, Type A (H1N1)	4.00 x 10^8 $CEID_{50}$/mL	No	No
Influenza virus, Type A (H3N2)	7.00 x 10^5 $TCID_{50}$/mL	No	No
Influenza virus, Type B	7.00 x 10^5 $TCID_{50}$/mL	No	No
Enterovirus 68	2.23 x 10^6 $TCID_{50}$/mL	No	No
Enterovirus 71	4.00 x 10^7 $TCID_{50}$/mL	No	No
Respiratory syncytial virus	2.23 x 10^6 $TCID_{50}$/mL	No	No
Rhinovirus 60	8.00 x 10^5 $TCID_{50}$/mL	No	No
Haemophilus influenzae	1.74 x 10^8 CFU/mL	No	No
Streptococcus pneumoniae	3.35 x 10^8 CFU/mL	No	No
Streptococcus pyogenes	5.98 x 10^8 CFU/mL	No	No
Candida albicans	1.19 x 10^8 CFU/mL	No	No
Bordetella pertussis	4.90 x 10^9 CFU/mL	No	No
Mycoplasma pneumoniae	6.75 x 10^7 CCU/mL	No	No
Chlamydia pneumoniae	4.25 x 10^7 CFU/mL	No	No
Legionella pneumophila	9.20 x 10^9 CFU/mL	No	No
Staphylococcus aureus	5.0 × 10^6 CFU/mL	No	No
Staphylococcus epidermidis	1.75 x 10^8 CFU/mL	No	No
Pooled human nasal wash	N/A	No	No

In-silico analysis was conducted for SARS-coronavirus, Human coronavirus HKU1, Mycobacterium tuberculosis, Pneumocystis jirovecii (PJP) and MERS coronavirus.

No sequence was found with significant homology with N protein sequence in both Mycobacterium tuberculosis and Pneumocystis jirovecii genomes, suggesting no 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; therefore, cross-reactivity cannot be ruled out. The N protein sequence of SARS coronavirus shares 79.01%- 97.61% sequence identity indicating that cross-reactivity is likely.

Endogenous Interfering Substances

To assess endogenous interference, potentially interfering substances which may be present in respiratory samples were tested to determine if interference may occur on the Healgen Rapid COVID-19 Antigen Test. A summary of the results is shown in **Table 6.**

Table 6. Results of Potential Interfering Substances

Substance	Concentration	Cross-Reactivity	Interference
Whole Blood	4%	No	No
Human Leukocytes	1 × 10^7 cells/mL	No	No
Mucin	0.5%	No	No
Chloraseptic (Menthol/Benzocaine)	3 mg/mL	No	No
Naso GEL (NeilMed)	5% v/v	No	No
CVS Nasal Drops (Phenylephrine)	15% v/v	No	No
Afrin (Oxymetazoline)	15% v/v	No	No
CVS Nasal Spray (Cromolyn)	15% v/v	No	No
Zicam	5% v/v	No	No
Homeopathic (Alkalol)	15% v/v	No	No
Sore Throat Phenol Spray	15% v/v	No	No
Tobramycin	4 µg/mL	No	No
Mupirocin	10 mg/mL	No	No
Fluticasone Propionate	15% v/v	No	No
Tamiflu (Oseltamivir Phosphate)	5 mg/mL	No	No

Hook Effect

The Hook Effect study tested up to 5.75 × 10^6 $TCID_{50}$/mL of heat-inactivated SARS-CoV-2 (USA-WA1/2020) on the Healgen Rapid COVID-19 Antigen Test. The test results show that there was no detectable hook effect even for the highest concentrated virus sample.

Precision/Reproducibility

A reproducibility study was performed to evaluate reproducibility of the Healgen Rapid COVID-19 Antigen Test. The study was performed at three external, CLIA-waived testing sites consisting 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 over 5 days, i.e., 3 replicates × 3 operators × 3 sites × 5 days= 135 replicates per concentration and a total of 405 data points collected. Three (3) test lots were used in this study, so lot-to-lot variability was also assessed. 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 7. Results of Multisite Precision Study (Reproducibility)

Site	Negative		Weak Positive		Positive	
	Correct Reads/Total	PPA	Correct Reads/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%**

