Rapid SARS-CoV-2 Antigen Test Card

Healthcare Provider Instructions for Use

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

1.1 Intended Use

The Rapid SARS-CoV-2 Antigen Test Card is a lateral flow immunoassay intended for the qualitative detection of nucleocapsid protein antigen from SARS-CoV-2. The test is authorized for non-prescription home use with self-collected anterior nasal (nares) swabs from individuals aged 14 years and older when tested twice over three days with at least 24 hours (and no more than 48 hours) between tests. The test is authorized for individuals aged 14 years and older with symptoms of COVID-19 within the first 6 days of symptom onset, or individuals without symptoms or other epidemiological reasons to suspect COVID-19.

This test is also authorized for non-prescription home use with adult-collected anterior nasal (nares) swab specimens from individuals aged 2 years and older when tested twice over three days with at least 24 hours (and no more than 48 hours) between tests. The test is authorized for individuals aged 2 years and older with symptoms of COVID-19 within the first 6 days of symptom onset, or individuals without symptoms or other epidemiological reasons to suspect COVID-19.

The Rapid SARS-CoV-2 Antigen Test Card does not differentiate between SARS-CoV or SARS-CoV-2 viruses.

Results are for the identification of SARS-CoV-2 nucleocapsid protein antigen. Antigen is generally detectable in anterior nasal (nares) swabs 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. The agent detected may not be the definite cause of disease. Individuals who test positive with the Rapid SARS-CoV-2 Antigen Test Card 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.

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. 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-like symptoms of fever, cough and/or shortness of breath may still have SARS-CoV-2 infection and should seek follow up care from their healthcare provider.

Individuals should provide all results obtained with this product to their healthcare provider for public health reporting. 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 Rapid SARS-CoV-2 Antigen Test Card is intended for non-prescription self-use and/or as applicable, for an adult lay user testing another aged 2 years or older. The Rapid SARS-CoV-2 Antigen Test Card is only for use under the Food and Drug Administration's Emergency Use Authorization.

1.2 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 Rapid SARS-CoV-2 Antigen Test Card is a rapid, qualitative immunochromatographic assay for the determination of the presence of SARS-CoV-2 antigens in anterior nasal swab specimens. Rapid SARS-CoV-2 Antigen Test Card employs a double antibody sandwich method. Colloidal gold conjugated anti-SARS-CoV-2 antibodies are dry-immobilized on the test device. When the specimen is added, it migrates by capillary diffusion through the strip to re-hydrate the gold conjugate complexes. If present at or above the limit of detection, SARS-CoV-2 viral antigens will react with the gold conjugate complexes to form particles, which will continue to migrate along the strip until the Test Zone (T) where they are captured by the immobilized anti-SARS-CoV-2 antibodies to form a visible pink/purple line. If there are no SARS-CoV-2 viral antigens in the specimen, no pink/purple line will appear in the Test Zone (T). The gold conjugate complexes will continue to migrate until being captured by immobilized antibody in the Control Zone (C) to form a pink/purple line, which indicates adequate fluid transfer past the test line to the control line of the test.

1.3 Materials Provided

Components	1 Test per Box	2 Tests per Box	4 Tests per Box	5 Tests per Box	8 Tests per Box	10 Tests per Box	20 Tests per Box	40 Tests per Box
Rapid SARS-CoV-2 Antigen Test Card (sealed foil pouch)	1	2	4	5	8	10	20	40
Sterilized Swab	1	2	4	5	8	10	20	40
Extraction Buffer Tube	1	2	4	5	8	10	20	40
Tube Holder	1 (packaging)	1 (packaging)	1	1	2	2	5	5
Quick Reference Instructions	1	1	1	1	2	2	5	5

1.4 Materials Required but not Provided

Clock or timer

1.5 Quality Control

Each Rapid SARS-CoV-2 Antigen Test Card has a built-in internal procedural control. The pink/purple 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. 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 Rapid SARS-CoV-2 Antigen Test Card in a home setting.

