Detect

COVID-19 Test

Instructions for Use

For Use Under an Emergency Use Authorization Only.

IVD

Emergency Use Authorization.

REV 1. January 2021

Contents

For Use Under an Emergency Use Authorization Only.

1. Proprietary name

Detect COVID-19 Test

2. Established Product Name

Detect COVID-19 Test

3. Intended Use

The Detect test is a molecular *in vitro* diagnostic test utilizing isothermal amplification and lateral flow technologies and is intended for the qualitative detection of RNA from the SARS-CoV-2 in self-collected anterior nares (nasal) swabs from individuals suspected of COVID-19 by their healthcare provider. 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 high, moderate, or waived complexity tests. The Detect 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.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in anterior nares specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient 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. Testing facilities within the United States and its territories are required to report all test results to the appropriate public health authorities.

The Detect test is intended for use by operators in a point of care professional environment. No specific operator training is required. The Detect test is only for use under the Food and Drug Administration's Emergency Use Authorization.

All prescribing healthcare providers will report all test results they receive from individuals who use the authorized product to relevant public health authorities in accordance with local, state, and federal requirements, using appropriate LOINC and SNOMED codes, as defined by the Laboratory In Vitro Diagnostics (LIVD) Test Code Mapping for SARS-CoV-2 Tests provided by CDC.

4. Summary and Explanation of the Test

An outbreak of pneumonia of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organisation (WHO) in December 2019. Chinese authorities identified a novel coronavirus SARS-CoV-2 (cause of COVID-19 respiratory disease) which has resulted in confirmed human infections worldwide, including the United States. Cases of severe respiratory illness and deaths have been reported. Patients can become infected with SARS-CoV-2 virus through contact with a contaminated environment or person.

Detect is a molecular *in vitro* diagnostic test that aids the diagnosis of COVID-19 through the detection of the SARS-CoV-2 RNA in nasal swab specimens from patients suspected of COVID-19 by their healthcare provider.

5. Principles of the Procedure

Detect is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis of SARS-CoV-2 infection and is based on nucleic acid amplification technology. The test contains primers and internal controls used for the *in vitro* qualitative detection of SARS-CoV-2 RNA in nasal swab specimens.

Sample collection can be performed by unskilled users, and sample processing requires no additional training and can be performed by an untrained healthcare professional.

Detect requires two swabs, a single-use disposable tube pre-loaded with Collection Buffer, a Test Cap with lyophilized reagents, a disposable tube in which an aliquot of patient sample is mixed with the lyophilized reagents, a reusable Warmer, an inert buffer, and a disposable Reader that contains a lateral flow strip for the detection of amplified viral RNA.

After pre-clearing the nose with the first swab, the patient collects a nasal sample using the second swab and passes the swab to the test administrator. The test administrator swishes the swab into the Collection Tube and then pipettes a portion of the sample into the Warmer Tube, adds the lyophilized reagents, and warms the sample to perform simultaneous lysis and isothermal amplification. After successful amplification, the tube is pressed into the single-use Reader primed with an inert buffer. Upon insertion, the tube is ruptured, and the amplification product is released onto a lateral flow strip. The presence of lines on the strip corresponds to the detection of amplified SARS-CoV-2 viral RNA, as well as the detection of the human internal control gene and the flow strip’s internal positive control.

6. Assay/Reagents

6.1 Materials

Each Detect COVID-19 Test contains enough reagents to process one sample.

Materials Provided in Test

- Nasal Swab (sterilized) x 2
- Collection Tube (contains Collection Buffer)
- Warmer Tube (empty, capped)
- Pipette
- Test Cap (contains lyophilized reagent bead)
- Tube Rack
- Dropper (contains inert buffer)
- Reader (lateral flow strip, plastic housing)
- Serial Number Stickers
- Personal Health Information Stickers
- Results Card

Materials Sold Separately

- Detect Single-Well Warmer (Model 21101)
  OR
  Detect Multi-Well Warmer (Model 21091)
- SeraCare AccuPlex™ SARS-CoV-2 Control Kit – FULL GENOME; Catalog # 0505-0229
- ZeptoMetrix NATrol™ SARS Associated Coronavirus 2 (SARS-CoV-2) Negative Control; Catalog # NATSARS(COV2)-NEG
- Biohazard Waste Receptacle

7. Warnings and Precautions

7.1 General

IVD

- For *in vitro* diagnostic use.
- 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 complexity/high 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.

- This test has been authorized only for the detection of nucleic acids from SARS-CoV-2, not for any other viruses or pathogens.
- 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 Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- Federal Law restricts this device for sale by or on the order of a licensed practitioner (US only).
- Performance characteristics of this test have been established with the specimen type listed in the Intended Use section only. The performance of this assay with other specimen types or samples has not been evaluated.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
- Only use the test components provided. Do not use swabs from other tests.

- Treat all biological specimens, including used test components, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be handled using standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention [1, 2] and the Clinical and Laboratory Standards Institute [3].
- Follow safety procedures set by your institution for working with chemicals and handling biological specimens.
- Consult your institution's environmental waste personnel on proper disposal of used test components, which may contain amplified material. This material may exhibit characteristics of federal EPA Resource Conservation and Recovery Act (RCRA) hazardous waste requiring specific disposal requirements. Check state and local regulations as they may differ from federal disposal regulations. Institutions should check the hazardous waste disposal requirements within their respective countries.

7.2 Storage & Handling

- Store all components at 50 °F to 86 °F (10 °C to 30 °C).
- Do not open components until you are ready to perform testing.
- Do not use Collection Tubes that are wet or have leaked.
- Do not use the black Test Cap if its storage pouch is punctured or not fully sealed. The cap contains a freeze-dried bead of reagents, including enzymes, that are sensitive to moisture.
- Do not use the Detect COVID-19 Test past the Use By date on the pouch label.
- All components other than the Warmer are single-use and should be disposed of properly in accordance with your institution's waste disposal requirements.
- See Section 9 (procedure for use) for detailed directions on reconstitution, mixing, and readout.
- See Section 10 for detailed instructions on the controls included with the test. These help indicate whether the reaction is taking place correctly.

7.3 Specimens

- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Section 9.2, Collection). Specimen stability under conditions other than those recommended has not been evaluated.

7.4 Components & Reagents

- Do not remove the black Test Cap after screwing it onto the Warmer Tube.
- Do not use a black Test Cap that has been dropped after removing it from the packaging.
- Do not shake the Warmer Tube except as described in Section 9.3 (Warmer Tube Preparation).
- Begin a warming cycle for each sample within 1 hour of collecting the sample.
- Place swabs immediately into the Collection Tube after collecting samples. Failure to do so may result in dried swabs and yield an incorrect test result.

