Hotgen™ COVID-19 Antigen Home Test

Healthcare Provider Instructions for Use (IFU)

For over-the-counter use

For in vitro diagnostic use only

For use under an Emergency Use Authorization (EUA) only

For use with anterior nasal (nares) swab samples

V. 2023-04.13[Eng.]

Intended Use

The Hotgen™ COVID-19 Antigen Home Test is a lateral flow immunoassay device intended for the qualitative detection of nucleocapsid protein antigen from the SARS-CoV-2 virus.

This test is authorized for non-prescription home use with self-collected anterior nasal (nares) swab samples from individuals aged 14 years or older or adult collected anterior nasal (nares) swab samples from individuals aged two years or older. This test is authorized for individuals with symptoms of COVID-19 within the first 7 days of symptom onset when tested at least twice over three days with at least 48 hours between tests, and for individuals without symptoms or other epidemiological reasons to suspect COVID-19, when tested at least three times over five days with at least 48 hours between tests.

The Hotgen™ COVID-19 Antigen Home Test does not differentiate between SARS-CoV and SARS-CoV-2.

Results are for the identification of SARS-CoV-2 nucleocapsid protein antigen, which is generally detectable in anterior nasal (nares) swab specimens during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with past medical history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Individuals who test positive with the Hotgen™ COVID-19 Antigen Home Test should self-isolate and seek follow-up care with their physician or healthcare provider as additional testing may be necessary.

All negative results are presumptive and confirmation with a molecular assay, if necessary for patient management, may be performed. Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control measures such as isolating from others and wearing masks. Negative results should be considered in the context of an individual's recent exposures, history, and the presence of clinical signs and symptoms consistent with COVID-19.

Individuals who test negative and continue to experience COVID-19 like symptoms of fever, cough and/or shortness of breath may still have SARS-CoV-2 infection and should seek follow up care with their physician or healthcare provider.

Individuals should provide all results obtained with this product to their healthcare provider for public health reporting and to receive appropriate medical care. All healthcare providers will report all test results they receive from individuals who use the authorized product to relevant public health authorities in accordance with local, state, and federal requirements using appropriate LOINC and SNOMED codes, as defined by the Laboratory In Vitro Diagnostics (LIVD) Test Code Mapping for SARS-CoV-2 Tests provided by CDC.

The Hotgen™ COVID-19 Antigen Home Test is intended for non-prescription self-use and/or as applicable an adult lay user testing another person 2 years of age or older in a non-laboratory setting.

The Hotgen™ COVID-19 Antigen Home Test is only for in vitro diagnostic use under the Food and Drug Administration's Emergency Use Authorization. The product has not been FDA cleared or approved.

Explanation of the Test

COVID-19 (short for 'Coronavirus Disease 2019') is a disease first recognized in 2019 that is caused by a type of novel coronavirus called SARS-CoV-2. Due to its rapid spread, the World Health Organization (WHO) recognized the disease as a global pandemic on March 11, 2020. Individuals infected with SARS-CoV-2 may have a range of symptoms from asymptomatic infection to severe respiratory illness and even death. The virus is spread primarily from person to person through respiratory particles, even by individuals without symptoms.

The Hotgen™ COVID-19 Antigen Home Test is a rapid, qualitative immunochromatographic assay for the determination of the presence of SARS-CoV-2 antigens in anterior nasal swab specimens. The total time required to perform one test is approximately 15 minutes from clinical specimen collection to result.

This kit is based on the colloidal gold immunochromatographic technology and uses double antibody sandwich method to detect the novel coronavirus antigen in anterior nasal swab samples. The detection line (T line) of the novel coronavirus antigen test cassette was coated with novel coronavirus antibody, and the quality control line (C line) was coated with goat anti-mouse antibody.