REFERENCES

1. Julien Favresse, Constant Gillot, Maxime Oliveria, Julie Cadrobbi, Marc Elsen, Christine Eucher, Kim Laffineur, Catherine Rosseels, Sandrine Van Eeckhoudt, Jean-Baptiste Nicolas, Laure Moiremont, Jean-Michael Dogné and Jonathan Douxfils. Head-to-Head Comparison of Rapid and Automated Antigen Detection Tests for the Diagnosis of SARS- CoV-2 Infection J. Clin. Med. 2021, 10, 265.
2. Ignacio Torres, Sandrine Poujois, Eliseo Albert, Gabriela Álverez, Javier Colomina and David Navarro. Point-of-care evaluation of a rapid antigen test for diagnosis of SARS- CoV-2 infection in symptomatic and asymptomatic individuals February 11, 2021
3. Public Health England. SARS-CoV-2 lateral flow antigen tests: evaluation of VOC1 (Kent UK) and VOC2 (South Africa) Published12 February 2021

INDEX OF SYMBOLS

	Do not reuse		See Instruction for Use		Expiration Date
Σ	Tests per Kit	2°C-30°C	Store Between 2-30°C (36-86°F)		Keep Dry
LOT	Batch Number	REF	Catalog#		Keep Away from Sunlight
UDI	Unique Device Identifier	IVD	For *in vitro* diagnostic use only		Manufacturer
℞ Only	Prescription Use Only				

TECHNICAL SUPPORT

For questions or technical support, please contact the Technical Support Number 866-982-3818 or send email to Customer.Support@healgen.com.

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Website: www.healgen.com

Revision Date: 2024-04-19
C-04112024-A1-IVD-D4-V3

HEALGEN RAPID COVID-19 ANTIGEN TEST

IVD | Rx Only

HEALTHCARE PROVIDER QUICK REFERENCE GUIDE

For *in vitro* diagnostic use
For prescription use

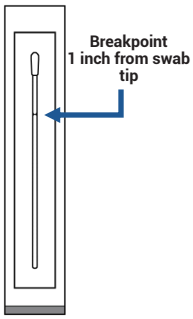
Revision Date: 2024-4-18
C-04122024-A1-IVD-D2-V1

IMPORTANT

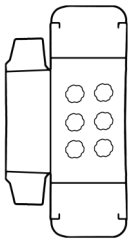
- Read all instructions carefully before performing the test. Failure to follow the instructions may result in inaccurate test results. Refer to "Instructions for Use" for additional information.
- The test is intended for use with nasal (anterior nares) swab specimens from individuals: suspected of COVID-19 by their healthcare provider within the first six (6) days of symptom onset.
- Negative results are presumptive and should be confirmed with a molecular test, if necessary.
- Serial testing should be performed in individuals with negative results at least twice over three days (with 48 hours between tests) for symptomatic individuals.
- All test materials must be at room temperature (15°C - 30°C) before use. Do not open the Test Cassette until you are ready to perform the test.
- Wear appropriate personal protective equipment when performing this test.
- Check expiration date of each test device before use. Do not use any test past the expiration date. Refer to test device pouch or outer box labeling for expiration date.
- External Controls may be used to demonstrate that the reagents and assay procedure perform properly. Refer to the COVID-19 Antigen Control Kit for additional information.

MATERIALS

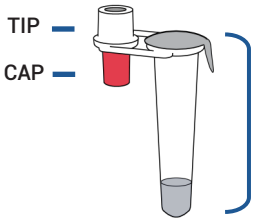
TEST CASSETTE POUCH



SWAB



TUBE HOLDER (unfolded)



TUBE (with liquid)

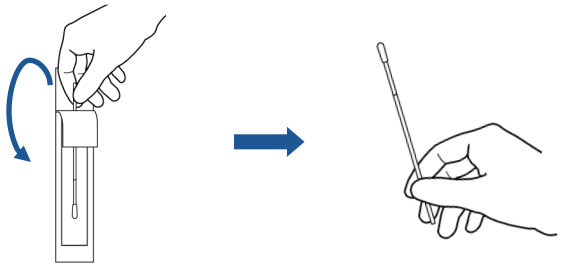
TIMER (not included)

SPECIMEN COLLECTION

See reverse side of page for TEST PROCEDURE

1 OPEN SWAB

Open the swab pouch by peeling the cover back. Hold the **plastic stick end** of the swab and remove from pouch. Do not touch the swab tip.