1.6 Test Procedures

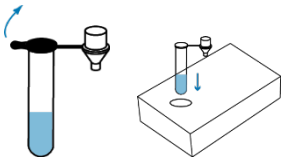
1 

Wash your hands with soap and water, or use hand sanitizer, before performing the test.

2 Check test expiration date on the test cassette pouch.

3 Bring the kit to room temperature when you are ready to begin the test.

4



When you are ready to perform the test, remove the seal from the buffer tube and place the tube in the tube holder.

Open it away from your face and be careful not to spill any of the liquid.

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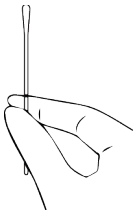

Peel open the swab packaging and gently take out the swab.

Be careful not to touch the soft, fabric tip of the swab.

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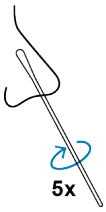

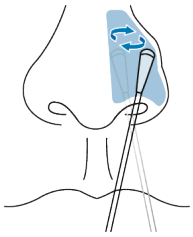

Holding the stick end of the swab, gently insert the entire absorbent tip of the swab into the nostril **no more than ½ to ¾ inch**. There is no need to go deeper.

Slowly rotate the swab in a circular motion **5 times** by firmly pressing against the inside walls of the nostril **for a total of 15 seconds. Do not just spin the swab.**

Gently remove the swab and repeat in the other nostril using the same swab.

WARNING: Inaccurate test results may occur if the nasal swab specimen is not properly collected.

When swabbing others, please wear a face mask. With children, you may not need to insert the swab as far into the nostril. For very young children, you may need another person to hold the child's head while swabbing.

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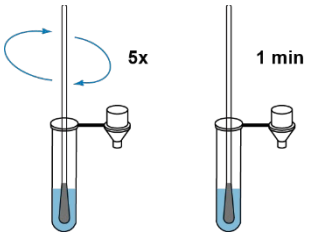

Place swab into buffer tube. **Rotate swab 5 times**.

Set a timer and leave swab in buffer tube for 1 minute.

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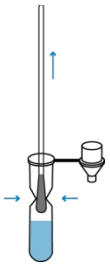

Pinch buffer tube with fingers and remove the solution from swab as much as possible.

WARNING: Failure to squeeze the tube can lead to incorrect results due to excess buffer in the swab.

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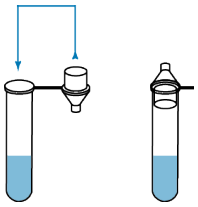
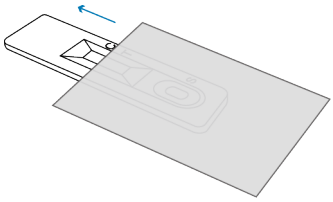


Press the cap onto the buffer tube until it is secure.

10



Open the pouch and remove the test cassette. Place the cassette on a flat and level surface.

WARNING: Once opened, the test cassette must be used within 30 minutes, otherwise inaccurate results may occur.

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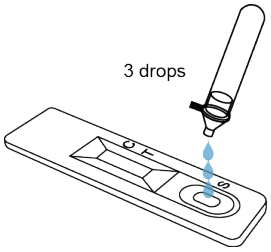

Invert the buffer tube and add 3 drops of test sample into the sample well (S) by gently squeezing the extraction tube. **Do not add to the rectangular results window.**

WARNING: Adding other then the recommended number of drops may result in inaccurate results.

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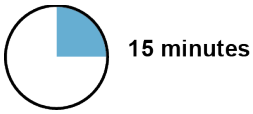


Set a timer and read the results at 15 minutes.

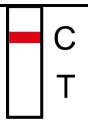
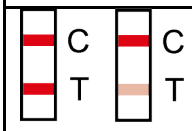
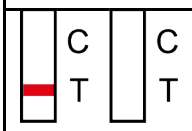
WARNING: Do not read the result before 15 minutes or after 30 minutes.

After test is completed, dispose of used materials in the trash.

1.7 Interpretation of Results

WARNING: Do not read the result before 15 minutes or after 30 minutes. Inaccurate test interpretations may occur.

Look at the result window and locate the letters C and T on the side of the window. A pink/purple line should always appear at the C position; this is a control line and signals that the test is working properly.

