DNA
DIAGNOSTIC

User manual

COVID-19 Antigen Detection Kit

SARS-CoV-2 N-Protein detection

Colloidal Gold Immunochromatography

25 tests / 50 tests / 100 tests

For Professional Use Only

PURPOSE OF THE TEST (INTENDED USE)

The kit is a 15-minute chromatographic immunoassay for the qualitative detection of nucleoprotein (N) antigen of SARS-CoV-2 from nasal swab samples from patients suspected of COVID-19 infection. It is used as a supplementary detection indicator for suspected cases of novel coronavirus, e.g. in conjunction with nucleic acid detection methods (like RT-PCR). It cannot be used as a sole basis for the diagnosis and exclusion of pneumonitis infected by novel coronavirus, and cannot be used as a home test (self-test).

The test is intended for professional use only – intended users are trained personnel instructed in the *in vitro* diagnostic procedure.

PRINCIPLE OF THE TEST

The COVID-19 Antigen Detection Kit is designed to detect the SARS-CoV-2 virus, which is responsible for the disease COVID-19.

The virus group Coronavirus (Coronavirus, CoV) are single-stranded, positive-stranded RNA virus. In addition to the new coronavirus that is causing COVID-19, a total of six coronaviruses are found to infect humans (HCoV-229E, HCoV-OC43, SARS-CoV, HCoV-NL63, HCoV-HKU1, and MERS-CoV)[1-3].

The swab is used to collect an upper respiratory tract sample. The content of the Buffer ampoule is transferred to an extraction tube, which is used for the elution of the swab sample. After addition of a Drip Lid, three (3) drops of sample is added to the Detection Card. The Detection Card pad is coated with an anti-SARS-CoV-2 N-Protein monoclonal IgY antibody which is labeled with colloidal gold. A second anti-SARS-CoV-2 N-Protein antibody is coated on the nitrocellulose membrane to make the Test line (T), and an anti-IgY secondary antibody is coated on the same nitrocellulose membrane to make a control line "C". The anti-SARS-CoV-2 N protein antibody reacts with the virus antigen (if present) and carry out chromatography along the nitrocellulose membrane, to react with the test line and control line, respectively. If the test result is valid, the control line is visual.

MAIN KIT COMPONENTS

Each box contains 25/50/100 pieces of each component. The main components are:

COMPONENT	MAIN CONTENTS
Detection card	Antibodies, a water absorbent pad, nitrocellulose membrane and a plastic case
Buffer Ampoule	Buffered solution for elution of upper respiratory samples (from swabs)
Extraction Tube	Single-use, non-capped extraction tubes
Drip Lid	Single-use drip lids (nozzle caps) for extraction tube
Swab	Sterile, individually packed, single-use specimen sampling swabs

STORAGE CONDITIONS & PERIOD OF VALIDITY

Store the kit dry at room temperature 2 ℃ to 30 ℃.

Do not freeze and keep out of direct sunlight.

Note that the detection card must be used within 1 hour after opening the detection card pouch.

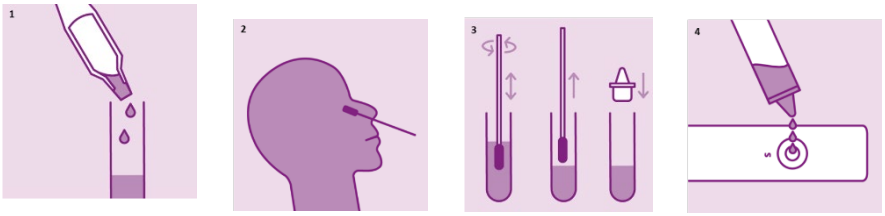
Do not mix the components of different lots.

Each component is stable under the specified conditions for the specified shelf life on the kit. See the label for the expiration date.

