The instructions for use in German for the self-test

Instruction of uses. Doc.# 0001

Saliva SARS-CoV-2 (2019-nCoV) Antigen Test Kit

(Nanocarbon Assay)

About the Test

This product detects SARS-CoV-2 protein (antigen).

A COVID-19 rapid antigen test is a chromatographic immunoassay based on the sandwich method of antibody-antigen binding using a saliva sample for self-administration in Germany up to **dd.mm .2021 (BfArM GZ: 5640-S-354/21)** without conformity assessment procedure completed.

Introduction

1. Coronavirus is a large family of viruses, known to cause colds, Middle East respiratory syndrome (MERS), severe acute respiratory syndrome (SARS) and other serious diseases. Novel Coronavirus is a new strain of coronavirus that has never been found in human body before.

2. This method is recommended for cases within 5 days after the onset of symptoms[1].

3. The common symptom of coronavirus infection include respiratory symptoms, fever, cough, shortness of breath and dyspnea. In more severe cases, infection can lead to pneumonia, severe acute respiratory syndrome, renal failure, and even death.

4. This method is a rapid chromatographic immunoassay based on the principle of sandwich antibody and antigen binding. Nano carbon Assay is more sensitive than colloidal gold Assay [2].

5. Antigen detection is earlier than antibody, and antigen is the direct evidence of virus infection, so antigen detection is more accurate than antibody detection. Saliva samples are easy to sample and suitable for continuous monitoring, and results could be obtained in 15 minutes. The tested person can operate and judge by himself, which is convenient, fast and no laboratory equipment required[3]. PCR is standard

method but inconvenient and time-consuming. The result of this method was consistent with that of PCR[4].

6. This product is only used for the initial screening of SARS-CoV-2. The diagnosis needs to be confirmed by virus nucleic acid detection and other methods.

Test Principle

One Step COVID-19 Antigen Test is a rapid chromatographic immunoassay based on the principle of sandwich antibody and antigen binding.

During testing, COVID-19 N antigen, if the Virus antigens present in the saliva specimen it will bind to the antibody conjugates to form a complex. And migrates upward by capillary. The complex will then be captured by the immobilized antibody coated at the Test line region on the NC membrane. A visible colored line will show up in the test line region. The colored line will not form in the test line region if there is no virus antigen in the sample.

A virus antigen-positive saliva specimen will generate a colored line in the test line region because of antigen binding, while a virus antigen-negative saliva specimen or a specimen containing a very low concentration will not generate a colored line in the test line region.

To serve as a procedural control, a colored line will always appear at the control line region (C line), indicating that proper volume of specimen has been added and membrane wicking has occurred.

Intended Use

One Step COVID-19 Antigen Test is a lateral flow, one-step immunoassay for the qualitative detection of N antigen in Saliva sample. This product is used to obtain a visual, qualitative results.

Because of its simple operation, laymen and professionals can use, do not need special skills and expertise.

This assay provides only a preliminary analytical test result, and more specific alternate method must be used to obtain a confirmed analytical result. PCR virus nucleic acid test is standard confirmatory method so far. The positive results of antigen detection can be used for early shunting and rapid management of suspected people, but the positive results only indicate that SARS-CoV-2 antigen exists in the samples, which can not be used as a diagnostic basis for SARS-CoV-2 infection. Negative results can not exclude SARS-CoV-2 infection, nor should it be used alone as a basis for treatment and disease management decisions. Further nucleic acid test should be carried out for both positive and negative results of antigen in suspected population.

This product is only used for the initial screening of SARS-CoV-2. The diagnosis needs to be confirmed by virus nucleic acid detection and other methods.

Materials Provided

- Test devices

- Drying agent

- Instruction for use

Materials required but not provided.

- Specimen collection container

- Timer

Active Ingredients of Main Components

Nanocarbon-labeled anti-N antigen monoclonal antibodies, anti-N antigen monoclonal antibodies goat anti-mouse IgG antibody.

Storage and Stability

1.Store as packaged in the sealed pouch at 2-30℃. Valid for one year.

Note: When stored in a refrigerator, all kit components must be brought to room temperature (15-30℃) for a minimum of 30 minutes prior to performing the test.

