COVID-19 Sero NP/RBD

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IFU-5824/EN/V02

***In vitro* rapid detection test of antibodies specific to NP/RBD antigens of SARS-CoV-2 coronavirus in human serum or plasma**

FOR IN VITRO USE ***EN***

FOR PROFESSIONAL USE ONLY

Reference: K-1224, 25 tests per kit, buffer

I. INTRODUCTION

The SARS-CoV-2 coronavirus that emerged late 2019 and disseminated worldwide leading to the COVID-19 pandemic had a huge impact on the healthcare system and on our lives. In the countries were epidemic has a decreasing trend, the epidemiological surveillance is of paramount importance to prevent a new increase of COVID-19 cases. Numerous serological assays have been developed and are available, but these tests focus on the detection of antibodies directed towards one single antigen. While the most sensitive tests target antibodies against the nucleocapsid protein (NP), the antibodies against the receptor binding domain (RBD) of the spike protein are expected to confer protection by inhibiting the virus entry into the host cell.
The COVID-19 Sero NP/RBD test is a serological assay that allows to detect and differentiate antibodies against NP and RBD in a single assay.

II. PRINCIPLE OF THE TEST

COVID-19 Sero NP/RBD test is a ready-to-use immunochromatographic assay. A nitrocellulose membrane is sensitized with reagents to catch antibodies of the samples and these are revealed with colloidal gold conjugates. Reagents are recombinant nucleocapsid protein (NP) and receptor binding domain (RBD) of the spike protein.

The sample is delivered directly into the sample well of the cassette. 4 drops of buffer are then added to the same sample well and migration starts. The solubilised conjugates react with antibodies present in the sample and migrate by passive diffusion. The conjugates and sample material come into contact with the reagents adsorbed onto the nitrocellulose. If the sample contains anti-RBD antibodies, the conjugate-antibodies complex will remain bound to the RBD (first) test line and a red line will develop. If the sample contains anti-NP antibodies, the conjugate-antibodies complex will remain bound to the NP (second) test line and a red line will develop. Solution continues to migrate to reach a third reagent that binds the migration control conjugate, thereby producing a red control line confirming that the test did work properly. The result is visible within 15 minutes.

III. REAGENTS AND MATERIALS

1. COVID-19 Sero NP/RBD (25)
Sealed pouches each containing one device and one desiccant. Each device contains one sensitized strip.
2. Instruction for use (1)
3. BL-A buffer (6 mL)
Saline dilution buffered to pH 7.5 containing TRIS, EDTA, NaN_3 (<0.1%), a detergent and blocking proteins.

IV. SPECIAL PRECAUTIONS

- All operations must be performed in accordance with Good Laboratory Practices (GLP).
- All reagents are for *in vitro* diagnostic use only.
- Pouch must be opened with care at the moment of the test.
- Avoid touching nitrocellulose with your fingers.
- Wear gloves when handling samples.
- Never use pouches or buffer from another kit.
- Green/Blue lines indicate reagents adsorption sites. Green/Blue color disappears during the test.
- Reagents quality cannot be guaranteed beyond their shelf-life dates or if reagents are not stored under required conditions as indicated on the packaging.

V. WASTE DISPOSAL

- Dispose of gloves, micropipette tips and used devices in accordance with GLP and biosecurity legislation (ref. C).
- Each user is responsible for the management of any waste produced, and must ensure that it is disposed of in accordance with the applicable legislation.

VI. STORAGE

- An unopened kit must be kept between 4 and 30 °C and used until the shelf-life date indicated on the packaging.
- Avoid freezing strips and buffer.

VII. SPECIMEN HANDLING AND COLLECTION

Specimen should be tested as soon as possible after collection. Serum or plasma may be stored at 2-8°C for 1 week or -20°C for longer periods of time.
No issue of inhibition was observed with neither EDTA nor heparinized collection tubes.

