I. Intended Use

The Biomerica COVID-19 Antigen Rapid Test is a lateral flow chromatographic immunoassay for rapid, qualitative detection of nucleocapsid protein antigen from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in swab specimens. The test is intended for professional *in vitro* diagnostic use only.

When testing for SARS-CoV-2 infections, antigens commonly can be detected in upper respiratory specimens during the acute phase of infection. Positive results indicate the presence of nucleocapsid protein antigen, but clinical correlation with patient history and additional diagnostic information is necessary to determine infection status. The agent detected may not be the definite cause of disease.

Negative results do not exclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions. Negative results should be treated as presumptive and if necessary, confirmed for patient management with a molecular assay. Negative results should be considered in the context of a patient's recent exposures, history, and presence of clinical signs and symptoms consistent with COVID-19.

II. Summary and Explanation

Coronaviruses (CoVs) belong to the subfamily Orthocoronavirinae in the family Coronaviridae and the order Nidovirales. Coronaviruses are a large family of viruses that are common in people and many different species of animals, including camels, cattle, cats, and bats. Rarely, animal coronaviruses can infect people and then spread between people. A human coronavirus (SARS-CoV) caused the severe acute respiratory syndrome coronavirus (SARS) outbreak in 2003 followed by MERS-CoV in 2012.[1] Most recently, a SARS-related CoV was implicated as the etiological agent responsible for the outbreak in Wuhan, central China. This outbreak is estimated to have started on 12th December 2019.[2] This virus has been called COVID-19, novel coronavirus (2019-nCoV), SARS-CoV-2, and other similar names. The virus has rapidly spread to other countries by travelers from China.[2] Typical symptoms are fever, malaise, shortness of breath, nasal congestion, runny nose, sore throat, myalgia, diarrhea, and in severe cases, pneumonia, severe acute respiratory syndrome, kidney failure and even death.[3-5] The disease was first called unidentified viral pneumonia.

The SARS-CoV-2 virus is a betacoronavirus, like MERS-CoV and SARS-CoV. All three of these viruses have their origins in bats. The sequences from U.S. patients are similar to the one that China initially posted, suggesting a likely single, recent emergence of this virus from an animal reservoir.[1]

Early on, many of the patients at the epicenter of the outbreak in Wuhan, Hubei Province, China had some link to a large seafood and live animal market, suggesting animal-to-person spread. Later, a growing number of patients reportedly did not have exposure to animal markets, indicating person-to-person spread. Person-to-person spread was subsequently reported outside Hubei and in countries outside China, including in the United States. Nearly all countries now have ongoing community spread of the virus that causes COVID-19, as do all states in the US. Community spread means some people have been infected and it is not known how or where they became exposed.[1]

III. Principle of the Test

Antibodies to SARS-CoV-2 nucleocapsid protein are coated on the test line region. When specimen is added to the test, it reacts with latex particles conjugated to antibodies to SARS-CoV-2 nucleocapsid protein. The mixture then migrates through the membrane via capillary action and reacts with the antibodies to SARS-CoV-2 on the test line. If the specimen contains SARS-CoV-2 antigens, a pink/red colored line will appear in the test line region. If the specimen does not contain antigens to SARS-CoV-2, no pink/red colored line will appear at the test line region, indicating a negative result. To serve as a procedural control, a colored line will always appear in the control line region to indicate sufficient volume of specimen has been added and membrane wicking has occurred.