	**Negative result** If a control line (C) is visible (regardless of how faint it is) and a test line (T) is not visible, this means that the result is negative. A negative test result means that antigen from the virus that causes COVID-19 was not detected in your sample. Negative results do not rule out SARS-CoV-2 infection. All individuals that test negative should be tested again with at least 24 hours and no more than 48 hours between tests. All negative results are considered presumptive, and confirmation with a molecular assay, if necessary for patient management, may be performed.
	**Positive result** If a test line (T) is visible together with a control line (C), this means that the result is positive. A Positive test result is interpreted as protein antigen from the virus that causes COVID-19 was detected in the specimen. The individual is positive for COVID-19. Test results should be considered in association with the patient's history and epidemiological data (such as local prevalence rates and current outbreak/epicenter locations). Note: The Test line (pink/purple line) may vary in shade and intensity (light or dark, weak or strong) depending on the concentration of antigen present in the sample. The intensity of the Control line should not be compared to that of the Test line for the interpretation of the test result. Any faint visible pink/purple Test line should be interpreted as positive, when the control line (C) line is also present.
	**Invalid result** If a line does not appear on the control line position (C) in **15 minutes**, the test result is **invalid**. The test is not working correctly and you should perform another test using a different test kit. You may have performed the test incorrectly. Carefully read the Quick Reference Instructions and repeat the test. If your test result is still invalid, please contact a doctor or visit a COVID-19 test center.

1.8 Storage and Stability

- Rapid SARS-CoV-2 Antigen Test Card should be stored between 2 to 30 °C (35.6 to 86 °F).
- Kit components in the Rapid SARS-CoV-2 Antigen Test Card are stable until the expiration date printed on the label.
- The Test Device must remain in the sealed foil pouch until use.
- The shelf-life of the Rapid SARS-CoV-2 Antigen Test Card is 6 months and it is stable until the expiration date marked on the packaging.

1.9 Warnings and Precautions

Read the Rapid SARS-CoV-2 Antigen Test Card instructions carefully before performing a test. Failure to follow directions may produce inaccurate test results.

- For in vitro diagnostic use only.
- This product has not been FDA cleared or approved but has been authorized by FDA under an Emergency Use Authorization (EUA).
- 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 the 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.
- The test is intended to aid in the diagnosis of a current SARS-CoV-2 infection. Please consult a healthcare professional to discuss your results and if any additional testing is required.
- Keep test kit and kit components away from children and pets before and after use.
- Do not use on anyone under 2 years of age.
- Do not open the kit contents until ready for use. Use within 30 minutes of opening pouch.
- Do not use this test kit beyond the expiration date printed on the outside of the box.
- Do not use if any of the test kit contents or packaging is damaged.
- All test components are single-use. Do not reuse.
- Make sure there is sufficient light when testing.
- Do not use nasal sprays for at least 30 minutes before collecting a nasal sample.
- Remove any piercings from the nose before starting the test.
- Do not use on anyone who is prone to nosebleeds or has had facial injuries or head injuries/surgery in the past 6 months.
- Inadequate or inappropriate specimen collection may yield false negative test results.
- Do not touch the swab tip when handling the swab sample.
- Test immediately, but no more than 1 hour after collecting the sample on the swab and no more than 30 minutes after mixing into buffer.
- Do not read the test before 15 minutes or after 30 minutes. Results read before 15 minutes or after 30 minutes may lead to a false negative, false positive, or invalid result.
- Do not ingest any kit components.
- Avoid exposure of your skin, eyes, nose, or mouth to the solution in the tube.
- The chemicals in the reagent solution are hazardous to the skin and eye. Please see the below table for safety recommendations for skin and eye irritation.
- In the event of spillage, ensure that it is cleaned thoroughly using a suitable disinfectant.