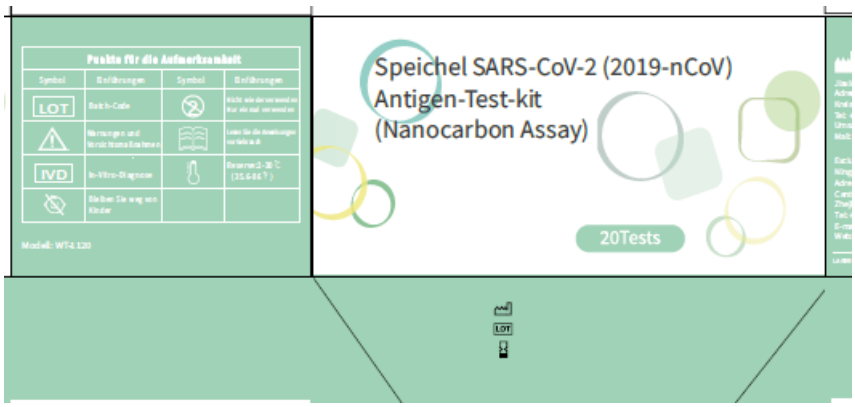
2.The test device is stable through the expiration date printed on the sealed pouch.

3.The test device must remain in the sealed pouch until use.

4. Do not freeze.

5.Do not use beyond the expiration date marked on the package.

6. Read the notes on the package carefully.



Warnings

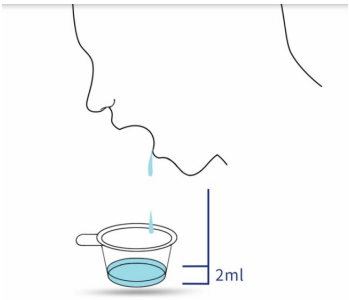
- For in vitro diagnostic use only.
- Disposable use.
- Please read the manual before use.
- Do not eat while collecting specimens.
- Please keep it out of children's reach.
- Wear protective gloves while collecting specimens and wash hands thoroughly afterwards.
- Avoid splashing or aerosol formation of specimen.
- Clean up spills thoroughly using an appropriate disinfectant.
- Decontaminate and dispose of all specimens, reaction kits and potentially

contaminated materials in a biohazard container as if they were infectious waste and dispose according to applicable local regulations.

- Do not mix or interchange different specimens.
- Do not mix reagent of different lots or those for other products.
- Do not use after the expiration date.
- Do not store the test kit in direct sunlight.
- To avoid contamination, do not touch the head of absorbing rod when opening the pouch.
- Before use, the test cassette should be kept in the original package.
- The used test Cassette should be discarded according to federal, state and local regulations.
- This test is only used to detect SARS-CoV-2 in saliva samples.

Test Procedure (Refer to Figure)

1. Put your tongue on your upper jaw and bow your head at comfortable position to make saliva secrete naturally and spit into a disposable plastic cup for about 2 ml.



2. Insert the absorbing rod vertically into the disposable plastic cup containing saliva (Ensure that half of the absorption rod can be submerged). Require the absorbing rod to be immersed in saliva for about 2 minutes.

Caution: During the period, the absorbing rod can be changed direction slightly to suck up the liquid.

3. When the liquid appears in the observation window, take out the kit and place it on a clean and flat table, and start the timing.
4. Read result at 15 minutes after the addition of samples. Do not read result after 30 minutes.

Test Preparation (Caution)

1. If the test kit were stored and kept in refrigerator or cold environment, test kit must be brought to room temperature (20-30℃) prior to testing at least 30 minutes.
2. Do not open pouches until ready to perform the assay. Place on a flat, horizontal and clean surface.
3. Remove the test cassette from its package and set to the clean and level surface.

Caution: If the amount of saliva is insufficient, an improper test result may occur.

Specimen Collection & Extraction

1. The saliva specimen could be collected in a disposable sample cup.

2. Saliva could be collected and used at any time of the day.

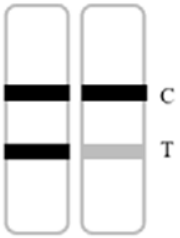
Reaction with Test Device

1. Open the cover at the bottom of the test strip to expose the sponge head.
2. Insert the absorbing rod vertically into the disposable plastic cup containing saliva.
3. Require the absorbing rod to be immersed in saliva at about 2 minutes.
4. When the liquid appears in the observation window, take out the kit and place it on a clean and flat table, and start the timing.

   Caution: Do not handle or move the test device until the test is complete and ready for reading.
5. Read results at 15 minutes after start of timing. Result will be invalid after 30 minutes.
6. Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials in a biohazard container as if they were infectious waste and dispose according to applicable local regulations..