IV. Materials Provided

1. Test device in sealed foil pouch with desiccant
2. Sterile swabs
3. Instructions for use
4. Workstation
5. Specimen collection tubes (Kit contains either A or B)
   A. Tubes pre-filled with extraction buffer (use specimen preparation procedure A in section IX)
   B. Unfilled tubes and vial of extraction buffer (use specimen preparation procedure B in Section IX)

V. Materials Required but Not Provided

1. Timer
2. Personal protective equipment, such as protective gloves, medical mask, eye protection and lab coat or gown
3. Appropriate biohazard waste container and disinfectants

VI. Warnings and Precautions

1. This test is for *in vitro* diagnostic use only.
2. This package insert must be read completely before performing the test. Failure to follow instructions for use may yield inaccurate test results.
3. Freshly collected specimens should be processed as soon as possible, but no later than one hour after specimen collection.
4. Extracted specimens for PCR tests or Viral Transport Media (VTM) specimens have not been validated and cannot be used with the assay.
5. Only Biomerica's Specimen collection tubes with extraction buffer have been validated with the assay.
6. Frozen specimens have not been validated and cannot be used with the assay.
7. All specimens should be treated as capable of transmitting diseases. Use appropriate precautions and follow Biosafety Level 2 or higher guidelines.
8. Wear appropriate personal protective equipment (e.g., gowns, gloves, masks, eye protection) in the collection, handling, storage and disposal of patient samples and used kit contents.
9. Proper specimen collection storage and transport are critical to the performance of this test.
10. Do not eat, drink, or smoke in the area where the specimens or kits are handled.
11. The test is single use only. Discard after use.
12. Do not touch the test window of the device.
13. Do not use test beyond the expiration date.
14. Do not use the device if the pouch is punctured or not sealed.
15. This test should only be used under ambient conditions. High humidity and temperature of the testing environment can adversely affect results.
16. This test should only be used by professionally trained staff using sample(s) that have been collected by qualified medical personnel.
17. The test result should be interpreted by a physician or qualified medical professional along with clinical findings and other laboratory test results.
18. Do not open the test device (cassette).
19. Wash hands thoroughly after handling specimens or used device components.
20. BIOHAZARDOUS WASTE DISPOSAL: All specimens and the used device components carry risk of infection. The process of disposing of used device components must follow the local regulations for biohazardous waste disposal.
21. Only the provided sterilized swab in the package should be used for specimen collection.

VII. Storage and Stability

Store the tests at 2-30°C; **DO NOT FREEZE**. The tests are usable through the expiration date printed on the sealed pouch. Do not use beyond the expiration date. The tests must remain in the sealed pouch until used.

VIII. Quality Control

A procedural control is included in the test. A colored line appearing in the control region (C) is considered the internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking, and correct procedural technique.

Positive and Negative Controls are not supplied with this kit. Users should follow Good Laboratory Practices which recommend the daily use of positive and negative controls to confirm the test procedure and to verify proper test performance. Users should follow all applicable federal, state, and local guidelines concerning the frequency of assaying external quality control materials.

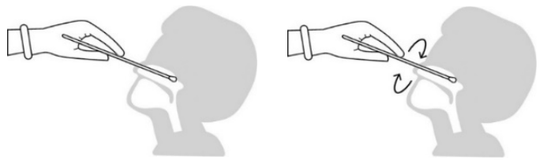
IX. Preparation of Materials and Sample Collection

If tests have been stored between 2-14°C, allow test device and extraction buffer to reach room temperature (15° - 30°C) for 30 minutes prior to testing. Use test device within one hour after removing from sealed pouch. It is critical that the specimen collection and preparation methods outlined in this insert are followed.

The Biomerica COVID-19 Antigen Rapid Test should be tested with swab specimens.
Only the extraction buffer and tubes provided in the kit are to be used for swab sample collection. A workstation has been provided with the kit. Use this workstation to facilitate collection and extraction of sample.

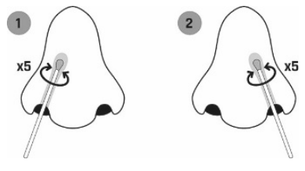
Nasopharyngeal Swab Specimen Collection:

1. Insert a sterile swab into the nostril of the patient reaching the surface of the posterior nasopharynx.
2. Swab over the surface of the posterior nasopharynx at least **two times**.
3. Withdraw the sterile swab from the nasal cavity.