- When collecting a sample, use only the nasal swab provided in the kit.
- Do not mix components of different test kit lots.
- A negative test result may occur if the level of antigen in a sample is below the detection limit of the test.
- All test materials must be at room temperature before use.
- Wear a face mask when collecting a specimen from another individual.
- Test devices that contain patient samples should be handled as though they could transmit disease. Follow universal precautions when handling samples, this kit, and its contents. Wear appropriate personal protection equipment (PPE) and gloves when running the test and handling a patient's test device. Change gloves between tests.
- Exposure to humidity may decrease the stability of the test. The test should be performed immediately after removing it from the pouch.
- Collect specimen and immediately perform test according to instructions.
- This test is read visually. Individuals with impaired vision or color-impaired vision may not be able to read the test.
- Wash hands thoroughly or use hand sanitizer after handling.
- Do not eat, drink, or smoke in the area where the specimens and kit contents are handled.
- Dispose of used specimens in in accordance with Federal, State, and Local requirements.
- This is a qualitative test, therefore quantitative values of SARS-CoV-2 antigen concentration cannot be determined.
- The immune response cannot be evaluated using this test. Other test methods are required for that purpose.
- The control line may show up within a few minutes of starting the test. It may take up to 15 minutes for the test line to show up.
- The solution in the tube contains a hazardous ingredient (see table below), which is harmful if inhaled, swallowed, or exposed to skin. Avoid contact with your skin, eyes, nose, or mouth. If the extraction solution contacts the skin or eye, flush with copious amounts of water. If irritation persists, seek medical advice: https://www.poison.org/contact-us or 1-800-222-1222

Hazard Category (mixture)	GHS Hazard Class for mixture	Labeling of Harm(s)	Hazardous Ingredients (%)	Recommended PPE Statement
Category 2/2A	Eye Irritation	Causes serious eye irritation (H319)	Sodium chloride 7647-14-5/1% TERGITOL 15-S-9/1%	Wear eye protection
Category 3	Skin Irritation	Causes mild skin irritation (H316)	TERGITOL 15-S-9/1%	NA

1.10 Limitations

- Children aged 2-13 years should be tested by an adult.

- Testing should be performed at least twice over three days, with at least 24 hours and no more than 48 hours between tests. You may need to purchase additional tests to perform this serial (repeat) testing.
- There is a higher chance of false negative results with home use tests than with laboratory-based molecular tests. This means that there is a higher chance this test will give you a negative result when you have COVID-19.
- Because it is not possible to know the viral load in a patient's sample prior to molecular testing, serial testing should be performed for all subjects (i.e., both symptomatic and asymptomatic) to increase the likelihood of detecting COVID-19.
- Serial testing (i.e., testing every day or every other day) is more likely to detect COVID-19.
- The 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.
- Failure to follow the test procedure correctly may result in false negative or false positive results and/or invalidate the test result.
- Test results must be evaluated in conjunction with other clinical data available to the physician.
- Positive test results do not exclude co-infection with other pathogens.
- Negative test results are not indicative of the presence/absence of other viral or bacterial pathogens.
- Negative results do not rule out COVID-19, should be treated as presumptive, and may need to be confirmed with an FDA-authorized molecular assay.
- Performance of this test in individuals with or without symptoms or other epidemiological reasons to suspect COVID-19 infection or for serial screening, when tested twice over two to three days with at least 24 but not more than 48 hours between tests has not yet been determined; a study to support use will be completed.
- Positive test results do not differentiate between SARS-CoV and SARS-CoV-2.
- If the differentiation of specific coronaviruses and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- False negative results may occur if a specimen is improperly collected or handled.
- This device is only used for testing direct human anterior nasal swab specimens. Viral transport media (VTM) should not be used with this test.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens collected between January 2022 to February 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.

2 Performance Characteristics

2.1 Analytical Sensitivity: Limit of Detection (LoD)

The Limit of Detection (LoD) of the Rapid SARS-CoV-2 Antigen Test Card was determined using serial dilutions of the gamma irradiated inactivated SARS-CoV-2 (USA-WA1/2020). Contrived samples were prepared by spiking the strain into the pooled negative nasal wash obtained from healthy volunteers confirmed negative by RT-PCR. The preliminary LoD initially determined by testing two-fold serial dilution series of 3 replicates was confirmed by testing in 20 replicates. The confirmed LoD for the Rapid SARS-CoV-2 Antigen Test Card was 1.4×10^2 $TCID_{50}$/mL.