Test Interpretation (Refer to Figure)

Positive: One Clear black line should be in the control region (C), and another visible black line should be in the test region (T). This positive result indicates that there are



Positive

COVID-19 N antigens in the saliva specimen.

In case of a positive test result:

-There is currently a suspicion of COVID-19 infection.

-Contact a doctor or a local health authority immediately.

-Follow the local guidelines for self-isolation.

-Have a PCR test performed for confirmation.

Negative: One black line appears in the control region (C). No visible line appears in the test region (T). This negative result indicates that there is no COVID-19 N antigen present in the saliva specimen or the antigen concentration is too low.

Negative

In case of a negative test result:

The instructions for use in German for the self-test

-Continue to observe all applicable rules regarding contact with other persons and regarding protective measures.

-An infection may also occur if the test is negative.

-Because the corona virus has not been accurately detected at all stages of infection. You should repeat the test in case of suspicion after one to two days.

Invalid: If the control line (C) is not visible within the result window after performing the test, the result is considered invalid or test kit was damaged.

Invalid

In case of invalid test result:

-An invalid result may be due to a faulty test.

-Repeat the test.

-Contact a doctor or a COVID-19 test centre if the test results are invalid again.

Note: The shade of black in the test line region (T) will vary, but it should always be considered as positive whenever there is even visible line.

Test Limitations

1. One Step COVID-19 Antigen Test provides only a preliminary analytical result. A secondary analytical method must be used to obtain a confirmed result. Virus nucleic acid test is the preferred confirmatory method.
2. One Step COVID-19 Antigen Test is a qualitative screening assay and cannot determine either the concentration of the antigen in the saliva or the level of the virus.

3. If there are not enough saliva to allow the sample to pass through the membrane and finish the test, the conjugate or coating antibodies will not react with the sample completely. At this situation, the sensitivity of the test can not be assured.
4. If the sample was contaminated during transportation or storage, such as microorganism or chemicals, it will change the reaction condition of the test kit to get a uncertain result.
5. Adulterants, such as bleach and/or alum, in saliva specimens may produce erroneous results regardless of the analytical method used.If adulteration is suspected, the test should be repeated with another saliva specimen.
6. A Positive Result indicates presence of the antigen but does not indicate level of the virus in saliva.
7. A Negative Result may not necessarily indicate virus-free saliva. Negative results can be obtained when virus is present but below the cutoff level of the Test.
8. Because of its simple operation, laymen and professionals can use, do not need special skills and expertise. This method can be used by laymen who suspect that they are at risk of infection.

Quality Control

1. Internal Quality Control:

Internal procedural controls are included in the test. A collared line appearing in the control region (C) is an internal positive control. It confirms sufficient specimen volume and correct procedural technique. A clear background is an internal negative procedural control. If the test is working properly, the background on the result area should be white to black and not interfere with the ability to read the read result.

2. External Quality Control:

Controls are not included in this kit. However, in compliance with Good Laboratory Practice (GLP) positive/negative controls are recommended.

Performance Characteristics

1. External evaluation of Saliva SARS-CoV-2(2019-nCoV) Antigen Test Kit （Nanocarbon Assay）

Saliva SARS-CoV-2(2019-nCoV)Antigen Test Kit（Nanocarbon Assay） has been evaluated with specimens obtained from the patients. RT-PCR is used as the reference method for the One Step COVID-19 Antigen Test. Specimens were considered positive if RT-PCR indicated a positive result. Specimens were considered negative if RT-PCR indicated a negative result.

SARS-CoV-2 Antigen Test	Reference RT-PCR Assay		
	Positive	Negative	Total
Positive	123	0	123
Negative	11	110	121
Total	134	110	244
Positive Agreement: 123/134=91.8% （95%C.I., 83%-95%）			
Negative Agreement: 110/110=100% （95%C.I., 93%-100%）			

COVID-19 Antigen Test Performance within 5 days of symptom onset against the Reference FDA approved RT-PCR Assay. The positive agreement rate of 244 samples was 91.8%, and the negative agreement rate was 100%

2. Detection Limit

One Step COVID-19 Antigen Test limit of detection (LoD) was determined by evaluating different concentrations of heat inactivated SARS-CoV-2 virus. Presumed negative natural saliva specimens were eluted in PBS. Saliva were combined and mixed thoroughly to create a clinical matrix pool to be used as the diluent. Inactivated SARS-CoV-2 virus was diluted in this natural saliva matrix pool to generate virus

dilutions for testing. Contrived saliva samples were prepared by absorbing 20 microliters of each virus dilution onto the saliva. The contrived saliva samples were tested according to the test procedure. The LoD was determined as the lowest virus concentration that was detected ≥95% of the time (i.e., concentration at which at least 19 out of 20 replicates tested positive). The One Step COVID-19 Antigen Test LoD in natural saliva matrix was confirmed as $1.25x10^2 TCID_{50}$/ml.