- Do not put anything into the chimney of the Reader until instructed to do so. Doing so may lead to indeterminate results.

- Each single-use Swab is used to process one test. Do not reuse processed Swabs.

- Each single-use Collection Tube is used to process one test. Do not reuse processed Collection Tubes.

- Each single-use black Test Cap is used to process one test. Do not reuse processed Test Caps.

- Each single-use Dropper is used to process one test. Do not reuse processed Droppers.

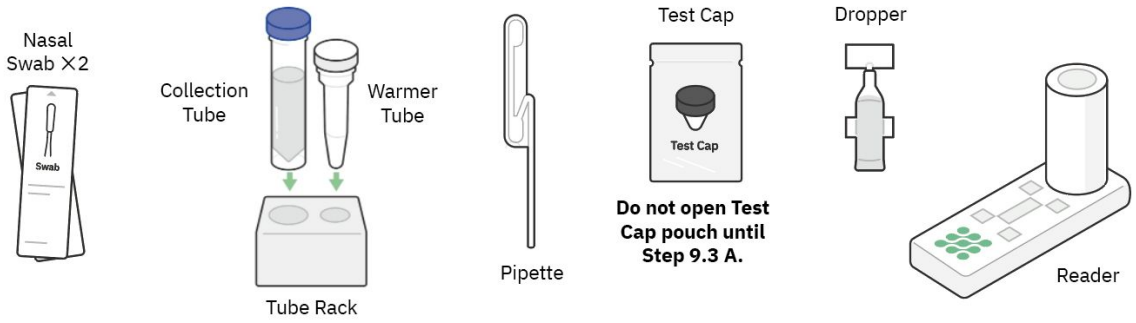
- Each single-use Reader is used to process one test. Do not reuse processed Readers.
- Do not warm the samples using any warming protocol other than the one described in Sections 9.4 and 9.5 as other protocols have not been tested.
- Do not attempt to interpret Reader results outside the time range described in Section 9.6 (10-60 minutes after fully inserting Warmer Tube into Reader). Doing so may yield inaccurate results.
- Do not tamper with the Reader after processing a test.

8. Operating Conditions

- The test should be used between 59 °F and 86 °F (15 °C and 30 °C). Failure to do so may yield invalid results.
- The test is best used in a room with adequate lighting and away from glare. Failure to do so may result in an inability to see the results on the test.
- The Warmer Tube must be inserted into the Reader within 60 minutes after completing the warming step. Failure to do so may yield invalid results.
- The test must be run on a level surface and should not be moved during operation. Failure to do so may yield invalid or inaccurate results.
- If a power failure occurs during the warming protocol or the Warmer is unplugged during the Warmer protocol, the test result is invalid, and the sample should be retested following the retest procedure laid out in Section 12.2.

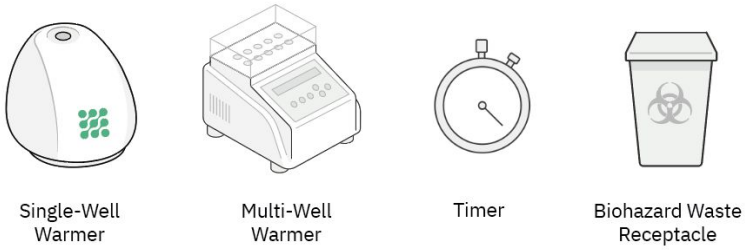
9. Procedure

9.1 Setup



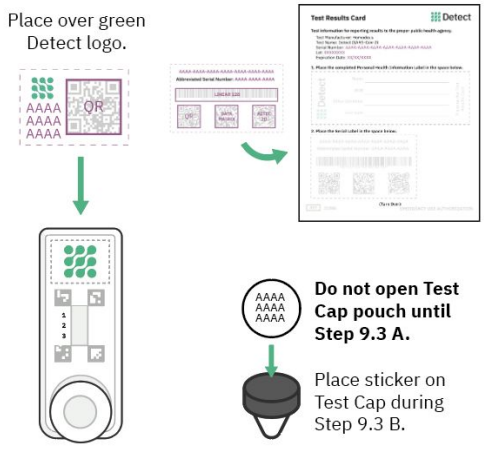
Not included

Note: See section 10.2 for information about external controls.



A. Clean work surface and label components

- Wipe down work surface with 10% bleach.
- Wash hands and wear appropriate PPE.
- Place included stickers on test components as shown.
- **Do not place any sticker on side of Warmer Tube.**
- Place both tubes inside Tube Rack.



9.2 Collection

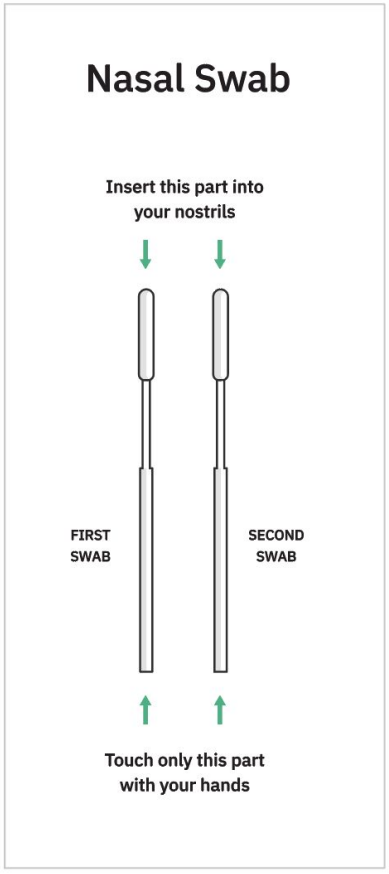
Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection or improper specimen handling and/or transport may yield a false result. Samples in Collection Buffer can be stored at room temperature (59–86 °F or 15–30 °C) for up to 1 hour until testing is performed on the Detect system. Refrigerate (36–46 °F or 2–8 °C) remnant sample in case a retest is needed. For specimen transport and storage requirements and additional information, refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) using the link provided below.

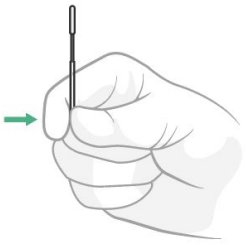
https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html

9.2.1 Anterior Nares Swab Self-Collection Procedure (2 Swabs)

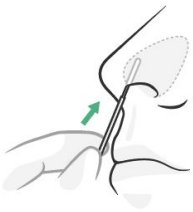
Important: Give the following directions to the patient who is being tested. Give the patient the print-out titled “Instructions for Patient Nasal Swab Self-Collection,” and give them a moment to read the directions.

