COVID-19 Ag *K*-SeT

Manufacturer:
Coris BioConcept
Science Park CREALYS
Rue Jean Sonet 4A
B – 5032 GEMBLOUX
BELGIUM
Tel.: +32(0)81.719.917
Fax: +32(0)81.719.919
info@corisbio.com

www.corisbio.com

IFU-5825/EN/V02

In vitro rapid diagnostic test for the detection of SARS-CoV-2 antigen in nasal secretions

FOR IN VITRO USE **EN**
FOR PROFESSIONAL USE ONLY
References: K-1525: 20 cassettes, 20 buffers, 20 droppers and 20 swabs

I. INTRODUCTION

The Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) responsible for the Coronavirus disease (COVID-19) first appeared in December 2019 in China. The appearance of the new SARS-CoV-2 coronavirus and its rapid dissemination throughout all continents has led to high concern among local and international health authorities, the scientific community and the media and general population. As of 1 July 2021, SARS-CoV-2 has spread worldwide to 192 countries/regions with over 182 million confirmed COVID-19 cases and 3.95 million fatalities, leading the WHO to declare the disease a pandemic. SARS-CoV-2 causes disease of the respiratory tract, leading to severe pneumonia in fragile patients, with most fatal cases occurring in the elderly.

The containment of epidemics relies mainly on rapid identification and isolation of COVID-19 patients. This strategy is based on the availability of rapid diagnostic tests to be performed on any suspect patient who presents with specific symptoms. It should be noted that asymptomatic carriage of the virus is reported in 20% of infected people, in addition to the possibility of prolonged shedding after recovery, which may hamper proper control of the epidemic, making the availability of diagnostic tests even more crucial. Development of point of care testing was recommended by a WHO expert panel on 11-12 February 2020 (COVID 19 Public health emergency of international concern. Global research and innovation forum: towards a research roadmap).

Serological antibody-detection assays do not fulfil the requirement of early detection after infection, as the average incubation period of 3-5 days is too short for developing an immune response. Antigen detection is the most suitable test for early detection of infection.

II. PRINCIPLE OF THE TEST

This test is ready to use and is based on a membrane technology with colloidal gold nanoparticles. A nitrocellulose membrane is sensitized with monoclonal antibodies directed against highly conserved SARS-CoV and SARS-CoV-2 nucleoprotein antigen. Another monoclonal antibody is conjugated to colloidal gold nanoparticles. The conjugate is immobilized on a membrane.
The purpose of this test is to detect SARS-CoV-2 in nasal secretions.

When the nasal secretions come into contact with the strip, the solubilized conjugate migrates with the sample by passive diffusion and the conjugate and sample material come into contact with the anti-SARS antibody adsorbed onto the nitrocellulose strip. If the sample contains SARS-CoV-2, the conjugate–SARS-CoV complex will remain bound to the anti-SARS-CoV-2 antibody immobilized on the nitrocellulose. The result is a red line that develops on the strip. The solution continues to migrate to encounter a control reagent that binds a control conjugate, thereby producing a second red line.

III. REAGENTS AND MATERIALS

1. **COVID-19 Ag *K*-SeT (20)**
20 sealed pouches containing one device and one desiccant. Each device contains one sensitized strip.
2. **Extraction Buffer tube: individual semi-rigid tube containing 0.5 ml of COVID-19 Ag *K*-SeT Extraction Buffer (20)**
Solution buffered to pH 7.5 containing Tris, NaN_3 (<0.1%) and a detergent.
3. **Instruction for use (1)**
4. The **droppers** (20) come in zip-locked plastic bag
5. **Sampling material**: 20 swabs (individually packaged)
6. **Tube rack (1)**

IV. SPECIAL PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices (GLP).
- All the reagents are for *in vitro* diagnostic use only.
- Green lines indicate immunoreagents adsorption sites. Green colour disappears during the test.
- Avoid touching the nitrocellulose with your fingers.
- Wear gloves, a FFP2 or FFP3 mask and lab safety glasses when handling samples. Where available, run the test under a laminar flow cabinet.
- Never use reagents from another kit.
- The quality of the reagents cannot be guaranteed beyond their shelf-life dates or if the reagents are not stored under the required conditions as indicated in the Instruction for use.
- Precipitate may appear in the buffer tube but it does not affect the performances.

