Rapid Response™

COVID-19 Antigen Nasal Pen Test

REF COV-19P, COV-19PPE, COV-19PPE1, COV-19PPE2, COV-19PPE5, COV-19PPE20, COV-19PPE25 Product Insert

This test is authorized for use at the Point-of-Care.

For *in vitro* diagnostic use only.

Intended Use

The Rapid Response™ COVID-19 Antigen Nasal Pen Test is a rapid *in vitro* immunochromatographic assay for the direct and qualitative detection of SARS-CoV-2 viral nucleocapsid protein antigens from nasal secretions within seven (7) days of symptom onset or without symptoms or other epidemiological reasons to suspect COVID-19 infection. This test is authorized for use at the Point of Care i.e., in patient care setting.

Results are for the identification of SARS-CoV-2 viral nucleoprotein antigen. Antigens are generally detectable in nasal secretions 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 are required to report all positive results to the appropriate public health authorities.

Negative results should be treated as presumptive, 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 persons recent exposures, history, and the presence of clinical signs and symptoms consistent with COVID-19, and confirmed with a molecular assay, if necessary.

The Rapid Response™ COVID-19 Antigen Nasal Pen Test is intended for use by trained laboratory personnel or health care professionals.

Principle

The Rapid Response™ COVID-19 Antigen Nasal Pen Test detects SARS-CoV-2 viral antigens through visual interpretation of color development. Anti-SARS-CoV-2 antibodies are immobilized at the test region of the nitrocellulose membrane. Anti-SARS-CoV-2 antibodies conjugated to colored particles are immobilized on the conjugated pad.

The nasal secretion collected by the intended user, is supposed to be mixed with the liquid extraction buffer contained in a foil-sealed compartment of the pen cap provided in the kit.

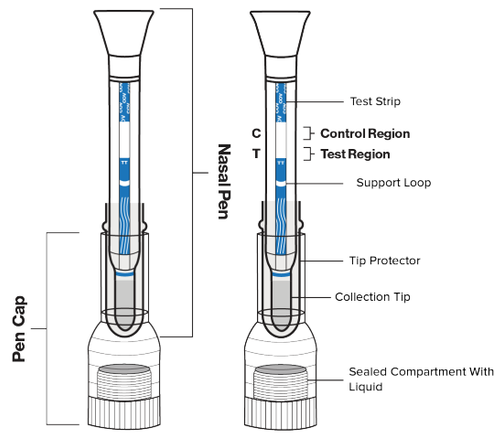
During testing, target antigens, if present in the nasal secretions, will be released into the extraction buffer. As the specimen migrates along the strip by capillary action and then interacts with reagents on the sample pad, the target antigens will bind to anti-SARS-CoV-2 antibodies on the conjugate pad. Consequently, the antigen-antibody complex will be captured by the anti-SARS-CoV-2 antibodies immobilized at the test region. Excess colored particles will be captured at the control region of the nitrocellulose membrane.

The presence of a colored line in the test region indicates a positive result for the SARS-CoV-2 viral antigens, while its absence indicates a negative result. A colored line at the control region serves as a procedural control, generally indicating that a proper volume of specimen has been added and membrane wicking is working. No colored line in the control region means that the test result is invalid.

Precautions

- For *in vitro* Diagnostic Use Only.
- Read the Product Insert prior to use. Directions should be read and followed carefully.
- Do not use kit or components beyond the expiration date.
- The device contains material of animal origin and should be handled as a potential biohazard.
- Do not use if pouch is damaged or open.
- Test devices are packaged in foil pouches that exclude moisture during storage. Inspect each foil pouch before opening. Do not use devices that have holes in the foil or where the pouch has not been completely sealed. Erroneous result may occur if test reagents or components are improperly stored.
- Do not use the Extraction Buffer if it is discolored or turbid. Discoloration or turbidity may be a sign of microbial contamination.
- All patient specimens should be handled and discarded as if they are biologically hazardous. All specimens must be mixed thoroughly before testing to ensure a representative sample prior to testing.
- Failure to bring specimens and reagents to room temperature before testing may decrease assay sensitivity. Inaccurate or inappropriate specimen collection, storage, and transport may yield false negative test results.
- Avoid skin contact with buffer.
- If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions and sent to state or local health departments for testing.
- Viral isolation in cell culture and initial characterization of viral agents recovered in cultures of SARS-CoV-2 specimens are NOT recommended, except in a BSL3 laboratory using BSL3 work practices.
- Keep the collection tip clean. Do not touch the tip and make sure it does not touch any surfaces before or after use.
- Use a separate test for each person.
- Once the test has begun, the device must be kept upright such that the pen is pointing downwards into the cap and the wide end of the cap is on a flat, level surface.
- If the patient has a nose piercing, swab the other nostril. If they are pierced on both sides, remove the piercing on one side before swabbing.
- Each device (pen and cap) is for a single use only. If repeat testing is required, discard the used test, and repeat with a new one.