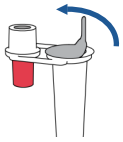


2 SWAB BOTH NOSTRILS

To collect the anterior nasal swab sample, tilt the patient's head back 70 degrees and insert the swab tip into one nostril of the patient, at about **1/2 to 3/4 inches deep.** For children, young children, swab should not be inserted more than 1/2 inch. Rotate the swab along the inside walls of nostril in a complete circle at least 5 times. Remove swab from the nostril and use the same swab to **repeat in another nostril.**

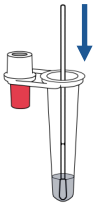
3 OPEN TUBE

While maintaining tube upright, remove seal from tube. Avoid spilling liquid.



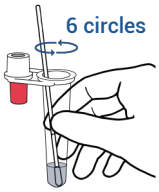
4 PLACE SWAB IN TUBE

Insert swab tip in tube opening.



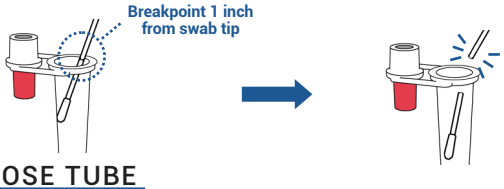
5 MIX WITH SWAB

Grab swab shaft and mix well by rolling swab in a circular motion **at least 6 times**. **Press the swab tip against the bottom and sides of tube while rolling swab.**



6 BREAK SWAB SHAFT

While holding the tube in your hand, place the swab's tip near the top of the tube. Gently bend the swab until it snaps at the breakpoint.



7 CLOSE TUBE

Hold tube upright. Close the flip cap of the tube tightly to ensure there is a tight fit so it does not leak. **Do not remove red cap or swab tip from tube**.

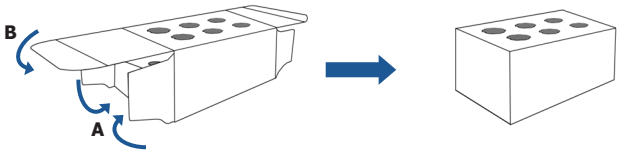
HEALGEN®

TEST PROCEDURE

See reverse side of page for SPECIMEN COLLECTION

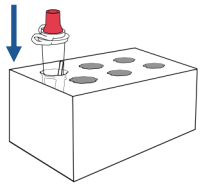
1 FOLD TUBE HOLDER

Fold tube holder by folding vertical flaps towards center (**A**). Hold vertical flaps in place and fold horizontal flap down and inward (**B**).



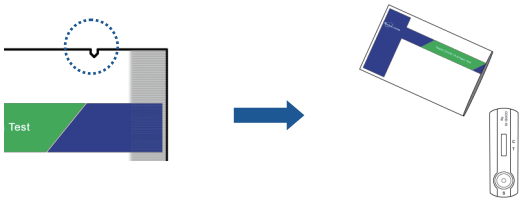
2 PLACE TUBE IN TUBE HOLDER

Allow the swab to sit in buffer tube for **at least 1 minute, but no longer than 30 minutes immediately after specimen collection**.



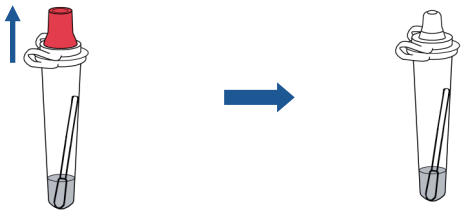
3 OPEN TEST DEVICE

Open the test cassette pouch by tearing the area circled below. Place the test device on a flat surface.



4 REMOVE CAP

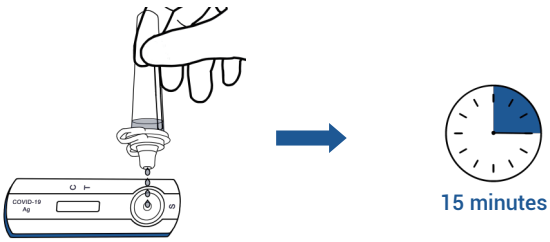
Gently remove red cap from tip. Ensure tip remains secure within tube opening.



5 ADD 4 DROPS OF SAMPLE

Invert the tube and gently squeeze, from the middle of the tube, to add **4 drops** of solution into the sample well, labeled as "S" on the test device.

Start timer for 15 minutes.



6 READ TEST RESULT

After 15 minutes find result window, labeled as "C" and "T" on the test device.