V. WASTE DISPOSAL

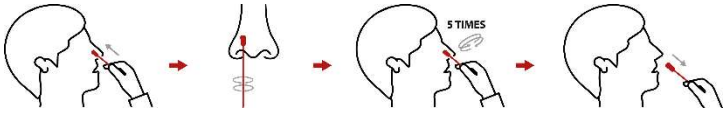
- Dispose of gloves, swabs tubes and used devices in accordance with GLP and biosecurity legislation (ref. C).
- Each user is responsible for the management of any waste produced and must ensure that it is disposed of in accordance with the applicable legislation.

VI. STORAGE

- An unopened pouch may be kept at between 4 and 30°C and used until the shelf-life date indicated on the packaging. Once the pouch is opened, run the test immediately.
- Avoid freezing the pouch containing the device and buffer.

VII. SPECIMEN HANDLING AND COLLECTION

Nasal swab
Insert the swab provided in the kit into one or both nostrils; the tip of the swab should be inserted up to 2.5 cm from the edge of the nostril. Gently roll the swab 5 times along the mucous membrane inside the nostril to ensure that the mucus and cells are well collected. Remove the swab from the nasal cavity and proceed with the specimen preparation procedure (Part VIII.)



Specimens must be tested as soon as possible after collection, with a maximum delay of 72 hours at 2-8°C prior to testing. Even if kept refrigerated, any additional delay may result in a low signal intensity, possibly leading to false-negative results.
Coris BioConcept recommends using the swabs provided in the kit in order to ensure the performance described in the IFU. The effectiveness of other swabs has not been established with this COVID-19 Ag *K*-SeT assay.

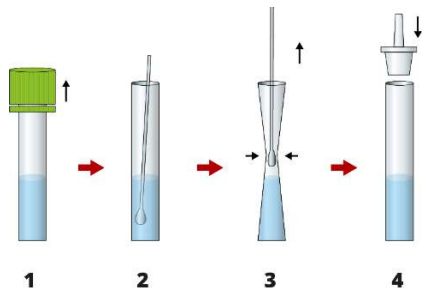
VIII. PROCEDURE

PREPARATION OF THE TEST:
Allow the kit's components, in the unopened packaging, and specimens to reach room temperature (15-30°C) before performing a test.
Open the pouch and remove the device. Once opened, run the test immediately. Indicate the patient's name or specimen number on the device (one device per sample).

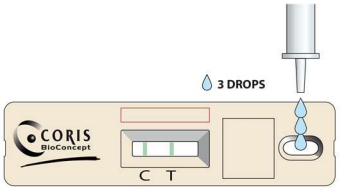
SPECIMEN PREPARATION PROCEDURE:
Performance claims with regard to sample types other than nasal secretions have not been established. We recommend using a fresh specimen for optimal performance of the test.
A highly viscous sample may affect the migration and/or reaction, resulting in weak or delayed colouration or no formation of the line because of sample retention.

Prepare an extraction tube

1. Open the Extraction Buffer tube
2. Dip the swab into the buffer and roll the swab 5 times in the buffer
3. Squeeze the swab several times by compressing the outside walls of the tube against the swab to mix well
   Wipe it against the tube wall
   Discard the swab according to biosecurity requirements
4. Tightly insert the dropper into the semi-rigid tube



Invert the test tube and slowly add 3 drops (100 µl) of diluted sample into the sample well of the cassette.

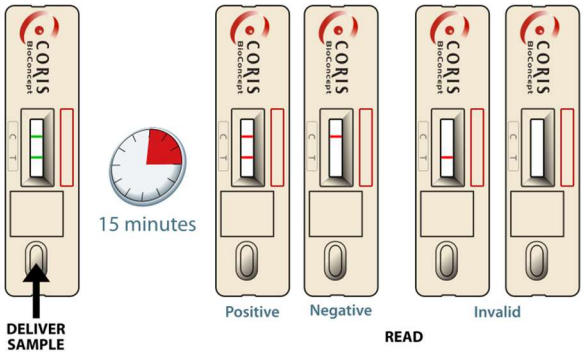


Read the results after 15 minutes

After reading, discard the test tube and the cassette according to biosecurity requirements.
Positive results may be reported sooner, the moment the test line becomes visible. The result must be read while the strip is still wet.