During the test, the sample is dropped into the sample well of the test cassette. The novel coronavirus antigen in the sample is first combined with the novel coronavirus antibody labelled with colloidal gold to form a novel coronavirus antigen - labelled novel coronavirus antibody - colloidal gold complex. This complex migrates on the membrane via capillary action, to form a solid phase novel coronavirus antibody - novel coronavirus antigen - labelled novel coronavirus antibody - colloidal gold complex at the T line position. As the chromatography continues, a solid phase goat anti-mouse antibody - labelled novel coronavirus antibody - colloidal gold complex is formed at the position of line C. After the test is completed, observe the colloidal gold color reaction of T line and C line to determine results of novel coronavirus antigen in anterior nasal swab samples.

Material Provided

The Hotgen™ COVID-19 Antigen Home Test is offered in a 1, 2, 5, 10, 20, or 40 Tests size, as detailed in Table 1.

Table 1. Kit configurations for the Hotgen™ COVID-19 Antigen Home Test.

Components	1 Test	2 Tests	5 Tests	10 Tests	20 Tests	40 Tests
SARS-CoV-2 Antigen Test Cassette	1	2	5	10	20	40
Sample Tube containing extraction buffer	1	2	5	10	20	40
Disposable Sterile Swab	1	2	5	10	20	40
Instructions For Use	1	1	1	1	1	1

Materials Required but Not provided

Timer.

Quality Control

Each SARS-CoV-2 Antigen Test Cassette has a built-in internal procedural control. The pink/red line appearing at the "C" position is an internal procedural control. This procedural control line indicates that sufficient flow has occurred, and the functional integrity of the test device has been maintained. A distinct reddish-pink Control line should always appear if the test has been performed correctly – independent of whether a Test line appears or does not appear on the test. If the Control line does not appear, the test result is invalid, and a new test should be performed.

External run controls are not required to use the SARS-CoV-2 Antigen Test Cassette in a home setting.

Storage and shelf life

- The Hotgen™ COVID-19 Antigen Home Test should be stored at 2-30°C (35.6~86°F). Do not freeze. Avoid direct sunlight.
- Kit components in the Hotgen™ COVID-19 Antigen Home Test are stable until the expiration date printed on the label.
- The test cassette must remain in the sealed foil pouch until use. Once the pouch has been opened, it should be used within 2 hours.
- Date of manufacture and expiration date see label.

Warnings, Precautions, and Safety Information

- Read all instructions carefully before performing the test. Failure to follow the instructions may result in inaccurate test results.
- In the USA, this product has not been FDA cleared or approved, but has been authorized by

FDA under an Emergency Use Authorization. This product has been authorized only for the detection of proteins from SARS-CoV-2, not for any other viruses or pathogens. The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

- **Serial testing should be performed in individuals with negative results at least twice over three days (with 48 hours between tests) for symptomatic individuals and three times over five days (with at least 48 hours between tests) for asymptomatic individuals. You may need to purchase additional tests to perform this serial (repeat) testing.**
- If you have had symptoms longer than 7 days you should consider testing at least three times over five days with at least 48 hours between tests.
- An anterior nasal swab sample can be self-collected by an individual age 14 years and older. Children 2 to 13 years of age should be tested by an adult.
- Do not use on anyone under 2 years of age.
- Wear a safety mask or other face-covering when collecting a specimen from a child or another individual.
- Do not use if any of the test kit contents or packaging is damaged.
- Test components are single use. Do not re-use.
- Do not use kit past its expiration date. For the most current expiration dates of this test, please refer to: **http://www.fda.gov/covid-tests**
- Do not touch the swab tip.
- **Once opened, the test cassette should be used within 2 hours.**
- **Do not read test results before 15 minutes or after 30 minutes. Results read before 15 minutes or after 30 minutes may lead to a false positive, false negative, or invalid result.**
- **Keep testing kit and kit components away from children and pets before and after use. Avoid contact with your skin or eyes. Do not ingest any kit components. The reagent solution contains harmful chemicals (see Table 2). If the solution contacts your skin or eyes, flush with large amounts of water. If irritation persists, seek medical advice: https://www.poisonhelp.org or 1-800-222-1222.**

Table 2. Harmful chemicals in the Sample Tube solution.