VIII. PROCEDURE

Preparation of the test

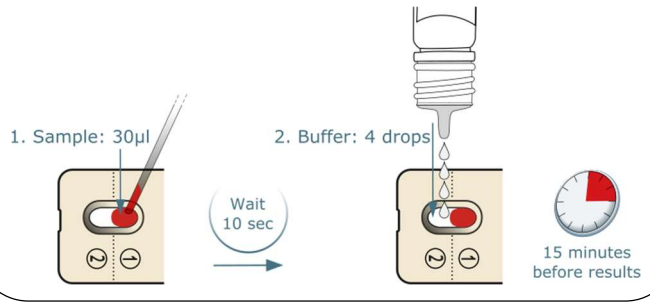
A. Allow kit components, in unopened packaging, and specimens to reach room temperature before performing a test
B. Open the pouch. Once opened, run the test immediately.
C. Indicate the patient's name or specimen number on the device (one device per sample).
D. Check if the three green lines are present in the reading window. If not, take another device.

Sampling

Serum/plasma
Take 30 µL of serum or plasma with a micropipette or with lab pipette (not provided).

Running the test

1. **Dispense the 30µl of serum/plasma** into the **upper** part of sample well (Zone labeled 1 on the cassette); let the sample be absorbed by the sample pad (wait 10 seconds)
2. Add **4 drops of the BL-A buffer** into the **lower** part of sample well (Zone labeled 2 on the cassette).
   Note: make sure that the buffer vial is held vertically and prevent touching the membrane
3. **Allow to react** for 15 minutes
4. After 15 minutes read the results of each test line
   Note:
   - a result can be considered as positive, as soon as the signal appears
   - a result has to be considered as negative if no signal appears after 15 minutes

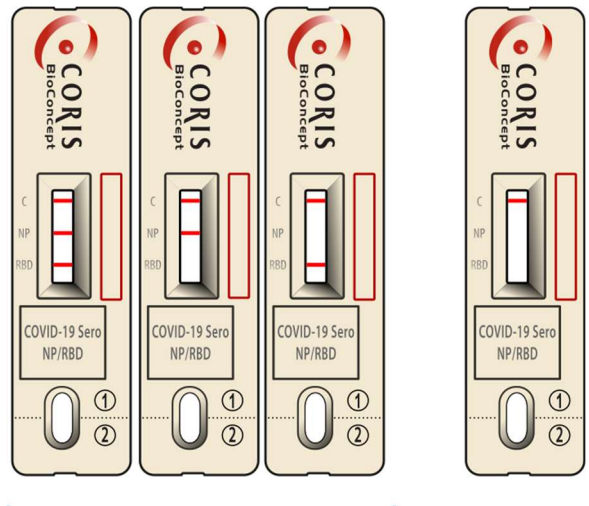


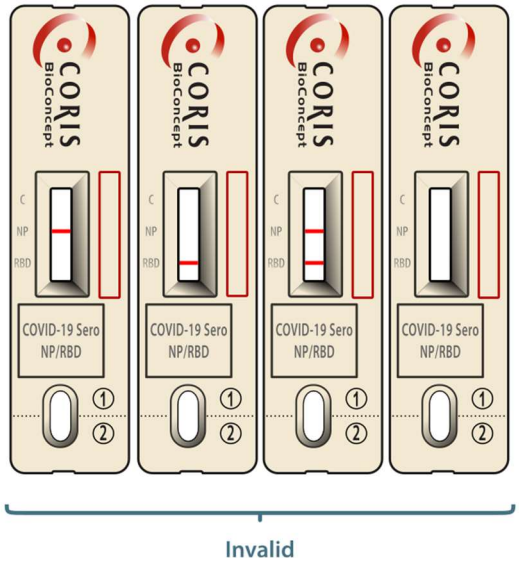
WARNINGS

Test must be read immediately after 15 minutes reaction. The result must be read on still wet strip.
Do not take the new faint lines into account after the reaction time is passed.
After reading, discard the test cassette according to biosecurity requirements.

IX. INTERPRETING RESULTS

The results are to be interpreted as follows:





Negative test result: a reddish-purple line appears across the reading window at the Control line (C) position. No other band is present.

Positive test result: in addition to a reddish-purple band at the Control line (C), a visible reddish-purple band appears at one or both test line positions (NP and/or RBD). Intensity of the test line may vary. Any reddish-purple NP and/or RBD line, even weak, should be considered as a positive result.

