LABORATORY NAME:	
LABORATORY ADDRESS:	
DATE OF THIS PACKET:	
INSERT REVISION:	IN195000 Rev.1 2020/08/25

BinaxNOW™ COVID-19 Ag Card

Part Number #195-000 (40 Test Kit) Laboratory Procedure

This procedure is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete package insert. **Any modifications to this document are the sole responsibility of the Facility.**

For Use Under an Emergency Use Authorization (EUA) Only
For use with nasal swab specimens
For *in vitro* Use Only
Rx Only

1. Intended Use

The BinaxNOW™ COVID-19 Ag Card is a lateral flow immunoassay intended for the qualitative detection of nucleocapsid protein antigen from SARS- CoV-2 in direct nasal swabs from individuals suspected of COVID-19 by their healthcare provider within the first seven days of symptom onset. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform moderate, high or waived complexity tests. This test is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

The BinaxNOW™ COVID-19 Ag Card does not differentiate between SARS- CoV and SARS-CoV-2.

Results are for the identification of SARS-CoV-2 nucleocapsid protein antigen. Antigen is generally detectable in nasal 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. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results from patients with symptom onset beyond seven days, should be treated as 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 decisions. Negative results should be considered in the context of a patient's recent exposures, history and the presence of clinical signs and symptoms consistent with COVID-19.

The BinaxNOW™ COVID-19 Ag Card is intended for use by medical professionals or trained operators who are proficient in performing rapid lateral flow tests. BinaxNOW™ COVID-19 Ag Card is only for use under the Food and Drug Administration's Emergency Use Authorization.

2. Summary and Explanation of the Test

Coronaviruses are a large family of viruses which may cause illness in animals or humans. SARS-CoV-2 is an enveloped, single-stranded RNA virus of the β genus. The virus can cause mild to severe respiratory illness and has spread globally, including the United States.

BinaxNOW™ COVID-19 Ag Card is a rapid lateral flow immunoassay for the qualitative detection and diagnosis of SARS-CoV-2 directly from nasal swabs, without viral transport media.

The BinaxNOW™ COVID-19 Ag Card kit contains all components required to carry out an assay for SARS-CoV-2.

3. Principles of the Procedure

The BinaxNOW™ COVID-19 Ag Card is an immunochromatographic membrane assay that uses highly sensitive antibodies to detect SARS-CoV-2 nucleocapsid protein from nasal swab specimens. SARS-CoV-2 specific antibodies and a control antibody are immobilized onto a membrane support as two distinct lines and combined with other reagents/pads to construct a test strip. This test strip and a well to hold the swab specimen are mounted on opposite sides of a cardboard, book-shaped hinged test card.

To perform the test, a nasal swab specimen is collected from the patient, 6 drops of extraction reagent from a dropper bottle are added to the top hole of the swab well. The patient sample is inserted into the test card through the bottom hole of the swab well, and firmly pushed upwards until the swab tip is visible through the top hole. The swab is rotated 3 times clockwise and the card is closed, bringing the extracted sample into contact with the test strip. Test results are interpreted visually at 15 minutes based on the presence or absence of visually detectable pink/purple colored lines. Results should not be read after 30 minutes.

4. Reagents and Materials

Materials Provided

COMPONENT	CONTENT	QUANTITY
TEST CARDS	A cardboard, book-shaped hinged test card containing the test strip	40
EXTRACTION REAGENT	Bottle containing 10 mL of extraction reagent	1
NASAL SWABS	Sterile swabs for use with BinaxNOW™ COVID-19 Ag Card test	40
POSITIVE CONTROL SWAB	Non-infectious recombinant SARS-CoV-2 nucleocapsid antigen dried onto a swab	1
NEGATIVE CONTROL SWAB	The use of a sterile patient swab ensures appropriate negative results are obtained	1
PACKAGE INSERT		1
PROCEDURE CARD		1

Materials Recommended But Not Provided

- Clock, timer or stopwatch

Materials Available as on Optional Accessory Item

- Swab Transport Tube Accessory Pack

5. Precautions

1. For *in vitro* diagnostic use.
2. This test has not been FDA cleared or approved; this test has been authorized by FDA under an EUA for use by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate, high, or waived complexity tests and at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.
3. Federal Law restricts this device to sale by or on the order of a licensed practitioner (US only).
4. This test has been authorized only for the detection of SARS-CoV-2 antigen, not for any other viruses or pathogens.
5. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
6. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health laboratories.
7. Treat all specimens as potentially infectious. Follow universal precautions when handling samples, this kit and its contents.
8. Proper sample collection, storage and transport are essential for correct results.
9. Leave test card sealed in its foil pouch until just before use. Do not use if pouch is damaged or open.
10. Do not use kit past its expiration date.
11. Do not mix components from different kit lots.
12. Do not reuse the used test card.
13. Inadequate or inappropriate sample collection, storage, and transport may yield false test results.
14. Do not store specimens in viral transport media for specimen storage.
15. All components of this kit should be discarded as Biohazard waste according to Federal, State and local regulatory requirements.
16. Solutions used to make the positive control swab are non-infectious. However, patient samples, controls, and test cards should be handled as though they could transmit disease. Observe established precautions against microbial hazards during use and disposal.
17. Wear appropriate personal protection equipment and gloves when running each test and handling patient specimens. Change gloves between handling of specimens suspected of COVID-19.
18. INVALID RESULTS can occur when an insufficient volume of extraction reagent is added to the test card. To ensure delivery of adequate volume, hold vial vertically, 1/2 inch above the swab well, and add drops slowly.
19. False Negative results can occur if the sample swab is not rotated (twirled) prior to closing the card.

20. Swabs in the kit are approved for use with BinaxNOW™ COVID-19 Ag Card.

    Do not use other swabs.

