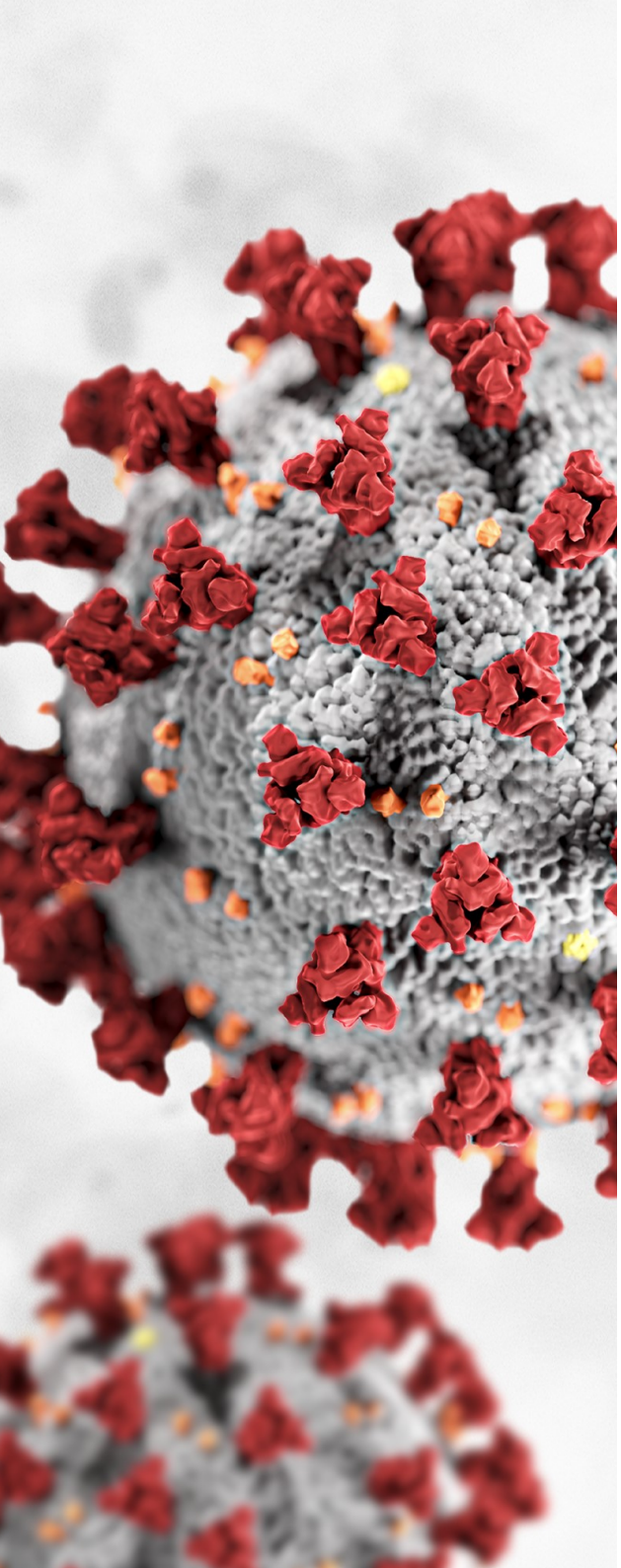

REF V13XX

Rapid Test Ag 2019-nCoV

Lateral Flow Test | IVD

For the detection of SARS-CoV-2 antigen in human nasal or nasopharyngeal specimen.

This Lateral Flow test kit is manufactured by ProGnosis Biotech S.A. and complies with the specifications on the Standard EN ISO 13485:2016

Use only the current version of Product Data Sheet enclosed with the kit.

Rapid Test Ag 2019-nCoV, V13XX, is a qualitative Lateral Flow test for the detection of SARS-CoV-2 antigen in nasal or nasopharyngeal specimen.

The Lateral flow kit contains all reagents required for the immunoassay method.

Specimen: Nasal or nasopharyngeal swab.

