For use under an Emergency Use Authorization (EUA) only
For use with anterior nasal swab specimens
For *in vitro* diagnostic use only

CareStart™

COVID-19 Antigen Home Test

Rapid Diagnostic Test for the Detection of SARS-CoV-2 Antigen

Healthcare Provider Instructions for Use

Intended Use

The *CareStart*™ 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 2 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 *CareStart*™ COVID-19 Antigen Home Test does not differentiate between SARS-CoV or SARS-CoV-2.

Results are for the identification of SARS-CoV-2 nucleocapsid protein antigen, which is generally detectable in anterior nasal (nares) samples during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient 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 and the agent detected may not be the definitive cause of disease. Individuals who test positive with the *CareStart*™ 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 Page 2 of 19 Mapping for SARS-CoV-2 Tests provided by CDC.

The *CareStart*™ COVID-19 Antigen Home Test is intended for non-prescription self-use and/or as applicable, for an adult lay user testing another aged 2 years or older in a non-laboratory

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

Principles 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. Many individuals with confirmed COVID-19 have developed fever and/or symptoms of acute respiratory illness (e.g., cough, dyspnea), although some individuals experience only mild symptoms or no symptoms at all. The virus is spread primarily from person to person through respiratory particles, even by individuals without symptoms.

The *CareStart*™ COVID-19 Antigen Home Test is a lateral flow immunochromatographic assay for the detection of extracted nucleocapsid protein antigens specific to SARS-CoV-2 in self-collected anterior nasal (nares) swab specimens.

Nasal swabs require a sample preparation step in which the sample is eluted into the extraction buffer solution. Extracted swab sample is added to the sample well of the test device to initiate the test. When the swab sample migrates in the test strip, SARS-CoV-2 viral antigens bind to anti-SARS-CoV-2 nucleocapsid protein antibodies conjugated to indicator and capture particles in the test strip forming an immune complex. The immune complex is then captured by the test line on the nitrocellulose membrane as it migrates through the strip.

The user should perform the test following the in-app self-paced, step-by-step instructions or Quick Reference Instructions.

Test results are interpreted visually at 10 minutes after sample loading followed by the instructions. The presence of two-colored lines in the control line region "C" and test line region "T" indicates COVID-19 positive. The presence of one colored line in the control line region "C" indicates COVID-19 negative. No appearance of a colored line in the control region "C" indicates an invalid test. Results should not be read after 15 minutes.

Quality Control

- The *CareStart*™ COVID-19 Antigen Home Test contains a built-in internal procedural control that is included in the test device. A purple-colored line appearing in the control region "C" is designed as an internal procedural control. The appearance of the procedural control line indicates that sufficient flow has occurred, and the functional integrity of the test device has been maintained. If the procedural control line does not develop in 10 minutes, the test result is considered invalid and retesting with a new device

is recommended. If the internal procedural control line is still absent in the retest, please contact the Technical Support at +1-888-898-1270 (Available Hours: Mon. to Fri.: 8 a.m. – 5 p.m.) or TShelp@accessbio.net (24/7 available).

- The unique barcode on the test device contains essential device information and captured during the test process using mobile application to ensure test validity. In the event the barcode is not valid for any reason, the user is presented with a final screen indicating the fail reason by one of the below:
  Invalid: Barcode Not Found
  Invalid: Test Expired
  Invalid: Test Barcode Invalid
  Invalid: Test Previously Used

Reagents and Materials

Materials provided

All following required components for single-use are packed and sealed in a tray.

- a test device: foil pouched test device containing one test strip which is encased in plastic device cassette with a desiccant.
- an extraction vial and cap: the extraction vial contains 500 µL of extraction buffer solution.
- a nasal swab: swab for anterior nasal specimen collection.

Quick Reference Instructions and Fact Sheet for Individuals are also included in each box.