21. The Extraction Reagent packaged in this kit contains saline, detergents and preservatives that will inactivate cells and virus particles. Samples eluted in this solution are not suitable for culture.
22. Do not store the swab after specimen collection in the original paper packaging, if storage is needed use a plastic tube with cap.

6. Storage and Stability

Store kit at 2-30°C. The BinaxNOW™ COVID-19 Ag Card kit is stable until the expiration date marked on the outer packaging and containers. Ensure all test components are at room temperature before use.

7. Quality Control

BinaxNOW™ COVID-19 Ag Card has built-in procedural controls. For daily quality control, Abbott suggests that you record these controls for each test run.

Procedural Controls:

A. The pink-to-purple line at the "Control" position is an internal procedural control. If the test flows and the reagents work, this line will always appear.

B. The clearing of background color from the result window is a negative background control. The background color in the window should be light pink to white within 15 minutes. Background color should not hinder reading of the test.

External Positive and Negative Controls:

Good laboratory practice suggests the use of positive and negative controls to ensure that test reagents are working and that the test is correctly performed. BinaxNOW™ COVID-19 Ag Card kits contain a Positive Control Swab and Sterile Swabs that can be used as a Negative Control Swab. These swabs will monitor the entire assay. Test these swabs once with each new shipment received and once for each untrained operator. Further controls may be tested in order to conform with local, state and/or federal regulations, accrediting groups, or your lab's standard Quality Control procedures.

If the correct control results are not obtained, do not perform patient tests or report patient results. Contact Technical Support during normal business hours before testing patient specimens.

8. Specimen Collection & Handling

Test specimens immediately after collection for optimal test performance. Inadequate specimen collection or improper sample handling/storage/ transport may yield erroneous results. 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

Nasal Swab

Only the swab provided in the kit is to be used for nasal swab collection.

To collect a nasal swab sample, carefully insert the swab into the nostril exhibiting the most visible drainage, or the nostril that is most congested if drainage is not visible. Using gentle rotation, push the swab until resistance is met at the level of the turbinates (less than one inch into the nostril).

Rotate the swab 5 times or more against the nasal wall then slowly remove from the nostril. Using the same swab, repeat sample collection in the other nostril.

9. Specimen Transport and Storage

Do not return the nasal swab to the original paper packaging.

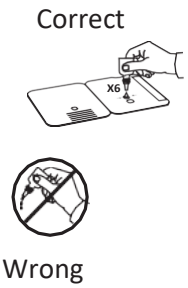
For best performance, direct nasal swabs should be tested as soon as possible after collection. If immediate testing is not possible, and to maintain best performance and avoid possible contamination, it is highly recommended the nasal swab is placed in a clean, unused plastic tube labeled with patient information, preserving sample integrity, and capped tightly at room temperature (15-30°C) for up to (1) hour prior to testing. Ensure the swab fits securely within the tube and the cap is tightly closed. If greater than 1-hour delay occurs, dispose of sample. A new sample must be collected for testing.

10. Test Procedure

Procedure for Patient Specimens

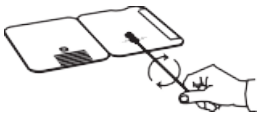
Open the test card just prior to use, lay it flat, and perform assay as follows. The test card must be flat when performing testing, do not perform testing with the test card in any other position.

1. Hold Extraction Reagent bottle vertically. Hovering 1/2 inch above the TOP HOLE, slowly add 6 DROPS to the TOP HOLE of the swab well. DO NOT touch the card with the dropper tip while dispensing.



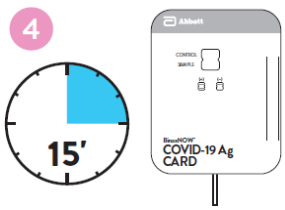
2. Insert sample into BOTTOM HOLE and firmly push upwards so that the swab tip is visible in the TOP HOLE.

3. Rotate (twirl) swab shaft 3 times CLOCKWISE (to the right). Do not remove swab.



Note: False negative results can occur if the sample swab is not rotated (twirled) prior to closing the card.

4. Peel off adhesive liner from the right edge of the test card. Close and securely seal the card. Read result in the window 15 minutes after closing the card. In order to ensure proper test performance, it is important to read the result promptly at 15 minutes, and not before. Results should not be read after 30 minutes.

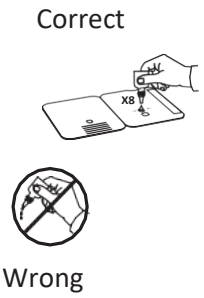


Note: When reading test results, tilt the card to reduce glare on the result window if necessary. Individuals with color-impaired vision may not be able to adequately interpret test results.

Procedure for BinaxNOW™ Swab Controls

Open the test card just prior to use, lay it flat, and perform assay as follows.

1. Hold Extraction Reagent bottle vertically Hovering 1/2 inch above the TOP HOLE, slowly add 8 DROPS to the TOP HOLE of the swab well. DO NOT touch the card with the dropper tip while dispensing.



2. Follow Steps 2 – 4 of the Test Procedure for Patient Specimens.

11. Result Interpretation

Note: In an untested BinaxNOW™ COVID-19 Ag Card there will be a blue line present at the Control Line position. In a valid, tested device, the blue line washes away and a pink/purple line appears, confirming that the sample has flowed through the test strip and the reagents are working. If the blue line is not present at the Control Line position prior to running the test, do not use and discard the test card.

Negative

A negative specimen will give a single pink/purple colored Control Line in the top half of the window, indicating a negative result. This Control Line means that the detection part of the test was done correctly, but no COVID-19 antigen was detected.

Positive

A positive specimen will give two pink/ purple colored lines. This means that COVID-19 antigen was detected.
Specimens with low levels of antigen may give a faint Sample Line. Any visible pink/ purple colored line is positive**.**

Invalid

If no lines are seen, if just the Sample Line is seen, or the Blue Control Line remains blue, the assay is invalid. Invalid tests should be repeated.