Limit of Detection (LoD) Study Results

Concentration $TCID_{50}$/ml	Number Positive/Total	% Detected
$1.25x10^2$	19/20	95

3. Hook Effect

No hook effect was observed when tested with the concentrations of S1($1.85×10^5$ $TCID_{50}$/mL) ,S2 ($1.85×10^4$ $TCID_{50}$/mL) and S3 ($1.85×10^3$ $TCID_{50}$/mL) with Saliva SARS-CoV-2 (2019-nCoV) Antigen Test Kit（Nanocarbon Assay）.

4. Cross Reactivity

Total 20 bacterial and viral isolates were used for the cross-reactivity evaluation. Each bacterial isolate was diluted to $1×10^8$ ORG/ml, and viral isolate was diluted to $1×10^5$ $TCID_{50}$/ml. Each isolate was test for 3 different lot test devices. The result showed all negative for 20 organisms in the the One Step COVID-19 Antigen Test listed below:

No.	Name	Concentration	Result
1	Human coronavirus 229E	$1×10^8$ ORG/ml	No cross reaction
2	Human coronavirus OC43	$1×10^8$ ORG/ml	No cross reaction

3	Human coronavirus NL63	1×10^8 ORG/ml	No cross reaction
4	Adenovirus	1×10^8 ORG/ml	No cross reaction
5	Human Metapneumovirus (hMPV)	1×10^8 ORG/ml	No cross reaction
6	Parainfluenza virus 1-4	1×10^8 ORG/ml	No cross reaction
7	Influenza A & B	1×10^8 ORG/ml	No cross reaction
8	Enterovirus	1×10^8 ORG/ml	No cross reaction
9	Respiratory syncytial virus	1×10^8 ORG/ml	No cross reaction
10	Rhinovirus	1×10^8 ORG/ml	No cross reaction
11	MERS-coronavirus	1×10^8 ORG/ml	No cross reaction
12	Human coronavirus HKU1	1×10^8 ORG/ml	No cross reaction
13	Haemophilus influenzae	1×10^5 $TCID_{50}$/ml	No cross reaction
14	Streptococcus pneumoniae	1×10^5 $TCID_{50}$/ml	No cross reaction
15	Bordetella pertussis	1×10^5 $TCID_{50}$/ml	No cross reaction
16	Mycoplasma pneumoniae	1×10^5 $TCID_{50}$/ml	No cross reaction
17	Chlamydia pneumoniae	1×10^5 $TCID_{50}$/ml	No cross reaction
18	Legionella pneumophila	1×10^5 $TCID_{50}$/ml	No cross reaction

19	Mycobacterium tuberculosis	1×10^5 $TCID_{50}$/ml	No cross reaction
20	Candida albicans	1×10^5 $TCID_{50}$/ml	No cross reaction

5. Interfering Substances

The following substances, naturally present in saliva specimens or that may be artificially introduced into mouth washes, were evaluated with the One Step COVID-19 Antigen Test at the concentrations listed below and were found not to affect test performance.

Substance	Active Ingredient	Concentration
Endogenous	Mucin.	100 ug/ml
	Bilirubin	100 ug/ml
	Hemoglobin	100 ug/ml
	Rheumatoid Factor (RF)	100 ug/ml
	Antinuclear Antibodies (ANA)	100 ug/ml
	Lipoproteins	100 ug/ml
	Whole Blood	1% v/v
Mouth Wash 1	Sodium Fluoride [1] (Fluoride ion[2])	[1] 0.05% w/v ([2] 0.02% w/v)
Mouth Wash 2	Cetylpyridinium chloride	0.075% w/v
Mouth Wash 3	ChlorhexidineGluconate	0.12% w/v
Mouth Wash 4	Hydrogen Peroxide	1.5% w/v

Substance	Active Ingredient	Concentration
Mouth Wash 5	Eucalyptol[3] Menthol[4] Methyl Salicylate[5] Thymol[6]	[3] 0.092% w/v [4] 0.042% w/v [5] 0.060% w/v [6] 0.064%w/v

6. Repeatability & Reproducibility

The enterprise reference materials R1 and R2 were tested, each repeated 10 times, should be positive.