CareStart™ COVID-19 Antigen Home Test is available in the following packaging configuration: 1 test (REF: RCPM-00171), 2 tests (REF: RCPM-00271), 4 tests (REF: RCPM-00471), 5 tests (REF: RCPM-00571), 20 tests (REF: RCPM-02071), 25 tests (REF: RCPM-02571) or 30 tests (REF: RCPM-03071)

Materials required but not provided

- Smartphone (supplied by the user): For a list of compatible smartphone OS systems, visit www.accessbio.net/app.
- Mobile application: Prior to testing, the user should download the free mobile application, Othena App, for iOS or Android smartphones.
- Timer

Warnings and Precautions

- Read all instructions carefully before performing the test. Failure to follow the instructions may result in inaccurate test results.
- For *in vitro* diagnostic use only.
- 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 aged 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 anterior nares swab 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 the kit past its expiration date.
- Do not touch the swab tip.
- Once opened, the test device should be used immediately.
- **Do not read test result before 10 minutes or after 15 minutes. Results read before 10 minutes or after 15 minutes may lead to a false positive, false negative, or invalid result.**
- This test is read visually. Users with impaired vision or color-impaired vision may not be able to read the test.
- Wash hands thoroughly for at least 20 seconds before and after handling nasal swab samples.
- Keep the test device on a flat surface during the testing.

- Excess blood or mucus on the swab specimen may interfere with test performance and may yield a false-positive result. Avoid touching any bleeding areas of the nasal cavity when collecting specimens.
- False negative results may occur if a specimen is incorrectly collected or handles, or inadequately stored.
- When collecting a nasal swab sample, use only the Nasal Swab provided in the kit.
- Keep foreign substances and household cleaning products away from the test during the testing process. Contact with foreign substances and household cleaning products may result in an incorrect test result.
- Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents.
- Handle all specimens as though they contain infectious agents.
- In the event of a spillage, ensure it is cleaned thoroughly using a suitable disinfectant.
- Do not operate your test outside of storage conditions.
- Do not close the App during processing as it may cause an error and you will need a new test kit.
- Do not use on anyone who is prone to nosebleeds or has had facial or head injury/surgery in the last 6 months.
- Do not interchange kit contents from different lots.
- **Keep testing kit and kit components away from children and pets before and after use. Avoid contact with your skin, eyes, nose, or mouth. Do not ingest any kit components. The reagent solution contains harmful chemicals (see table below). If the solution contacts your skin, eyes, nose, or mouth, flush with large amounts of water. If irritation persists, seek medical advice: https://www.poisonhelp.org or 1-800-222-1222.**

Chemical Name/CAS	**Harms (GHS Code) for each Ingredient**	**Concentration**
Boric Acid/10043-35-3	H360 May damage fertility or the unborn child.	0.38%
Ethylenediaminetetraacetic acid (EDTA)/13235-36-4	H302 Harmful if swallowed. H318 Causes serious eye damage.	0.08%
Sodium Chloride (NaCl)/ 7647-14-5	None	4.38%
Triton X-100/9002-93-1	H302 Harmful if swallowed. H315 Causes skin irritation. H318 Causes serious eye damage. H410 Very toxic to aquatic life with long-lasting effects.	1.50%
N-Lauroylsarcosine sodium salt/137-16-6	H315 Causes skin irritation. H318 Causes serious eye damage. H330 Fatal if inhaled.	0.15%

- 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

Storage and Stability

- Store the test kit as packaged between 1 ~ 30°C.
- The test device must remain in the sealed pouch until use.
- Do not freeze any contents of the kit.
- For information about current expiration dates for at-home OTC COVID-19 diagnostic tests, visit http://www.fda.gov/covid-tests.