12. Limitations

- This test detects both viable (live) and non-viable, SARS-CoV and SARS- CoV-2. Test performance depends on the amount of virus (antigen) in the sample and may or may not correlate with viral culture results performed on the same sample.
- A negative test result may occur if the level of antigen in a sample is below the detection limit of the test.
- The performance of the BinaxNOW™ COVID-19 Ag Card was evaluated using the procedures provided in this product insert only. Modifications to these procedures may alter the performance of the test.
- False negative results may occur if a specimen is improperly collected, transported, or handled.
- False results may occur if specimens are tested past 1 hour of collection. Specimens should be tested as quickly as possible after specimen collection.
- False negative results may occur if inadequate extraction buffer is used (e.g., <6 drops).
- False negative results may occur if specimen swabs are not twirled within the test card.
- False negative results may occur if swabs are stored in their paper sheath after specimen collection.
- Positive test results do not rule out co-infections with other pathogens.
- Positive test results do not differentiate between SARS-CoV and SARS- CoV-2.
- Negative test results are not intended to rule in other non-SARS viral or bacterial infections.
- The presence of mupirocin may interfere with the BinaxNOW™ COVID-19 Ag test and may cause false negative results.
- Negative results, from patients with symptom onset beyond seven days, should be treated as presumptive and confirmation with a molecular assay, if necessary, for patient management, may be performed.
- If the differentiation of specific SARS viruses and strains is needed, additional testing, in consultation with state or local public health departments, is required.

Conditions of Authorization for Laboratory and Patient Care Settings

The BinaxNOW™ COVID-19 Ag Card Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www. fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use- authorizations-medical-devices/vitro-diagnostics-euas.

However, to assist clinical laboratories using the BinaxNOW™ COVID-19 Ag Card, the relevant Conditions of Authorization are listed below:

- Authorized laboratories[1] using your product will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using your product will use your product as outlined in the "BinaxNOW™ COVID-19 Ag Card" Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
- Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

- Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Abbott Diagnostics Scarborough, Inc. (via email: ts.scr@abbott.com, or via phone by contacting Abbott Diagnostics Scarborough, Inc. Technical Service at 1-800-257- 9525 any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
- All operators using your product must be appropriately trained in performing and interpreting the results of your product, use appropriate personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.
- Abbott Diagnostics Scarborough, Inc., authorized distributors, and authorized laboratories and patient care settings using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high, moderate, or waived complexity tests. This test is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation." as "authorized laboratories."

13. Performance Characteristics

Clinical Performance

Clinical performance characteristics of BinaxNOW™ COVID-19 Ag Card was evaluated in a multi-site prospective study in the U.S in which patients were sequentially enrolled and tested. A total of seven (7) investigational sites throughout the U.S. participated in the study. Testing was performed by operators with no laboratory experience and who are representative of the intended users at CLIA waived testing sites. In this study testing was conducted by thirty-two (32) intended users. No training on the use of the test was provided to the operators. To be enrolled in the study, patients had to be presenting at the participating study centers with suspected COVID-19. Patients who presented within 7 days of symptom onset were included in the initial primary analysis, as only seven asymptomatic patients were enrolled.

Of the seven asymptomatic patients, only two patients were positive for SARS-CoV-2. Two nasal swabs were collected from patients and tested using the BinaxNOW™ COVID-19 Ag Card at all study sites. An FDA Emergency Use Authorized real-time Polymerase Chain Reaction (RT-PCR) assay for the detection of SARS-CoV-2 was utilized as the comparator method for this study.

At all sites, one nasal swab was tested directly in the BinaxNOW™ COVID-19 Ag Card test according to product instructions and the other swab was eluted in viral transport media (VTM). Swabs were randomly assigned to testing with the BinaxNOW™ or RT-PCR testing and were tested by minimally trained operators who were blinded to the RT-PCR test result. All sites shipped the VTM sample to a central testing laboratory for RT-PCR.

External control testing, using BinaxNOW™ COVID-19 Ag Card Positive and Negative Controls, was performed prior to sample testing each day, at all study sites.

The performance of BinaxNOW™ COVID-19 Ag Card was established with 102 nasal swabs collected from individual symptomatic patients (within 7 days of onset) who were suspected of COVID-19.

BinaxNOW™ COVID-19 Ag Card Performance against the Comparator Method

BinaxNOW™ COVID-19 Ag Card	Comparator Method		
	Positive	Negative	Total
Positive	34	1	35
Negative	1	66	67
Total	35	67	102
Positive Agreement: 34/35	97.1% (95% CI: 85.1% - 99.9%)		
Negative Agreement: 66/67	98.5% (95% CI: 92.0% - 100%)		

Patient Demographics

Patient demographics (gender, age, time elapsed since onset of symptoms) are available for the 102 samples used in the analysis. The table below shows the positive results broken down by age of the patient:

Age	BinaxNOW™ COVID-19 Ag Card		
	Total #	Positive	Prevalence
≤ 5 years	0		
6 to 21 years	0		
22 to 59 years	77	28	36.4%
≥ 60 years	25	7	28.0%

Positive results broken down by days since symptom onset:

Days Since Symptom Onset	Cumulative RT-PCR Positive (+)	Cumulative BinaxNOW™ COVID-19 Ag Card Positive (+)	PPA	95 % Confidence Interval	Prevalence

1	**4**	**4**	**100.0%**	**39.8%**	**100.0%**
2	**10**	**10**	**100.0%**	**69.2%**	**100.0%**
3	**15**	**15**	**100.0%**	**78.2%**	**100.0%**
4	**18**	**18**	**100.0%**	**81.5%**	**100.0%**
5	**23**	**22**	**95.7%**	**78.1%**	**99.9%**
6	**27**	**26**	**96.3%**	**81.0%**	**99.9%**
7	**35**	**34**	**97.1%**	**85.1%**	**99.9%**

The following data is provided for informational purposes:

The performance of BinaxNOW™ COVID-19 Ag Card with positive results stratified by the comparator method cycle threshold (Ct) counts were collected and assessed to better understand the correlation of assay performance to the cycle threshold, estimating the viral titer present in the clinical sample. As presented in the table below, the positive agreement of the BinaxNOW™ COVID-19 Ag Card is higher with samples of a Ct count <33.

