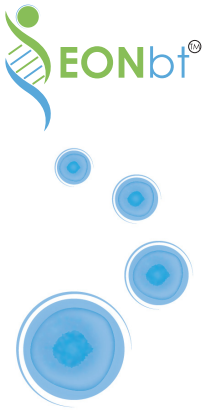


EONbt™ COVID-19 Antigen Saliva Test

Rapid card Test (Immunochromatographic Assay)

Features:

Principle	**Lateral Flow Chromatographic Immunoassay**
Target Antigen	**Nucleocapsid protein**
Sample Type	**Fresh Saliva Specimen**
Cross- Reactivity & Interferences	**Other pathogenic viral, bacterial, fungal organisms and interferences tested do not cross-react or interfere.**
Test Duration	**20 minutes**

1. INTRODUCTION:

EONbt™ COVID-19 Antigen Saliva Test is a rapid and convenient Immunochromatographic assay for the qualitative detection of COVID-19 antigen (viral nucleoprotein) from Saliva samples, obtained from Asymptomatic subjects and suspected patients with signs and symptoms of respiratory infection. The device is designed to aid in the rapid differential diagnosis of COVID-19 Virus infection. This assay provides only a preliminary result. Negative results should be confirmed by Real- Time Reverse Transcriptase (RT)-PCR Diagnostic kit; they do not preclude COVID-19 Virus infection and should not be used as the sole basis for treatment or other management decisions. The test is intended for Health care professional and laboratory use.

2. SUMMARY:

COVID-19(Corona Virus Disease) is the infectious disease caused by the most recently discovered corona virus. This new virus and disease were unknown before the outbreak began in Wuhan, China, s in December 2019. The most common symptoms of COVID-19 are fever, tiredness, and dry cough. Some patients may have aches and pains, nasal congestion, runny nose, sore throat or diarrhoea. These symptoms are usually mild and begin gradually. Some people become infected but don't develop any symptoms and don't feel unwell. Most people (about 80%) recover from the disease without needing special treatment. Around 1 out of every 6 people who gets COVID-19 becomes seriously ill and develops difficulty breathing. Older people, and those with underlying medical problems like high blood pressure, heart problems or diabetes, are more likely to develop serious illness. About 2% of people with the disease have died. People with fever, cough and difficulty breathing should seek medical attention. People can catch COVID-19 from others who have the virus. The disease can spread from person to person through small droplets from the nose or mouth which are spread when a person with COVID-19 coughs or exhales. These droplets land on objects and surfaces around the person. Other people then catch COVID-19 by touching these objects or surfaces, then touching their eyes, nose or mouth. People can also catch COVID-19 if they breathe in droplets from a person with COVID-19 who coughs out or exhales droplets. Most estimates of the incubation period for COVID-19 range from 1-14 days.
EONbt™ COVID-19 Antigen Saliva Test is a Rapid chromatographic immunoassay for the qualitative detection of specific antigens to SARS-CoV-2 Present in human Salivary glands and in saliva. This test is administration by healthcare workers and labs only, as an aid to early diagnosis of SARS-CoV-2 infection in patient with onset of clinical symptoms with in 5 days of SARS-CoV-2 infection. It provided only an initial screening test result. More specific alternative diagnosis methods should be performed in order to obtain the confirmation of SARS-CoV-2 infection. Please check your national health authority guidelines.

3. TEST PRINCIPLE:

EONbt™ COVID-19 Antigen Saliva Test is an antigen-capture immunochromatographic assay, detecting presence of COVID-19 viral nucleoprotein antigen in Saliva samples. This assay utilizes the chemical extraction of viral antigens followed by solid phase immunoassay technology for the detection of extracted antigen. COVID-19 Monoclonal antibodies specifically against COVID-19 antigen are conjugated with colloidal gold, deposited on the conjugate pad, and immobilized on the Test Zone of the nitrocellulose membrane. When a sample is added, the gold-antibody conjugate is rehydrated and the COVID-19 antigen, if any in the sample, will interact with the gold conjugated antibodies. The antigen- antibody-gold complex will migrate towards the test window until the Test Zone where they will be captured by immobilized antibodies, forming a visible pink line (Test band) indicative a positive result. If COVID-19 antigen is absent in the sample, no pink line will appear in the Test Zone (T). To serve as an internal process control, a control band was designed to indicate that the test is performed properly. By utilizing the different antigen/antibody reaction, this control line should always be seen after test is completed. Absence of a pink control line in the control region is an indication of an invalid result.