Do not read result after 20 minutes.

Below are results examples for positive, negative and invalid results.

External positive and negative control swabs are supplied separately as "COVID-19 Antigen Control Kit". These controls provide additional quality control material to assess that the test kit reagents perform as expected. Process the controls in the same manner as a clinical sample swab, and conduct the assay as described in "Test Procedure" section.

Used test materials should be discarded as biohazard waste according to Federal, State and local regulatory requirements.

7 RESULTS INTERPRETATION

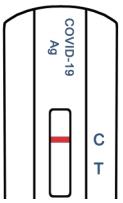
POSITIVE

If the Control (C) line and the Test (T) line are visible, the test is positive. Any visible faint red or pink test (T) line with a visible control (C) line should be read as positive. Repeat testing is not needed for individuals with a positive result.

NEGATIVE

If the Control (C) line is visible, but the Test (T) line is not visible, the test is negative. A negative test result indicates that the virus that causes COVID-19 was not detected in the sample.

NOTE: Negative results are presumptive and may be confirmed with a molecular assay, if necessary, for patient management. Individuals with symptoms of COVID-19 and initial negative results should be tested again after 48 hours or followed up with a molecular test.



INVALID

If a control (C) line is not visible, the test is not valid. Invalid tests should be repeated with a new test.

TECHNICAL SUPPORT

For questions or technical support, please contact the Technical Support Number 866-982-3818 or send email to Customer.Support@healgen.com.

INDEX OF SYMBOLS

IVD	For *in vitro* diagnostic use only	℞ Only	Prescription Use Only

Healgen Scientific, LLC.
3818 Fuqua Street Houston, TX, 77047
Phone: 713-733-8088
www.healgen.com

COVID-19 Antigen Control Kit

REF: GCCOV(Ag)-PN10 or GCCOV(Ag)-PN20

Configurations: (PN10: 5 positive swabs, 5 negative swabs)
(PN20: 10 positive swabs, 10 negative swabs)

For in vitro diagnostic use only. For prescription use only. For professional use only.

Instructions for use must be carefully followed when performing the test. Failure to follow the instructions may result in inaccurate results.

INTENDED USE

The COVID-19 Antigen Control Kit is a ready-to-use external control kit for use with the Healgen® Rapid COVID-19 Antigen Test to ensure that the reagents and materials are working properly and that the test procedure is correctly performed.

CONTENTS

The COVID-19 Antigen negative control swab is composed of negative control buffer dried onto a swab, with a blue shaft, containing a preservative.

The COVID-19 Antigen positive control swab is composed of a SARS-CoV-2 recombinant antigen extract dried onto a swab, with a red shaft, containing a preservative.

The preservative is 0.1% sodium azide.

The COVID-19 Antigen Control swabs are designed to be used to verify proper test procedure and performance of the Healgen® Rapid COVID-19 Antigen Test.

PRECAUTIONS

- For in vitro diagnostic use only.
- For professional use only.
- Do not use after expiration date.
- The COVID-19 Antigen Control Kit is only for use with the Healgen® Rapid COVID-19 Antigen Test. They have not been validated for use with other tests.
- The swab should remain in the sealed pouch until use.
- Do not use the swab if pouch is damaged.
- Do not re-use any contents in the product.
- Follow Good Laboratory Practices, wear protective clothing and use disposal disposable gloves when working with these controls. Do not eat, drink or smoke in the area.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The control swab and test device should be discarded in a proper biohazard container after testing.

STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperatures (2-30°C/36-86°F). The swab is stable through the expiration date printed on the sealed pouch. The swab must remain in the sealed pouch until use. Do not freeze.

MATERIALS

MATERIALS PROVIDED	MATERIALS REQUIRED BUT NOT PROVIDED
- 5 or 10 Negative control swab (blue) - 5 or 10 Positive control swab (red) - Instructions for use	- Disposable gloves - Timer - Healgen® Rapid COVID-19 Antigen Test

CONTROL KIT TEST PROCEDURE

Allow the test materials to reach room temperature (15-30°C/59-86°F) prior to testing. Do not open pouches until ready to perform the test.

For full instructions for use on how to use these controls, please refer to Healgen® Rapid COVID-19 Antigen Test Healthcare Provider Instructions for Use (IFU). The swab samples should be tested in the same manner as patient swab specimens.