The reference materials R1 and R2 were taken to test three batches of trial products respectively, and the test results of each batch of trial products were 100% positive.

PREPARATION

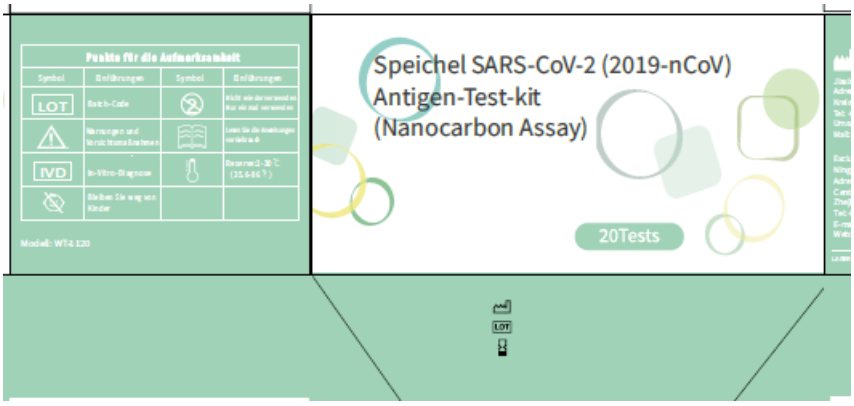
1. Allow all kit components to reach a temperature between 15-30℃ prior to testing for 30 minutes.

Note: Healthcare professionals should comply with personal safety guidelines including the use of personal protective equipment.

2. Open the package and look for the following:

- Test device with desiccant in individual foil pouch.
- Instructions for use.
- Carefully read these instructions prior to using Saliva SARS-CoV-2(2019-nCoV) Antigen Test Kit.
- Look at the expiration date of the kit box (Refer the red box in the figure). If the expiration date has passed, use another kit.

The instructions for use in German for the self-test



3. Open the foil pouch and look for the following:

- Test strip

- Desiccant package

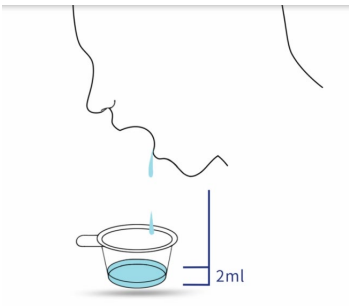
Label the device with the patient identifier.

The instructions for use in German for the self-test

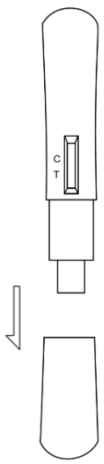
TEST PROCEDURE

1. Put your tongue on your upper jaw and bow your head to make saliva secrete naturally into a disposable plastic cup.

   Caution: Children use it under the guidance of adults. We recommend adults wear PPE when instructing children to use it.



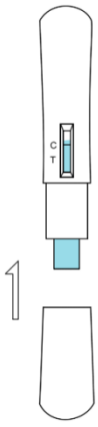
2. Open the cover at the bottom of the test strip to expose the absorbing rod. of the test strip to expose the absorbing rod.



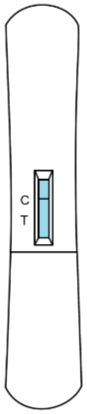
3. Insert the absorbing rod vertically into the disposable plastic cup containing saliva (about 2ml). Require the absorbing rod to be immersed in saliva at about 2 minutes.

   Caution: During the period, the absorbing rod can be changed slightly to suck up the liquid. Our test kit is specially designed for the nonprofessional people, which not use nasal swab to take the sample, no any buffer to dilute the sample. The person just wears a glove and mask during the test procedure. No special protection needed.

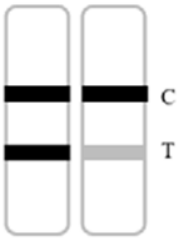
4. When the liquid appears in the observation window, take out the kit, close the cover at the bottom of the test strip and place it on a clean and flat table, and start the timing.



5. Read result at 15 minutes after the addition of samples. Do not read result after 30 minutes.



TEST INTERPRETATION



Positive

Positive: One black line should be in the control region (C), and another visible black line should be in the test region (T). This positive result indicates that there are COVID-19 N antigens in the saliva specimen.