BinaxNOW™ COVID-19 Ag Card Performance against the Comparator Method – by Cycle Threshold Counts

BinaxNOW™ COVID-19 Ag Card	**Comparator Method (POS by Ct Category)**	
	POS (Ct < 33)	**POS (Ct ≥ 33)**
Positive	29	5
Negative	0	1
Total	29	6
Positive Agreement (95% CI)	100.0 (88.1, 100.0)	83.3 (35.9, 99.6)

A limited cohort of patients who presented with symptom onset greater than seven days were enrolled in the clinical study (n = 28). Although the sample size was relatively small, the positive agreement in this cohort was 75% (9/12) and negative agreement was 92% (11/12). Therefore, negative results in patients with symptom onset greater than seven days should be treated as presumptive and confirmed with a molecular assay if needed for clinical management.

Analytical Performance:

Limit of Detection (Analytical Sensitivity)

BinaxNOW™ COVID-19 Ag Card limit of detection (LOD) was determined by evaluating different concentrations of heat inactivated SARS-CoV-2 virus. Presumed negative natural nasal swab specimens were eluted in PBS. Swab eluates were combined and mixed thoroughly to create a clinical matrix pool to be used

as the diluent. Inactivated SARS-CoV-2 virus was diluted in this natural nasal swab matrix pool to generate virus dilutions for testing.

Contrived nasal swab samples were prepared by absorbing 20 microliters of each virus dilution onto the swab. The contrived swab samples were tested according to the test procedure.

The LOD was determined as the lowest virus concentration that was detected ≥ 95% of the time (i.e., concentration at which at least 19 out of 20 replicates tested positive).

The BinaxNOW™ COVID-19 Ag Card LOD in natural nasal swab matrix was confirmed as 22.5 $TCID_{50}$/swab.

Limit of Detection (LoD) Study Results

Concentration $TCID_{50}$/Swab	Number Positive/Total	% Detected
22.5	20/20	100%

Cross Reactivity (Analytical Specificity) and Microbial Interference

Cross reactivity and potential interference of BinaxNOW™ COVID-19 Ag Card was evaluated by testing 37 commensal and pathogenic microorganisms (8 bacteria, 14 viruses, 1 yeast and pooled human nasal wash) that may be present in the nasal cavity. Each of the organism, viruses, and yeast were tested in triplicate in the absence or presence of heat inactivated SARS-CoV-2 virus (45 $TCID_{50}$/swab). No cross-reactivity or interference was seen with the following microorganisms when tested at the concentration presented in the table below.

Potential Cross-Reactant		Test Concentration
Virus	Adenovirus	1.0 x 10^5 TCID50/mL
	Human metapneumovirus (hMPV)	1.0 x 10^5 TCID50/mL
	Rhinovirus	1.0 x 10^5 PFU/mL
	Enterovirus/Coxsackievirus B4	1.0 x 10^5 TCID50/mL
	Human coronavirus OC43	1.0 x 10^5 TCID50/mL
	Human coronavirus 229E	1.0 x 10^5 TCID50/mL
	Human coronavirus NL63	1.0 x 10^5 TCID50/mL
	Human parainfluenza virus 1	1.0 x 10^5 TCID50/mL
	Human parainfluenza virus 2	1.0 x 10^5 TCID50/mL
	Human parainfluenza virus 3	1.0 x 10^5 TCID50/mL
	Human parainfluenza virus 4	1.0 x 10^5 TCID50/mL
	Influenza A	1.0 x 10^5 TCID50/mL
	Influenza B	1.0 x 10^5 TCID50/mL
	Respiratory Syncytial Virus A	1.0 x 10^5 PFU/mL

Potential Cross-Reactant		Test Concentration
Bacteria	*Bordetella pertussis*	1.0 x 10^6 cells/mL
	Chlamydia pneumoniae	1.0 x 10^6 IFU/mL
	Haemophilus influenzae	1.0 x 10^6 cells/mL
	Legionella pnuemophila	1.0 x 10^6 cells/mL

	Mycoplasma pneumoniae	1.0 x 10^6 U/mL
	Streptococcus pneumoniae	1.0 x 10^6 cells/mL
	Streptococcus pyogenes (group A)	1.0 x 10^6 cells/mL
	Mycobacterium tuberculosis	1.0 x 10^6 cells/mL
	Staphylococcus aureus	1.0 x 106 org/mL
	Staphylococcus epidermidis	1.0 x 106 org/mL
	Pooled human nasal wash	N/A
Yeast	*Candida albicans*	1.0 x 10^6 cells/mL

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.

- For *P. jirovecii* one area of sequence similarity shows 45% homology across 18% of the sequence, making cross-reactivity in the BinaxNOW™ COVID-19 Ag Card highly unlikely.
- No protein sequence homology was found between M. tuberculosis, and thus homology-based cross-reactivity can be ruled out.
- The comparison between SARS-CoV-2 nucleocapsid protein, MERS-CoV and human coronavirus HKU1 revealed that cross-reactivity cannot be ruled out. Homology for KHU1 and MERS-CoV is relatively low, at 37.8% across 95% of the sequence and 57.14% across 87% of the sequence, respectively.

High Dose Hook Effect

No high dose hook effect was observed when tested with up to a concentration of 1.6 x 10^5 $TCID_{50}$/mL of heat inactivated SARS-CoV-2 virus with the BinaxNOW™ COVID-19 Ag Card.

Endogenous Interfering Substances

The following substances, naturally present in respiratory specimens or that may be artificially introduced into the nasal cavity or nasopharynx, were evaluated with the BinaxNOW™ COVID-19 Ag Card at the concentrations listed below and were found not to affect test performance.