PRECAUTIONS

1. The test must comply with the requirements of laboratory management specifications and strictly prevent cross-contamination. All samples, washes and wastes should be treated as infectious agents. Follow relevant biosafety regulations and use appropriate personal protective equipment. Consult with current national and WHO guidelines on biosafety for COVID-19 testing [4,5].
2. Inactivated samples cannot be used (56 ℃ for 30 min, 75% ethanol or otherwise inactivated samples after processing).
3. Perform test under the protection of a biological safety cabinet or personal protective equipment. Consult with current national and WHO guidelines on biosafety for COVID-19 testing.
4. Read the operating instruction carefully before operation, and strictly follow the operation procedures of the instruction.
5. Specimen Collection and Preparation: Acceptable specimens for testing with this kit include nasal swab specimens obtained by the dual nares collection method. Correct specimen collection and preparation methods must be followed. Specimens obtained early during symptom onset will contain the highest viral titers; specimens obtained after five days of symptoms are more likely to produce negative results when compared to an RT-PCR assay. Inadequate specimen collection, improper specimen handling and/or transport may yield a falsely negative result; therefore, training in specimen collection is highly recommended due to the importance of specimen quality for generating accurate test results.
6. Specimen Transport and Storage: Freshly collected specimens should be processed as soon as possible, but no later than one hour after specimen collection. Correct specimen collection and preparation methods must be followed.
7. The reaction time is 15 minutes ± 1 minute. After the reaction has completed, the results are invalid after more than 5 additional minutes.
8. Open the detection card pouch immediately before use, do not leave on the table to reduce exposure to humidity, light and strong convection, as prolonged exposure can compromise test performance.
9. Reuse of the items in the kit is not permitted.
10. Do not use damaged products, and do not use if the packaging is damaged.

PROTOCOL AT-A-GLANCE



DETAILED PROTOCOL

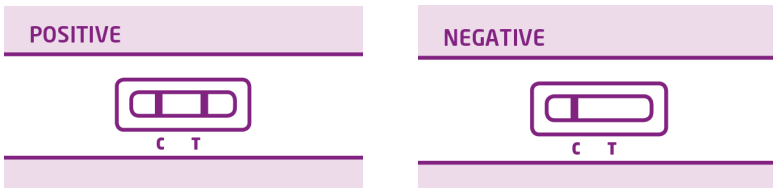
1. Take out a Buffer Ampoule. Twist off the top and dispense all of the buffer into an Extraction Tube.
2. Collect nasal sample by the dual nares collection method: Insert the swab head into a nostril of the person, approximately 2 cm from the nostril edge. With one finger, apply pressure on the outside of the nostril so that the swab head is pressed against both inside nasal surfaces (mucosa). Rotate the swab approximately 5 times. Repeat the procedure with the same swab in the second nostril.
   Note: In case of high secretion in the nose, ask the testee to blow their nose prior to sample collection.
3. Insert the swab head into the extraction tube. Compress the bottom of the tube around the swab head, so that the swab head is immersed in buffer. Rotate the swab several times to elute the sample. Additionally, move the swab up-and-down into the solution 5 times. Carefully remove and discard the swab. Close the tube with the drip lid.
   Note: Process the sample as soon as possible after sample collection. Should be processed within one hour.
4. Open the pouch with the Detection Card. Place the detection card on a horizontal surface. From the extraction tube with drip lid, gently squeeze out three (3) drops of liquid sample into the sample spot of the detection card.
   Note: The test kit and the sample must be room temperature before testing.
5. Incubate for 15 minutes at room temperature. Do not exceed 20 minutes. Go to 'interpretation of results'.

INTERPRETATION OF RESULTS

The test results of the kit should only be used for clinical auxiliary diagnosis, not as the sole basis for clinical diagnosis, and should be comprehensively judged in combination with clinical symptoms and other detection indicators.

6. Visually inspect the detection card:

   POSITIVE: Band on both the control line (C) and the test line (T).
   NEGATIVE: Band on the control line (C) and no band on the test line (T).
   INVALID: No band on the control line (C).