Chemical Name	Subcomponent	GHS Code for each Ingredient	Concentrations
KV-300	5-Chloro-2-methyl-4-isothiazolin-3-one	H316, Skin Sensitization	0.00255%
	2-Methyl-4-isothiazolin-3-one	H316, Skin Sensitization	0.00081%

- For more information on EUAs please visit: https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization

- For the most up to date information on COVID-19, please visit: www.cdc.gov/COVID19

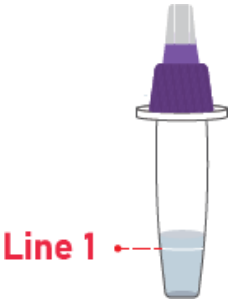
Test Procedure

1. Please wash and dry your hands thoroughly before the test.

2. Read the Instructions for use carefully.
Note: Check kit components and confirm test is not expired prior to use. Use within 2 hours after opening the foil pouch (step 11).

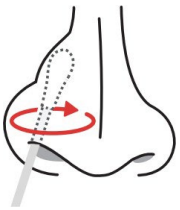
For the most current expiration dates of this test, please refer to: http://www.fda.gov/covid-tests

3. Confirm the liquid level is at or above Line 1 in the sample tube. Then proceed with collecting the nasal swab sample.
Note: If the liquid level is below Line 1 in the sample tube, DO NOT proceed with the test. The test result will not be accurate. If the sample tube liquid level is below Line 1, use a new kit.

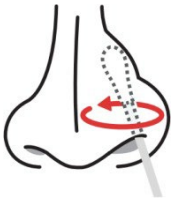


4. Take the swab out of the package and do not touch the sampling end.
Note: Do not touch the swab tip with your fingers.

5. Carefully insert the swab 1/2 to 3/4 of an inch into the nostril. Under moderate pressure, swab the nostril at least 5 times for at least 15 seconds total.
Note: For young children, the swab may not need to be inserted so far. Stop pushing the swab in if you feel any kind of resistance.



6. Repeat sampling with the same swab in the other nostril.
Note: Failure to swab properly may cause false negative results.



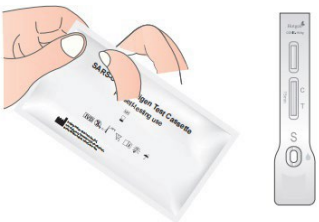
7. Tap the sample tube vertically several times on the table. Open the larger cap on the sample tube.

8. Insert the swab and soak in the liquid for at least 15 seconds, stir the swab several times, and squeeze the tube walls onto the swab tip 3 times.

9. Pinch tube walls while removing swab to squeeze excess liquid from tip.

10. Close the sample tube with the larger tube cap.

11. Open the foil pouch and place the test cassette on a flat surface.
Note: Locate sample well on the test cassette. It is marked with the letter S and an image of a drop.



12. Open the small cap at the front end of the sample tube, and place exactly 4 drops into the sample well (S) of the test cassette.
Note: False negative or invalid results may occur if too little sample is added. Do not touch or move the test cassette during this time.

13. Start a timer and read the result at 15 minutes. Do not read test before 15 minutes, even if the Control (C) line appears sooner. Do not read after 30 minutes.

Note: A false negative or false positive result may occur if the test result is read before 15 minutes or after 30 minutes.

14. Throw away all used test components in the trash.

Test Interpretation

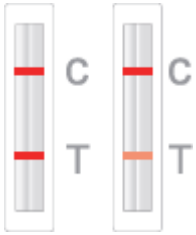
Repeat testing is needed to improve test accuracy. Please follow Table 3 when interpreting test results.

Table 3. Test interpretation table for serial (repeat) testing.