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. The clinical specimens used to prepare this dilution series were not identical to the previous specimen pools prepared 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 Rapid SARS-CoV-2 Antigen Test Card detected 100% of live virus Omicron samples at a Ct-value of 24.8 (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 25.8) were not detected by the Rapid SARS-CoV-2 Antigen Test Card 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)**	**Rapid SARS-CoV-2 Antigen Test Card Percent Positive (n=5)**
Omicron-Dilution 1	19.8	100	100	100
Omicron-Dilution 2	20.8	100	100	100
Omicron-Dilution 3	21.5	100	100	100
Omicron-Dilution 4	22.7	100	100	100
Omicron-Dilution 5	23.6	100	0	100
Omicron-Dilution 6	24.0	60	0	100
Omicron-Dilution 7	24.8	0	0	100
Omicron-Dilution 8	25.8	0	0	0

Omicron Pool 2 – Live Omicron Clinical Samples	Average N2 Ct (n=9)	Assay #1 Percent Positive (n=5)	Assay #2 Percent Positive (n=5)	Rapid SARS-CoV-2 Antigen Test Card Percent Positive (n=5)
Omicron-Dilution 9	27.4	0	0	0
Omicron-Dilution 10	28.1	0	0	0
Omicron-Dilution 11	29.1	0	0	0

2.2 High-dose hook effect

The Rapid SARS-CoV-2 Antigen Test Card was tested up to 2.8 x 10^5 $TCID_{50}$/mL of gamma irradiated inactivated SARS-CoV-2 (USA-WA1/2020) and no high-dose hook effect was observed.

2.3 Endogenous Interfering Substances

The Rapid SARS-CoV-2 Antigen Test Card 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 were tested with the addition of potentially interfering substances. The performance of Rapid SARS-CoV-2 Antigen Test Card was not affected by any of the potentially interfering substances listed in the table below at the concentrations tested.

Potentially Interfering Substance	Concentration Tested	Potentially Interfering Substance	Concentration Tested
Human Whole Blood (EDTA tube)	4% v/v	Mupirocin	10 mg/mL
Mucin (porcine stomach, type II)	0.5%	Tamiflu (Oseltamivir Phosphate)	5 mg/mL
Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL	Fluticasone Propionate	5% v/v
Naso GEL (NeilMed)	5% v/v	Body & Hand lotion (Cerave)	0.5%w/v
Nasal Drops (Phenylephrine)	15% v/v	Body Lotion with 1.2% dimethicone	0.5%w/v
Nasal Spray (Oxymetazoline)	15% v/v	Hand Lotion (Eucerin)	5% w/v
Nasal Spray (Cromolyn)	15% v/v	Hand Sanitizer with Aloe, 62% ethyl alcohol	5% v/v
Zicam	5% v/v	Hand Sanitizer cream lotion (vaseline)	15% v/v
Homeopathic (Alkalol)	10% v/v	Hand Sanitizer, 80% ethanol, fast drying	15% v/v
Sore Throat Phenol Spray	15% v/v	Hand Soap liquid gel (soft soap)	10% w/v
Tobramycin	4 µg/mL		

2.4 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 and virus (13 bacteria and 16 viruses) was tested in both the absence and presence of gamma irradiated inactivated SARS-CoV-2 (SARS-CoV-2 isolate USA-WA1/2020) at 3x LoD. All testing samples were prepared in the pooled negative nasal wash (PNW). No cross reactivity or interference was observed for any of the organisms tested, except for SARS-coronavirus which exhibited cross-reactivity when tested as 7.9×10^3 $TCID_{50}$/mL. A titration of SARS-CoV was performed to find the concentration at which cross-reactivity was no longer observed. Cross reactivity was no longer observed for SARS-CoV at 7.9×10^0 $TCID_{50}$/mL. These results are not unexpected in that the Rapid SARS-CoV-2 Antigen Test Card targets the nucleocapsid protein which is present on both SARS-CoV and SARS-CoV-2 viruses.