Invalid test result: The absence of a Control line indicates a failure in the test procedure, even if a Test line is present. Repeat invalid tests with a new test device.

X. QUALITY CONTROL

In accordance with Good Laboratory Practices, we recommend checking the test's performance regularly according to the laboratory's requirements.

XI. PERFORMANCE (on serum/plasma samples)

A. Positive rate detection

1. A first trial was conducted on 62 plasma/serums samples from patients infected by SARS-CoV-2 as diagnosed by qRT-PCR and confirmed as serologically positive

Target:	*NP*	*RBD*	Total
Positivity	95.2% (85.6 to 98.7%)[1]	91.9% (81.4 to 97%)[1]	98.4% (90.2 to 99.9%)[1]

[1]: 95% Confidence Interval

2. A second trial was conducted on 106 plasma samples from patients infected by SARS-CoV-2 and diagnosed by qRT-PCR (more than 15 days between RT-PCR positivity and blood sampling).

Days after RT-PCR positivity:	≥ 15 days		
Target:	*NP*	*RBD*	Total
Positivity	84% (75.3 to 90.1%)[1]	78.3% (69 to 85.5%)[1]	92.5% (85.2 to 96.4%)[1]

[1]: 95% Confidence Interval

B. Specificity

Specificity was evaluated on 271 repository serum/plasma samples from 2019 and regarded as pre-COVID-19 samples.

Target:	*NP*	*RBD*	*Total*
Specificity	98.5% (96 to 99.5%)[1]	100% (98.3 to 100%)[1]	98.5% (96 to 99.5%)[1]

[1]: 95% Confidence Interval

C. Repeatability and reproducibility

To check intra-batch accuracy (repeatability), same positive samples (one for each target: NP and RBD) and a negative sample were processed 15 times on kits of the same production batch in the same experimental conditions. All observed results were confirmed as expected.
To check inter-batch accuracy (reproducibility), tests with positive and negative samples were processed from three different production batches. All results were confirmed as expected

D. Interference

Cross-reactivity to serum samples from patients positive for seasonal human pathogenic coronaviruses HCoV-HKU1, -NL63, -OC43, and -229E were tested and found to be negative.
The effect of the acute bacterial pneumonia with *Mycoplasma pneumoniae* and pneumonia as a complication of mononucleosis (EBV) were also evaluated and the serum samples from these patients were found to be negative.
Cross-reactivity to samples containing antibodies directed against HIV (HIV-1 including O group and HIV-2 types) was tested and found to be negative.

XII. LIMITS OF THE KIT

The test is qualitative and cannot predict the quantity of antibodies present in the sample. Clinical presentation and other test results must be taken into consideration to fix a diagnosis.
Immunocompromised patients usually have a delayed antibody response to SARS-CoV-2 compared to immunocompetent patients; this can lead to a negative result which does not rule out the possibility of a past SARS-CoV-2 infection.

XIII. TECHNICAL PROBLEMS / COMPLAINTS

If you encounter a technical problem or if performances do not correspond with those indicated in this package insert:

1. Record the kit batch number
2. If possible, keep the clinical sample in the freezer during the complaint management
3. Contact Coris BioConcept (client.care@corisbio.com) or your local distributor

XIV. BIBLIOGRAPHIC REFERENCES

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H. **Tai W, et al.** Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cell Mol Immunol. 2020 Jun;17(6):613-620
I. **Wrapp D, et al.** Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. 2020 Mar 13;367(6483):1260-1263

Ref C. Belgian biosecurity legislation https://www.biosecurite.be/content/utilisation-confinee-dogm-et-pathogenes

[1] Newcombe, Robert G. "Two-Sided Confidence Intervals for the Single Proportion: Comparison of Seven Methods," *Statistics in Medicine,* **17**, 857-872 (1998).

Last update: 01 DECEMBER 2020

REF	Catalogue number		Manufacturer
IVD	*In vitro* diagnostic medical device		Temperature limits
Σ	Contains sufficient for <n> tests	LOT	Batch code
	Consult instructions for use	2	Single use
	Keep dry		Use by
DIL SPE	Diluent specimen	CONT NaN_3	Contains Sodium azide