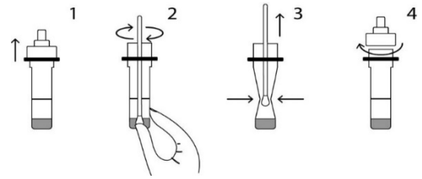
Nasal Swab Specimen Collection:
(NOTE: Nasal Swab Specimen Collection can be performed by professionally trained staff or by patient while being observed by professionally trained staff.)

1. Remove the swab from the wrapper, being careful not to touch the soft end, which is the absorbent tip. Break the swab in half at the score mark. Discard the half without the absorbent tip.
2. Insert a sterile swab about 2 cm into a nostril until there is a gentle resistance.
3. Slowly rotate the swab in a circular path 5-10 times against the inside of the nostril wall.
4. Withdraw the swab from the nostril and repeat in the other nostril.



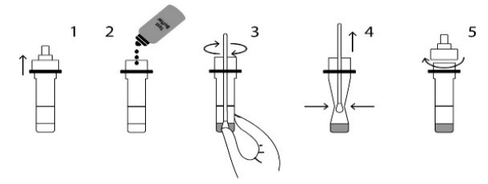
A. Specimen Preparation using specimen collection tubes WITH extraction buffer:
NOTE: Freshly collected specimens should be processed as soon as possible.

1. Unscrew the cap of the specimen collection tube.
2. Insert the swab specimen into the specimen collection tube. Press against the inner wall of the tube and stir the swab for approximately 10 seconds while pressing the swab head against the inner wall of the tube to release antigens into the collection tube.
3. Remove the swab while squeezing the sides of the tube to extract any liquid from the swab.
4. Tighten the cap onto the specimen collection tube.
5. Mix gently by inversion 2-3 times after the extraction. Do not shake or vortex.



B. Specimen Preparation using specimen collection tubes WITHOUT extraction buffer:
NOTE: Freshly collected specimens should be processed as soon as possible.

1. Unscrew the cap of the specimen collection tube.
2. Add 10 drops of Extraction Buffer to the collection tube.
3. Insert the swab specimen into the specimen collection tube. Press against the inner wall of the tube and stir the swab for approximately 10 seconds while pressing the swab head against the inner wall of the tube to release antigens into the collection tube.
4. Remove the swab while squeezing the sides of the tube to extract any liquid from the swab.
5. Tighten the cap onto the specimen collection tube.
6. Mix gently by inversion 2-3 times after the extraction. Do not shake or vortex.



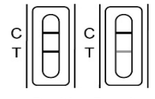
NOTE: If samples cannot be run immediately, the extracted sample is stable for up to 2 hours at room temperature or 24 hours at 2-8°C. **DO NOT FREEZE** extracted specimens.

X. Test Procedure

NOTE: If extracted specimen has been stored at 2-8°C**,** bring to room temperature (15°C to 30°C) prior to testing.

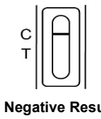
1. Remove the test device from its sealed pouch and place the device on a level surface.
2. Set a timer for 15 minutes.
3. Invert the specimen collection tube and add **3 drops** of the extracted specimen to the specimen well (S) and then start the timer.
4. Do not move the test while it is running.
5. Wait for the colored line(s) to appear. Read the result at **15 minutes**. Do not interpret the result after 20 minutes.

XI. Interpretation of Results



Positive Results

POSITIVE: If the sample contains SARS-CoV-2 antigens, a pink/red-colored band next to the "T" in the test window will appear.
NOTE: Any shade of pink/red color next to the "T" should be considered positive.



Negative Result

NEGATIVE: A single colored band appears next to the "C". No pink/red-colored band appears next to the "T".

Invalid Results

INVALID: An absence of a colored band next to the "C" regardless of the appearance of a colored band next to "T". Note: Insufficient sample volume, most common reasons for invalid results. The sample should be retested using a new test device.