Status on first day of Testing	First Result Day 1	Second Result Day 3	Third Result Day 5	Interpretation
With Symptoms	Positive	N/A	N/A	Positive for COVID-19
	Negative	Positive	N/A	Positive for COVID-19
	Negative	Negative	N/A	Negative for COVID-19
Without Symptoms	Positive	N/A	N/A	Positive for COVID-19
	Negative	Positive	N/A	Positive for COVID-19
	Negative	Negative	Positive	Positive for COVID-19
	Negative	Negative	Negative	Negative for COVID-19

Results should be considered in the context of an individual's recent exposures, history, and the presence of clinical signs and symptoms consistent with COVID-19.

COVID-19 Positive (+): if both the Control (C) line and the Test (T) line are visible, the test is positive. Any faint visible red/purple test (T) line with the control line (C) should be read as positive.



Note: Repeat testing does not need to be performed if patients have a positive result at any time.

A positive test result means that the virus that causes COVID-19 was detected in the sample, and it is very likely the individual has COVID-19 and is contagious. Please contact the patient's doctor/primary care physician (if applicable) and the local health authority immediately and instruct your patient to adhere to the local guidelines regarding self-isolation. There is a very small chance that this test can give a positive result that is incorrect (a false positive).

Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Individuals who test positive with the Hotgen™ COVID-19 Antigen Home Test should self-isolate and seek follow up care with their physician or healthcare provider as additional confirmatory testing with a molecular test for positive results may also be necessary, if there is a low likelihood of COVID-19, such as in individuals without known exposures to COVID-19 or residing in communities with low prevalence of infection.

COVID-19 Negative (-): if the Control (C) line is visible, but the Test (T) line is not visible, the test is negative.

Note: To increase the chance that the negative result for COVID-19 is accurate, you should:

- **Test again in 48 hours if the individual has symptoms on the first day of testing.**
- **Test 2 more times at least 48 hours apart if the individual does not have symptoms on the first day of testing.**

A negative test result indicates that the virus that causes COVID-19 was not detected in the sample. A negative result does not rule out COVID-19. There is a higher chance of false negative results with antigen tests compared to laboratory-based tests such as PCR tests. If the test is negative but COVID-19-like symptoms, e.g., fever, cough, and/or shortness of breath continue, follow up testing for SARS-CoV-2 with a molecular test or testing for other respiratory disease should be considered. If applicable, seek follow up care with the primary health care provider.

All negative results should be treated as presumptive and confirmation with a molecular assay may be necessary if there is a high likelihood of SARS-CoV-2 infection, such as in an individual with a close contact with

COVID-19 or with suspected exposure to COVID-19 or in communities with high prevalence of infection. Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions.

Invalid: if the Control (C) line is not visible, the test is invalid. Re-test with a new swab and new test device.

Limitations

- The performance of this test was established based on the evaluation of a limited number of clinical specimens collected between February 2022 and June 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.
- 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.
- All COVID-19 antigen test negative results are presumptive and confirmation with a molecular assay may be necessary.
- If the patient continues to have symptoms of COVID-19, and both the patient's first and second tests are negative, the patient may not have COVID-19, however additional follow-up may be needed.
- If the test is positive, then proteins from the virus that causes COVID-19 have been found in the sample and the individual likely has COVID-19.
- This test is read visually and has not been validated for use by those with impaired vision or color-impaired vision.
- Incorrect test results may occur if a specimen is incorrectly collected or handled.
- This test detects both viable (live) and nonviable SARS-CoV-2. 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.