- For professional use only
- For in vitro diagnostic use only
- Rapid Test Ag 2019-nCoV is a sensitive screening assay for the detection of SARS-CoV-2. Results should not be used as the only source to diagnose or to determine infection status.
- Negative result do not rule out SARS-CoV-2 infection
- Test should only be conducted by medical personnel
- Test time (incubation time after specimens preparation): 15 min
- Shelf life: 2 years
- Storage: 4-30°C

1. Description

Rapid Test Ag 2019-nCov is a qualitative, lateral flow immunoassay designed to detect the presence or absence of nucleocapsid protein of SARS-CoV-2 in nasal or nasopharyngeal swab specimens directly collected. The antigen (Nucleocapsid Protein, NP) is generally detectable during the acute phase of infection.

2. Principal of the assay

In this test, antibodies specific to the NP are coated on the test line region of the nitrocellulose membrane. During testing, antigens of SARS-CoV-2 in the specimen react with the antibodies that are coated onto gold nanoparticles. The mixture migrates up the membrane to react with the antibodies immobilized on the membrane and generate one colored line in the test region. The presence of this colored line indicates a positive result. To serve as a procedural control, a colored line will always appear in the control region if the test has been performed properly.

3. Introduction

A novel coronavirus (identified as 2019-nCoV) emerged in the Chinese province of Hubei (Wuhan) in December 2019, which has resulted in hundreds of thousands of confirmed human infections worldwide. Cases of severe illness and deaths have been reported. On February 11, 2020 the International Committee for Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2. The median incubation time is estimated to be approximately 5 days with symptoms estimated to be present within 12 days of infection. The most common symptoms of COVID-19 (according to WHO), are similar to other viral respiratory diseases and include fever, dry cough and tiredness. The virus spreads primarily through droplets of saliva or discharge from the nose when an infected person coughs or sneezes.

Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses with a nucleocapsid of helical symmetry and are composed of several proteins including the Spike (S), Envelope (E), Membrane (M) and Nucleocapsid (N) proteins (Figure 1).

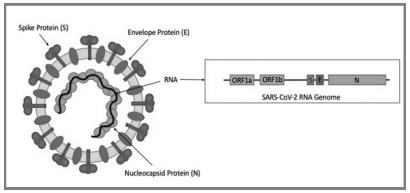

Figure 1. SARS-CoV-2 Structure and Molecular Targets. Common SARS-CoV-2 virus antigenic targets include spike, envelope, and nucleocapsid proteins.

Molecular and antigen testing are the only techniques capable of detecting the SARS-CoV-2 virus. Nucleocapsid protein is a most abundant protein of coronavirus. During virion assembly, N protein binds to viral RNA and leads to formation of the helical nucleocapsid. Nucleocapsid protein is a highly immunogenic phosphoprotein also implicated in viral genome replication and in modulating cell signaling pathways. Because of the conservation of N protein sequence and its strong immunogenicity, the N protein of coronavirus is chosen as a diagnostic tool.

4. Test's formats

The Rapid Test Ag 2019-nCoV is available in two different formats:

◊ The stick format: this involves the reagent test sticks into a vial

◊ The cassette format: this involves a reagent test stick inside a plastic case.

Both formats have the same characteristics, the only difference is the way the test is undertaken.

These instruction for use apply to any commercial reference of the product: V13XX.YYY

5. Reagents Provided

5.1 Stick format

	V1310	V1330	V1360
Vial containing Lateral Flow test sticks	1	1	2
Running Buffer dropper bottle	1	1	-
Extraction tubes in clear zipper bags	1	1	-
Sterile swabs	10	30	60
Positive control	-	1	1
Negative control	-	1	1
Prefilled extraction tube with Running Buffer	-	-	60
Tube rack	1	1	1

5.2 Cassette format

	V1301	V1302	V1304	V1305	V1320	V1340
Reaction device	1	2	4	5	20	40
Prefilled extraction tube with Running Buffer	1	2	4	5	20	40
Sterile swabs	1	2	4	5	20	40
Positive control	-	-	-	-	1	1
Negative control	-	-	-	-	1	1

Note 1: The prefilled tubes on V1320 have an aluminum foil closure. On V1340 & V1360 they are closed with a screw cap.