Disposal

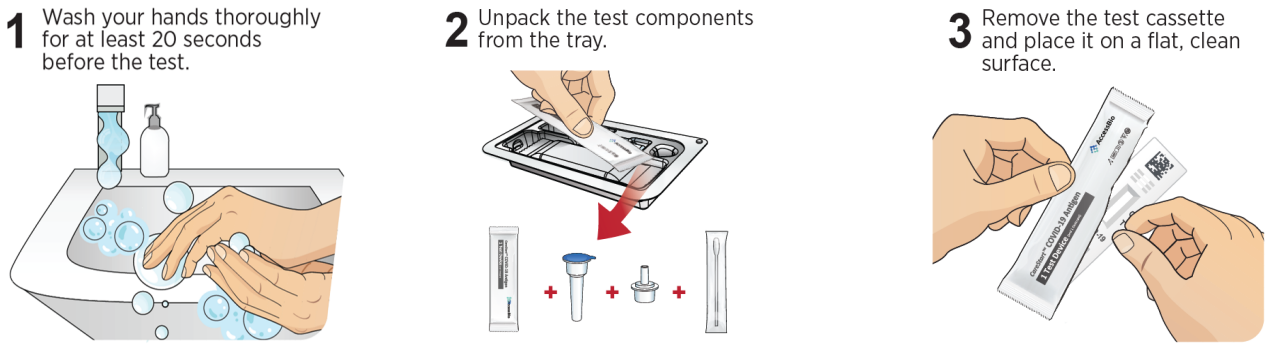
Dispose of all used test kit components and patient samples in household trash.

Specimen Collection and Handling

Acceptable specimen type for testing with the *CareStart*™ COVID-19 Antigen is a direct anterior nasal (nares) swab specimen. It is essential that correct specimen collection and preparation methods be followed. Inadequate specimen collection, improper specimen handling and/or transport may yield false results. Process the test swab sample immediately after collection (specimens are stable up to 4 hours in extraction buffer). Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)
https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html

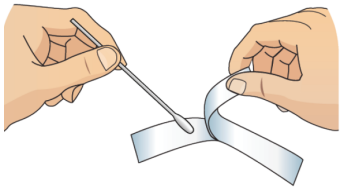
Instructions for Running the Test

IMPORTANT: Do not open kit components until instructions to do so. Follow the in-app self-paced, step-by-step instructions or paper instructions printed on the QRI as below.

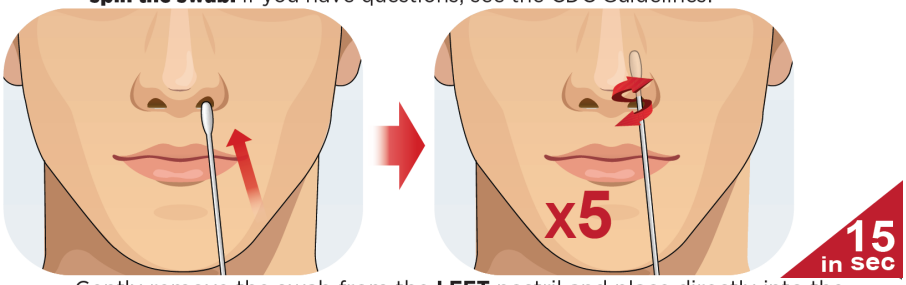


4 Locate the extraction vial and gently peel off the aluminum foil seal, being sure to keep the vial upright and place it in the packaging tray.

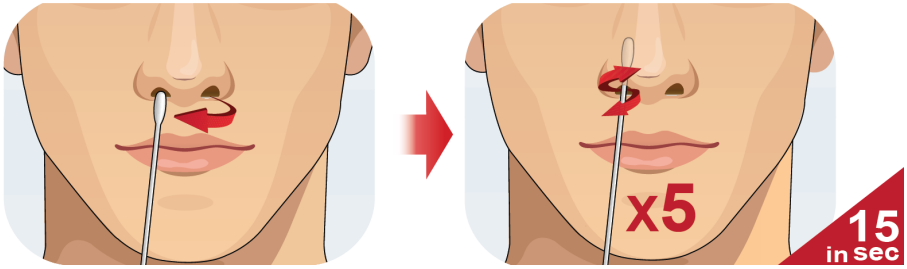
5 Locate a nasal swab and remove from the pouch. Be careful not to touch the swab tip.



