CE

CoviRAT™

Rapid Test for Detection of SARS-CoV-2 Antigen in Human Nasopharyngeal Swab Specimens

DEVICE

INTENDED USE

CoviRAT™ is an invitro, rapid, qualitative immunoassay for the detection of nucleocapsid protein antigens expressed by the SARS-CoV-2 virus present in human nasopharyngeal swab specimens. It is to be used for screening or to aid in the diagnosis of COVID-19 disease. This rapid antigen detection test takes 15-30 minutes for producing a positive or negative test result. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. Presumptive positive or negative result may need to be further confirmed with a molecular test.

SUMMARY

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the strain of coronavirus that causes Coronavirus disease 2019 (COVID-19), the respiratory illness responsible for the COVID-19 pandemic. This virus was first identified in the respiratory tract of patients with pneumonia in Wuhan, Hubei China, in December 2019 which was then indicated as a newly identified β-coronavirus (nCoV). SARS-CoV-2 is an enveloped, non-segmented, positive sense RNA virus that is included in the sarbecovirus, ortho corona virinae subfamily which is broadly distributed in humans and other mammals . Its diameter is about 65-125 nm, containing single strands of RNA and provided with crown-like spikes on the outer surface. SARS-CoV-2 is a novel β-coronavirus after the previously identified SARS-CoV and MERS-CoV which led to pulmonary failure and potentially fatal respiratory tract infection and caused outbreaks mainly in Guandong, China and Saudi Arabia. Severe Acute Respiratory Syndrome Coronavirus 2 can attack lung cells because there are many conserved receptor entries, namely Angiotensin Converting Enzyme-2. The presence of this virus in host cells will initiate various protective responses leading to pneumonia and Acute Respiratory Distress Syndrome.

The most characteristic symptom of COVID-19 patients is respiratory distress. Most people infected with SARS-CoV-2 virus do not have symptoms, but when present they are usually mild and last less than seven days. Common symptoms of COVID-19 infection are fever, headache, nausea and vomiting. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death. Older people and people with severe chronic conditions are at higher risk of developing serious COVID-19 illness.

PRINCIPLE

CoviRAT™ utilizes the principle of agglutination of antibodies with respective antigen in immuno-chromatography format along with use of nano indicator colloidal particles as agglutination revealing agent. Mouse monoclonal anti-SARS-CoV-2 antibodies are coated as capture in the Test region 'T, Goat anti-mouse IgG as assay control in the control region 'C and mouse monoclonal anti-SARS-CoV-2 antibodies conjugated with color particles are used as detectors in this device. As the test specimen flows through the membrane assembly within the test device, the colored Mouse monoclonal anti-SARS-CoV-2 antibodies- indicator colloidal particles complexes with SARS-CoV-2 antigen, if present in the specimen. This complex moves further on the membrane to the Test region where it is immobilized by the Mouse monoclonal anti-SARS-CoV-2 antibodies coated as capture on the nitrocellulose membrane leading to formation of a colored band in the Test region 'T' which confirms a positive test result. Absence of the colored band in the Test region 'T' indicates a negative test result.

The unreacted conjugate and the unbound complex move further on the membrane and are subsequently immobilized by the Goat anti-mouse IgG coated at the control region 'C', forming a colored band.

This control band serves to validate the test results.

REAGENT AND MATERIAL SUPPLIED

CoviRAT™ test kit comprises of,

A. Individual pouches, each containing:
   1. DEVICE : Membrane assembly pre-dispensed with Mouse monoclonal anti-SARS-CoV-2 antibodies -indicator colloidal particles, Mouse monoclonal anti- SARS-CoV-2 antibodies -capture at test region 'T' and Goat anti-mouse IgG at control region 'C'.
   2. Desiccant pouch.

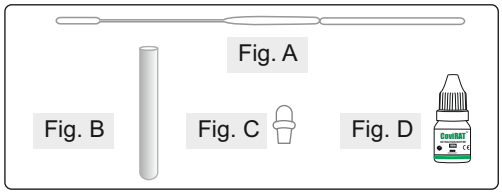
B. Accessories box containing:
   - Sterile nasopharyngeal swabs - **Fig. A**
   - Extraction Buffer tubes - **Fig. B**
   - Nozzles for Extraction Buffer tubes - **Fig. C**
   - Stand for Extraction Buffer tubes

C. BUF: Extraction Buffer bottle-**Fig.D**

D. Package Insert.

E. Test Record Sheet.

REF	502100025
Σ	25 T



ADDITIONAL MATERIAL REQUIRED

Stop watch, Disposable gloves, Micropipette and tips.

STORAGE AND STABILITY

The test kit (including sealed pouches) may be stored between 4ºC to 30ºC till the duration of the shelf life as indicated on the pouch/ carton. DO NOT FREEZE the kit or its components. After first opening of the Extraction Buffer bottle, it can be stored between 4ºC to 30ºC for remaining duration of its shelf life.