Materials



Materials provided

- Test device individual packaged in a foil pouch:
  - Pen containing test strip and collection tip
  - Pen cap containing sealed compartment with liquid (extraction buffer)
  - Tip protector
- Product insert

Materials required but not provided

- Clock, timer, or stopwatch

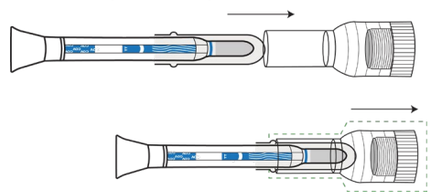
Storage and Stability

- Store the Rapid Response™ COVID-19 Antigen Nasal Pen Test at 35.6~86°F (2~30°C) when not in use.
- Do not open the sealed pouch until just before you are ready to use the test.
- **Do not freeze.**
- Do not use after the expiration date marked on the packaging.

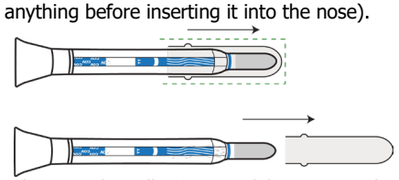
Test Procedure

1. Bring devices, reagents, and specimens and/or controls to room temperature (59~86°F; 15~30°C) before use.
2. Remove the test device from its packaging. Label the device with patient identification. For the best results, the assay should be performed within one hour.
3. Have the person being tested gently blow their nose into a tissue to get rid of excess mucus and dispose of the tissue away in a closed waste bin.
4. The operators must wash their hands thoroughly with soap and water or use hand sanitizer for 20 seconds. Dry hands before starting. If more than one test are being conducted, wash hands between each test.

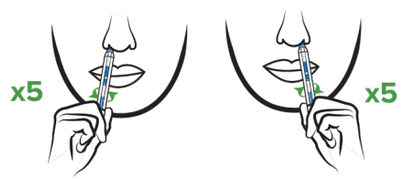
5. Remove the cap from the pen.



6. Remove the protector from the collection tip of the pen. (**NOTE:** Keep the tip clean. Be careful not to touch the tip to anything before inserting it into the nose).



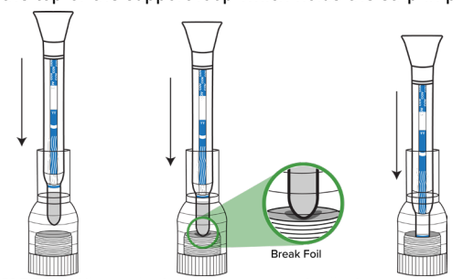
7. Gently insert the collection tip of the pen into the nose until resistance is met (about 1-2 cm into the nostril, roughly the length of the collection tip). Swab thoroughly, rotating the top at least five times against the nasal walls. Remove from the nostril and **repeat the sample collection procedure for the other nostril to ensure that sufficient specimen be collected from BOTH nasal cavities.**



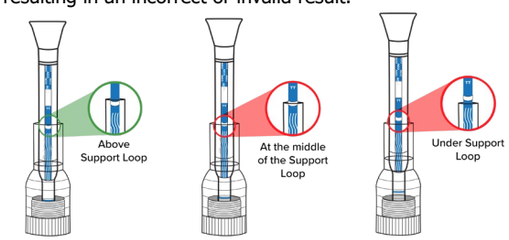
NOTE: Swabbing may feel uncomfortable but it should not be painful. It is important to obtain as much secretion as possible. Do not insert the collection tip any deeper if strong resistance is felt. When swabbing a small child, we

recommend doing so with the help of a second adult so that one adult holds and reassures the child while the other takes the swab. Use caution when swabbing a small child, as the maximum depth of insertion may be less than 2 cm (3/4 of an inch).

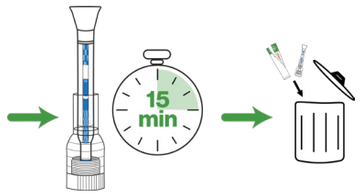
8. Place the white end of the cap on a clean, level surface. Holding the cap in place, insert the pen vertically into the cap and press down to break the foil seal inside the cap, releasing the liquid and pushing it upwards so that it reaches the test strip inside the pen. Push the pen down firmly into the cap until the top edge of the cap reaches the top of the support loop which holds the strip in place.