IX. INTERPRETING THE RESULTS

The results should be interpreted as follows, **after 15 min of incubation:**



Negative test result: a reddish-purple line appears across the central reading window at the Control line (C) position. If no other band is present after 15 minutes, the test must be regarded as negative.
Positive test result: in addition to a reddish-purple band at the Control line (C), a visible reddish-purple band appears at the Test line (T) position. The intensity of the test line may vary according to the detectable antigens found in the sample. Any reddish-purple line (T), even if it is weak, should be considered as a positive result.
Invalid test result: the absence of a Control line indicates a failure in the test procedure. Repeat invalid tests with a new test device.

Do not take the appearance of new lines into account after the reaction time has passed.

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

A. Detection Limit:

The detection limit was determined with quantified SARS-CoV-2 virus and has been evaluated at 140 $TCID_{50}$/ml.

B. Sensitivity – Specificity:

The kit was evaluated in two clinical laboratories with a panel of 272 nasal specimens (sample from one nostril). Sample status was confirmed using RT-PCR.

RT-PCR COVID-19 Ag *K*-SeT	Positive	Negative	Total
Positive	95	0	95
Negative	3	174	177
Total	98	174	272

95% Confidence Interval[1]

Sensitivity:	96.9%	(90.7 to 99.2%)
Specificity:	100%	(97.3 to 100%)
Positive predictive value:	100%	(95.2 to 100%)
Negative predictive value:	98.3%	(94.7 to 99.6%)
Agreement:	98.9%	(269/272)

The kit was evaluated with nasal samples taken from patients (sample from both nostrils). A commercial molecular test was used as a reference method. The study included 176 samples (36 confirmed positive and 140 negative). The analysis also showed that 99.3% of the different types of results were correctly interpreted by patients *versus* health care provider (HCP), and that 96% (144/148) of patients completed their test analysis without assistance.

qRT-PCR COVID-19 Ag *K*-SeT	Positive	Negative	Total
Positive	35	0	35
Negative	1	140	141
Total	36	140	176

95% Confidence Interval[1]

Sensitivity (CT≤25):	100 % (82.2 to 100 %)
Sensitivity (CT≤35):	97.2 % (83.8 to 99.9 %)
Specificity:	100 % (96.7 to 100 %)
Positive Predictive value:	100 % (87.7 to 100 %)
Negative predictive value:	99.3 % (95.5 to 99.9 %)
Agreement:	99.4 % (175/176)

C. Repeatability and reproducibility:

To check intra-batch accuracy (repeatability), the same positive samples and a buffer solution were processed 20 times on kits of the same production batch under the same experimental conditions. All the observed results were confirmed as expected.
To check inter-batch accuracy (reproducibility), some samples (positive and buffer) were processed on kits from three different production batches. All the results were confirmed as expected.

D. Cross-reactivity:

Cross-reactivity to samples positive for the following viruses at the concentration of $1x10^6$ $TCDI_{50}$/ml was tested and found to be negative: Influenza A, Influenza B, Respiratory Syncytial Virus (RSV), Adenovirus, Rhinovirus, Parainfluenza virus, Metapneumovirus, Enterovirus and Coronaviruses (229E, OC43, NL63). Cross-reactivity to samples positive for the following bacteria at the concentration of $1.5x10^6$ $TCDI_{50}$/ml was tested and found to be negative: *Streptococcus pneumoniae, Streptococcus pyogenes, Pseudomonas aeruginosa, Acinetobacter baumannii, Nocardia asteroides, Moraxella catarrhalis, Klebsiella pneumoniae, Aspergillus niger, Candida albicans, Haemophilus influenzae, Staphylococcus aureus, Staphylococcus epidermidis, Mycobacteria tuberculosis*, *Legionella pneumophila* and *Bordetella pertussis*.
No false-positive results were obtained with *Chlamydia pneumoniae* and *Mycoplasma pneumoniae.*
Nucleoprotein from HKU-1 Coronavirus was tested at the highest common concentration of 100 ng/ml and does not lead to any signal on the COVID-19 Ag *K*-SeT. As expected, COVID-19 Ag *K*-SeT detects both SARS-CoV and SARS-CoV-2 viruses.