NOTE

1. For *in vitro* diagnostic use and for professional use only. NOT FOR MEDICINAL USE.
2. Do not use the kit beyond expiry date and do not re-use the test device.
3. Read the instructions carefully before performing the test.
4. Any modification to the above procedure and / or use of other reagents will invalidate the test results.
5. Do not inter mix the reagent or devices from different lots.
6. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.
7. Wear personal protective equipment, such as gloves and lab coats when handling kit reagents. Wash hands thoroughly after the tests are done.
8. Clean up spills thoroughly using an appropriate disinfectant.
9. Handle all specimens as if potentially infectious. Follow standard bio-safety guidelines for handling and disposal of potentially infective material.
10. The Extraction buffer contains Sodium Azide (< 0.1%), avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing and form highly explosive metal oxides. Flush with large volumes of water to prevent azide build up in the plumbing.

SPECIMEN COLLECTION AND PREPARATION

1. Use the appropriate personal protective equipment while collecting specimen.

Nasopharyngeal swab specimen collection

2. Insert a sterile nasopharyngeal swab into the nostril of the patient, reaching the surface of the posterior nasopharynx. Refer **Fig.1**
3. Gently rotate the swab and slowly pushing the swab a little further, rotate the swab a few more times against the nasopharyngeal wall.
4. Remove the swab with specimen, from the nostril carefully.
5. The specimen should be tested as soon as possible after collection.
6. Specimens may be stored at room temperature for up to 1 hour or at 2-8°C/ 36-46°F for up to 4 hours prior to testing.

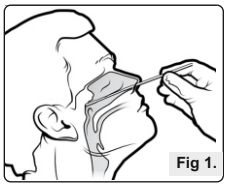


Fig 1.

TEST PROCEDURE

1. Bring the **CoviRAT**™ kit components to room temperature before testing.
2. Place the required number of Extraction Buffer tubes, according to the number of specimens to be tested, in the tube stand provided.
3. Next open the Extraction Buffer bottle by tightening its cap in clockwise direction to pierce the bottle nozzle. Refer **Fig.3**
4. Dispense 8 drops of the Extraction Buffer into Extraction Buffer tube.
   Note: For each patient sample a new tube should be used.
5. Label each Extraction Buffer tubes with the patient's name/ identity.
6. Using the swab provided, collect the nasopharyngeal specimen as mentioned in **Specimen Collection and Preparation** section above

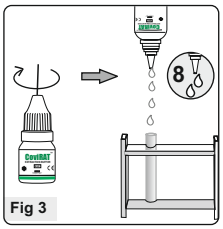


Fig 3

7. Insert the swab with collected specimen into the Extraction Buffer tube.
8. Roll the swab more than **5 times** within Extraction Buffer tube, squeeze against inside of tube, let stand for 1 minute, and squeeze several more times. Refer **Fig.4**

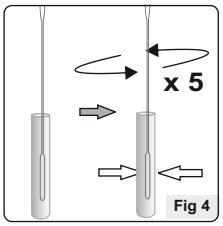


Fig 4

9. Safely remove the swab while squeezing the sides of the Extraction Buffer tube to extract the liquid from the swab completely. Discard the swab into gluteraldehyde or hypochlorite solution following Biohazard protocols.

10. Press the nozzle cap tightly onto the Extraction Buffer tube. The extracted specimen is ready for the test. Refer **Fig.5.**

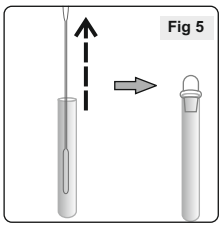


Fig 5

Running the test

11. Next open a **CoviRAT**™ test device pouch by tearing along the "notch".
12. Retrieve the device and desiccant pouch. Check the color of the desiccant. It should be blue. If it has turned colorless or pink, discard that test device and use another device.
13. ***Once opened, the device must be used immediately.***
14. Label the test device with patient's identity.
15. Place the device on a flat horizontal surface.

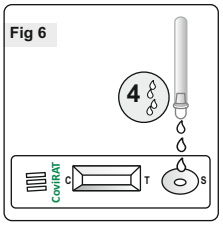


Fig 6

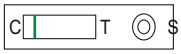
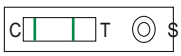
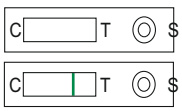
Specimen addition

16. Add 4 drops of the extracted specimen into the specimen port of the device. Refer **Fig. 6**

Read Results

17. Read the test results in 15-30 minutes. Do not read test results beyond 30 minutes.

INTERPRETATION OF RESULT

	**Negative result**: The presence of only one pink-purple coloured band in the control area marked 'C', indicates absence of SARS CoV-2 antigen in the nasopharyngeal specimen.
	**Positive Result:** In addition to the band in the control area marked 'C', appearance of a pink-purple coloured band in the test region 'T', indicates the presence of SARS-CoV-2 antigen in the nasopharyngeal specimen.
	**Invalid Result**: The test result is invalid if no bands appear on the device. The test should also be considered invalid if only the test band appears and no control band appears. In such cases, verify the test procedure and repeat the test with a new device.