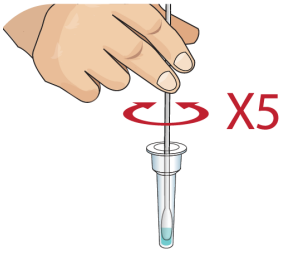
6 Gently insert the swab no more than 3/4 inch into the **LEFT** nostril. Then, slowly rotate the swab at least **5 times** in a circular path pressing gently against the inside walls of your nostrils for a total of **15 seconds. Do not just spin the swab.** If you have questions, see the CDC Guidelines.



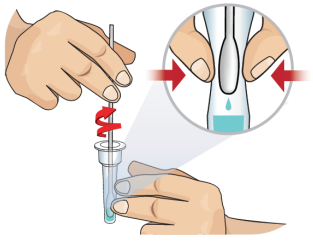
7 Gently remove the swab from the **LEFT** nostril and place directly into the **RIGHT** nostril, repeating the process of rotating at least **5 times** in a circular path pressing gently against the inside walls of your nostrils" for a total of **15 seconds. Do not just spin the swab.** Remove the swab from the RIGHT nostril.



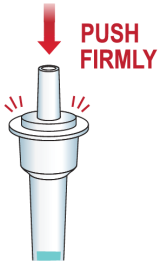
8 Place the swab into the extraction vial and rotate the swab at least 5 times **against the walls of the vial**.



9 Remove the swab by rotating against the extraction vial while squeezing the sides of the vial to release the liquid from the swab. Discard the swab in trash.



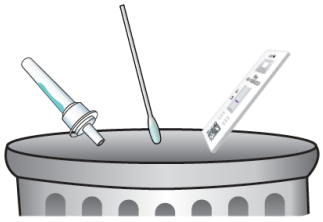
10 Close the vial by pushing the cap firmly onto the vial.



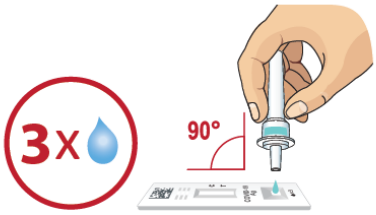
11 With your finger, mix thoroughly by flicking the bottom of the vial.

Disposal

Dispose of all used test kit components and swab samples in household trash.



12 Invert the extraction vial and hold the sample vertically above the sample well. Squeeze the vial gently. Allow **THREE (3)** drops of sample to fall into the sample well.



13 **Start a timer.**

Read the result at **10 minutes**. The test result should not be read after 15 minutes.

IMPORTANT

Do not move or lift the test cassette during this time. Do not exit the mobile app during this process.

Interpretation of Results

The test results will be interpreted by visual reading following the in-app interpretation instructions or provided Quick Reference Instructions.

NOTE: The test results should be read by visual and interpreted at 10 minutes after the sample application and the reading and interpretation of the results should not exceed 15 minutes as it may yield inaccurate results.

Serial Testing:

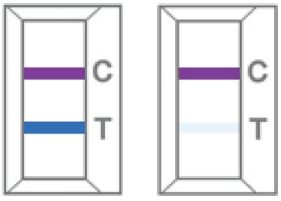
Repeat testing is needed to improve test accuracy. Please follow the table below when interpreting test results.

Status on first day of Testing	First Result Day 1	Second Result Day 2	Third Result Day 3	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 Detected (Positive *(+)*):

If the Purple-colored Control (C) line and the Blue-colored Test (T) line are visible, the test is positive. Any faint visible, Blue-colored Test (T) line with the Purple-colored Control line (C) should be read as positive.



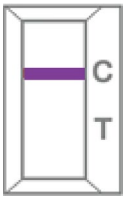
Repeat testing does not need to be performed if patients have a positive result at any time.

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 *CareStart*™ 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 Not Detected (Negative *(-)*):

If the Purple-colored Control (C) line is visible, but the Blue-colored Test (T) line is not visible, the test is negative.