LIMITATIONS

1. Sample collection and processing have a great impact on virus detection.
2. Negative test results do not exclude the possibility of virus infection. If the test result is negative and the patient has clinical symptoms, it is recommended to use nucleic acid testing, and a comprehensive diagnosis by the attending physician.
3. This product is only suitable for a qualitative test and auxiliary diagnosis.
4. The test results are only for clinical reference and should not be the only basis for clinical diagnosis and treatment. The clinical management of patients should be considered in combination with their symptoms, physical signs, medical history, other laboratory tests, therapeutic reaction, and epidemiological information.
5. A false-negative test result may occur if the level of viral antigen in a sample is below the detection limit of the test or if the sample was collected or transported improperly. Therefore, a negative test result does not eliminate the possibility of SARS-CoV-2 infection.
6. The amount of antigen in a sample may decrease as the duration of illness increases. Specimens collected after day 5 of illness are more likely to be negative, compared to an RT-PCR SARS-CoV-2 assay.
7. The kit detects antigen load and may not correlate with other diagnostic methods.
8. The performance of this test has not been evaluated for use in patients without signs and symptoms of respiratory infection and performance may differ in asymptomatic individuals.
9. Specimen stability recommendations are based upon stability data from influenza testing and performance may be different from SARS-CoV-2. Users should test specimens as quickly as possible after specimen collection, and within one hour after specimen collection.

CLINICAL PERFORMANCE

A clinical study was performed between August and November 2020: 370 nasal swab samples were collected from individual symptomatic patients (within 13 days of symptom onset). Samples were collected by qualified personnel only. Nasal swabs were collected following the dual nares method and handled as described in the instruction of the kit. All specimens blinded before being tested. The results of the kit were compared to results of a pharyngeal swab tested with a validated commercial molecular assay (RT-PCR).

Table 1: Clinical Study Result

		RT-PCR		
		Positive	Negative	Total
COVID-19 Antigen detection test	Positive	135	1	136
	Negative	9	225	234
	Total	144	226	370

Positive Percentage Agreement (PPA): (135/144) *100 = **93,75%**
Negative Percentage Agreement (NPA):(225/226) *100 = **99.56%**
Total Percentage Agreement (Accuracy):(360/370) * 100 = **97.30%**
95% confidence interval of positive percentage agreement= 89,80 % - 97.70%
95% confidence interval of negative percentage agreement= 98.69% - 100.0%
Kappa: 0.94.

Clinical Sensitivity = 93,75 %. Clinical Specificity = 99.56%

ANALYTICAL PERFORMANCE

Assay Cross-Reactivity

Cross-Reactivity: There was no cross-reaction with potentially cross-reactive species, except SARS-coronavirus

Table 2: Cross-reactivity Results

Potential Cross- Reactive Substances	Concentration Tested	Cross-Reactivity (Yes/No)
Influenza A	1.6 x 10^5 $TCID_{50}$ /mL	NO
Influenza B	1.6 x 10^5 $TCID_{50}$ /mL	NO
Human coronavirus HKU1	1.6 x 10^5 $TCID_{50}$ /mL	NO
Human coronavirus OC43	1.6 x 10^5 $TCID_{50}$ /mL	NO
Haemophilus influenza	2.2 x 10^5 $TCID_{50}$ /mL	NO
MERS-coronavirus	2.1 x 10^5 $TCID_{50}$ /mL	NO
SARS-coronavirus	3.2 x 10^5 PFU/mL	YES
Adenovirus C1	1.5 x 10^5 $TCID_{50}$ /mL	NO
Adenovirus 71	1.5 x 10^5 $TCID_{50}$ /mL	NO
Candida albicans	4.2 x 10^5 CFU/mL	NO
Respiratory syncytial virus	5.1 x 10^5 $TCID_{50}$ /mL	NO
Enterovirus	5.4 x 10^5 $TCID_{50}$ /mL	NO
Malaria	2.2 x 10^6 CFU/mL	NO
Dengue	1.2 x 10^5 $TCID_{50}$ /mL	NO
Human coronavirus NL63	1.7 x 10^5 $TCID_{50}$ /mL	NO
Human coronavirus 229E	2.2 x 10^5 $TCID_{50}$ /mL	NO
Streptococcus pneumonia	1.1 x 10^6 CFU/mL	NO
Pneumocystis	1.0 x 10^5 CFU/mL	NO
Jirovecii	1.0 x 10^5 CFU/mL	NO
Legionella pneumophila	1.4 x 10^6 CFU/mL	NO
Chlamydia pneumonia	1.1 x 10^6 IFU/mL	NO
Human Metapneumovirus (hMPV)	1.1 x 10^5 $TCID_{50}$ /mL	NO
Parainfluenza virus 1	1.0 x 10^5 $TCID_{50}$ /mL	NO
Parainfluenza virus 2	1.0 x 10^5 $TCID_{50}$ /mL	NO
Parainfluenza virus 3	3.5 x 10^5 $TCID_{50}$ /mL	NO
Parainfluenza virus 4	1.4 x 10^5 $TCID_{50}$ /mL	NO
Rhinovirus	1.3 x 10^5 PFU/mL	NO
Mycoplasma pneumonia	1.8 x 10^6 CFU/mL	NO
Bordetella pertussis	1.5 x 10^6 CFU/mL	NO
Mycobacterium tuberculosis	1.0 x 10^6 CFU/mL	NO
Streptococcus pyogenes	1.0 x 10^6 CFU/mL	NO
Pooled human nasal wash representative of normal respiratory microbial flora	100%	NO