4. STORAGE AND STABILITY:

The test kit can be stored at temperatures between 4 to 30°C in the sealed pouch to the date of expiration. The test kit should be kept away from direct sunlight, moisture and heat. Do not Freeze the kit.

5. SPECIMEN COLLECTION & PRESERVATION

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.
1. To collect a saliva specimen, Use the Saliva Sampler to collect up to 500µL of Saliva into the Eppendorf. The calibration on the walls of the Eppendorf will guide you.
2. Specimen should be tested as soon as possible after collection.
3. Do not use transport media, use the collected specimen and extraction buffer immediately. Be careful of contamination.

6. TESTING PROCEDURE:

1. Carefully read the instructions before using the EONbt™ COVID-19 Antigen Saliva Test.
2. Allow the Test Kit to attain room temperature before use.
3. Use the Saliva Sampler to collect up to 500µL of Saliva into the Eppendorf. Please check the calibration on the walls of the Eppendorf to ensure the quantity is accurate.
4. Add 500µL (10 drops) of buffer solution to the Eppendorf using the dropper. The recommended ratio of Saliva: Buffer solution is 1:1
5. Close the cap of the Eppendorf. Shake & incubate it (Let it stand still) for 5 minutes
6. Add 3 drops of extracted specimen to the Cassette sample well using the dropper provided.
7. Read the test result in 10-15 minutes. Do not read result after 20 minutes.

7. PERFORMANCE CHARACTERISTICS:

An independent study collected Saliva samples from 577 prospective subjects, of which 149 subjects were positive and 428 subjects were negative. The method of confirmation was RT-PCR

EONbt™ COVID-19 Antigen Saliva Test	Comparator EUA RT PCR test (ARGENE SARS-CoV-2 R-GENE)		
	Positive	Negative	Total
Positive	137	3	140
Negative	12	425	437
Total	149	428	577
PPA	137/149		91,95%
NPA	425/428		99,3%

PPA: Positive Percent Agreement = True Positives / True Positives + False Negatives
NPA: Negative Percent Agreement = True Negatives / True Negatives + False Positive

8. PRODUCT VALIDATION AND CLINICAL VERIFICATION STUDY *CLINICAL TRIAL*

Statistic	Value	95% CI
Sensitivity	91.95%	86.35% to 95.77%
Specificity	99.27%	86.35% to 95.77%
Positive Likelihood Ratio	126.27	40.85 to 390.30
Negative Likelihood Ratio	0.08	0.05 to 0.14
Disease prevalence (*)	3.10%	
Positive Predictive Value (*)	80.16%	56.65% to 92.59%
Negative Predictive Value (*)	99.74%	99.56% to 99.85%
Accuracy (*)	99.04%	97.84% to 99.67%

(*) These values are dependent on disease prevalence.

8.1 DEFINITIONS

• Sensitivity: probability that a test result will be positive when the disease is present (true positive rate).
= a / (a+b)
• Specificity: probability that a test result will be negative when the disease is not present (true negative rate).
= d / (c+d)
• Positive likelihood ratio: ratio between the probability of a positive test result given the presence of the disease and the probability of a positive test result given the absence of the disease,i.e.
= True positive rate / False positive rate = Sensitivity / (1-Specificity)
• Negative likelihood ratio: ratio between the probability of a negative test result given the presence of the disease and the probability of a negative test result given the absence of the disease,i.e.
= False negative rate / True negative rate = (1-Sensitivity) / Specificity
• Positive predictive value: probability that the disease is present when the test is positive.

9. CROSS-REACTIVITY

EONbt's Rapid Antigen markers cross-reactivity test plan covers a wide range of organisms and their strain variations from samples sourced from standard suppliers and that are internally maintained with the hospital collaborators. The analysis currently covers multiple organisms in vitro and in silico. The study will be extended with more organisms
based on ease of access to samples and sources.