XII. Performance Characteristics

Sensitivity and Specificity Summary Table – Nasopharyngeal Swab Specimen

Method		RT-PCR		Total Results
		Positive	Negative	
COVID-19 Antigen Test	Positive	143	1	144
	Negative	8	296	304
Total Results		151	297	448

Sensitivity: 94.7% (95%CI*: 89.8% to 97.7%)
Specificity: 99.7% (95%CI*: 98.1% to 99.9%)**
Accuracy: 98.0% (95%CI*: 96.2% to 99.1%)
*Confidence Interval
**Specificity study contains n=150 asymptomatic individuals without known exposure to SARS-CoV-2

Sensitivity and Specificity Summary Table – Nasal Swab

Method		RT-PCR		Total Results
		Positive	Negative	
COVID-19 Antigen Test	Positive	74	0	74
	Negative	6	61	67
Total Results		80	61	141

Sensitivity: 92.5% (95%CI*: 84.4% to 97.2%)
Specificity: 100.0% (95%CI*: 94.1% to 100.0%)
Accuracy: 95.7% (95%CI*: 91.0% to 98.4%)
*Confidence Interval

Specificity Testing with Various Viral Strains
The COVID-19 Antigen Rapid Test was evaluated with the following viral strains. No test line was observed at the concentrations listed:

Description	Test Level	Description	Test Level
Adenovirus type 3	3.16 x 10^4 $TCID_{50}$/ml	Influenza A H1N1	3.16 x 10^5 $TCID_{50}$/ml
Adenovirus type 7	1.58 x 10^5 $TCID_{50}$/ml	Influenza A H3N2	1 x 10^5 $TCID_{50}$/ml
Human coronavirus 229E	5 x 10^5 $TCID_{50}$/ml	Influenza B	3.16 x 10^6 $TCID_{50}$/ml
Human coronavirus HKU1	1 x 10^6 $TCID_{50}$/ml	Measles	1.58 x 10^4 $TCID_{50}$/ml
Human coronavirus NL63	1 x 10^6 $TCID_{50}$/ml	Mumps	1.58 x 10^4 $TCID_{50}$/ml
Human coronavirus OC43	1 x 10^6 $TCID_{50}$/ml	Parainfluenza virus 2	1.58 x 10^7 $TCID_{50}$/ml
Human rhinovirus 14	1.58 x 10^6 $TCID_{50}$/ml	Parainfluenza virus 3	1.58 x 10^8 $TCID_{50}$/ml
Human rhinovirus 16	8.89 x 10^6 $TCID_{50}$/ml	Respiratory syncytial virus	8.89 x 10^4 $TCID_{50}$/ml
Human rhinovirus 2	2.81 x 10^4 $TCID_{50}$/ml		

Cross-reactivity
Specimens that tested positive to the following potentially cross-reacting organisms were tested with the Biomerica COVID-19 Antigen Rapid Test without any effect on the expected results.

Arcanobacterium	*Pseudomonas aeruginosa*
Candida albicans	*Staphylococcus aureus subspaureus*
Corynebacterium	*Staphylococcus epidermidis*
Escherichia coli	*Streptococcus pneumoniae*
Moraxella catarrhalis	*Streptococcus pygenes*
Neisseria lactamica	*Streptococcus salivarius*
Nesseria subllava	*Streptococcus sp group F*

SARS-CoV cross-reactivity has not been established. There have been no cases of SARS-CoV since 2004 (only 8098 cases total worldwide were identified in mid-July 2003).[6,7]

MERS-CoV cross-reactivity has not been established. MERS-CoV is limited to the Arabian Peninsula and to recent travelers from that part of the world. Fewer than 2600 cases have been identified worldwide. 62 cases identified from 01 Jan through 02 Dec 2020 were reported in Saudi Arabia, Qatar, and the UAE. If SARS-CoV-2 is suspected and the patient is from the Arabian Peninsula or has recently traveled there (especially if the patient had any contact with camels), MERS-CoV should be tested for as well.[8]