Substance	Active Ingredient	Concentration
Endogenous	Mucin	2% w/v
	Whole Blood	1% v/v
OTC Nasal Drops	Phenylephrine	15% v/v
OTC Nasal Gel	Sodium Chloride (i.e. NeilMed)	5% v/v
OTC Nasal Spray 1	Cromolyn	15% v/v
OTC Nasal Spray 2	Oxymetazoline	15% v/v
OTC Nasal Spray 3	Fluconazole	5% w/v
Throat Lozenge	Benzocaine, Menthol	0.15% w/v
OTC Homeopathic Nasal Spray 1	Galphimia glauca, Sabadilla, Luffa opperculata	20% v/v
OTC Homeopathic Nasal Spray 2	Zincum gluconium (i.e., Zicam)	5% v/v

OTC Homeopathic Nasal Spray 3	Alkalol	10% v/v
OTC Homeopathic Nasal Spray 4	Fluticasone Propionate	5% v/v
Sore Throat Phenol Spray	Phenol	15% v/v
Anti-viral Drug	Tamiflu (Oseltamivir Phosphate)	0.5% w/v
Antibiotic, Nasal Ointment	Mupirocin[1]	0.25% w/v
Antibacterial, Systemic	Tobramycin	0.0004% w/v

1 Testing demonstrated false negative results at concentrations of 5 mg/mL (0.5% w/v). Standard dose of nasal ointment: 20 mg (2% w/w) of mupirocin in single-use 1-gram tubes.

Symbols

℞ Only	Prescription Only

Ordering and Contact Information
Reorder numbers:
195-000: BinaxNOW™ COVID-19 Ag Card (40 Tests)
195-080: BinaxNOW™ COVID-19 Ag Control Swab Kit
190-010: Swab Transport Tube Accessory Pack

US + 1 877 441 7440
Technical Support Advice Line
Further information can be obtained by contacting Technical Support on:

US
+ 1 800 257 9525 ts.scr@abbott.com

Abbott Diagnostics Scarborough, Inc.
10 Southgate Road
Scarborough, Maine 04074 USA
www.globalpointofcare.abbott

IN195000 Rev.1 2020/08

Abbott

Test Procedure Approval and Review Sheet

PREPARED BY:	
DATE:	
SUPERVISOR REVIEW:	
DATE:	
LABORATORY DIRECTOR OR DESIGNEE APPROVAL:	
IMPLEMENTATION DATE:	
SUPERSEDES PROCEDURE DATED:	
DATE PROCEDURE RETIRED:	

LABORATORY DIRECTOR OR DESIGNEE	DATE REVIEWED	LABORATORY DIRECTOR OR DESIGNEE	DATE REVIEWED

Method Comparison Study for BinaxNOW™ COVID-19 Ag Card Confirming BinaxNOW™ COVID-19 Ag Card Performance Using Patient Samples

1. Regulatory Background

The BinaxNOW™ COVID-19 Ag Card (195.000) does not require verification per the package insert/manufacturer recommendations, but some facilities may choose to complete.

This protocol should be followed as directed when performing a method comparison study of BinaxNOW™ COVID-19 Ag Card using patient samples.

2. Objective

The primary objective of this study is to estimate the positive percent agreement and negative percent agreement of the BinaxNOW™ COVID-19 Ag Card against the comparator method, in patients suspected of COVID-19 infection using healthcare worker-collected nasal swabs specimens tested directly (i.e. without dilution in viral transport media).

3. Study Population

Inclusion Criteria:

- Test direct nasal swabs from individuals suspected of COVID-19 by their healthcare provider within the first seven days of symptom onset.
- Select study sites with a good positivity rate and/or where clinicians are experienced in the diagnosis of COVID-19 (as good clinical sensitivity will increase the prevalence in the sample of patients) so that the study can be completed in a timely fashion.

Exclusion Criteria:

- Do not try to find previously positive patients to quickly obtain positive samples.
- Subjects with active nose bleeds or acute facial injuries/trauma.

4. Sample Size

Generally, 20 samples (10 positive and 10 negative) are a minimum requirement[1], but low positivity rates can drive this number up. Please contact your inspecting agency (CAP, COLA, TJC, or State) for current requirements. The depth of the verification study is determined by the laboratory director.

5. Recommendations on Study Design and Personnel Training

Study design and parameters should be established in order to ensure the proper training for healthcare staff on sample collection, handling, transport, and storage. Abbott Technical Consultants are available to assist with study design in addition to providing sample collection and end-user training (if facility staff is unable).

Two (2) patient swabs must be taken at the same time. One swab will be tested directly using the BinaxNOW™ COVID-19 Ag Card immediately after collection. The other swab will be tested on the chosen comparator method. Depending on the manufacturer's instructions, this swab will likely need to be put into transport media and transported to the lab. If verifications will be conducted at multiple locations, the study design should be reviewed to gain insight from all participating sites.

During study design, it is important to consider the order of collection. For the best study comparison, nasal swabs should be tested by both the BinaxNOW™ COVID-19 Ag Card and the comparator method. The study design should randomize the sequence of collection for each paired swab collected from a patient as assay performance can be impacted by unequal viral distribution due to differences in sample concentration.

Randomization of collection can ensure that this factor does not impact performance of the two methods. For instance, from Patient 1 the first paired swab should be tested on the BinaxNOW™ COVID-19 Ag Card and the second on the comparator method. For Patient 2 the first paired swab should be tested on the comparator method and the second on the BinaxNOW™ COVID-19 Ag Card.

These factors are critical components to ensure optimal study design. However, depending on specific situations other factors may also be critical to take into consideration. To ensure optimal study design, Abbott Technical Consultants are available to assist.

6. Sample Collection, Handling, and Storage

As with any diagnostic test, the quality of sample collection, handling and storage are critical to assay performance. Freshly collected specimens should be used for optimal performance. 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.