Note: The Self-Collection Procedure Guide can be found on the Detect website at: www.detectnow.com/resources

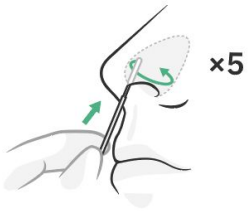




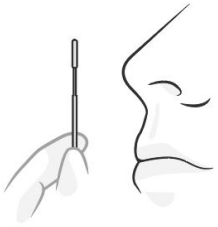
01
Take hold of the first swab, making sure not to touch the tip.



02
Insert the swab into your nostril, about 1-2 cm deep. Make sure the swab tip is in the nostril.



03
Run the swab in a circle, around the inner surface of the nostril 5 times, while applying light contact to the inside of the nostril.



04
Gently remove the swab.

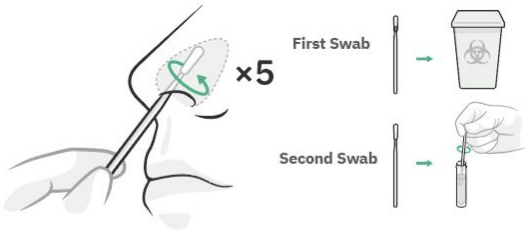
05
Insert the swab into the opposite nostril, and swab 5 times the same way as before.

06
Hand the swab to the test coordinator, making sure not to touch the tip of the swab. **Repeat with the second swab.**

9.2.2 Sample Collection

A. Instruct patient to swab nostrils

- **Complete this step within 15 minutes.**
- **First Swab:** Instruct patient to swab in a circle around each nostril 5 times. This clears the nose of excess material. Discard first Swab.
- **Second Swab:** Instruct patient to swab again with second Swab. This Swab is to be used for the test.

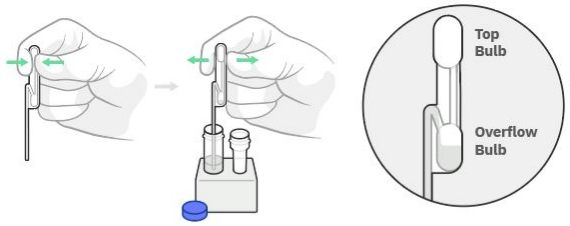


B. Stir Swab in liquid

- Remove blue cap from Collection Tube.
- Submerge Swab tip into liquid in Collection Tube.
- Vigorously stir for 15 seconds.
- Discard Swab.
- Remove and discard cap from Warmer Tube.

C. Draw liquid into Pipette

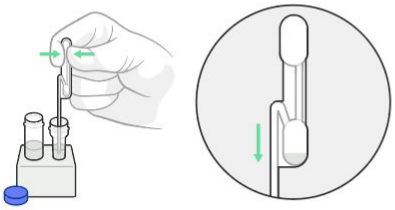
- Firmly squeeze and hold **top bulb** of Pipette **before** submerging Pipette into Collection Tube.
- Submerge Pipette into Collection Tube. Release top bulb to draw liquid into Pipette until some liquid is in overflow bulb.
- If no liquid enters overflow bulb, repeat entire step then release until some liquid enters overflow bulb.



Squeeze and hold. Submerge and release. **Note:** A small amount of liquid must flow into overflow bulb.

D. Transfer liquid to Warmer Tube

- Place Pipette into Warmer Tube.
- Squeeze **only top bulb** to dispense liquid.
- **Do not squeeze overflow bulb.** Some liquid may remain in overflow bulb.
- Discard Pipette.
- Replace blue cap on Collection Tube. Label with patient information. Refrigerate (2 to 8° C) in case a retest is needed.



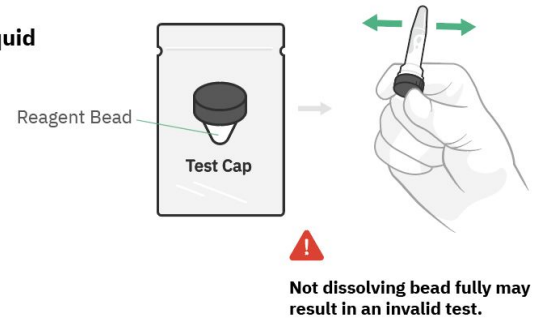
Note: Perform liquid transfer only once.

Note: Do not squeeze overflow bulb at any time.

9.3 Warmer Tube Preparation

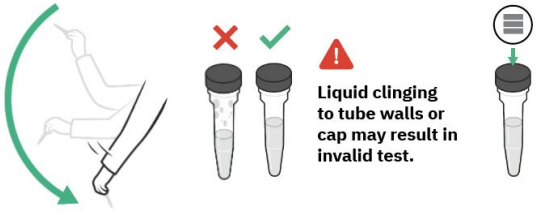
A. Apply Test Cap and dissolve Reagent Bead in liquid

- **Be careful opening pouch** as contents may spill. Remove Test Cap.
- Firmly screw cap onto Warmer Tube. **Ensure that it is tight.**
- **Turn Warmer Tube upside down.**
- **Holding by Cap**, shake vigorously for **10 seconds.**



B. Force all liquid to bottom of Warmer Tube

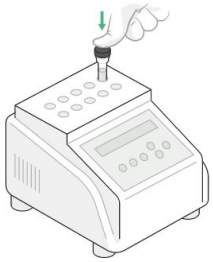
- Collect **all liquid to bottom** of Warmer Tube by **forcefully moving arm** as shown.
- **Repeat until all liquid has pooled at bottom of tube.**
- Place the round serial number sticker on top of Test Cap.



9.4 Warming with Multi-Well Warmer

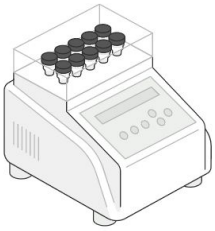
A. Place Warmer Tube(s) in Warmer.

- Plug in and turn on power switch on back of Warmer.
- Remove clear cover and place Warmer Tube(s) in Warming Block.
- **Put clear cover back onto Warmer.**



B. Press "Start/Stop" and wait 55 minutes.

- Press "Start/Stop" button on Warmer.
- Set timer for **55 minutes.**
- Leave Tubes in Warmer and proceed to Step 9.6.
- **Complete Step 9.6 within one hour of warming cycle completion.**



Note: Warming is not complete until the screen displays "Finished."