9.1. IN SILICO ANALYSIS:

To estimate the likelihood of cross-reactivity with SARS-CoV-2 of organisms that were not available for wet testing, in silico analysis using the Basic Local Alignment Search Tool (BLAST) managed by the NCBI (National Center for Biotechnology Information) was used to assess the degree of protein sequence homology using sequence identity by protein protein BLAST search.
1. The homology between SARS-CoV-2 nucleocapsid protein and human coronavirus HKU1 nucleocapsid protein is relatively low, at 36.7% across 86.4% of sequences, but cross-reactivity cannot be ruled out.
2. The homology between SARS-CoV-2 nucleocapsid protein and Mycobacterium tuberculosis total protein (3,991 proteins) is relatively low, and No significant similarity founds o, homology-based cross-reactivity can be ruled out.
3. The homology between SARS-CoV-2 nucleocapsid protein and Pneumocystis jirovecii total protein (3,745 proteins) is relatively low, and No significant similarity found so, homology-based cross-reactivity can be ruled out.
4. The homology between SARS-CoV-2 nucleocapsid protein and human coronavirus 229E nucleocapsid protein is relatively low, at 28.8% across 72.1% of sequences, with highest sequence identity of 31% with 229E Bat related Coronavirus, but cross-reactivity cannot be ruled out.
5. No homologous protein was detected as a result of in silico assay with all the
proteins (686 proteins) of Mycoplasma pneumoniae and the nucleocapsid protein (NP) of SARS-CoV-2.

9.2. IN-VITRO ANALYSIS:

Cross reactivity and potential interference of EONbt™ COVID-19 Antigen Saliva Test was evaluated by wet testing against normal and pathogenic organisms that may be present in the Oral cavity. No cross-reactivity or interference was seen with the following microorganisms.

Organisms	Cross-Reactivity
Human coronavirus 229E	No
Human coronavirus OC43	No
Human coronavirus HKU1	No
Human Metapneumovirus (hMPV)	No
Parainfluenza virus 1-4	No
Influenza A	No
Influenza B	No
Enterovirus (EV68)	No
Respiratory syncytial virus	No
Rhinovirus	No
Chlamydia pneumoniae	No
Haemophilus influenzae	No
Legionella pneumophila	No
Mycobacterium tuberculosis	No
Streptococcus pneumoniae	No
Streptococcus pyrogenes	No
Bordetella pertussis	No
Mycoplasma pneumoniae	No
Pneumocystis jirovecii (PJP)	No
Human coronavirus NL63	No
SARS-coronavirus	No
MERS-coronavirus	No
Adenovirus (C1 Ad. 71)	No

10. WARNINGS AND PRECAUTIONS

1. Do not re-use the test kit.
2. Do not use the test kit if the pouch is damaged or the seal is broken.
3. Do not use the extraction buffer tube of another lot.
4. Do not smoke, drink or eat while handling specimen.
5. Wear personal protective equipment, such as gloves and lab coats when handling kit reagents. Wash hands thoroughly after

he tests are done.
6. Clean up spills thoroughly using an appropriate disinfectant.
7. Handle all specimens as if they contain infectious agents.
8. Observe established precautions against microbiological hazards throughout the testing procedure.
9. Dispose of all specimens and materials used to perform the test as biohazard waste. Laboratory chemical and biohazard wastes must be handled and discarded in accordance with all local, state, and national regulations.

10.1. CRITICAL INSTRUCTIONS

10. Do not lift or hold the cassette during lateral flow after extracted sample is dispensed into the cassette. Ensure flow is not vertical or gravity based.
11. Please dispose the used material after disinfection using either alcohol or sodium hypochloride. Please get the disinfection vessel ready before initiation of the test.
12. Use the test kit Pouch as disposal unit after disinfection.
13. Please ensure that extraction buffer incubation time in swab is followed strictly for right results.

11. LIMITATION OF THE TEST

1. The test procedure, precautions and interpretation of results for this test must be followed strictly when testing.
2. The test should be used for the detection of SARS-CoV-2 antigen in human Saliva specimens.
3. Neither the quantitative value nor the rate of SARS-CoV-2 antigen concentration can be determined by this qualitative test.
4. Failure to follow the test procedure and interpretation of test results may adversely affect test performance and/or produce invalid results.
5. A negative test result may occur if the level of extracted antigen in a specimen is below the sensitivity of the test or if a poor-quality specimen is obtained.
6. For more accuracy of immune status, additional follow-up testing using other laboratory methods is recommended.
7. The test result must always be evaluated with other data available to the physician.
8. A negative result may occur if the concentration of antigen or antibody in a specimen is below the detection limit of the test or if the specimen was collected or transported improperly, therefore a negative test result does not eliminate the possibility of SARS-CoV-2 infection and should be confirmed by a molecular assay or ELISA.
9. Positive test results do not rule out co-infections with other pathogens.
10. Negative test results are not intended to rule in other coronavirus infection except the SARS-CoV-2 Virus.
11. Children tend to shed virus for longer periods of time than adults, which may result in differences in sensitivity between adults and children.