We recommend controls be run once for:

- Each new kit lot,

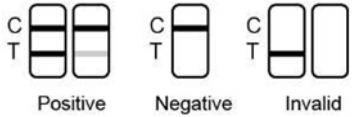
- Each new operator,

- Each new shipment,

- As required by site quality control procedures and in accordance with local, state and federal regulations or accreditation requirements.

If either or both external quality controls give invalid results, repeat the external controls with new swabs on the Healgen® Rapid COVID-19 Antigen Test.

INTERPRETATION OF RESULTS



POSITIVE:

If the Control (C) line and the Test (T) line are visible, the test is positive. Any visible faint red or pink test (T) line with a visible control (C) line should be read as positive. Repeat testing is not needed for individuals with a positive result.

NEGATIVE:

If the Control (C) line is visible, but the Test (T) line is not visible, the test is negative. A negative test result indicates that the virus that causes COVID-19 was not detected in the sample.

NOTE: Negative results are presumptive and may be confirmed with a molecular assay, if necessary, for patient management. Individuals with symptoms of COVID-19 and initial negative results should be tested again after 48 hours or followed up with a molecular test.

INVALID:

If the control line (C) is not visible within the result window after performing the test, the result is invalid. Some causes of invalid results include failure to correctly follow directions or the test was used beyond the expiration date. It is recommended that the control swab be re- tested using a new test device.

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Revision Date: 2024-04-19

510(k) Summary:

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Healgen Scientific LLC
Tel: 713-733-8088
Email: bingliang.fang@healgen.com

Date prepared: April 19, 2024

A. 510(k) Number:
K232377

B. Purpose for Submission:
New 510(k) device clearance for the Healgen Rapid COVID-19 Antigen Test

C. Measurand:
SARS-Coronavirus 2 (SARS-CoV-2) Nucleocapsid Protein Antigen

D. Type of Test:
Qualitative lateral flow immunoassay

E. Device

Proprietary and Established Name:	Healgen Rapid COVID-19 Antigen Test
Common Name:	Healgen Rapid COVID-19 Antigen Test
Classification Name:	Simple Point-Of-Care Device To Directly Detect SARS-Cov-2 Viral Targets From Clinical Specimens In Near-Patient Settings
Regulation Number:	21 CFR 866.3982
Classification:	Class II

Product Code:	QVF
Panel:	Microbiology
Predicate Device:	Sofia 2 SARS Antigen+ FIA, Sofia 2 SARS Antigen+ FIA Control Swab Set (DEN220039; granted March 8, 2023)

F. Intended Use:

1. Intended use(s):

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.

2. Indication(s) for use:

Same as the intended use

3. Special condition for use statement(s):

For prescription use only

For in vitro diagnostic use only

4. Special instrument requirements:

None

G. Device Description:

The Healgen Rapid COVID-19 Antigen Test is a lateral flow immunochromatographic assay that uses highly sensitive monoclonal antibodies to detect nucleocapsid protein from SARS-CoV-2 virus in nasal swab collected specimens. The test strip is composed of the following components: sample

pad, reagent pad, reaction membrane, and absorbing pad housed within a test cassette. The reagent pad contains colloidal-gold-conjugated monoclonal antibody that 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 of SARS-CoV-2.

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.

H. Test Principle:

The Rapid COVID-19 Antigen Test uses immunochromatographic-based technology and highly sensitive antibodies in a lateral flow design to detect nucleocapsid protein from SARS-CoV-2 in direct nasal (anterior nares) swabs.

To begin the test, nasal swab specimens are collected by a health care 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 are loaded onto the test cassette sample well, and the test result is read by the HCP after 15 minutes. If SARS-CoV-2 nucleocapsid antigen is present in the sample, a complex would form between the colloidal-gold-monoclonal antibody and the viral antigen, which is then captured by the second anti-SARS-2 antibody coated on the test line region (T).

The sample migrates along the test strip across 2 distinct areas: the test line and the 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 at the test line at the "T" site, showing a positive result. If SARS-CoV-2 nucleocapsid protein antigen is absent in the sample, a test line would not appear at the "T" site, indicating a negative result. A control line would always appear at the "C" site in a test that functions normally and when the test procedure is followed. If no line is seen at the "C" site after 20 minutes, then the result is invalid and should be repeated with new test materials.