Note: The complete Quick Reference Guide for the Detect COVID-19 Test employing the Detect Multi-Well Warmer can be found on the Detect website at: www.detectnow.com/resources

9.5 Warming with Single-Well Warmer

A. Plug in Warmer and Insert Warmer Tube. Wait 55 minutes.

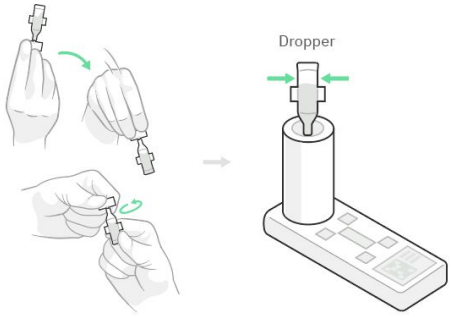
- Ensure Warmer is plugged in and green light is solid green. If green light is not on, unplug and plug in again.
- Insert Warmer Tube **fully** into Warmer.
- Warmer will beep and green light will start blinking when Tube is fully inserted.
- Warming is complete after **55 minutes.** Warmer will beep twice and green light will stop blinking.
- Leave Tube in Warmer and proceed to Step 9.6.
- **Complete Step 9.6 within one hour of warming cycle completion.**

Note: The complete Quick Reference Guide for Detect COVID-19 Test that employs the Detect Single-Well Warmer can be found on the Detect website at: www.detectnow.com/resources

9.6 Results

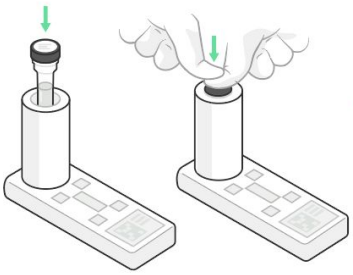
A. Open Dropper and insert in Reader

- Hold Dropper by tip and snap wrist downward to **collect liquid at the bottom**.
- Twist off and discard tip of Dropper.
- Insert Dropper into Reader chimney.
- **Squeeze entire contents of Dropper into center of Reader chimney.**
- Discard Dropper and immediately proceed to Step B.



B. Push Warmer Tube into Reader

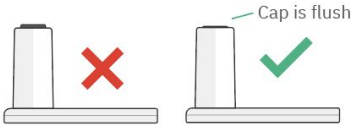
- Remove Tube from Warmer and place into Reader chimney.
- **Press down firmly on Tube using both thumbs.** You may hear a "pop."



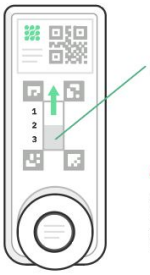
Ensure Tube and Reader are labeled for the same patient.

C. Initiate liquid flow

- Keep pressing until top of Warmer Tube **is flush** with top of Reader chimney.
- Liquid flow should begin within 10 seconds. If not, **tap Reader firmly against work surface 3 times to initiate flow**. Repeat if necessary.
- Start a timer for 10 minutes. After 10 minutes, read results.



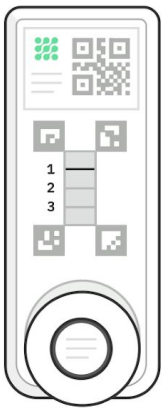
Note: Cap not being flush with Reader chimney may result in an invalid test.



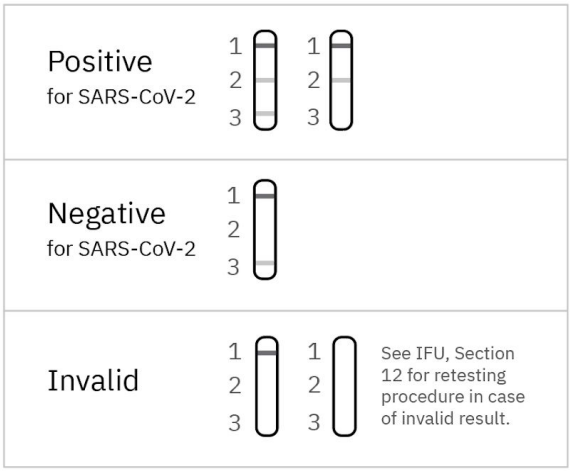
Once in Reader, Tube should not be opened or tampered with.

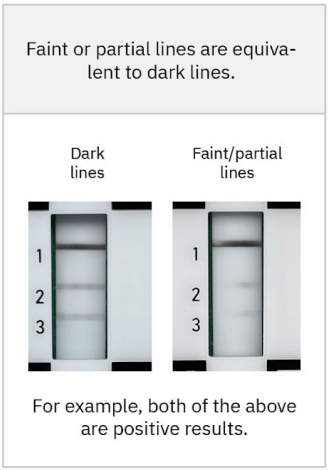
Note: Look closely when interpreting results. Line darkness and fullness may vary. It is common for Line 1 to have a darker, fuller appearance than Lines 2 and 3. The following table illustrates possible test results:

D. Read Results



Example result shown is positive.





After reading results, dispose of all used test kit components according to institution's standard practices.
Do NOT remove Tube from Reader. Do NOT discard Warmer as it is reusable.

Result	Readout Check Control (Line 1)	Test Band (Line 2)	Sample Processing Control (Line 3)
SARS-CoV-2 Positive	+	+	+/-
SARS-CoV-2 Negative	+	-	+
Invalid (1)	+	-	-
Invalid (2)	-	+/-	+/-

- The appearance of Line 1 and Line 3 alone is a SARS-CoV-2 Negative result.
- The appearance of Line 1, Line 2, and Line 3 is a SARS-COV-2 Positive result.
- The appearance of Line 1 and Line 2 alone is a SARS-CoV-2 Positive result.
- The appearance of Line 1 alone is an Invalid result.
- The appearance of Line 2 and/or Line 3 alone is an Invalid result.
- If no lines appear, the result is invalid.
- For detailed interpretation of results see Section 11 (Interpretation of Results).

10. Quality Control

10.1 Internal Controls

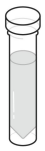
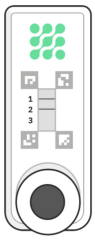
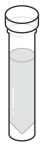
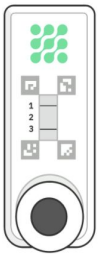
CONTROL Each reaction includes a Sample Processing Control (SPC) and Readout Check Control (RCC).

Sample Processing Control (SPC, Line 3) - Ensures that the sample was processed correctly. The Sample Processing Control is designed to detect and amplify a human control gene that will be present in a swab sample collected from a patient. The SPC determines whether sample collection was performed correctly and whether amplification reaction conditions were appropriate (temperature, time, and reagent mixing). The SPC should be positive in a human sample that tests negative and positive in a human sample that tests positive; however, a positive sample that lacks the SPC is still valid.

Readout Check Control (RCC, Line 1) - Ensures that the Reader functions properly. When the Warmer Tube is correctly inserted into the Reader, a blade will rupture the bottom of the Warmer Tube, causing its contents to flow onto the lateral flow strip housed within. When the sample wicks up the lateral flow strip, the RCC will appear. The RCC should be positive for all tests.