6. Materials required but not provided

- Clock or Timer
- Gloves and face protection shield
- Container for biohazardous waste

7. Storage Instructions

Store kit components between 4 and 30°C (39.2 - 96°F). Do not freeze any components provided. Reseal the unused sticks in the storage vial together with the desiccant bag provided. Expiry of the kit and reagents is stated on their labels and no quality guarantee is accepted after the expiration date. The expiry of the kit components can only be guaranteed if the components are stored properly and the reagent is not contaminated prior handling.

8. Safety and Precautions for use

8.1 Health and safety precautions

- Use gloves, protective clothing and eye/face protection and handle appropriately with the requisite Good Laboratory Practices. The product must only be used by qualified personnel.
- WASTE MANAGEMENT: 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.
- During the specimen collection human source material spills should be also treated as potentially infectious. Spills should be immediately decontaminated, including the spill area, materials and any contaminated surfaces, with an appropriate chemical disinfectant and should be wiped away.
- Do not re-use any of the kit components as they are single-use only.
- Sterile swabs must be used only for nasal or nasopharyngeal specimen. Avoid to touch the tip of the swab.
- Do not eat, drink or smoke in the area where the specimens and the kit are stored and handled.
- All positive results should be processed following local news and regulations.

8.2 Precautions related to the procedure

- In accordance with Article 1, Paragraph 2b of European Directive 98/79/EC, the use of in-vitro diagnostic medical devices is envisaged by the manufacturer to ensure the suitability, performance, and safety of these products. Consequently, the testing procedure, information, precautions, and warnings in the instructions for use must be followed rigorously. No changes to the test procedure are permitted, nor is any use in combination with other products not approved by the manufacturer. The user is solely responsible for any such changes. The manufacturer is not responsible for false results nor incidents arising as a result of these.
- Do not use the kit if the packaging of components is damaged, if there is an expired reagent or if the desiccant bag is absent inside the vial containing the sticks.
- All reagents should be warmed in room temperature before use.
- Cover or cap all reagents when not in use.
- Do not mix and interchange different specimens.
- Do not interchange individual reagents between kits of different lot numbers.

9. Specimen collection

9.1 Nasal Mid-Turbinate specimen collection

Tilt the patient's head back 70 degrees. Remove a sterile swab from the pouch. While gently rotating it, insert the swab less than one inch (about 2 cm) into patient's nostril (until resistance is met at the turbinates). Rotate the swab five times against the nasal wall then slowly remove from the nostril. Using the same swab repeat the collection procedure with the second nostril. (Figure 2.)

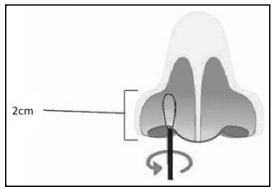

Figure 2. Nasal swab procedure

9.2 Nasopharyngeal specimen collection

Tilt the patient's head back 70 degrees. Remove a sterile swab from the pouch. Place the swab into one of the patient's nostrils. When it reaches the posterior nasopharynx rotate 3 to 5 times over the surface and then remove it slowly while rotating it (Figure 3.)

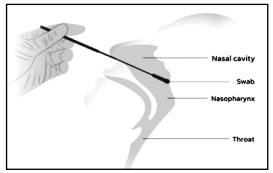

Figure 3. Nasopharyngeal swab procedure

10. Method Procedure

10.1 Calculate the number of swabbing sticks and tubes needed, according to the number of samples to collect.

10.2 Mark the extraction tubes according to the specimens you intend to collect and add Running Buffer (300µL) to each one till the second line from the tube's base (300µL).

ATTENTION: In Cassette format the extraction tubes are pre-filled.