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

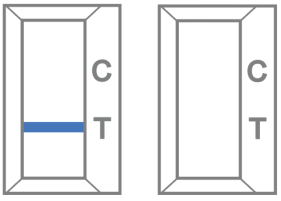
- **Test again in 48 hours if the individual has symptoms on the first day of testing.**
- **Test 2 more times at least 48 hours apart if the individual does not have symptoms on the first day of testing.**

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 Purple-colored Control (C) line is not visible or invalid barcode appears, the test is invalid. An invalid test result indicates that the test assay has experienced an error and unable to interpret the test result. Re-test with a new swab and new test device.



Report the test result(s) at MakeMyTestCount.Org (https://makemytestcount.org) – this voluntary and anonymous reporting helps public health teams understand COVID-19 spread in your area and across the country and informs public health decisions.

Limitations

1. The performance of this test was established based on the evaluation of a limited number of clinical specimens collected between March 2021 and April 2021. 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.
2. 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.
3. All COVID-19 antigen test negative results are presumptive and confirmation with a molecular assay may be necessary.
4. 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.
5. 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.
6. This test is read visually and has not been validate for use by those with impaired vision or color-impaired vision.
7. Incorrect test results may occur if a specimen is incorrectly collected or handled.
8. 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.
9. The detection of SARS-CoV-2 nucleocapsid antigen is dependent upon proper specimen collection, handling, storage, and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
10. False negative results may occur in patients who have indicated or whose clinical status or history would indicate they are currently taking high doses of biotin (> 10 mg per day). Biotin levels of 2.5 µg/mL and greater have been demonstrated to result in false negative test results.
11. Failure to follow the instructions for use may adversely affect test performance and/or invalidate the test result.
12. False negative results are more likely after seven days or more of symptoms.

13. Interpretation of any result after 15 minutes may yield inaccurate test results.
14. This test and the results from this test do not establish that the user has acquired immunity to COVID-19.
15. Extracted specimens are stable for 4 hours in extraction buffer at room temperature.
16. Results from antigen testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to determine infection status.
17. This device has been evaluated for use with human specimen material only.
18. Viral transport media (VTM) should not be used with this test.
19. False-negative results may occur if the concentration of the target antigen in the clinical specimen is below the detection limits of the device.
20. This device is a qualitative test and does not provide information on the viral concentration present in the specimen.
21. This test cannot rule out diseases caused by other bacterial or viral pathogens.
22. The prevalence of infection will affect the test's predictive values.
23. False positive results may occur, particularly in individuals without COVID-19 symptoms and/or individuals who live in areas with low numbers of COVID-19 and without known exposure to COVID-19.
24. Positive and negative predictive values are highly dependent on prevalence. False-negative test results are more likely during peak activity when the prevalence of the disease is high. False-positive test results are more likely during the periods of low SARS-CoV-2 activity when prevalence is moderate to low.
25. If the differentiation of specific SARS viruses and strains is needed, additional testing, in consultation with state or local public health departments, is required.

Performance Characteristics

Clinical Performance

The clinical performance characteristics of the *CareStart*™ COVID-19 Antigen Home Test using anterior nasal swab specimen were evaluated at seven (7) geographically diverse study sites in the U.S. between March 2021 and May 2021 against an FDA Emergency Use Authorized RT-PCR molecular assay as a comparator method. Subjects self-sampled and self-tested using the *CareStart*™ COVID-19 Antigen Home Test in a simulated home setting utilizing only the labeling provided with the test. A total of 153 subjects were evaluated in this study. The CareStart™ COVID-19 Antigen Home Test when conducted by a lay user correctly identified 87% of positive samples and 98% of negative samples. The overall clinical performance is shown in the following tables.