I. Substantial Equivalence Information

Device & Predicate Device(s):	**Healgen Rapid COVID-19 Antigen Test (K232377) (Device)**	**Sofia 2 SARS Antigen+ FIA, Sofia 2 SARS Antigen+ FIA Control Swab Set (DEN220039) (Predicate)**
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	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

	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.	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 SARS-CoV-2 were established during the 2021-2022 SARS-CoV-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.
SIMILARITIES		
Intended Use Population	Symptomatic individuals within 6 days DPSO	Same
Target Analyte	Nucleocapsid protein antigen from SARS-CoV-2	Same
Sample Type	Anterior nasal swab specimen	Same
Test Time	15 minutes after the extracted sample is added to the test cassette sample well	Same
Results Reported	Qualitative (positive, negative, invalid)	Same
Serial Testing	Yes	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).
DIFFERENCES		
Test Design/ Principle	Lateral flow immunochromatographic assay	Lateral flow immunofluorescence sandwich assay
Instrumentation	None	Sofia 2 Instrument
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.

Reagent Storage	Store at room temperature or refrigerate, 36 to 86°F (2 to 30°C).	Store the kit at room temperature, 59°F to 86°F (15°C to 30°C).

J. Performance Characteristics

1. Analytical Performance:

a. Precision/Reproducibility:

A reproducibility study was performed at three (3) external testing sites, each site with three (3) operators. Each of the three (3) operators per site tested a panel of samples in replicates of three (3) over the course of five (5) days. Panel members consisted of inactive SARS-CoV-2 virus added into negative matrix at each of three different levels representing negative, low positive (1x LoD) and moderate positive (3x LoD) samples. Three (3) test lots were used in this study, so lot-to-lot variability was also assessed. Results are shown in the tables below.

Table 1: Summary Results of Multisite Precision Study

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

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%**

b. Limit of Detection

For the limit of detection study, serially diluted samples were tested using three lots of test devices to determine the limit of detection (LoD), which is determined to be the lowest concentration of SARS-CoV-2 that the Healgen Rapid COVID-19 Antigen Test can reliably detect 95% of the time. A preliminary LoD was first established using 3 replicates per lot of 1:500, 1:1,000, 1:2,000, and 1:4,000 serial dilution preparations of SARS-CoV-2 (USA-WA1/2020) on 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. The LoD of the Healgen Rapid COVID-19 Antigen Test was determined as 5.75 x 10^3 $TCID_{50}$/mL at the 1:2000 dilution. The results are summarized in the table below.

Table 3: Summary Results for the Limit of Detection Study

Dilution	Analyte Concentration ($TCID_{50}$/mL)	$TCID_{50}$/swab	# Positive	% Positive
	Range Finding			
1:500	2.3×10^4	11.5 x 10^2	9/9	100.0%
1:1000	1.15×10^4	5.75 x 10^2	9/9	100.0%
1:2000	**5.75×10^3**	**2.875 x 10^2**	**9/9**	**100.0%**
1:4000	2.875×10^3	1.438 x 10^2	4/9	44.4%
	Confirmatory			
1:1000	1.15×10^4	5.75 x 10^2	60/60	100.0%
1:2000	**5.75×10^3**	**2.875 x 10^2**	**58/60**	**96.7%**
1:4000	2.875×10^3	1.438 x 10^2	51/60	85.0%

A study was also performed to determine the LoD of the candidate device with the WHO International Standard for SARS-CoV-2 Antigen (NIBSC 21/368) in real clinical matrix. The results are shown below, and the LoD for the WHO International Standard antigen was determined to be 250 IU.

Table 4. Results of LoD Study with WHO SARS-CoV-2 Standard

Dilution	Analyte Concentration (IU/mL)	IU/swab	# Positive	% Positive
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. Linearity/assay reportable range:

Not applicable. This is a qualitative assay.

d. High dose hook effect study:

The hook effect study was conducted to determine whether the presence of a high concentration of the SARS-CoV-2 virus (USA-WA1/2020) in a sample would result in a negative test result, a phenomenon known as the hook effect. A heat-inactivated SARS-CoV-2 virus sample was used in

this study. The highest concentration tested was 5.75×10^6 $TCID_{50}$/mL, and no high-dose hook effect was observed.

e. Inclusivity:

A total of six COVID-19 variants (Delta, Beta, Alpha, Omicron, P1 and Kappa) were tested to determine if the test can reliably detect these variants. A 3-dilution series for each lineage was prepared, and each dilution was tested in replicates of three (3) by three (3) operators using three (3) kit lots. All variants gave positive results.