10.3 After the specimen collection (see Chapter 9), place the swab in the extraction tube, rotate the swab forcefully against the side of the tube for 1min. Best results are obtained when the specimen is vigorously extracted in the solution.

10.4 Remove the swab, squeezing the sides of the tube to extract as much liquid as possible.

10.5 Discard the swab.

10.6 The stick format: Immerse the test stick following the direction shown by the arrows, so the uncovered area of the sticks gets soaked.

Note: In case the test stick gets inserted in the wrong direction (arrows pointing up) and gets wet at the top label area, it becomes useless and has to be replaced with a new test stick.

10.7 The cassette format: close the extraction tube with the dropper cup. Add 3 drops in the circular window of the cassette.

10.8 After 15 minutes, the test stick can be visually read and interpreted according to the corresponding figure.

Note: The test result should not be read and interpreted after 15 minutes.

POSITIVE CONTROL:

The stick format: Immerse the stick directly into the positive control tube.

The cassette format: Insert the swab into the positive control tube. Afterwards place it in the extraction tube and follow the described procedure. **(10.3-10.8)**

NEGATIVE CONTROL:

The stick format: Immerse the stick directly into the negative control tube.

The cassette format: Insert the swab into the negative control tube. Afterwards place it in the extraction tube and follow the described procedure. **(10.3-10.8)**

11. Interpretation of results

Note: *For internal procedure purposes two colored lines (blue & green) are present on the result window of the Rapid Ag 2019-nCov. The colored lines (blue& green) have no effect on the product's performance since they are washed away during the experiment.*

Positive: Two visible colored bands appear at both Test (T) and Control (C) line. It indicates a positive result for the SARS-CoV-2 Nucleocapsid Protein in the specimen..

Negative: One visible colored band appears at Control line. It indicates that the concentration of the SARS-CoV-2 NP is zero or below the detection limit of the test.

Invalid: No colored band appears at Control line no matter whether it appears at Test line or not.

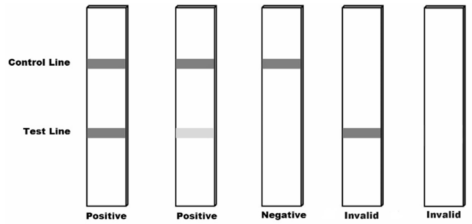

Figure 4: Interpretation of results

12. Limitations

- The test procedure, precautions and interpretation of results for this test must be followed strictly when testing.
- After specimen collection the swab must be extracted as soon as possible. Otherwise they may be stored dry in their original packaging for up to 2 hours at RT. When using a viral transport medium, extract AGAIN the swab in the extraction tube that contains Prognosis Running Buffer. When using the transport media, the test sensitivity can be reduced due to excessive dilution of specimen.

  Note: the transport medium should not contain chaotropic substances such as Guanidinium thiocyanate.
- After the extraction specimens should be tested as soon as possible. Otherwise they can be stored at room temperature 20-25°C (68-77°F) for two hours.
- The test should be used for the detection of SARS-CoV-2 antigen ONLY in nasal or nasopharyngeal swab specimens.
- Failure to follow the guidelines for proper specimen collection, test procedure and interpretation of test results may adversely affect test performance and/or produce invalid result.
- USE ONLY the sterile swabs that are provided in the kit for the specimen collection.
- During specimen collection avoid contact with bleeding areas and excess of mucus as both of them may give a false positive result due to interference with the test performance.
- Positive results indicate the presence of SARS-CoV-2 antigens but a diagnosis of an infection should only be made by a physician evaluating all clinical and laboratory findings and must be based in the correlation of the results with further clinical observations.
- 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.
- Positive test results do not rule out co-infection with other pathogens.
- The Rapid Test Ag will indicate the presence of SARS-CoV-2 NP in the specimen from both viable and non-viable virus.

13. Immunoassay Performance

13.1 Cross-reactivity

In order to determine the cross reactivity of Rapid Test Ag, an evaluation was performed; no cross reactivity against organism, pathogens that could cause infections was observed.