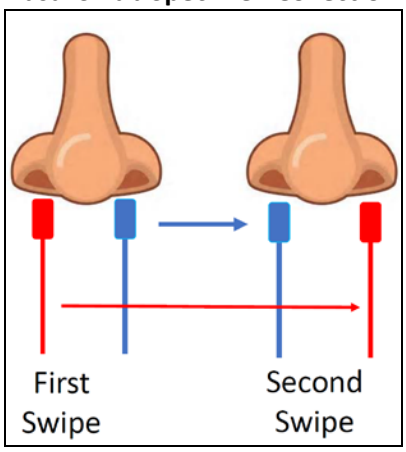
Two (2) paired swabs should be freshly collected in succession by a healthcare worker. The recommended procedures for collecting nasal swabs are provided below. Direct nasal should be tested as soon as possible after collection. If immediate testing is not possible the nasal swab should be placed in a clean, unused plastic tube and capped tightly at room temperature (15-30°C) for up to 1 hour prior to testing.

6.1 Nasal Swab Specimen Collection

With the **first** swab, use gentle rotation to push the swab into the **right nostril** until resistance is met at the level of the turbinates (less than one inch into the nostril). Rotate the swab five times against the nasal wall then slowly remove from the nostril. Using the **second** swab, conduct sample collection in the **left nostril**.
With the **second** swab, use gentle rotation to push the swab into the **right nostril** until resistance is met at the level of the turbinates (less than one inch into the nostril). Rotate the

swab five times against the nasal wall then slowly remove from the nostril. Using the **first** swab, repeat sample collection in the **left nostril**.
Cross over collection allows for equal loading of both swabs. Swab randomization accounts for difference in viral load between the left and right nostril.

Nasal Swab Specimen Collection[2]



7. Comparator Testing

A comparator method should be selected for which study staff are trained and the same sample type can be evaluated as selected BinaxNOW™ COVID-19 Ag Card. As all products have specific requirements, study design should consider the specific product package insert to optimize performance of both test methods. Quality and procedural controls should be followed as recommended by the specific product manufacturer and package insert.

8. Data Analysis

It is recommended that statistical analysis for positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement are used to analyze and report study data. An example of how to calculate and present the study results is shown below.

2 x 2 Contingency Table When Using a Comparative Method[3]

BinaxNOW™ COVID-19 Ag Card	**Comparative Method**		
	Positive	**Negative**	**Total**
Positive	a	b	a + b
Negative	c	d	c + d
Total	a + c	b + d	n

Percent positive agreement (candidate method) = 100 x a / (a + c)
Percent negative agreement (candidate method) = 100 x d / (b + d)
Overall percent agreement = 100 x (a + d) / n

9. BinaxNOW™ COVID-19 Ag Card Test Procedure[4]

Refer to the BinaxNOW™ COVID-19 Ag Card product insert.

If you have any questions or require further assistance on the BinaxNOW™ COVID-19 Ag Card, please contact Abbott Technical Support at: 800-257-9525 ts.scr.@abbott.com

[1]CAP All Common Checklist 09172019 COM.40350
[2]Clinical Evaluation of the Investigational ID NOW™ COVID-19 Assay. Protocol Number: 2012601. Original (Version 1): 16 June 2020 Version 2: 01 July 2020
[3]CLSI EP12-A2:2008 User Protocol for Evaluation of Qualitative Test Performance, 2nd Edition. Jan 2008 Vol 28 (3). ISBN 1-56238-654-9 ISSN 0273-3099
[4]BinaxNOW™ COVID-19 Ag Card Part Number # 195.000 Test Kit Laboratory Procedure

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BinaxNOW™ COVID-19 Ag Card Verification Form

ACCOUNT NAME: _____

ADDRESS: _____

_____

TELEPHONE: _____

BINAXNOW™ COVID-19 Ag Card LOT #/EXP: _____

DATE: _____

SUPERVISOR SIGNATURE: _____

Record the results from reference specimens below.

Record the Sample #, the BinaxNOW™ COVID-19 Ag Card results, Tester's Initials, and any comments. After the BinaxNOW™ COVID-19 Ag Card results have been recorded (positive or negative) then record the Expected Results (positive or negative).

SAMPLE #	EXPECTED RESULTS	BinaxNOW™ COVID-19 Ag Card RESULT	TESTER'S INITIALS	COMMENTS

BinaxNOW™ COVID-19 Ag Card Verification Form (Continued)

SAMPLE #	EXPECTED RESULTS	BinaxNOW™ COVID-19 Ag Card RESULT	TESTER'S INITIALS	COMMENTS

REVIEW: _____ **DATE:** _____

LABORATORY DIRECTOR REVIEW AND APPROVAL FOR CLINICAL USE: _____

DATE: _____

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BinaxNOW™ COVID-19 Ag Card External Quality Control Log

Abbott recommends that external positive and negative controls be run:
-Once with each new shipment received
-Once for each untrained operator
-When required by local, state, and/or federal regulations, accrediting groups, or your lab's Quality Control procedures

If the expected external control results are not obtained, do not report patient results. Contact Technical Service at 800-257-9525.

INSTRUMENT SERIAL NUMBER: _____

DATE	BINAXNOW™ COVID-19 Ag CARD KIT LOT/EXP	POSITIVE CTRL LOT/EXP	NEGATIVE CTRL LOT/EXP	POSITIVE RESULT	NEGATIVE RESULT	CONTROL LINE PRESENT: Y/N	TESTER'S INITIALS	CORRECTIVE ACTION / COMMENTS

REVIEWED BY: _____ DATE: _____

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BinaxNOW™ COVID-19 Ag Card Internal Controls and Patient Record

LOT NUMBER _____ **EXP. DATE** _____ **NAME OF FACILITY:**_____

Record the Date, Patient's Name, Patient Test Result, Internal Control Results and the tester's initials.
Positive Internal Control = The pink-to-purple line at the "Control" position can be considered an internal positive procedural control.
Negative Internal Control = The background color in the window should be light pink to white within 15 minutes.