10.2 External Controls

Positive and negative controls should be used to confirm that the test is correctly performed and that all components of the assay are working as intended. Both controls should be tested upon completion of training for a new operator, receipt of a new shipment of test kits, and in accordance with guidelines or requirements of local, state and/or federal regulations or accrediting organizations and the laboratory's internal Quality Control procedures.

Product code	Unit	Control Key
Positive Control - SeraCare AccuPlex™ SARS-CoV-2 Control Kit – FULL GENOME; Catalog # 0505-0229	Six (6) x 0.6 mL vials per package 	Valid Positive Control Run 
Negative Control - ZeptoMetrix SARS Associated Coronavirus 2 (SARS-CoV-2) Negative Control; Catalog # NATSARS(COV2)-NEG	Six (6) x 0.5 mL vials per package 	Valid Negative Control Run 

To run either the positive or negative external control, follow the testing procedure outlined below:

1. Prepare a Detect COVID-19 Test for use as outlined in the 9.1 Setup section of this guide.
2. Omit the patient swab collection step 9.2.1.

3. Instead, completely submerge the Swab tip in the liquid contained within the external control vial and twirl in the external control vial for 15 seconds.
4. Resume the standard Detect COVID-19 Test procedure at the sample resuspension step 9.2.2 B, treating the Swab submerged in the external control as if it were a patient nasal Swab.
5. Cap and discard the used external control vial in the appropriate specimen waste container according to the Institution's standard practice.

If the positive or negative external control fails, repeat with a new external control vial and a new test. If the repeat test fails, please contact Detect Customer Support at +1 (855) 322-3692 or support@detectnow.com

11. Interpretation of Results

Results are to be interpreted according to the chart listed in Section 9.6 (Results), step D. See the table below to interpret result statements from the Detect COVID-19 Test.

Result	Interpretation
SARS-CoV-2 Positive	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are detected. ● The SARS-CoV-2 signal for the nucleic acid target(s) has an endpoint above the minimum threshold of detection. ● SPC is ignored because SARS-CoV-2 target amplification occurred. ● RCC must be positive, indicating that readout was performed correctly, for result to be valid.
SARS-CoV-2 Negative	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are not detected. ● The SARS-CoV-2 signal for the nucleic acid target(s) has an endpoint below the minimum threshold of detection. ● SPC must be positive, indicating that sample collection and test preparation were performed correctly, for result to be valid. ● RCC must be positive, indicating that readout was performed correctly, for result to be valid.
Invalid (1)	SPC does not meet acceptance criteria. Presence or absence of 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. SPC failure may indicate one or more of the following:

	● The sample was not collected properly. ● Amplification did not occur. ● Readout was not performed correctly. Repeat test according to the Retest Procedure in Section 12.2.
Invalid (2)	RCC does not meet acceptance criteria. Presence or absence of 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. RCC fail may indicate the following: ● Readout step was not performed correctly. Repeat test according to the Retest Procedure in Section 12.2.

12. Retests

12.1 Reasons to Retest

If an **INVALID** test result occurs, repeat the test once according to instructions in 12.2 (Retest Procedure). If after retesting the test result is still invalid, perform the test with the external controls and a new test. If the repeat external control test fails to produce a valid result, please contact Detect Customer Support at +1 (855) 322-3692 or support@detectnow.com

12.2 Retest Procedure

Note: If an invalid test result occurs, retest immediately. Failure to do so may result in the loss of the fidelity of the reserved sample in the Collection Tube.

1. Prepare the work surface as detailed in Section 9.1 (Setup).
2. Retrieve the stored Collection Tube of the patient whose test was invalid.
3. Obtain a new Detect COVID-19 Test.
4. Dispose of the Swabs and Collection Tube, as they will not be used.
5. Using the sample previously resuspended in the Collection Tube, place the Collection Tube (blue cap) and the Warmer Tube (empty, capped) in the Tube Rack.

6. Remove the blue cap from the reserved Collection Tube.

7. Repeat the test procedure outlined in Section 9 (Procedure) beginning at Step C of Section 9.2.2 (“Draw liquid into Pipette”).

13. Limitations

- Performance has only been evaluated for self-collected anterior nares swab specimens. Use of the Detect COVID-19 Test with other specimen types has not been assessed,and performance characteristics are unknown.
- A false negative result may occur if a specimen is improperly collected, transported, or handled. False negative results may also occur if inadequate numbers of SARS-CoV-2 virions are present in the specimen.
- Invalid results may occur in patients currently taking high dose biotin (vitamin B7) supplements. Biotin levels higher than 0.13 µg/mL have been demonstrated to result in invalid test results in some cases.
- As with any molecular test, mutations within the Detect target regions of SARS CoV-2 could affect primer binding, resulting in failure to detect the presence of virus.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

14. Performance Characteristics

14.1 Analytical Sensitivity (Limit of Detection)

The Limit of Detection (LoD) was determined by testing the Detect test’s sensitivity with contrived positive samples created through serial dilution of heat-inactivated SARS-CoV-2 in pooled nasal matrix. The LoD of the Detect COVID-19 Test was determined to be 8,250 copies/swab, which is equivalent to 5 copies per µL if all virus is transferred from the swab to the buffer.

Limit of Detection using heat-inactivated SARS-CoV-2 (BEI, USA-WA1/2020):

Viral Load (genomic copies/swab)	Concentration (genomic copies /µL Collection Buffer)	LoD Study Detection Rate	% Detected
4,125	2.5	8/20	40%
8,250	5	20/20	100%
16,500	10	20/20	100%

14.2 Analytical Reactivity (Inclusivity)

An *in silico* inclusivity study was performed to analyze the Detect test's primer binding sequences in the SARS-CoV-2 genome to demonstrate that the primers will detect all variants of the SARS-CoV-2 virus identified to date (November 2020) and predict inclusivity of the Detect COVID-19 Test. A total of 170,190 sequences from the GISAID EpiCoV database (www.gisaid.org) were evaluated in the study.

97.4% of genomes contained no mismatches. Based on an *in silico* analysis, at least 99% of all 114,757 genomes are expected to be robustly detected by the Detect test's SARS-CoV-2 primer set.

In addition, an *in silico* inclusivity analysis of the Detect's test's SARS-CoV-2 primer set was performed with the two emerging variants listed by the US Centers for Disease Control and Prevention (CDC) in January 2021: B.1.1.7 lineage (also known as 20B/501Y.V1 Variant of Concern (VOC) 202012/01) and B.1.351 lineage (also known as 20C/501Y.V2). Neither of the two emerging variants have any lineage-defining mutations in the SARS-CoV-2 genome region targeted by the Detect's test's SARS-CoV-2 primer set; it is therefore expected that the Detect test will robustly detect both of these emerging variants.