Rapid Test Ag 2019-nCov could have some cross reaction with SARS and very low with MERS.

Table 13.1

Microorganism	Concentration	Result
Adenovirus	$1x10^6$ PFU/ml	Negative
Astrovirus	$1x10^5$ PFU/ml	Negative
Alpha coronavirus 229E	$1x10^5$ PFU/ml	Negative
Alpha coronavirus NL63	$1x10^6$ PFU/ml	Negative
Beta coronavirus OC43	$1x10^5$ PFU/ml	Negative
Beta coronavirus HKU1	$1x10^6$ PFU/ml	Negative
Escherichia Coli O157	$6.4x10^6$ CFU/ml	Negative
Influenza A virus	$1x10^6$ PFU/ml	Negative
Influenza B virus	$1x10^5$ PFU/ml	Negative
Listeria monocytogenes	$2.5x10^6$ CFU/ml	Negative
Salmonella enteritidis	$3.6x10^6$ CFU/ml	Negative
Streptococcus pneumococcal	$4.2x10^5$ CFU/ml	Negative
Streptococcus pyogenes	$3.6x10^6$ CFU/ml	Negative

13.2 Interference Data

The following substances showed no significant interference on the test results of Rapid Test Ag 2019n -CoV.

Table 13.2

No	Interfering Substances	Final Test
1	Azithromycin	84 mg/ml
2	Amoxicillin	54 mg/L
3	Albuterol	0.05 mg/L
4	Acarbose	0.3 mg/L
5	Chlorpheniramine	0.8 mg/L
6	Chlorothiazide	27 mg/L
7	Rheumatoid factor	200 IU/ml
8	Triglycerides	1.5 mg/L
9	Hemoglobin	100 mg/L
10	Human Chorionic Gonadotropin Hormone (pregnancy)	10-fold dilution
11	Ibuprofen	219 mg/L
12	Xylometazoline (Otriven)	10%
13	Acetylsalicylic Acid	3 mg/ml
14	Mucin	0.5%

13.3 Limit of Detection

The lowest detectable concentration of an analyte in a method is known as LOD. In this case, we check the concentration of heat inactivated SARS-CoV-2 isolate USA-WA1/2020 in Rapid Test Ag 2019-nCov. The LOD is the level at which 95% of the replicates are characterized as positive. The results of 20 replicates of 10 dilutions with heat inactivate virus are shown at the table below.

LOD : 358.75 TCID50/mL

Concentration	Positive Replicates	Visual Interpretation of results
1.15×10^7	20 / 20	Strong positive
1.15×10^6	20 / 20	Strong positive
1.15×10^5	20 / 20	Strong positive
1.15×10^4	20 / 20	Strong positive
5.75×10^3	20 / 20	Positive
2.87×10^3	20 / 20	Positive
1.435×10^3	20 / 20	Positive
717.5	20 / 20	Positive
358.75	20 / 20	Positive
179	3 / 20	Negative

13.4 High Dose Hook Effect

No high dose hook effect was observed up to 1.15 x 107 TCID50/mL of inactivated SARS-CoV-2 or with the Rapid Test Ag 2019-nCoV.

13.5 Clinical performance characteristics

13.5.1 Nasal specimens

In order to determine the clinical performance of the Rapid Test Ag 2019-nCoV, 386 negative and 142 positive nasal specimens confirmed with RT-PCR assay **LightCycler Multiplex RNA Virus Master (ROCHE)**, were tested. The results are presented at the table below.