CareStart™ COVID-19 Antigen Home Test clinical performance against the comparator method

CareStart™ COVID-19 Antigen Home Test	Comparator		
	Positive	Negative	Total
Positive	26	3[a]	29
Negative	4[b]	120	124
Total	30	123	153
Positive Percent Agreement (PPA)	87% (26/30) (95% CI: 70%-95%)		
Negative Percent Agreement (NPA)	98% (120/123) (95% CI: 93%-99%)		

[a]*COVID-19 was detected in 0/3 False Positive specimens using another FDA authorized RT-PCR assay.*
[b]*COVID-19 was not detected in 2/4 False Negative specimens using another FDA authorized RT-PCR assay.*

Patient Demographics

Age Group	*CareStart*™ COVID-19 Antigen Home Test		
	Female	Male	Positivity Rate % (total positive / total tested)
2-13 Years of Age	6	2	0.0% (0/8)
14-24 Years of Age	16	10	15.4% (4/26)
25-64 Years of Age	69	34	22.3% (23/103)
≥65 Years of Age	9	7	12.5% (2/16)
Total	100	53	13.9% (29/153)

Positive results are broken down by days since onset of symptoms:

Days Since Symptom Onset	PPA (95% CI)	NPA (95% CI)
Asymptomatic	70.0% (7/10) (95% CI: 39.7%-89.2%)	97.6% (123/126) (95% CI: 93.2%-99.2%)
0-1	100% (5/5) (95% CI: 56.6%-100%)	96.8% (30/31) (95% CI: 83.8%-99.4%)
0-2	100% (11/11) (95% CI: 74.1%-100%)	94.8% (55/58) (95% CI: 85.9%-98.2%)
0-3	100% (20/20) (95% CI: 83.9%-100%)	96.3% (78/81) (95% CI: 89.7%-98.7%)
0-4	92.0% (23/25) (95% CI: 75.0%-97.8%)	97.1% (100/103) (95% CI: 91.8%-99.0%)

Days Since Symptom Onset	PPA (95% CI)	NPA (95% CI)
0-5	92.6% (25/27) (95% CI: 76.6%-97.9%)	97.3% (108/111) (95% CI: 92.4%-99.1%)
0-6	89.7% (26/29) (95% CI: 73.6%-96.4%)	97.3% (109/112) (95% CI: 92.4%-99.1%)
0-7	86.7% (26/30) (95% CI: 70.3%-94.7%)	97.5% (116/119) (95% CI: 92.9%-99.1%)

Invalid Test Rate: The overall invalid result rate within a total of 172 subjects that performed testing in a clinical study was 2.9% (5/172).

Clinical Performance (Asymptomatic Population)

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 the antigen test with serial testing in individuals is described in the table below.

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 Test	3 Test	1 Test	2 Test	3 Test
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.

Analytical Sensitivity: Limit of Detection (LoD)

The LoD for direct nasal swab was established using gamma-irradiated SARS-CoV-2 isolate USA-WA1/2020 (NR-52287). To prepare the positive sample, the strain was spiked into the pooled human nasal swab matrix obtained from multiple healthy volunteers eluted in PBS and confirmed as SARS-CoV-2 negative by RT-PCR. 50 µl of each positive sample dilution was dispensed onto a dry swab and was tested. The estimated LoD found from the initial two-fold serial dilution test was confirmed by testing 20 replicates. The confirmed LoD for direct swab was 2.8×10^3 $TCID_{50}$/ml. Based upon the testing procedure for this study the LOD of 2.8×10^3 $TCID_{50}$/ml equates to 1.4×10^2 $TCID_{50}$/swab.

NIH/ RADx® Variant Testing

The performance of this test 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 *CareStart*™ COVID-19 Antigen Home Test detected 100% of live virus Omicron samples at a Ct-value of 23.6 (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 23.6) were not detected by the *CareStart*™ COVID-19 Antigen Home Test in this study.