14.3 Analytical Specificity/Exclusivity (Cross-Reactivity)

The Detect COVID-19 Test's cross-reactivity with closely related pathogens, common disease agents, and normal and pathogenic flora was tested by spiking genomic material from the organism or the organism itself directly into triplicate Detect reactions at the concentrations listed in the table below. The Detect test showed no interaction with all of the 31 organisms tested. The Detect test was also tested repeatedly with pooled human nasal matrix (115 replicates in all) and showed no cross-reactivity.

Organism	**Target**	**Concentration Tested (in final reaction)**	**SARS-CoV-2** # Detected /# tested	**Human Control Gene** # Detected /# tested	**Cross-reactivity with Detect**
Human coronavirus 229E	Synthetic RNA	1.00E+06 copies/mL	0/3	0/3	No
Human coronavirus OC43	Synthetic RNA	1.00E+06 copies/mL	0/3	0/3	No
Human coronavirus HKU1	Synthetic RNA	1.00E+06 copies/mL	0/3	0/3	No
Human coronavirus NL63	Virus	4.00E+04 TCID50/mL	0/3	0/3	No
MERS-coronavirus	Synthetic RNA	1.00E+06 copies/mL	0/3	0/3	No
SARS-coronavirus	Synthetic RNA	1.00E+06 copies/mL	0/3	0/3	No
Adenovirus (Adenoid 71)	Virus	1.00E+05 TCID50/mL	0/3	1/3 ǂ	No
Human Metapneumovirus	Virus	1.00E+05 TCID50/mL	0/3	0/3	No
Parainfluenza virus 1	Synthetic RNA	1.00E+06 copies/mL	0/3	0/3	No
Parainfluenza virus 2	Virus	1.00E+05 TCID50/mL	0/3	0/3	No
Parainfluenza virus 3	Virus	1.00E+05 TCID50/mL	0/3	0/3	No
Parainfluenza virus 4	Synthetic RNA	1.00E+06 copies/mL	0/3	0/3	No
Influenza A H1N1	Synthetic RNA	1.00E+06 copies/mL	0/3	0/3	No
Influenza A H3N3	Synthetic RNA	1.00E+06 copies/mL	0/3	0/3	No
Influenza B	Synthetic RNA	1.00E+06 copies/mL	0/3	0/3	No

Enterovirus 68	Synthetic RNA	1.00E+06 copies/mL	0/3	0/3	No
Respiratory syncytial virus (Subgroup A)	Virus	1.00E+05 PFU/mL	0/3	3/3 †	No
Rhinovirus 89	Synthetic RNA	1.00E+06 copies/mL	0/3	0/3	No
Chlamydia pneumoniae	Bacteria	1.00E+06 IFU/mL	0/3	3/3 †	No
Haemophilus influenzae	Bacteria	1.00E+06 CFU/mL	0/3	0/3	No
Legionella pneumophila	Bacteria	1.00E+06 CFU/mL	0/3	0/3	No
Mycobacterium tuberculosis	Genomic DNA	1.00E+06 copies/mL	0/3	0/3	No
Streptococcus pneumoniae	Genomic DNA	1.00E+06 copies/mL	0/9	1/9*	No
Streptococcus pyogenes	Genomic DNA	1.00E+06 copies/mL	0/3	0/3	No
Bordetella pertussis	Genomic DNA	1.00E+06 copies/mL	0/3	0/3	No
Mycoplasma pneumoniae	Genomic DNA	1.00E+06 copies/mL	0/3	0/3	No
*Pneumocystis jirovecii (PJP)-S. cerevisiae***	Yeast	1.00E+06 CFU/mL	0/3	0/3	No
Candida albicans	Yeast	1.00E+06 CFU/mL	0/3	0/3	No
Pseudomonas aeruginosa	Genomic DNA	1.00E+06 copies/mL	0/3	0/3	No
Staphylococcus epidermis	Genomic DNA	1.00E+06 copies/mL	0/3	0/3	No
Streptococcus salivarius	Genomic DNA	1.26E+02 ng/mL	0/9	1/9*	No

† The human control gene was detected due to the viral or bacterial stock available containing cell culture lysate.

* Initial cross-reactivity testing showed human control gene detection in one replicate, but follow-up testing did not confirm the interaction, and the source is believed to have been environmental contamination.

** Due to limited pathogen availability, cross-reactivity was tested with a recombinant version of *S. cerevisiae* containing genomic material from PJP.

In addition, *in silico* cross-reactivity analysis of Detect primer sequences was performed by comparing them to representative genomic sequences of the specific respiratory microorganisms below, downloaded from the NCBI database. The table below details all detected instances of ≥80% homology between a primer and respiratory microorganism genome.

Greater than 80% homology was only apparent for the SARS-CoV-2 B3 primer (with *Candida albicans*) and the human internal control gene F3 primer (with *Mycobacterium tuberculosis* (2 sites), *Pneumocystis jirovecii* (PJP) and *Pseudomonas aeruginosa*). Thus, only 1 primer from each set displayed ≥80% homology with any of these respiratory microorganisms, and none of the microorganisms displayed ≥80% homology with more than 1 primer. Further, none of the labelled primers required for detection of the amplified nucleic acid target showed ≥80% homology with any of the listed respiratory microorganism genomes. Therefore, *in silico* analysis identified no potential unintended cross-reactivity of the Detect test with the listed respiratory pathogens, including other coronaviruses.

Organism	**SARS-CoV-2 primer set**	**Human control gene primer set**
Human coronavirus 229E	no alignment found	no alignment found
Human coronavirus OC43	no alignment found	no alignment found
Human coronavirus HKU1	no alignment found	no alignment found
Human coronavirus NL63	no alignment found	no alignment found
MERS-CoV	no alignment found	no alignment found
SARS-CoV	no alignment found	no alignment found
Adenovirus (e.g. C1 Ad. 71)	no alignment found	no alignment found
Human Metapneumovirus (hMPV)	no alignment found	no alignment found
Parainfluenza virus 1	no alignment found	no alignment found
Parainfluenza virus 2	no alignment found	no alignment found
Parainfluenza virus 3	no alignment found	no alignment found
Parainfluenza virus 4	no alignment found	no alignment found
Influenza A	no alignment found	no alignment found