DATE	PATIENT NAME	PATIENT ID NUMBER	PATIENT RESULTS	CONTROL LINE PRESENT?		INTERNAL CONTROL RESULTS		COMMENTS	TESTER'S INITIALS
				NO	YES	+	-		

REVIEWED BY: _____ **DATE:** _____

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Quality Assessment Review Form and Checklist

QUALITY ASSESSMENT ACTIVITY	COMMENTS	DATE	INITIALS
Patient test management: evaluate criteria for specimen submission, handling, and rejection; test results requisitions and reporting, accuracy and reliability of reports.			
Quality control: Assess control data, reference range verification, errors in reporting results, and corrective actions taken with appropriate documentation records.			
Proficiency testing: review the effectiveness of corrective actions taken for unsatisfactory performance or failures.			
Comparison of test results: review at least semi-annually comparative results for multiple methods, instruments, or site correlations when more than one procedure exists.			
Relationship of patient test information to test results*:* evaluate patient test reports for accuracy of patient information, test results, and normal ranges. Identify and evaluate results inconsistent with patient's age, sex, diagnosis, and other test parameters.			
Personnel: evaluate the effectiveness of policies and procedures for assuring employees competence of testing and reporting test results.			
Communications: evaluate documented problems and corrective actions that occur between the laboratory and the authorized individual who orders or receives the test result.			
Complaint investigation: evaluate documented complaints and corrective actions.			
Quality assessment reviews with staff: document discussion with staff regarding identified problems and corrective actions during the QA review.			

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Corrective Action Form

PROBLEM/ERROR	CORRECTIVE ACTION

TECHNOLOGIST: _____ **DATE:** _____

SUPERVISOR: _____ **DATE:** _____

LABORATORY DIRECTOR: _____ **DATE:** _____

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Temperature Log

EQUIPMENT: Binax Ag cards
NAME OF FACILITY: Ulster County DOH

TO BE RECORDED AT THE BEGINNING OF EACH WORKDAY. TEMPERATURE RANGE:35.6-86 degrees F

DATE	°F	INITIALS	ADJUSTMENTS	DATE	°F	INITIALS	ADJUSTMENTS

Tips for Successful Proficiency Testing (PT) Performance

- Strictly follow the PT provider's storage or handling requirement ***before testing PT specimens***.
- Analyze PT specimens ***within the time frame*** provided by the PT provider.
- Contact the PT provider ***promptly*** when specimens are received damaged. You may be able to receive a replacement immediately.
- Avoid clerical error when filling out PT answer sheets. Be sure to ***enter the correct result next to the correct analyte*** on the answer form.
- Remember to identify the instrument or method you are using to perform your PT so you are ***graded among your peer group***.
- Make copies of all answer forms ***before submitting them*** to your PT provider.
- Please contact Technical Support at 800-257-9525 or ts.scr@abbott.com for further information on proficiency providers.

BinaxNOW™ COVID-19 Ag Card - Training Checklist

FACILITY/LABORATORY:_____

USER NAME: _____ **USER ID:** _____

ITEM DETAILS		
BinaxNOW™ COVID-19 Ag CARD - KIT OVERVIEW	**USER'S INITIALS**	**DATE**
The user acknowledges being shown and understands the following kit components and precautions in the package insert: • Kit Storage temperature • Lot number and expiration date • Package Insert including Precautions and Limitations • BinaxNOW™ COVID-19 Ag Card Procedure Card • Extraction Reagent • Swabs provided in the BinaxNOW™ COVID-19 kit • Patient samples, controls, and test cards should be handled as though they could transmit disease. Observe established precautions against microbial hazards during use and disposal. • Wear appropriate personal protection equipment and gloves when running each test and handling patient specimens. Change gloves between handling of specimens suspected of COVID-19.		
SPECIMEN COLLECTION, STORAGE AND HANDLING	**USER'S INITIALS**	**DATE**
The user acknowledges being shown; sample collection and storage conditions in the package insert: • For use with direct nasal swab specimens with swabs provided in the kit ONLY • To collect a nasal swab sample, carefully insert the swab into the nostril exhibiting the most visible drainage, or the nostril that is most congested if drainage is not visible. Using gentle rotation, push the swab until resistance is met at the level of the turbinate (less than one inch into the nostril). Rotate the swab 5 times or more against the nasal wall then slowly remove from the nostril. Using the same swab, repeat sample collection in the other nostril. • Direct nasal swabs should be tested as soon as possible after collection If immediate testing is not possible, and to maintain best performance and avoid possible contamination, it is highly recommended the nasal swab is placed in a clean, unused plastic tube labeled with patient information, preserving sample integrity, and capped tightly at room temperature (15-30°C) for up to (1) hour prior to testing. Ensure the swab fits securely within the tube and the cap is tightly closed. If greater than 1-hour delay occurs, dispose of sample. A new sample must be collected for testing. DO NOT RETURN THE SWAB TO ITS ORIGINAL PACKAGING		
SAMPLE PREPARATION **TEST PROCEDURE FOR QUALITY CONTROL & PATIENT TESTING**	**USER'S INITIALS**	**DATE**
The User follows instructions for QC and Patient Testing of BinaxNOW™ COVID-19 Ag Card.		