Caution: The shade of black in the test line region (T) will vary, but it should always be considered as positive whenever there is even a faint black line.

Negative

Negative: One black line appears in the control region (C). No visible line appears in the test region (T). This negative result indicates that there is no COVID-19 N antigen present in the saliva specimen or the antigen concentration is too low.

Invalid

Invalid: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test using a new test device. If the problem persists, discontinue using the lot immediately and contact your local distributor.

Test card T-line color(negative/positive)

Interpretation shows

Method of operation

1.The reagent card shall operate according to the product instructions and interpret the results within the rescribed time.

2.Compare the test line(T line) of the reagent card after the reaction with the color chart and record the interpretation score.

Standard colour chart of test line (T line)

The interpretation of results

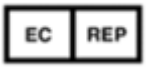
Reagent card test results—The color of detection line (T line) is compared with the color chart of this standard.

Interpretation score	<C3.5	⩾C3.5
Result interpretation	**The result is negative.** It indicates that the concentration of the SARS-CoV-2 antigen is zero or below the detection limit of the kit.	**The result is pasitive.** It indicates that there is SARS-CoV-2 antigen in specimen.

Company Name/Address/…

 **Manufacturer**

JIAXING WISETEST BIO-TECH CO., LTD.
Address: No.268-F, Kaiyuan Avenue, Xitang Town, Jiashan County, Jiaxing City, Zhejiang Province, China 314102

 **Global Exclusive Distributor**

NINGBO ZHUOYUE IMPORT & EXPORT CO., LTD
Address: Room 1614, Block 6, Dream Apartment, Century Town, Hangzhou Bay New Zone, Ningbo, Zhejiang Province, China 315336
Tel: +86-137 7716 1718
Mail: ZhouYue@jiabisen.com

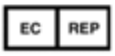
German customer service provider
TriPart-Logistic GmbH
Street: Heckhofweg 146 50739
Postcode: 50667
City: Köln
Hotline: +49 (0) 221 33 77 1-0
E-mail: info@tripart-logistic.com

Lotus NL B.V.
Company Address：Koningin Julianaplein 10, 1e Verd, 2595AA, The Hague, Netherlands.
Tel：+31 6441 6899 9
Email：peter@lotusnl.com

【Approval Date and Version】
Approval Date: Apr.22.2021
Version: A0

GLOSSARY OF SYMBOLS

The instructions for use in German for the self-test

	USE BY		KEEP DRY
	CAUTION		BIOLOGICAL RISKS
	CONSULT INSTRUCTIONS FOR USE		BATCH CODE
	DO NOT REUSE		IN VITRO DIAGNOSTIC MEDICAL DEVICE
	TEMPERATURE LIMITATION		DATE OF MANUFACTURE
	MANUFACTURER		SUFFICIENT FOR
	AUTHORISED REPRESENTATIVE IN THE EUROPEAN COMMUNITY		

Safety instructions

1.Do not swallow.

2.Do not exposure to sunlight.

3.The unpacked reagent strip cannot be immersed in water.

4.Children use it under the guidance of adults. Adults should wear PPE when instructing children to use it.

Disposal of the product

1. Collect used packages, samples, reagent strips and plastic cups.

2.Clean the operating table with paper towel.

3.Put 1. And 2. in a biohazard container.

4. Whether the sample is negative or positive, the collection is considered as infectious waste

5. Dispose according to applicable local regulations.

Reference

1. Food and Drug Administration. In vitro diagnostics EUAs. Silver Spring, MD: US Department of Health and Human Services, Food and Drug Administration; 2020. https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations- medical-devices/vitro-diagnostics-euas.

2. Wei YQ, Duan YC, Bi YH, et al. A novel carbon nanoparticle probe-based ultrasensitive lateral flow assay for rapid detection of Ebola virus. Chin J Biotech, 2018, 34(12):2025–2034.

3. Sri Santosh T, Parmar R, Anand H, et al. A Review of Salivary Diagnostics and Its Potential Implication in Detection of Covid-19. Cureus 12(4): e7708. DOI 10.7759/cureus.7708(April 17, 2020).

4. To, K. K.-W. et al. Consistent Detection of 2019 SARS-CoV-2 in Saliva. Clin. Infect. Dis.(2020) doi:10.1093/cid/ciaa149.