Influenza B	no alignment found	no alignment found
Enterovirus (e.g. EV68)	no alignment found	no alignment found
Respiratory syncytial virus	no alignment found	no alignment found
Rhinovirus A	no alignment found	no alignment found
Rhinovirus B	no alignment found	no alignment found
Rhinovirus C	no alignment found	no alignment found
Chlamydia pneumoniae	no alignment found	no alignment found
Haemophilus influenzae	no alignment found	no alignment found
Legionella pneumophila	no alignment found	no alignment found
Mycobacterium tuberculosis	no alignment found	F3 primer only, 82%
Streptococcus pneumoniae	no alignment found	no alignment found
Streptococcus pyogenes	no alignment found	no alignment found
Bordetella pertussis	no alignment found	no alignment found
Mycoplasma pneumoniae	no alignment found	no alignment found
Pneumocystis jirovecii (PJP)	no alignment found	F3 primer only, 88%
Candida albicans	B3 primer only, 82%	no alignment found
Pseudomonas aeruginosa	no alignment found	F3 primer only, 82%
Staphylococcus epidermidis	no alignment found	no alignment found
Staphylococcus salivarius	no alignment found	no alignment found

14.4 Analytical Specificity (Interfering Substances)

Common endogenous and exogenous substances that might be present in clinical nasal swab samples were tested for interference with the Detect test. Each potentially interfering substance was spiked into both negative pooled nasal matrix and contrived positive pooled nasal matrix spiked with heat-inactivated SARS-CoV-2 virus at 2X LoD. From these pools, triplicate swabs were tested using the Detect test. The interfering substances and their concentrations are listed in the table below. The results show that the Detect test is robust to a wide range of potentially interfering substances, with the exception of biotin at concentrations above 0.13 µg/mL. Labeling is included in the Detect IFU to advise users of this potential interaction.

Interfering Substance	Final Concentration in Nasal Matrix Pool	Negative Samples #Negative /# tested	Positive Samples # Positive /# tested
Rhinocort Allergy	15% v/v	3/3	3/3
Afrin Nasal Congestion Relief Spray	15% v/v	3/3	3/3
Zicam Cold Remedy Nasal Spray	15% v/v	3/3	3/3
Chloraseptic Sore Throat Spray	15% v/v	3/3	3/3
Flonase Allergy Relief Nasal Spray	15% v/v	3/3	3/3
Mupirocin	5 mg/mL	3/3	3/3
Neo-Synephrine	15% v/v	3/3	3/3
Nasal Saline Spray	15% v/v	3/3	3/3
Tobramycin	600 µg/mL	3/3	3/3
Fresh whole blood	10%	3/3	3/3
Biotin	3.5 µg/mL	0/3*	0/3*
	1.17 µg/mL	3/3	2/3*
	0.39 µg/mL	2/3*	3/3
	0.13 µg/mL	3/3	3/3
Dexamethasone	15% v/v	3/3	3/3
Flunisolide	15% v/v	3/3	3/3
Mucin	1 mg/mL	3/3	3/3
Triamcinolone	15% v/v	3/3	3/3
Mometasone nasal spray	1 mg/mL	3/3	3/3

*Undetected replicates were all invalid.

14.5 Clinical Evaluation

A prospective, multi-center clinical study was conducted across four sites in the United States that are representative of a typical Point of Care setting. Testing at the sites was performed by untrained operators. The study enrolled symptomatic subjects, each of whom self-collected two nasal swab samples. For each subject, the first swab was collected and sent to a reference laboratory and run through a high sensitivity FDA-authorized SARS-CoV-2 RT-PCR test by trained laboratory personnel as a comparator, while the second swab was run through the Detect test by an operator at the clinical site.

Comparing the Detect COVID-19 Test's results to those produced by the high sensitivity FDA-authorized SARS-CoV-2 RT-PCR test, the Positive Percent Agreement (PPA) was 93.5% (43/45) and the Negative Percent Agreement (NPA) was 100% (62/62).

Clinical Study Results Summary

	FDA-authorized SARS-CoV-2 RT-PCR Assay	
Detect	Positive	Negative
Positive	43	0
Negative	2*	62

*All reference-arm remnant samples were also processed with the Roche Cobas SARS-CoV-2 PCR test, which produced results in agreement with the original comparator assay, except for one sample which gave a negative on the Detect test, a negative on the Roche Cobas, and a positive on the original comparator. This suggests that one of the two apparent Detect false negative results may have in fact been a true negative that produced a false positive on the original comparator assay.

Positive Percent Agreement (PPA): 95.6% (43/45), (95% CI: 85.2%-98.8%)
Negative Percent Agreement (NPA): 100% (62/62), (95% CI: 94.2%-100.0%

Detect, Inc.
530 Old Whitfield St.
Guilford, CT 06437 USA
Customer Support: support@detectnow.com or call toll-free +1 855-322-3692

15. Bibliography

1 Centers for Disease Control and Prevention. “Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19).” (Refer to latest edition.) https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

2 Centers for Disease Control and Prevention. “Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition.” (Refer to latest edition.) https://www.cdc.gov/labs/BMBL.html

3 Clinical and Laboratory Standards Institute. “Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline.” Document M29 https://clsi.org/standards/products/microbiology/documents/m29/ (Refer to latest edition).

16. Symbols and Abbreviations

The following symbols are used throughout this manual:

Symbol	Definition
	Biohazard – Potentially infectious materials. Precautions must be observed.
	Consult the instructions for use for important cautionary information such as warnings and precautions that cannot, for a variety of reasons, be presented on the medical device itself.
IVD	For *in vitro* diagnostic use
CONTROL	Positive or negative control
	Temperature limitation
	Do not re-use
	Manufacturer

17. Quick Reference Guide: For Professional Use

Quick Reference Guide: Single-Well

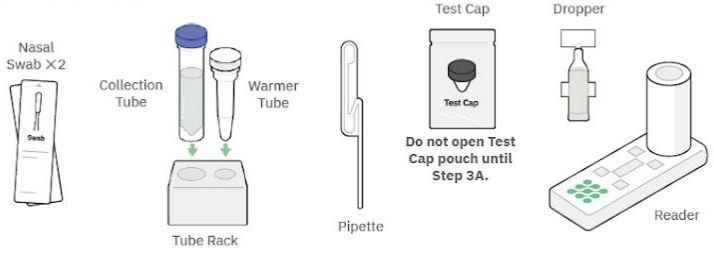
Detect

Full instructions can be found at detectnow.com/ifu
Please read complete instructions carefully before proceeding with the test.
Call 855-322-3692 for assistance.

detectnow.com/resources

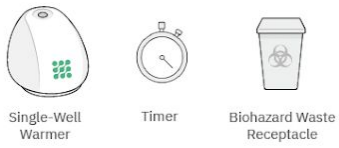
1. Setup

Included in Test Kit



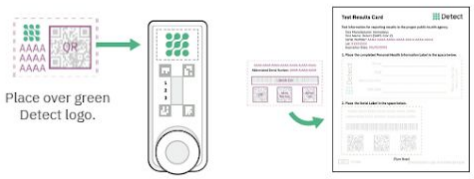
Not included

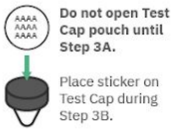
Note: See Instructions for Use (IFU) for information about external controls.