Performance characteristics

Analytical Sensitivity: Limit of Detection (LoD)

The LoD of the Hotgen™ COVID-19 Antigen Home Test was determined by serial dilutions of the UV-inactivated SARS-CoV-2 virus (USA-WA1/2020). Contrived samples were prepared by spiking the strain into pooled negative clinical matrix (NCM) collected from multiple healthy volunteers confirmed COVID-19 negative by RT-PCR. The preliminary LoD initially determined by testing six concentrations ranging from 1.15×10^6 $TCID_{50}$/mL to 2.88×10^3 $TCID_{50}$/mL was tested in 20 replicates. For each replicate, 50 µL of sample was spiked onto the swab. When only 90% of those replicates were positive, the 2-fold higher concentration was tested in 20 replicates. The confirmed LoD for the Hotgen™ COVID-19 Antigen Home Test was 1.15×10^4 $TCID_{50}$/mL. Based upon the testing procedure for this study, the LoD of 1.15×10^4 $TCID_{50}$/mL equates to 575 $TCID_{50}$/swab.

NIH/RADx Variant Testing

The performance of this device in the detection of the Omicron variant of SARS-CoV-2 was evaluated in a dilution series of clinical specimens which were positive for the Omicron variant. This testing was conducted by the National Institutes of Health (NIH) as a component of the Rapid Acceleration of Diagnostics (RADx®) initiative. Specimen pools were prepared by the RADx® team using clinical pooled samples from currently circulating Omicron strains and tested by RADx® to assess performance with the Omicron variant. Results from this dilution series cannot be compared to other specimen pools and do not indicate that a test will have different clinical performance compared to other EUA authorized tests. Compared to an EUA authorized RT-PCR method, the Hotgen™ COVID-19 Antigen Home Test detected 100% of live virus Omicron samples at a Ct-value of 25.4 (n=5). Testing was also compared to two additional EUA-authorized OTC antigen tests (Assay #1 and Assay #2). Omicron dilutions at lower viral concentrations (Ct-values greater than 25.4) were not detected by the Hotgen™ COVID-19 Antigen Home Test in this study.

Omicron Pool 1 - Live	**Average N2 Ct (n=9)**	**Assay #1 Percent Positive (n=5)**	**Assay #2 Percent Positive (n=5)**	**Hotgen™ COVID-19 Antigen Home Test Percent Positive (n=5)**
Dilution 1	18	100	100	100
Dilution 2	19.4	100	100	100
Dilution 3	20	100	100	100
Dilution 4	21.5	100	100	100
Dilution 5	23	100	100	100
Dilution 6	24.4	100	100	100
Dilution 7	25.4	0	0	100
Dilution 8	26.7	0	0	0
Dilution 9	27.7	0	0	0

Dilution 10	28.7	0	0	0
Dilution 11	29.8	0	0	0
Dilution 12	30.6	0	0	0

High-Dose Hook Effect

The Hotgen™ COVID-19 Antigen Home Test was tested with up to 1.15 x 10^7 $TCID_{50}$/mL of UV-inactivated SARS-CoV-2 virus (USA-WA1/2020) and no hook effect was observed.

Exogenous/Endogenous Interference Substances

The Hotgen™ COVID-19 Antigen Home Test was evaluated for performance in the presence of potentially interfering substances that may be found in a respiratory specimen. The positive (3x LoD SARS-CoV-2) and negative specimens prepared in NCM were tested with the addition of potentially interfering substances. The performance of Hotgen™ COVID-19 Antigen Home Test was not affected by any of the potentially interfering substances listed in Table 4 at the concentrations tested.

Table 4. List of potentially interfering substances tested.

Potentially Interfering Substance	Concentration Tested	Potentially Interfering Substance	Concentration Tested
Human Whole Blood (EDTA tube)	4% v/v	Tobramycin	4 µg/mL
Mucin (porcine stomach, type II)	0.5% w/v	Mupirocin	10 mg/mL
Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL	Tamiflu (Oseltamivir Phosphate)	5 mg/mL
Naso GEL (NeilMed)	5% v/v	Fluticasone Propionate	5% v/v
CVS Nasal Drops (Phenylephrine)	15% v/v	Body & Hand Lotion with 1.2% dimethicone	0.5% v/v
Afrin (Oxymetazoline)	15% v/v	Daily Moisture Lotion (Walgreens)	5% v/v
CVS Nasal Spray (Cromolyn)	15% v/v	Purell Hand Sanitizer	5% v/v
Zicam	5% v/v	Hand sanitizer, 75% isopropanol, fast-drying	15% v/v
Homeopathic (Alkalol)	10% v/v	Hand soap liquid gel	10% v/v
Sore Throat Phenol Spray	15% v/v	Dial Complete	10% v/v