Microorganism	Concentration	Microorganism	Concentration
Human coronavirus 229E	1.43×10^5 $TCID_{50}$/mL	Rhinovirus	1.43×10^5 $TCID_{50}$/mL
Human coronavirus OC43	8.50×10^4 $TCID_{50}$/mL	*Haemophilus influenzae*	1.0×10^6 cfu/mL
Human coronavirus NL63	5.85×10^4 $TCID_{50}$/mL	*Streptococcus pneumonia*	1.0×10^6 cfu/mL
SARS-coronavirus*	7.9×10^0 $TCID_{50}$/mL	*Streptococcus pyogenes*	1.0×10^6 cfu/mL
MERS-coronavirus	1.0×10^6 $TCID_{50}$/mL	*Candida albicans*	1.0×10^6 cfu/mL
Adenovirus	1.43×10^5 $TCID_{50}$/mL	*Bordetella pertussis*	5.0×10^3 cfu/mL
Human metapneumovirus 4 Type B2	1.43×10^5 $TCID_{50}$/mL	*Mycoplasma pneumonia*	1.0×10^6 cfu/mL
Parainfluenza virus 1	1.43×10^5 $TCID_{50}$/mL	*Chlamydia pneumoniae*	1×10^6 ifu/mL
Parainfluenza virus 2	1.43×10^5 $TCID_{50}$/mL	*Legionella pneumophila*	1.0×10^6 cfu/mL
Parainfluenza virus 3	1.43×10^5 $TCID_{50}$/mL	*Mycobacterium tuberculosis*	1.0×10^6 cfu/mL
Parainfluenza virus 4b	1.43×10^5 $TCID_{50}$/mL	*Pneumocystis carinii*	1.0×10^6 nuclei/mL
Influenza A	1.43×10^5 $CEID_{50}$/mL	*P. jiroveci-S. cerevisiae*	1.0×10^6 cfu/mL
Influenza B	1.43×10^5 $CEID_{50}$/mL	*Staphylococcus aureus* subsp. *Aureus*	1.0×10^6 cfu/mL
Enterovirus 68	1.43×10^5 $TCID_{50}$/mL	*Staphylococcus epidermidis*	1.0×10^6 cfu/mL
Respiratory syncytial virus	1.0×10^5 pfu/mL	Pooled Negative Matrix	N/A

*Cross reactivity was observed when testing SARS-coronavirus concentrations at 7.9×10^3, 7.9×10^2, and 7.9×10^1 $TCID_{50}$/mL.

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. HKU1 nucleocapsid phosphoproteins was analyzed and the results are below.

- The homology between SARS-CoV-2 nucleocapsid protein and human coronavirus HKU1 nucleocapsid phosphoproteins is relatively low, 36.74% across 82% of sequences, but cross-reactivity cannot be ruled out.

2.5 Flex Study

A robust use of Rapid SARS-CoV-2 Antigen Test Card was demonstrated by seven (7) flex studies as follows;

1) Non-level positioning of Test Device
2) Varying the swab rotation number
3) Sample volume variability
4) Result reading time variability
5) Temperature and humidity
6) Lighting conditions
7) Disturbance while testing