PERFORMANCE EVALUATION

External Evaluation

a) In an independent evaluation performed in the USA, **CoviRAT** ™ was tested with RT- PCR confirmed nasopharyngeal specimens of symptomatic patients and asymptomatic patients. Following is the summary of the evaluation results.

	Symptomatic Patients			Asymptomatic Patients			Total Patients		
	RT-PCR	**CoviRAT**™		RT-PCR	**CoviRAT**™		RT-PCR	**CoviRAT**™	
		Sensitivity	Specificity		Sensitivity	Specificity		Sensitivity	Specificity
Positive	23 Nos	95.7%	-	4 Nos	100%	-	27 Nos	96.3%	-
Negative	-	-	-	1 No.	-	100%	1 No.	-	100%

b) At an evaluation performed in a National Reference Centre, using 108 RT-PCR positive and 110 RT-PCR negative nasopharyngeal specimens, **CoviRAT**™ yielded a Sensitivity of 87.96%, Specificity of 96.36%, PPV equal to 95.9% and NPV equal to 89.83%. Another evaluation at the same centre using 87 RT-PCR positive (with Ct values<30) and 110 RT-PCR negative nasopharyngeal specimens, **CoviRAT**™ yielded a Sensitivity of 95.4%, Specificity of 96.36%, PPV equal to 95.4% and NPV equal to 96.36%.

Internal Evaluation

c) **Specificity:** In an in-house evaluation with 202 Nos. RT-PCR COVID-19 negative samples, **CoviRAT** ™ showed 100% specificity.

d) **Limit of Detection (LoD):** The study used "SARS-Related Coronavirus 2 (SARS-CoV-2) Culture Fluid (Heat Inactivated) " USA-WA1/2020 strain (Zeptomatrix # 0810587CFHI). The inactivated virus is diluted in extraction buffer. Based on the study LoD observed is $1.4 \times 10^{3.0}$ $TCID_{50}$ / ml.

LIMITATIONS OF THE TEST

(1). **CoviRAT** ™ test is used for the detection of SARS-CoV-2 antigen in human nasopharyngeal specimens. It should not be used as sole criteria for the treatment and management of COVID-19 infection. (2).This test is a qualitative test therefore neither the quantitative value or rate of SARS-COV-2 antigen concentration can be determined by this qualitative test. (3). As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test but should rather be made by a clinician after all clinical findings have been evaluated. (4). A negative result may occur with **CoviRAT** ™ if the concentration of the antigen in the specimen is below the detection limit of the test or if the specimen was not collected or transported properly, therefore a negative test does not eliminate the possibility of SARS-CoV-2 infection, and should be confirmed by real-time reverse transcriptase-polymerase (RT)-PCR method. (5). Serological cross reactivity across the other coronavirus group may occur in certain patients with prior exposure to HKU1 or NL63 or OC43 or 229E or SARS-CoV or MERS-CoV etc. (6). There is always a possibility that false results may occur due to presence of interfering substances in the specimen or factors beyond the control of the manufacturer, such as technical or procedural errors associated with the testing. (7). Do not interpret the test results beyond 30 minutes. (8).This test is meant for and validated for testing human nasopharyngeal samples only. This test is not meant for testing of pooled samples. (9). Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. The result of this test should not be the sole basis for the diagnosis. (10). *Streptococcus pneumoniae* ATCC 6305, *Staphylococcus epidermidis* ATCC 12228 and *Streptococcus pyogenes* ATCC 19615 did not interfere with the results. (11). Children tend to shed virus for longer periods of time than adults, which may result in differences in sensitivity between adults and children.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

(1). Indwiani Astuti, Ysrafil, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response, Diabetes & Metabolic Syndrome: Clinical Research & Reviews, Volume 14, Issue 4, July–August 2020, Pages 407-412. (2). Interim Guidance for Rapid Antigen Testing for SARS-CoV-2, https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antigen-tests-guidelines.html. (3). Data on file: Viola Diagnostic Systems.

SYMBOL KEYS

Temperature Limitation	Do not use if package is damaged	Consult Instructions for use	REF	Catalogue Number	Σ	Contains sufficient for <n> tests
Manufacturer	Do not reuse	IVD	*In vitro* Diagnostic Medical Device	LOT	Batch Number / Lot Number		
Use by	Date of Manufacture	DEVICE	Device		This side up	EC REP	Authorised Representative in the European Community

Manufactured by: **Viola Diagnostic Systems**
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