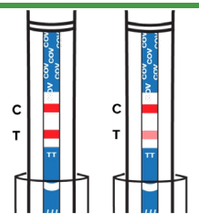
NOTE: When placing the pen vertically into the cap, the edge of the cap must line up with the top of the support loop. There is a thin white line on the surface of the pen where the top of the loop is. The cap will meet this line when the pen is fully inserted. Not pushing the pen far enough into the cap may lead to lateral flow failure, resulting in an incorrect or invalid result.



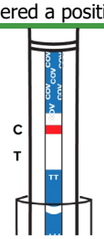
9. Start a timer and read the results at **15 minutes**. Do not read the results 30 minutes after starting the test.
10. When the test is finished, dispose of the used test components with regular waste.



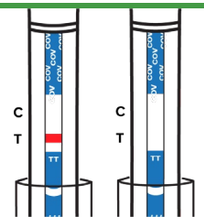
Results Interpretation



POSITIVE: COVID-19 Detected
Two colored lines appear on the membrane. One line appears in the control region (C), and another line appears below it in the test region (T).\
Important: Look very closely! Any faint colored line in the test region is considered a positive result.



NEGATIVE: COVID-19 Not Detected
Only one colored line appears in the control region (C). No apparent colored line appears below it in the test region (T).
Negative results do not rule out SARS-CoV-2 infection. Individuals without symptoms that test negative should be tested again with at least 24 hours and no more than 36 hours between tests. All negative results are considered presumptive, and confirmation with a molecular assay, if necessary for patient management, may be performed. The amount of antigen in a sample may decrease as the duration of illness increases. 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 and confirmed with a molecular assay, if necessary, for patient management.



INVALID: Control line fails to appear. Results from any test which has not produced a control line at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The color intensity in the test region (T) may vary depending on the concentration of analytes present in the specimen. Note that this is a qualitative test only and cannot determine the concentration of analytes in the specimen.
2. Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for control line failure.
3. For questions or to report a problem, please call technical support at 1-888-339-9964 (MON-FRI 9AM – 5:30PM EST) or email support@btnx.com.

Quality Control

Internal Procedural Controls

The Rapid Response™ COVID-19 Antigen Nasal Pen Test has built-in (procedural) controls. Each test device has an internal standard zone to ensure proper sample flow. The user should confirm that the colored line located at the "C" region is present before reading the result.

External Positive and Negative Controls

Good laboratory practice suggests that positive and negative external controls are run routinely to ensure that the test is correctly performed. External positive and negative controls should be used in accordance with applicable accrediting organizations. However, BTNX recommends that labs receiving this test execute a control test for each lot of kits that they receive.

COVID-19 Antigen Controls

Positive and Negative controls are provided upon request with the kit. These controls should be used according to the nasopharyngeal swab test procedure provided in this package insert.

Limitations

1. The Rapid Response™ COVID-19 Antigen Nasal Pen Test is for professional *in vitro* diagnostic use and should only be used for the qualitative detection of SARS-CoV-2 antigens from nasal specimens. The intensity of color in a positive band should not be evaluated as "quantitative or semi-quantitative".
2. Both viable and nonviable SARS-CoV-2 viruses are detectable with the Rapid Response™ COVID-19 Antigen Nasal Pen Test.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test but should only be made by the physician after all clinical and laboratory findings have been evaluated.
4. Failure to follow the test procedure and result interpretation may adversely affect test performance and/or invalidate the test result.
5. Results obtained with this assay, particularly in the case of weak test lines that are difficult to interpret, should be used in conjunction with other clinical information available to the physician.
6. Negative results do not preclude SARS-CoV-2 infection and should be confirmed via molecular assay.
7. The performance of the device has not been assessed on specimens from individuals who have been infected with emerging variants of SARS-CoV-2 of public health concern.
8. The performance of this device has not been assessed in a population vaccinated against COVID-19.
9. This assay is not intended for home testing (or self-testing).

Performance Characteristics

Analytical Sensitivity (Limit of Detection)

The limit of detection was determined with a quantified SARS-CoV-2 virus and has been evaluated at $1x10^{2.4}$ $TCID_{50}$/mL.

Clinical Evaluation

A total of 580 clinical specimens were collected from both symptomatic individuals (within 7 days of symptom onset) and asymptomatic individuals to verify the performance of the Rapid Response™ COVID-19 Antigen Nasal Pen Test. Specimens were collected by operators in the intended use settings of Point of care in multiple sites across the United States of America and the Netherlands. There were a total of 173 positive and 409 negative clinical specimens confirmed by RT-PCR. The results are summarized below:

Table: Rapid Response™ COVID-19 Antigen Nasal Pen Test vs RT-PCR

		RT-PCR		Total
		Positive	Negative	
Rapid Response™ COVID-19 Antigen Nasal Pen Test	Positive	160	1	161
	Negative	13	406	419
Total		173	407	580

Relative Sensitivity: 92.49 % (87.49% ~ 95.94%)*
Relative Specificity: 99.75 % (98.64% ~ 99.9%)*
Overall Agreement: 97.59 % (95.98% ~ 98.67%)*
*95% Confidence Interval

Cross Reactivity

Cross reactivity with the following organisms has been studied. Samples positive for the following organisms were found negative when tested with the Rapid Response™ COVID-19 Antigen Nasal Pen Test.