A. Clean work surface and label components

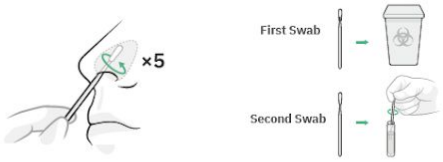
- Wipe down work surface with 10% bleach.
- Wash hands and wear appropriate PPE.
- Place included stickers on test components as shown.
- Place both tubes inside Tube Rack.
- **Do not place any sticker on side of Warmer Tube.**





2. Collection

A. Instruct patient to swab nostrils

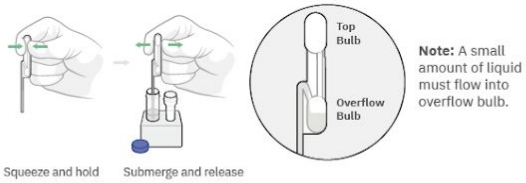


- **Complete Step 2 within 15 minutes.**
- **First Swab:** Instruct patient to swab in a circle around each nostril 5 times. This clears the nose of excess material. Discard first Swab.
- **Second Swab:** Instruct patient to swab again with second Swab. This Swab is to be used for the test.

B. Stir Swab in liquid

- Remove blue cap from Collection Tube.
- Submerge Swab tip into liquid in Collection Tube.
- Vigorously stir for 15 seconds.
- Discard Swab.
- Remove and discard cap from Warmer Tube.

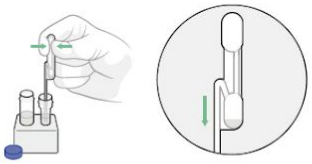
C. Draw liquid into Pipette



Note: A small amount of liquid must flow into overflow bulb.

- Firmly squeeze and hold **top bulb** of Pipette **before** submerging Pipette into Collection Tube.
- Submerge Pipette into Collection Tube. Release top bulb to draw liquid into Pipette until some liquid is in overflow bulb.
- If no liquid enters overflow bulb, repeat entire step then release until some liquid enters overflow bulb.

D. Transfer liquid to Warmer Tube



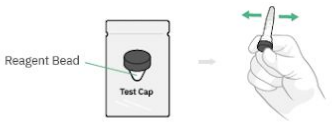
Note: Perform liquid transfer only once.

Note: Do not squeeze overflow bulb at any time.

- Place Pipette into Warmer Tube.
- Squeeze **only top bulb** to dispense liquid.
- **Do not squeeze overflow bulb.** Some liquid may remain in overflow bulb.
- Discard Pipette.
- Replace blue cap on Collection Tube. Label with patient information. Refrigerate (2 to 8° C) in case a retest is needed.

3. Warmer Tube Preparation

A. Apply Test Cap and dissolve Reagent Bead in liquid



Not dissolving bead fully may result in an invalid test.

- **Be careful opening pouch** as contents may spill. Remove Test Cap.
- Firmly screw cap onto Warmer Tube. **Ensure that it is tight.**
- **Turn Warmer Tube upside down.**
- **Holding by Cap**, shake vigorously for **10 seconds** to dissolve and mix reagent bead into liquid.

B. Force all liquid to bottom of Warmer Tube

Liquid clinging to tube walls or cap may result in invalid test.

- Collect **all liquid to bottom** of Warmer Tube by **forcefully moving arm** as shown above.
- **Repeat until all liquid has pooled at bottom of tube.**
- Place the round serial number sticker on top of Test Cap.

4. Warming

A. Plug in Warmer and Insert Warmer Tube. Wait 55 minutes.

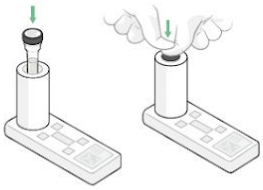
- Ensure Warmer is plugged in and green light is solid green. If green light is not on, unplug and plug in again.
- Insert Warmer Tube **fully** into Warmer.
- Warmer will beep and green light will start blinking when Tube is fully inserted.
- Warming is complete after **55 minutes.** Warmer will beep twice and green light will stop blinking.
- Leave Tube in Warmer and proceed to Step 5.
- **Complete Step 5 within one hour of warming cycle completion.**

5. Results

A. Open Dropper and insert in Reader

- Hold Dropper by tip and snap wrist downward to **collect liquid at the bottom**.
- Twist off and discard tip of Dropper.
- Insert Dropper into Reader chimney.
- **Squeeze entire contents of Dropper into center of Reader chimney.**
- Discard Dropper and immediately proceed to Step B.

B. Push Warmer Tube into Reader



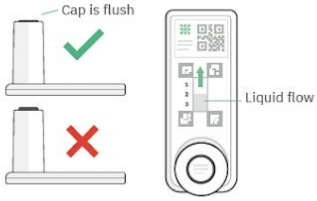
Ensure Tube and Reader are labeled for the same patient.

- Remove Tube from Warmer and place into Reader chimney.
- **Press down firmly on Tube using both thumbs.** You may hear a "pop."

C. Initiate liquid flow

Note: Cap not being flush with Reader chimney may result in an invalid test.

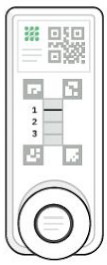
Once in Reader, Tube should not be opened or tampered with.



- Keep pressing until top of Warmer Tube **is flush** with top of Reader chimney.
- Liquid flow should begin within 10 seconds. If not, **tap Reader firmly against work surface 3 times to initiate flow**. Repeat if necessary.
- Start a timer for 10 minutes. After 10 minutes, read results.

D. Read results

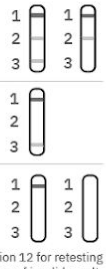
Example result shown is positive.



Positive for SARS-CoV-2

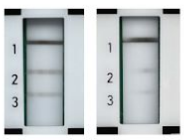
Negative for SARS-CoV-2

Invalid



See IFU, Section 12 for retesting procedure in case of invalid result.

Faint or partial lines are equivalent to dark lines.



For example, both of the above are positive results.

After reading results, dispose of all used test kit components according to institution's standard practices. **Do NOT remove Tube from Reader. Do NOT discard Warmer as it is reusable.**

For Use Under an Emergency Use Authorization Only.

Effective Date: January 2021
Need assistance? Call 855-322-3692

The complete Quick Reference Guide for the Detect COVID-19 Test can be found on the Detect website at: www.detectnow.com/resources