ITEM DETAILS		
The User follows instructions for Patient testing as outlined in the package insert and BinaxNOW™ COVID-19 Ag Card Procedure Card: • Bring all materials and patient sample to room temperature • Label test card with appropriate QC or Patient Identification information. **For External QC** 1. **Follow instructions for external controls.** Hold Extraction Reagent bottle vertically. Hovering 1/2 inch above the **TOP HOLE,** slowly adds **8 DROPS** to the **TOP HOLE** of the swab well. DO NOT touch the card with the dropper tip while dispensing. 2. Insert the (+) or (-) control swab into **BOTTOM HOLE** and firmly push upwards so that the swab tip is visible in the **TOP HOLE.** 3. Rotate (twirl) swab shaft 3 times **CLOCKWISE** (to the right). Do not remove swab. 4. Peel off adhesive liner from the right edge of the test card. Close and securely seal the card. 5. Read result in the window 15 minutes after closing the card. It is important to read the result promptly at 15 minutes, and not before. Results should not be read after 30 minutes. **For Patient Testing** 1. Hold Extraction Reagent bottle vertically. Hovering 1/2 inch above the **TOP HOLE,** slowly add **6 DROPS** to the **TOP HOLE** of the swab well. DO NOT touch the card with the dropper tip while dispensing. **2.** Insert sample swab into **BOTTOM HOLE** and firmly push upwards so that the swab tip is visible in the **TOP HOLE.** 3. Rotate (twirl) swab shaft 3 times **CLOCKWISE** (to the right). Do not remove swab. 4. Peel off adhesive liner from the right edge of the test card. Close and securely seal the card. 5. Read result in the window 15 minutes after closing the card. It is important to read the result promptly at 15 minutes, and not before. Results should not be read after 30 minutes.		

USER SIGNATURE_____

DATE_____

TRAINER SIGNATURE_____

DATE _____

Certification of Training

This is to verify that personnel responsible for running the BinaxNOW™ COVID-19 Ag Card at _____ have been thoroughly in-serviced on the test and the test procedure. This has included:

- **Review of the package insert**
- **Demonstration of the product assay**
- **Successful performance of the BinaxNOW™ COVID-19 Ag Card and interpretation of results**

Names of the personnel who have been trained with the BinaxNOW™ COVID-19 Ag Card and are responsible for reporting patient results:

PRINT NAME	SIGNATURE	DATE

Signature of Laboratory Director(s) responsible for personnel and testing:

_____ _____

SIGNATURE **DATE**

_____ _____

SIGNATURE **DATE**

_____ _____

TRAINER **DATE**

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Testing Personnel Training Assessment

Test Method: BinaxNOW™ COVID-19 Ag Card

PROCEDURE	SATISFACTORY	UNSATISFACTORY	NOT APPLICABLE	COMMENTS / CORRECTIVE ACTIONS
Observation of Test Performance:				
PATIENT SAMPLE PREPARATION (IF APPLICABLE)				
SPECIMEN HANDLING/PROCESSING				
TESTING				
RECORDING/REPORTING RESULTS				
ASSESSMENT OF TEST PERFORMANCE USING KNOWN SAMPLES				
Review of Records:				
PATIENT/QUALITY CONTROL LOG SHEET RECORDS				
PROFICIENCY TESTING RECORDS				
ASSESSMENT OF PROBLEM SOLVING SKILLS				

(Attach all supporting documents)

EVALUATOR:_____ **DATE:**_____

EMPLOYEE:_____

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BinaxNOW™ COVID-19 Ag Card Quiz

Name:_____

Date: _____

Circle T (True) or F (False) for each Question:

1.	For testing patient direct nasal swabs, hold Extraction Reagent bottle vertically Hovering 1/2 inch above the TOP HOLE, slowly add 8 DROPS to the TOP HOLE of the swab well.	T	F
2.	The BinaxNOW™ COVID-19 Ag Card device should not be removed from the foil pouch until just before use.	T	F
3.	A nasopharyngeal swab is an acceptable sample type for testing on the BinaxNOW™ COVID-19 Ag Card.	T	F
4.	Direct nasal swab samples may be stored at room temperature for 24 hours.	T	F
5.	It is acceptable to return the nasal swab after patient collection to its original packaging.	T	F
6.	False negative results can occur if the sample swab is not rotated (twirled) prior to closing the card.	T	F
7.	If no lines are seen, or if just the Sample Line is seen, the assay is negative.	T	F
8.	Results should be read promptly at 15 minutes, and not before. Results should not be read after 30 minutes.	T	F
9.	The appearance of a pink-to-purple Control Line and a pink-to-purple Sample Line below it is a positive result.	T	F
10.	Test using only with swabs provided in the kit, and collect direct nasal swabs from individuals suspected of COVID-19 by their healthcare provider within the first seven days of symptom onset.	T	F

BinaxNOW™ COVID-19 Ag Card Quiz Answer Key

	Answer Key	Explanation
1.	F	For testing patient direct nasal swabs, hold Extraction Reagent bottle vertically Hovering 1/2 inch above the TOP HOLE, slowly add **6 DROPS** to the TOP HOLE of the swab well.
2.	T	The BinaxNOW™ COVID-19 Ag Card device should be left in the foil pouch until just before use.
3.	F	A direct nasal swab specimen is the only accepted sample type for the BinaxNOW™ COVID-19 Ag Card kit. Other sample types are unacceptable.
4.	F	If immediate testing is not possible, the nasal swab must be returned to a clean, unused plastic tube labeled with patient information and can be held at room temperature (15- 30°C) for up to one (1) hour prior to testing.
5.	F	Do not store the swab after specimen collection in the original paper packaging, if storage is needed use a clean, unused plastic tube with cap.
6.	T	False negative results can occur if the sample swab is not rotated (twirled) prior to closing the card.
7.	F	If no lines are seen, or if just the Sample Line is seen, the assay is invalid. Invalid tests should be repeated.
8.	T	In order to ensure proper test performance, it is important to read the result promptly at 15 minutes, and not before. Results should not be read after 30 minutes.
9.	T	A positive BinaxNOW™ COVID-19 Ag Card will have a pink-to-purple control line and a second pink-to-purple sample line appears below it.
10.	T	Test using only with swabs provided in the kit, and collect direct nasal swabs from individuals suspected of COVID-19 by their healthcare provider within the first seven days of symptom onset.