Table 5: Summary of Inclusivity Results

SARS-CoV-2 Variant	Sublineage	Lowest Concentration Tested Positive for All 3 Replicates in 3 Lots
Delta	B.1.617.2	1.10×10^2 $TCID_{50}$/mL
Beta	B.1.351	3.83×10^2 $TCID_{50}$/mL
Alpha	B.1.1.7	1.0×10^2 $TCID_{50}$/mL
Omicron	B.1.1.529	2.51×10^2 $TCID_{50}$/mL
Gamma	P1	6.30×10^2 $TCID_{50}$/mL
Kappa	B.1.617.1	1.90×10^2 $TCID_{50}$/mL

f. Analytical specificity:

Cross Reactivity and Microbial Interference:

The microbial cross reactivity and interference studies were performed to determine whether microorganisms that may be present in respiratory samples will cross-react or interfere with device performance.

Three replicates each of 1:1 dilutions of microorganism stock prepared in negative 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 was applied to kit swab and eluted into the extraction buffer tube. No cross-reactivity or microbial interference was observed with the organisms tested.

Table 6: Cross-Reactivity and Microbial Interference Results

Microorganism	Concentration Tested	Cross-Reactivity	Interference
Human coronavirus 229E	8.00×10^5 $TCID_{50}$/mL	No	No
Human coronavirus OC43	7.00×10^6 $TCID_{50}$/mL	No	No
Human coronavirus NL63	2.93×10^4 $TCID_{50}$/mL	No	No
MERS-coronavirus	7.0×10^5 $TCID_{50}$/mL	No	No
Adenovirus 21	2.39×10^6 $TCID_{50}$/mL	No	No

Microorganism	Concentration Tested	Cross-Reactivity	Interference
Adenovirus 10	1.14×10^6 $TCID_{50}$/mL	No	No
Human Metapneumovirus	3.95×10^5 $TCID_{50}$/mL	No	No
Parainfluenza virus Type 1	2.23×10^6 $TCID_{50}$/mL	No	No
Parainfluenza virus Type 2	2.23×10^5 $TCID_{50}$/mL	No	No
Parainfluenza virus Type 3	4.00×10^6 $TCID_{50}$/mL	No	No
Parainfluenza virus Type 4a	7.0×10^4 $TCID_{50}$/mL	No	No
Influenza virus, Type A (H1N1)	4.0×10^8 $CEID_{50}$/mL	No	No
Influenza virus, Type A (H3N2)	7.00×10^5 $TCID_{50}$/mL	No	No
Influenza virus, Type B	7.00×10^5 $TCID_{50}$/mL	No	No
Enterovirus 68	2.23×10^6 $TCID_{50}$/mL	No	No
Enterovirus 71	4.00×10^7 $TCID_{50}$/mL	No	No
Respiratory syncytial virus	2.23×10^6 $TCID_{50}$/mL	No	No
Rhinovirus 60	8.00×10^5 $TCID_{50}$/mL	No	No
Haemophilus influenzae	1.74×10^8 CFU/mL	No	No
Streptococcus pneumoniae	3.35×10^8 CFU/mL	No	No
Streptococcus pyogenes	5.98×10^8 CFU/mL	No	No
Candida albicans	1.19×10^8 CFU/mL	No	No
Bordetella pertussis	4.90×10^9 CFU/mL	No	No
Mycoplasma pneumoniae	6.75×10^7 CCU/mL	No	No
Chlamydia pneumoniae	4.25×10^7 CFU/mL	No	No
Legionella pneumophila	9.20×10^9 CFU/mL	No	No
Staphylococcus aureus	5.0×10^6 CFU/mL	No	No
Staphylococcus epidermidis	1.75×10^8 CFU/mL	No	No
Pooled human nasal wash	N/A	No	No

In-silico analysis was conducted for SARS-coronavirus, Human coronavirus HKU1, *Mycobacterium tuberculosis, Pneumocystis jirovecii* (PJP) and, for comparison, MERS coronavirus. 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 *in-silico* analysis.