Rapid Test Ag 2019-nCoV	Real-time RT PCR		
	Positive	*Negative*	*Total*
Positive	140	1	141
Negative	2	385	387
Total	142	386	528

	Mean Value	95% confidence interval
Sensitivity	98.59%	95.00% to 99.83%
Specificity	99.74%	98.57% to 99.99%
PPV	99.29%	95.18% to 99.90%
NPV	98.86%	97.54% to 99.58%

CT cycles	RT-PCR positive	Rapid Test Ag positive	Positive Agreement (95% CI)
15-20	53	53	100% (92.28% to 100.00%)
21-25	44	44	100% (91.96% to 99.99%)
26-30	27	27	100% (87.23% to 100.00%)
31-35	18	16	88.89% (65.29% to 98.62%)

Clinical Diagnostic Specificity: 99.74%

Clinical Diagnostic Sensitivity: 98.59%

13.5.2 Nasopharyngeal specimens

In order to determine the clinical performance of the Rapid Test Ag 2019-nCoV, 478 negative and 135 positive nasopharyngeal specimens confirmed with RT-PCR assay **LightCycler Multiplex RNA Virus Master (ROCHE)** were tested. The results are presented at the table below.

Rapid Test Ag 2019-nCoV	Real-time RT PCR		
	Positive	*Negative*	*Total*
Positive	129	2	131
Negative	6	476	482
Total	135	478	613

	Mean Value	95% confidence interval
Sensitivity	95.56%	90.58% to 98.35%
Specificity	99.58%	98.50% to 99.95%
PPV	98.47%	94.18% to 99.61%
NPV	98.76%	97.32% to 99.43%

CT cycles	RT-PCR positive	Rapid Test Ag positive	Positive Agreement (95% CI)
15-20	48	48	100% (92.60% to 100.00%)
21-25	43	43	100% (91.78% to 100.00%)
26-30	23	23	100% (85.18% to 100.00%)
31-35	21	15	71.43% (47.82% to 88.72%)

Clinical Diagnostic Specificity: 99.58%

Clinical Diagnostic Sensitivity: 95.56%

14. Method Summary

Total procedure time (after specimens preparation): 15 min.

ONLY for V1310/V1330 (Running Buffer dropper bottle):

Add Running Buffer into the extraction tube till the second line from the tube's base (300μL)

⇩

Specimen collection (nasal or nasopharyngeal) according to specific instruction (chapter 9)

⇩

Insert the swab into the extraction tube and rotate it forcefully against the side of the tube for 1min

⇩

Remove the swab, squeezing the sides of the tube to extract as much liquid as possible

⇩

Stick Format: Place the test stick into the extraction tube with the arrows pointing down, so the uncovered area gets soaked

⇩

Cassette Format: close the extraction tube with the dropper cup. Add 3 drops in the circular window of the device.

⇩

(Wait 15 mins)

Formation of 2 lines indicates presence of SARS-CoV-2 antigen in the specimen

15. References

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IVD In Vitro Diagnostics Medical Device

Σ_n Content sufficient for tests

REF Catalog number

Manufacturer

4° 30° Storage Conditions

Instructions for use

EXP Expiration Date

CE

VERSION 27/ 2022-04-15

REF V13XX

RAPID TEST Ag 2019-nCoV

Lateral Flow Test | IVD

For the detection of SARS-CoV-2 antigen in human nasal or nasopharyngeal specimen.

This Lateral Flow test kit is manufactured by ProGnosis Biotech S.A. and complies with the specifications on the Standard EN ISO 13485:2016

Use only the current version of Product Data Sheet enclosed with the kit.

Rapid Test Ag 2019-nCoV, V13XX, is a qualitative Lateral Flow test for the detection of SARS-CoV-2 antigen in nasal or nasopharyngeal specimen.

The Lateral flow kit contains all reagents required for the immunoassay method.

Specimen: Nasal or nasopharyngeal swab.

- For professional use only.
- For in vitro diagnostic use only
- Rapid Test Ag 2019-nCoV is a sensitive screening assay for the detection of SARS-CoV-2. Results should not be used as the only source to diagnose or to determine infection status.
- Negative result do not rule out SARS-CoV-2 infection
- Test should only be conducted by medical personnel
- Test time (incubation time after specimens preparation): 15 min
- Shelf life: 2 years
- Storage: 4-30°C

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