Omicron Pool 2 - Live Omicron Clinical Samples	Average N2 Ct (n=9)	Assay #1 Percent Positive (n = 5)	Assay #2 Percent Positive (n = 5)	CareStart COVID-19 Antigen Home Test Percent Positive (n=5)
Dilution 1	19.8	100	100	100
Dilution 2	20.8	100	100	100
Dilution 3	21.5	100	100	100
Dilution 4	22.7	100	100	100
Dilution 5	23.6	0	100	100
Dilution 6	24.0	0	60	40
Dilution 7	24.8	0	0	0
Dilution 8	25.8	0	0	0
Dilution 9	27.4	0	0	0
Dilution 10	28.1	0	0	0
Dilution 11	29.1	0	0	0

Analytical Specificity: Cross Reactivity (Exclusivity) and Microbial Interference

The potential cross-reactivity (exclusivity) of a panel of common organisms was evaluated with SARS-CoV-2 negative samples using the *CareStart*™ COVID-19 Antigen Home Test. Potential microbial interference was evaluated with samples containing gamma-irradiated SARS-CoV-2 isolate USA-WA1/2020 at approximately 3x LoD. A total of 10 bacteria were tested at a target concentration of approximately 10^7 cfu/ml with the exception of *Mycoplasma pneumoniae*, which was tested at a final concentration of 1.5 x 10^3 cfu/ml. The 18 viruses were tested at concentrations between $10^{5.2}$ and $10^{7.9}$ $TCID_{50}$/ml. All negative samples gave negative results at the concentrations of the potentially cross-reactive common organisms tested showing no cross-reactivity with *CareStart*™ COVID-19 Antigen Home Test assay. All samples with SARS-CoV-2 strain tested positive showing no microbial interference at the concentrations of the potentially interfering common organisms tested.

Potential Cross-Reactant		
Adenovirus 1	MERS-Coronavirus, Irradiated Lysate	*Bodetella pertussis*
Adenovirus 7	Parainfluenza virus type 1	*Candida albicans*

Potential Cross-Reactant		
Enterovirus 71, Tainan/4643/1998	Parainfluenza virus type 2	*Chlamydophila pneumoniae*
Human coronavirus (OC43)	Parainfluenza virus type 3	*Haemophilus influenzae*
Human coronavirus (229E)	Parainfluenza virus type 4	*Legionella pneumophila*
Human coronavirus (NL63)	Respiratory syncytial virus Type B	*Mycoplasma pneumoniae*
Human metapneumovirus (hMPV)	Rhinovirus	*Staphylococcus aureus*
Influenza A/Michigan/45/2015	SARS-Coronavirus	*Staphylococcus epidermidis*
Influenza B/Wisconsin/01/2010	Pooled human nasal wash	*Streptococcus pneumoniae*
		Streptococcus pyogenes, Group A

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

https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins&PROGRAM=blastp&BLAST_PROGRAMS=blastp&PAGE_TYPE=BlastSearch&BLAST_SPEC=blast2seq&DATABASE=n/a&QUERY=&SUBJECTS=

- The homology between SARS-CoV-2 nucleocapsid protein and human coronavirus HKU1 nucleocapsid protein is relatively low, at 36.7% across 86.4% of sequences, but cross-reactivity cannot be ruled out.
- The homology between SARS-CoV-2 nucleocapsid protein and *Mycobacterium tuberculosis* total protein (3,991 proteins) is relatively low, homology-based cross-reactivity cannot be ruled out.
- The homology between SARS-CoV-2 nucleocapsid protein and *Pneumocystis jirovecii* total protein (3,745 proteins) is relatively low, homology-based cross-reactivity cannot be ruled out.
- The homology between SARS-CoV-2 nucleocapsid protein and human coronavirus 229E nucleocapsid protein is relatively low, at 28.8% across 72.1% of sequences, but cross-reactivity cannot be ruled out. However, a result of the cross-reactivity wet study showed that CareStart™ COVID-19 Antigen Home Test had no cross-reactivity against human coronavirus 229E.
- No homologous protein was detected as a result of in silico assay with all the proteins (686 proteins) of *Mycoplasma pneumoniae* and the nucleocapsid protein (NP) of SARS-CoV-2, however cross-reactivity cannot be ruled out.