Limit of Detection (LoD)

The LoD for COVID-19 Antigen Detection Kit is **1.6 x 10^2 $TCID_{50}$ /mL**.

The LoD was established using dilutions of a viral sample inactivated by gamma irradiation. The material was supplied at a concentration of 1.3 x 10^6 $TCID_{50}$/mL. The study was designed to estimate the LoD of the assay when using a direct nasal swab. An initial study was performed testing in triplicate using a 10-fold dilution series. At each dilution, 50 µL samples were added to swabs and then tested using the procedure for patient nasal swab specimens. A concentration was chosen between the last dilution to give three all positive results, and the first concentration to give three all negative results. Using this concentration spectrum, the LoD was further refined with a 2-fold dilution series. The last dilution demonstrating 100% positive results was then tested in an additional 20 replicates. The LoD was found to be 1.6 x 10^2 $TCID_{50}$ /mL

Hook Effect

No Hook Effect was found for virus concentrations up to 1.3 x 10^6 $TCID_{50}$/mL (highest concentration tested)

Mark	Explanation	Mark	Explanation	Mark	Explanation	Mark	Explanation	Mark	Explanation
IVD	IN VITRO DIAGNOSTIC MEDICAL DEVICE		CAUTION	LOT	BATCH CODE		MANUFACTURER	EC REP	EC AUTHORIZED REPRESENTATIVE
CE	CE MARK		CONSULT INSTRUCTIONS FOR USE	2°-30 °C	TEMPERATURE LIMITATION		DO NOT REUSE		USE BY

REFERENCES

[1] Van Der Hoek L, Pyrc K, Jebbink MF, Vermeulen Oost W, Berkhout RJ, Wolthers KC, Wertheim Van Dillen PM, Kaandorp J, Spaargaren J, Berkhout B. Identification of a new human coronavirus[J]. Nature medicine, 2004, 10(4): 368-373.

[2] Hu qian, Tan wenjie. Research progress of human coronavirus HCoV-OC43 [J]. Chinese Journal of Preventive Medicine, 2013, 47(7): 661-664.

[3] Dong xiaochun. Research progress of human coronavirus HCoV-229E [J]. Occupation and Health, 2014, 30(24): 3625-3627, 3631.

[4] WHO: Laboratory biosafety guidance related to coronavirus disease (COVID-19) (Link: https://www.who.int/publications/i/item/laboratory-biosafety-guidance-related-to-coronavirus-disease-(COVID-19))

[5] WHO: Laboratory Biosafety manual, third edition (link: https://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf?ua=1)

DNA Diagnostic A/S is a Danish Biotech company established in 1992. We invest all our professional knowledge and experience in developing the best, and most user friendly test tools imaginable.

Our vision is to improve global health and safety, by producing fast and easy user friendly tests, to the highest standards.

USER MANUAL

REF CV19IC

Revision

09.02.2021

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