To estimate the likelihood of cross-reactivity with SARS-CoV-2 virus in the presence 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.[9]

- No protein sequence homology was found between *M. tuberculosis* and SARS-CoV-2; thus homology-based cross-reactivity can be ruled out.
- Homology between the nucleocapsid proteins of SARS-CoV-2 and MERS-CoV is relatively low, at 48.5% across the sequence.
- Homology for SARS-CoV-2 and SARS-CoV is high, at 90.3% across the sequence; therefore, cross-reactivity cannot be ruled out.

Interference Study
The following substances were found not to interfere with the test:

Budesonide Nasal Spray	200 µL/mL	Phenylephrine	12 mg/mL
Dexamethasone	0.8 mg/mL	Rebetol	4.5 µg/mL
Flunisolide	6.8 ng/mL	Relenza	282 ng/mL
Mucin	50 µg/mL	Tamiflu	1.1 µg/mL
Mupirocin	12 mg/mL	Tobryamycin	2.43 mg/mL
Oxymetazoline	0.6 mg/mL	Whole Blood	20 µL/mL

Limit of Detection
The limit of detection for the test is 100 pg/mL of recombinant nucleocapsid protein expressed and purified from mammalian cells.

Inter-day, Inter-lot, Study
A negative specimen, a weak positive specimen, and a strong positive specimen were run in triplicates in 3 separate lots of COVID-19 Antigen Rapid Test. Ten replicates of each level were tested each day for 3 consecutive days. The specimens were correctly identified >99% of the time.

XIII. Limitations of the Test

1. The COVID-19 Antigen Rapid Test should be used for the qualitative detection of SARS-CoV-2 in human swab specimens. This test is not intended to determine the quantitative concentration or rate of increase of SARS-CoV-2 antigens.
2. The COVID-19 Antigen Rapid Test will only indicate the presence of SARS-CoV-2 antigen in the specimen and should not be used as the sole criteria for the diagnosis of SARS-CoV-2 infections. As with all diagnostic tests, all results must be considered with other clinical information available to the physician (e.g., signs and symptoms of COVID-19 and other diagnostic tests for SARS-CoV-2).
3. In conjunction with antigen test results, it is recommended to use nucleic acid detection for confirmation of SARS-CoV-2 infection. A definitive diagnosis should only be made after all clinical and laboratory findings have been evaluated.
4. The accuracy of the test depends on the sample collection process. Improper sample collection, improper sample storage, or repeated freezing and thawing of the sample will affect the test results.
5. Extracted specimens for PCR tests or Viral Transport Media (VTM) specimens have not been validated and cannot be used with the assay.
6. Freshly collected specimens should be processed as soon as possible, but no later than one hour after specimen collection.
7. Only Biomerica's Specimen collection tubes with extraction buffer have been validated with the assay.
8. Frozen specimens have not been validated and cannot be used with the assay.
9. Positive test results do not rule out co-infections with other pathogens including other coronavirus species.
10. A negative result of this test can be caused by:
    a) Improper sample transfer or handling.
    b) The level of SARS-CoV-2 antigens is below the detection limit of the test.
    c) SARS-CoV-2 antigens have not appeared at the time of the sample was collected.
    d) Variations in viral genes may cause changes in antibody affinity for the SARS-CoV-2 test reagent.
11. If the test results are negative and clinical symptoms persist, additional follow-up testing using other clinical and laboratory methods is suggested.
12. Color blind operators should not read or interpret test results.
13. Cross-reactivity between SARS-CoV-2 and SARS-CoV or MERS-CoV cannot be ruled out.

XIV. References

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For Export Use Only

COVID-19 Antigen Rapid Test
(Nasopharyngeal Swab)

April 2021

REF **1509A**

Immunoassay test kit for the qualitative detection of SARS-CoV-2 nucleocapsid protein antigen in human swab specimens.

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