2.6 Clinical Evaluation

A prospective study was completed at three (3) sites in the United States for clinical validation of the Boson Rapid SARS-CoV-2 Antigen Test Card in subject-collected anterior nasal (AN) swab samples. The study evaluated the investigational test's performance in symptomatic individuals (those suspected of COVID-19). A total of 186 symptomatic subjects were enrolled and each were currently experiencing symptoms associated with COVID-19, **within 6 days of symptom onset**. Each enrolled subject either self-collected one sample from their anterior nasal passages (from both nostrils), or had one sample collected from him/her by another individual. Each subject then had a mid-turbinate nasal swab sample collected from him/her by one of the study personnel. Test results from the Boson Rapid SARS-CoV-2 Antigen Test Card (candidate test) were compared to highly sensitive molecular FDA EUA Authorized SARS-CoV-2 assays to determine test performance. An analysis was performed which identified that 21/72 (29%) of study subjects had low viral loads based on the Ct values from a comparator method RT-PCR test. This may be associated with the Omicron variant since the low positive percentage in this study is higher than that observed in prior clinical studies for previously authorized COVID-19 rapid antigen tests. Antigen test performance decreases as the percent of low positives increases since the comparator method is significantly more sensitive than the candidate test. Therefore, to be consistent with previous studies, the analysis for the primary performance calculation was conducted to reflect a study population with 10-20% low positives. Multiple Percent Positive Agreement (PPA) are presented below for the positive samples cohort when a range of low positive samples was included (10% to 20%). At 10% low positives, the PPA was 82.7% and the negative percent agreement (NPA) was 99% with 95% confidence interval bounds of 71.1% to 90.4% for PPA and 95.2% to 99.6% for NPA respectively. This was the basis of the authorization. At 20% low positives, the PPA was 73.8% with 95% confidence interval bounds of 62.0% to 83.0%.

Primary Analysis					
	10% Low Positive	**12.5% Low Positive**	**15% Low Positive**	**17.5% Low Positive**	**20% Low Positive**
High Positive Samples	52	52	52	52	52
Low Positive Samples	6	8	10	12	13
Total Comparator Positive for PPA Calculation	58	60	62	64	65
Total Test Positives for PPA Calculation	48	48	48	48	48
PPA (%)	82.7%	80.0%	77.4%	75.0%	73.8%
95% CI (XX% - XX%)	71.1% - 90.4%	68.2% - 88.2%	65.6% - 86.0%	63.2%- 84.0%	62.0%- 83.0%
NPA (%)	99.1% (112/113)				
95% CI (XX%-XX%)	95.2%-99.8%				

When all study participants are included, the PPA is 67.1% and the NPA is 99.1% with the 95% confidence interval bounds of 55.7% to 76.8% for the PPA and 95.2% to 99.8% for the NPA, respectively

Age Distribution and Positive Rate for Symptomatic Subjects within First 6 Days of Symptom Onset			
	Positivity Rate		
Age Group	**Number of Specimens**	**Number of Positives**	**Positivity Rate**
2 to 13 years	42	11	26.2%
14 to 24 years	35	8	22.9%
25 to 64 Years	91	26	28.6%
65 Years and older	18	5	27.8%
Total	186	50	26.9%

Percent Agreement of the Boson Rapid SARS-CoV-2 Antigen Test Card vs Composite Comparator by Cumulative Symptoms Onset Day				
Days of COVID-19 Symptoms	**Number of Specimens Tested**	**Boson Positives (TP)**	**Composite Comparator Positives**	**Percent Agreement**
Day 0	13	0	6	PPA: 0%
Day 1	55	16	22	PPA: 72.7%
Day 2	54	18	22	PPA: 81.8%
Day 3	27	9	10	PPA: 90%

Day 4	17	3	7	PPA: 42.9%
Day 5	10	2	3	PPA: 66.7%
Day 6	10	1	3	PPA: 33.3%
Total	186	49	73	PPA: 67.1%

3.1 Technical Support

For questions, or to report a problem, please call Technical Support at 888-352-8394 (Available Hours: Mon. to Fri.: 9 a.m. – 5 p.m. PST) or support@bosoncovt.com.

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).

3.2 Ordering and Contact Information

Xiamen Boson Biotech Co., Ltd.
Tel: +1-888-352-8394
Email: info@bosoncovt.com

3.3 International Symbol Usage

You may see one or more of these symbols on the labelling/packaging of this product:

	Use-by date		Batch code		*In vitro* diagnostic medical device
	Catalog number		Consult instructions for use		Manufacturer
	Contains sufficient for <n> tests		Temperature limit		Do not reuse

Manufacturer: 

Xiamen Boson Biotech Co., Ltd.
90-94 Tianfeng Road, Jimei North Industrial Park,
Xiamen, Fujian, 361021, P.R.China.
Tel: +1-888-352-8394
Email: support@bosoncovt.com
www.bosoncovt.com

Number: XXXXXX

Effective Date: 2022-04-06