There was no sequence with significant homology with this N protein sequence in both *Mycobacterium tuberculosis* and *Pneumocystis jirovecii* genomes, suggesting that cross-reactivity would not occur. Homologous sequences (N proteins) were identified in SARS-coronavirus, Human coronavirus HKU1, and MERS coronavirus. The N protein of Human coronavirus HKU1 shares 41.18% to 49.00% homology or identity with that of the SARS-CoV-2 Omicron N protein sequence whereas that of 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. Since cross reactivity with SARS-CoV, MERS-CoV and HKU1 cannot be ruled out for SARS-CoV-2, the intended use states that it cannot distinguish SARS-CoV-2 and SARS-CoV.

Endogenous/Exogenous Interference:

Substances which may be present in respiratory samples were tested to determine if interference may occur on the Healgen Rapid COVID-19 Antigen Test in an endogenous/exogenous interference study. The potential interfering substances were tested with negative samples and low positive samples (2x LoD) SARS-CoV-2 (USA-WA1/2020) in triplicate, with three lots of tests at the listed concentrations. The results showed that none of the tested endogenous/exogenous substances interfered with the test at the tested concentrations.

Table 7: Summary of Results for Potential Interfering Substances

Substance	Concentration	Cross-Reactivity	Interference
Whole Blood	4%	No	No
Human Leukocytes	1×10^7 cells/mL	No	No
Mucin	0.5%	No	No
Chloraseptic (Menthol/Benzocaine)	3 mg/mL	No	No
Naso GEL (NeilMed)	5% v/v	No	No
CVS Nasal Drops (Phenylephrine)	15% v/v	No	No
Afrin (Oxymetazoline)	15% v/v	No	No
CVS Nasal Spray (Cromolyn)	15% v/v	No	No
Zicam	5% v/v	No	No
Homeopathic (Alkalol)	15%	No	No
Sore Throat Phenol Spray	15% v/v	No	No
Hand soap	1%	No	No
Hand sanitizer	1%	No	No
Tobramycin	4 μg/mL	No	No
Mupirocin	10 mg/mL	No	No
Fluticasone Propionate	15% v/v	No	No
Tamiflu (Oseltamivir Phosphate)	5 mg/mL	No	No

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

External Controls:

The Healgen Rapid COVID-19 Antigen Test is required to be run with external quality controls that are sold separately as the COVID-19 Antigen Control Kit. Three (3) external control lots were evaluated 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 3: 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

Control	Control Lot #	Test Lot #	n	# of Neg	# of Pos	% Agreement
	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
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

Specimen Stability:

The objective of the specimen stability study is to demonstrate that test performance is not impacted by using specimens that are not immediately tested after collection. Three (3) replicates each of low positive (prepared at 2x LoD) and negative samples were prepared and incubated at room temperature for 0, 2, 4 and 6 hours and then tested on three (3) test lots. The study results showed that the samples were stable for up to 4 hours when stored at room temperature.

2. Clinical Performance:

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. Enrolled subjects were symptomatic subjects within six (6) days post symptom onset (DPSO) and exhibiting symptoms at the time of collection. A total of 806 evaluable subjects were collected and tested.

Two swabs were collected, the 1st was the anterior nares (AN) swab, it was tested on the Healgen Rapid COVID-19 Antigen device, and the 2nd was nasopharyngeal (NP) swab, it was collected into 3 mL of viral transport media (VTM) and tested on the composite reference method. For comparator testing, samples were tested on two highly sensitive RT-PCR comparator assays; testing on a third comparator would be performed only if discordant results were observed between the first two RT-PCR tests. The comparator result for a sample was determined based on a 2 out of 3 rule. Results obtained on the candidate device were compared to the comparator method to determine a positive percent agreement (PPA) of 85.4% and a negative percent agreement (NPA) of 99.7% for the symptomatic cohort.

Table 9: Summary of Clinical Performance

Healgen Rapid COVID-19 Antigen Test	Composite RT-PCR Comparator		Total
	Positive	Negative	
Positive	140	2	142
Negative	24	640	664
Total	164	642	806

PPA: 85.4% (140/164, 95% CI: 79.1% - 90.0%)

NPA: 99.7% (640/642, 95% CI: 98.9% - 99.9%)

Table 4: Clinical Performance Based on DPSO

Days of COVID-19 Symptoms	Number of Subject Samples Tested	Antigen Positives	RT-PCR Positives	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	100.0%

3. Clinical Cut-off:

Not applicable

4. Expected Value/Reference Range:

Not applicable

K. Proposed Labeling

The labeling supports a finding of substantial equivalence for the candidate device.

L. Conclusion

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