Analytical Specificity: Cross-Reactivity and Microbial Interference

Cross-reactivity and interference studies were performed for related pathogens, high prevalence disease agents, and normal or pathogenic flora that are reasonably likely to be encountered in the clinical specimen of the nasal cavity. Each organism or virus (11 bacteria, 16 viruses) was tested in both the presence and absence of UV-inactivated SARS-CoV-2 (isolate USA-WA1/2020) at 3x LoD. All testing samples were prepared in the pooled negative clinical matrix (NCM). No cross reactivity or interference was observed for any of the organisms in Table 5 at the concentrations tested.

Table 5. List of potentially cross-reactive or interfering microorganisms tested.

Microorganism	Concentration	Microorganism	Concentration
Adenovirus 1	3.09×10^5 $TCID_{50}$/mL	Parainfluenza virus 3	8.51×10^5 $TCID_{50}$/mL
Enterovirus Type 68 Major Group	1.00×10^5 $TCID_{50}$/mL	Parainfluenza virus 4A	1.15×10^5 $TCID_{50}$/mL
HCoV-229E	1.40×10^5 $TCID_{50}$/mL	Respiratory syncytial virus A	1.00×10^5 $TCID_{50}$/mL
HCoV-OC43	1.00×10^5 $TCID_{50}$/mL	Rhinovirus Type 1A	1.00×10^5 $TCID_{50}$/mL
HCoV-NL63	1.00×10^5 $TCID_{50}$/mL	*Bordetella pertussis*	1.96×10^7 CFU/mL
MERS-coronavirus	1.05×10^5 $TCID_{50}$/mL	*Candida albicans*	4.76×10^6 CFU/mL
SARS-coronavirus	1.00×10^5 PFU/mL	*Chlamydia pneumoniae*	1.70×10^6 IFU/mL
Human metapneumovirus 16 (hMPV-16) Type A1	1.26×10^5 $TCID_{50}$/mL	*Haemophilus influenzae*	6.97×10^6 CFU/mL
Influenza A (H1N1)	1.00×10^5 $TCID_{50}$/mL	*Legionella pneumophila*	1.91×10^7 CFU/mL
Influenza A (H1N1pdm) A/California/07/2009	5.20×10^5 $CEID_{50}$/mL	*Mycoplasma pneumoniae*	2.70×10^6 CCU/mL
Influenza A (H3N2) Texas/50/12	1.00×10^5 $TCID_{50}$/mL	*Staphylococcus aureus*	2.51×10^6 CFU/mL
Influenza B Colorado/6/17	1.00×10^5 $TCID_{50}$/mL	*Staphylococcus epidermidis*	6.07×10^6 CFU/mL
Influenza B Utah/9/14	3.80×10^5 $TCID_{50}$/mL	*Streptococcus pneumoniae*	1.34×10^6 CFU/mL
Influenza B Washington/02/19	1.00×10^5 $TCID_{50}$/mL	*Streptococcus pyogenes*	2.39×10^6 CFU/mL
Parainfluenza virus 1	1.26×10^5 $TCID_{50}$/mL	Pooled negative clinical matrix (NCM)	N/A
Parainfluenza virus 2	1.00×10^5 $TCID_{50}$/mL		

To estimate the likelihood of cross-reactivity of organisms that were not readily available for wet testing with SARS-CoV-2, *in silico* analysis using the Protein-Protein Basic Local Alignment Search Tool (Blastp) managed by the National Center for Biotechnology Information (NCBI) was used.