REAGENT & MATERIAL

Reagents & Materials Provided

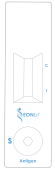
Instruction for use

Eppendorf tube

Pipette/ Dropper

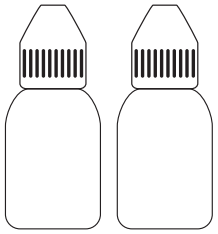
Saliva sampler

Antigen Cassette



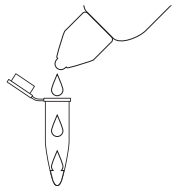
Material required but not provided: Timer, Clock or Stopwatch

2x Buffer Solution Bottles (each 5ml) per Box of 25 cassettes



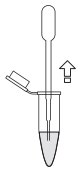
TEST PROTOCOL & PROCEDURE

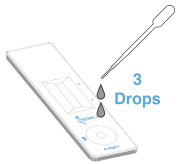
1. 
Use the Saliva Sampler to collect up to 500µL of Saliva into the Eppendorf. Please check the calibration on the walls of the Eppendorf to ensure the quantity is accurate.

2. 
Add 500µL (10 drops) of buffer solution to the Eppendorf using the dropper. The recommended ratio of Saliva: Buffer solution is 1:1

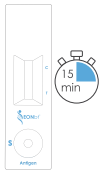
3. 

Close the cap of the Eppendorf. Shake & incubate it (Let it stand still) for 5 minutes

4. 
Take a sample from the Eppendorf Tube through the Pipette

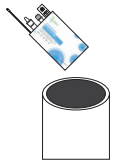
5. 

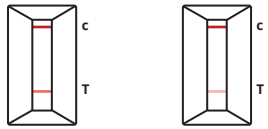
Add 3 drops of extracted specimen to the Cassette sample well using the dropper provided.

6. 

Read the test result in 10-15 minutes.

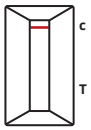
Do not read result after 20 minutes.

7. 
Dispose the used equipment in biohazardous waste (e.g., Hypo-chloride containing disposal unit).



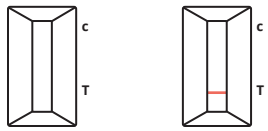
POSITIVE RESULT

There are two coloured line on the test cassette (C&T).
Both coloured test line (T) and coloured control line (C) appear on the test cassette.
Within the specified observation time, a weak coloured test line should be judged as a positive result.



NEGATIVE RESULT

Only the coloured control line (C) appears on the test cassette.
The absence of the test line indicates a negative result.



INVALID RESULT

There should always be a coloured control line (C) in the control region regardless of test result.
If control line (C) is not seen, repeat the assay with a new test cassette.

A Product of **Eon Biotechnology Limited**, registered in UK
Licensed & Manufactured by:
Angstrom Biotech Pvt. Ltd.
Plot No. G1-1035, RIICO Industrial Area,
Bhiwadi, Alwar, Rajasthan- 301019, India
Mfg Lic No.: MFG/IVD/2020/000069

Please contact us for any complaints/inquiries/suggestions via email (support@eonbt.com)
Or call +44-203-7610121

IFU ver. Eonangsal-1
Issued March 2021

CE IVD

REF	Reference Number	!	Caution
	Use By	LOT	Batch code
i	Consult Instructions for USE (IFU)	2	Do not re-use
IVD	*In Vitro Diagnostics*		Note
	Manufacturer		Date of Manufacture
Σ	Contains Sufficient for <n> tests		Keep away from sunlight
	Indicate that you should keep the product dry		To indicate the temperature limitation in which the transport package has to be kept and handled
CE	Fulfil the requirements of Directive 98/79/EC on In Vitro Diagnostic Medical devices		Do not use if packaging is damaged