Adenovirus 1	*Bordetellaparapertussis*
Adenovirus 2	SARS-coronavirus
Bordetella pertussis	Adenovirus 3
Human metapneumovirus	*Candida albicans*
Adenovirus 4	Influenza A (H1N1)
pdm09	*Chlamydia pneumoniae*
Adenovirus 5	Influenza A (H3N2)
Group C Streptococcus	Adenovirus 7
Influenza B Victoria lineage	*Hemophilus influenza*
Adenovirus 55	Influenza B Yamagata lineage
Legionella pneumophila	Epstein-Barr virus
Norovirus	*Mycoplasma pneumoniae*
Enterovirus EV70	Parainfluenza virus 1
Mycobacterium tuberculosis	Enterovirus EV71
Parainfluenza virus 2	*Staphylococcus aureus*
Enterovirus A16	Parainfluenza virus 3
Staphylococcus epidermidis	Enterovirus A24
Parainfluenza virus 4	*Streptococcus agalactiae*
Enterovirus B1	Respiratory syncytial virus A
Streptococcus pneumoniae	Echovirus 6
Respiratory syncytial virus B	*Streptococcus pyogenes*
HCoV-229E	Rhinovirus A30
HCoV-OC43	Rhinovirus B52
HCoV-NL63	

Microbial Interference Study

Potential microbial interference was evaluated to demonstrate that false negatives will not occur when SARS-CoV-2 is present in a specimen with other microorganisms. Low concentration of SARS-CoV-2 (2 X LOD) was spiked into the higher concentrations of interfering organisms, and it was found that there is no microbial interference for following organisms.

Adenovirus 1	*Bordetellaparapertussis*
Bordetella pertussis	Human metapneumovirus
Candida albicans	Influenza A (H1N1)
pdm09	*Chlamydia pneumoniae*
Influenza A (H3N2)	Influenza B Victoria lineage
Hemophilus influenza	Influenza B Yamagata lineage
Legionella pneumophila	*Mycoplasma pneumoniae*
Parainfluenza virus 1	*Mycobacterium tuberculosis*
Enterovirus EV71	Parainfluenza virus 2
Staphylococcus aureus	Enterovirus A16
Parainfluenza virus 3	*Staphylococcus epidermidis*
Parainfluenza virus 4	Respiratory syncytial virus A
Streptococcus pneumoniae	Respiratory syncytial virus B
Streptococcus pyogenes	HCoV-229E
HCoV-OC43	Rhinovirus B52
HCoV-NL63	

Interfering Substances

The following substances, naturally present in respiratory specimens or that may be artificially introduced into the respiratory tract, were evaluated at the concentrations listed below. None of them were found to affect test performance of the Rapid Response™ COVID-19 Antigen Nasal Pen Test.

Substance	Con.
3 OTC nasal sprays	10%
3 OTC mouthwashes	10%
3 OTC throat drops	10%
4-acetamidophenol	10mg/mL
Acetylsalicylic acid	10mg/mL
Albuterol	10mg/mL
Chlorpheniramine	5 mg/mL
Dexamethasone	50µg/mL
Dextromethorphan	10µg/mL
Diphenhydramine	5 mg/mL
Doxylamine succinate	1 mg/mL
Flunisolide	25µg/mL
Guaiacol glyceryl ether	20mg/mL
Mucin	1%
Whole blood	4%
Mupirocin	250µg/mL
Oxymetazoline	25µg/mL
Phenylephrine	10 mg/mL
Phenylpropanolamine	1mg/mL
Zanamivir	10mg/mL
Adamantanamine	500 ng/mL
Oseltamivir phosphate	10mg/mL
Tobramycin	10mg/mL
Triamcinolone	14mg/mL

High Dose Hook Effect

No high dose hook effect was observed when tested with up to a concentration of 1 x $10^{6.4}$ $TCID^{50}$/mL of heat inactivated SARS-CoV-2 virus with the Rapid Response™ COVID-19 Antigen Nasal Pen Test.

Glossary of Symbols

Consult instructions for use
Test per Kit
EC REP Authorized Representative
Store between 35.6°F to 86°F
Use by
Do Not Reuse
LOT Lot Number
IVD For *in vitro* diagnostic use only
REF Catalogue #

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