Endogenous Interfering Substances Effect

To assess substances with the potential to interfere with the performance of the *CareStart*™ COVID-19 Antigen Home Test, positive and negative samples were tested with the addition of potentially interfering substances. The SARS-CoV-2 target concentration in the positive samples

was approximately 2x LoD. All samples tested produced expected results, demonstrating that the *CareStart*™ COVID-19 Antigen Home Test performance was not affected by any of the 35 potentially interfering substances listed in the table below at the concentrations tested.

Potential Interfering Substances	Concentration	Potential Interfering Substances	Concentration
Acetaminophen	10 mg/ml	Oseltamivir Phosphate (Tamiflu)	5mg/ml
Acetyl salicylic acid	15 mg/ml	OTC Nasal Spray (Alkaol)	1:10 dilution
Beclomethasone	0.5 mg/ml	OTC Nasal Spray (Cromolyn Sodium)	15%
Benzocaine	5 mg/ml	OTC Naso GEL (NeilMed)	5%
Budesonide	2 mg/ml	OTC Sore Throat Phenol Spray	5%
Chlorpheniramine maleate	5 mg/ml	OTC Throat drop (Halls)	15%
Dexamethasone	1 mg/ml	OTC Throat drop (Ricola)	15%
Dextromethorphan HBr	2 mg/ml	OTC Nasal spray (Afrin)	15%
Diphenhydramine HCl	5 mg/ml	OTC Nasal spray (VicksSinex)	15%
Ephedrine HCl	10 mg/ml	OTC Nasal spray (Zicam)	15%
Flunisolide	5 mg/ml	Oxymetazoline HCl	10 mg/ml
Fluticasone	1 mg/ml	Phenylephrine HCl	5 mg/ml
Guaiacol Glyceryl Ether	20 mg/ml	Phenylpropanolamine	5 mg/ml
Histamine Dihydrochloride	10 mg/ml	Tobramycin	1 mg/ml
Menthol	10 mg/ml	Triamcinolone	1 mg/ml
Mometasone	1 mg/ml	Whole Blood	4%
Mucin	2%	Zanamivir	1 mg/ml
Mupirocin	1 mg/ml		

The interfering effects of biotin concentrations ranging between 625 ng/mL and 10 µg/mL were tested in a separate study. Biotin concentrations up to 1.25 µg/ml did not lead to false results. Biotin concentrations ≥2.5 µg/ml can cause false-negative COVID-19 results with the *CareStart*™ COVID-19 Antigen Home Test.

High-dose Hook Effect

The *CareStart*™ COVID-19 Antigen Home Test was tested up to 10^6 $TCID_{50}$/ml of gamma-irradiated SARS-CoV-2 isolate USA-WA1/2020 strain and no high-dose hook effect was observed.

Technical Support

For questions, or to report a problem, please call Technical Support at +1-888-898-1270 (Available Hours: Mon. to Fri.: 8 a.m. – 5 p.m.) or TShelp@accessbio.net (24/7 available).

Description of Symbols

Symbol	Descriptions
IVD	*In vitro* diagnostic medical device Indicates a medical device that is intended to be used as an *in vitro* diagnostic medical device.
	Consult instructions for use Indicates the need for the user to consult the instructions for use.
	Manufacturer Indicates the medical device manufacturer.
LOT	Batch code Indicates the manufacturer's batch code so that the batch or lot can be identified.
	Do not re-use Indicates a medical device that is intended for one use, or uses on a single patient during a single procedure.
	Use by date Indicates the date after which the medical device is not to be used.
Rx	Prescription-only

Symbol	Descriptions
REF	Catalog number Indicates the manufacturer's catalog number so that the medical device can be identified.
	Caution Indicates the need for the user to consult the instructions for use for important cautionary information such as warnings and precautions that cannot, for a variety of reasons, be presented on the medical device itself.
	Date of manufacture Indicates the date when the medical device was manufactured.
	Temperature limit Indicates the temperature limits to which the medical device can be safely exposed.
	Do not use if the package is damaged Indicates a medical device that should not be used if the package has been damaged or opened.
Σ	Contains sufficient for <n> tests Indicates the total number of IVD tests that can be performed with the IVD.

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