- Human coronavirus HKU1: 45.99% homology was found between the amino acid sequences of SARS-CoV-2 nucleocapsid protein and that of human coronavirus HKU1 nucleocapsid protein. Therefore, the cross-reactivity with human coronavirus HKU1 cannot be completely ruled out.
- *Pneumocystis jirovecii*: No significant similarity was found between *P. jirovecii* and amino acid sequences of SARS-CoV-2 nucleocapsid protein.
- *Mycobacterium tuberculosis*: No significant similarity was found between *M. tuberculosis* and amino acid sequences of SARS-CoV-2 nucleocapsid protein.

Clinical Evaluation

Based on the result of a clinical study conducted during February 2022 through June 2022 to determine the performance of the Hotgen™ COVID-19 Antigen Home Test compared to a highly sensitive RT-PCR assay authorized for emergency use by the FDA, the Hotgen™ COVID-19 Antigen Home Test correctly identified 81.7% positive specimens and 99.8% negative specimens. A total of 500 individuals with signs and symptoms of COVID-19 within the first seven (7) days of symptom onset who had a valid result on the Hotgen™ COVID-19 Antigen Home Test were enrolled across 9 distinct locations in the US. The breakdown of enrollment, positivity rate as determined by the RT-PCR, and the positive percent agreement (PPA) of the Hotgen™ COVID-19 Antigen Home Test by Days Post Symptom Onset (DPSO) is shown in Table 6.

Table 6. Hotgen™ COVID-19 Antigen Home Test performance by DPSO.

DPSO	Subjects	RT-PCR Positive	Positivity Rate	Hotgen Positive	PPA
0	20	2	10.0%	1	50.0%
1	107	27	25.2%	20	74.1%
2	141	28	19.9%	25	89.3%
3	105	15	14.3%	13	86.7%
4	51	9	17.6%	7	77.8%
5	37	6	16.2%	4	66.7%
6	19	1	5.3%	1	100.0%
7	20	5	25.0%	5	100.0%
Total	**500**	**93**	**18.6%**	**76**	**81.7%**

Usability

The usability of the Hotgen™ COVID-19 Antigen Home Test and the ability of the labeling to direct untrained users to perform self-testing was evaluated by observation in a dedicated usability study. A total of 35 subjects were enrolled in the study; 20 subjects self-collected a sample from themselves while 15 subjects collected a sample from a child. All subjects completed the required procedural steps and interpreted the test unassisted in a simulated home

environment. The overall success of every task completed by all subjects enrolled was determined by professional observation.

Subjects performed 94.6% (364/385) steps/tasks correctly. The 5% of tasks for which failures were observed were all non-critical tasks. After the completion of the test, the subject (or Parent/Legal Guardian) completed a questionnaire to assess usability of the test. The subjects scored the test positively on 98.4% (482/490) possible measures on the subject questionnaire.

Importance of Serial (Repeat) Testing

A prospective clinical study was conducted between January 2021 and May 2022 as a component of the Rapid Acceleration of Diagnostics (RADx) initiative from the National Institutes of Health (NIH). A total of 7,361 individuals were enrolled via a decentralized clinical study design, with a broad geographical representation of the United States. Per inclusion criteria, all individuals were asymptomatic upon enrollment in the study and at least 14 days prior to it and did not have a SARS-CoV-2 infection in the three months prior to enrollment. Participants were assigned to one of three EUA authorized SARS-CoV-2 OTC rapid antigen tests to conduct serial testing (every 48 hours) for 15 days. If an antigen test was positive, the serial-antigen testing result is considered positive.

At each rapid antigen testing time point, study subjects also collected a nasal swab for comparator testing using a home collection kit (using a 15-minute normalization window between swabs). SARS-CoV-2 infection status was determined by a composite comparator method on the day of the first antigen test, using at least two highly sensitive EUA RT-PCRs. If results of the first two molecular test were discordant a third highly sensitive EUA RT-PCR test was performed, and the final test result was based upon the majority rule.