E. Interference

There was no direct interference from the potential interfering substances listed below:

Substance	Active Ingredient	Concentration	Interference
Chloraseptic	Benzocaine, menthol	1.5 mg/ml	None
NasoGel	n/a	5% (v/v)	None
Phenylephrine Nasal Spray	Phenylephrine HCl	15% (v/v)	None
Afrin Nasal Spray	Oxymetazoline HCl	15% (v/v)	None
OTC Nasal spray	Cromolyn sodium	15% (v/v)	None
Zicam	Homeopathic	5% (v/v)	None
Sore Throat Spray	Phenol	15% (v/v)	None
Alkalol	Homeopathic	10% (v/v)	None
Tobramycin	Tobramycin	4 µg/ml	None
Mupirocin	Mupirocin	10 mg/ml	None
Fluticasone Propionate	Fluticasone propionate	1% (v/v)	None
Tamiflu	Oseltamivir phosphate	5 mg/ml	None
Blood	n/a	4% (v/v)	None
Mucin		0.3%	None

A SARS-CoV-2 nucleoprotein preparation was spiked at the LOD with interfering substances in extraction buffer before testing with COVID-19 Ag *K*-SeT. The substances and results are listed below:

Substance	Active ingredient	Concentration	Interference
Rhinospray	Tramazoline hydrochloride	118 µg/ml	None
Xylometazoline	Xylometazoline	0.01% (v/v)	None
Mometasone	Mometasone furoate	5 µg/ml	None
Soframycin	Framycetin sulphate	50 µg/ml	None
Aerius	Desloratadine	5 µg/ml	None
Paracetamol	Paracetamol	20 µg/ml	None
Paracodin	Dihydrocodeine	1 mg/ml	None
Ibuprofen	Ibuprofen	0.6 mg/ml	None
Medica	Chlorhexidine HCl	33 µg/ml	None
	Lidocaine HCl	6.7 µg/ml	None
Strepsil	Dichlorobenzyl alcohol	12 µg/ml	None
	Amylmetacresol	6 µg/ml	None
	Lidocaine hydrochloride	20 µg/ml	None
Thymol	Thymol	0.07% (v/v)	None
Budesonide	Budesonide	25 µg/ml	None
Atrovent	Ipratropium bromide	12.5 µg/ml	None
Ventolin	Salbutamol sulphate	0.5 mg/ml	None
Vasocedine	Naphazoline nitrate	0.01% (v/v)	None
Allergodil	Azelastine hydrochloride	0.01% (v/v)	None
Livostin	Levocabastine	5 µg/ml	None
Oxis	Formoterol fumarate dihydrate	3.3 µg/ml	None

XII. LIMITS OF THE KIT

The test is qualitative and cannot predict the quantity of antigens present in the sample. Clinical presentation and other test results must be taken into consideration to establish a diagnosis.
A positive test does not rule out the possibility that other pathogens may be present.
The COVID-19 Ag *K*-SeT test is an acute-phase screening test. Specimens that are collected after this phase may contain antigen titres below the reagent's sensitivity threshold. If a sample gives a negative result despite the observed symptoms, another relevant test should be run to check the sample.

XIII. TECHNICAL PROBLEMS / COMPLAINTS

If you encounter a technical problem or if the performance does not correspond with that indicated in this package insert:

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

XIV. BIBLIOGRAPHIC REFERENCES

A. **Na Zhu, Dingyu Zhang, et.al.**; *A Novel Coronavirus from Patients with Pneumonia in China, 2019;* N Engl J Med. 2020 Feb 20;382(8):727-733. doi: 10.1056/NEJMoa2001017. Epub 2020 Jan 24.
B. **Chan J. F.-W., Kok K.-H., et al.;** *Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan.*; EMI 2020, doi.org/10.1080/22221751.2020.1719902.
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: 05 OCTOBER 2021

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		Do not reuse
	Keep dry		Use by
		CONT NaN_3	Contains Sodium azide