Study participants reported symptom status throughout the study using the MyDataHelps app. Two-day serial antigen testing is defined as performing two antigen tests 36 - 48 hours apart. Three-day serial antigen testing is defined as performing three antigen tests over five days with at least 48 hours between each test.

Out of the 7,361 participants enrolled in the study, 5,609 were eligible for analysis. Among eligible participants, 154 tested positive for SARS-CoV-2 infection based on RT-PCR, of which 97 (62%) were asymptomatic on the first day of their infection, whereas 57 (39%) reported symptoms on the first day of infection. Pre-symptomatic subjects were included in the positive percent agreement (PPA) of asymptomatic individuals, if they were asymptomatic on the first day of antigen testing, regardless of whether they developed symptoms at any time after the first day of testing.

Performance of all antigen tests with serial testing in individuals is described in Table 7.

Table 7. Data establishing PPA of COVID-19 antigen serial testing compared to the molecular comparator single day testing throughout the course of infection with serial testing. Data is from all antigen tests in study combined.

DAYS AFTER FIRST PCR POSITIVE TEST RESULT	ASYMPTOMATIC ON FIRST DAY OF TESTING			SYMPTOMATIC ON FIRST DAY OF TESTING		
	Ag Positive / PCR Positive (Antigen Test Performance % PPA)					
	1 Test	2 Tests	3 Tests	1 Test	2 Tests	3 Tests
0	9/97 (9.3%)	35/89 (39.3%)	44/78 (56.4%)	34/57 (59.6%)	47/51 (92.2%)	44/47 (93.6%)
2	17/34 (50.0%)	23/34 (67.6%)	25/32 (78.1%)	58/62 (93.5%)	59/60 (98.3%)	43/43 (100%)
4	16/21 (76.2%)	15/20 (75.0%)	13/15 (86.7%)	55/58 (94.8%)	53/54 (98.1%)	39/40 (97.5%)
6	20/28 (71.4%)	21/27 (77.8%)	16/18 (88.9%)	27/34 (79.4%)	26/33 (78.8%)	22/27 (81.5%)
8	13/23 (56.5%)	13/22 (59.1%)	4/11 (36.4%)	12/17 (70.6%)	12/17 (70.6%)	7/11 (63.6%)
10	5/9 (55.6%)	5/8 (62.5%)	-	4/9 (44.4%)	3/7 (42.9%)	-

1 Test = one (1) test performed on the noted days after first PCR positive test result. Day 0 is the first day of documented infection with SARS-CoV-2.
2 Tests = two (2) tests performed an average of 48 hours apart. The first test performed on the indicated day and the second test performed 48 hours later.
3 Tests = three (3) tests performance an average of 48 hours apart. The first test performed on the indicated day, the second test performed 48 hours later, and a final test performed 48 hours after the second test.

Technical Support

For questions, or to report a problem, please call Technical Support at 1-800-966-2919 (Available Hours: Mon. to Fri., 9 a.m. – 5 p.m. PST) or cs@hotgen.info.

Test system problems may also be reported to the FDA using the MedWatch reporting system: (phone: 1-800-FDA-1088; fax: 1-800-FDA-1078) or http://www.fda.gov/medwatch.

Ordering and Contact Information

Beijing Hotgen Biotech Co., Ltd.
9th Building, No. 9 Tianfu Street, Biomedical Base,
Daxing District, Beijing, 102600, P.R. China.
Phone: 1-800-966-2919
Email: cs@hotgen.info
www.hotgen.info

Description of Symbols

	Use-by date		Batch code		Consult instructions for use
	Contents sufficient for <n> tests		Temperature limit		Catalogue number
	Date of manufacture		Warnings		Do not re-use
	Manufacturer		Keep away from sunlight		Keep dry
	In vitro diagnostic medical device		Do not use if the packaging is damaged		Over the counter