PROGNOSIS BIOTECH

RAPID TEST
2019-nCoV Total Ig

REF V1210/V1230

Lateral Flow Test | IVD

for the detection of Total Immunoglobulins (IgA, IgG and IgM) against SARS-CoV-2 in whole blood, human serum or plasma specimens.

www.prognosis-biotech.com

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 2019-nCoV Total Immunoglobulins, V120/V1230, is a qualitative Lateral Flow test for the detection of IgA, IgM and IgG against SARS-CoV-2 in human serum, plasma or whole blood specimens. The Lateral flow kit contains all reagents required for the immunoassay method.

Samples: Human serum, plasma or whole blood specimens.

- For *in vitro* diagnostic use only
- For a medical diagnosis, the serological test result should always be interpreted together with the clinical symptoms of the patient and other result.
- Negative results do not rule out SARS-CoV-2 infection.
- Test should only be conducted by a medical personnel.
- This test has not been reviewed by the FDA.
- Results from antibody testing should not be used to diagnose or exclude SARS-CoV -2 infection or to inform infection status.
- Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
- Not for the screening of donated blood

- Test time (incubation time after specimens preparation): 10 min
- Shelf life: 12 months
- Storage: 4-30°C

1. Description

Rapid Test 2019-nCoV Total Immunoglobulins (Ig) is a qualitative Lateral flow test for the detection of IgA, IgM and IgG antibodies against the Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) in human serum, plasma or whole blood specimens.

2. Intended Use

The Rapid Test 2019-nCoV Total Ig S is an in vitro diagnostics immunoassay, assisting in the diagnosis of patients with symptoms suggestive of SARS-CoV-2 infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. In combination with the clinical picture, results of RT-PCR and CT-Scan, Rapid Test 2019-nCoV Total Ig could be used as an aid in the diagnosis of COVID-19 disease. This immunoassay is a method for the determination of SARS-CoV-2 exposure levels, giving valuable information about the individuals' immune status. However, the interpretation of the results should not be used as the unique base of the SARS-CoV-2 infection status diagnosis.

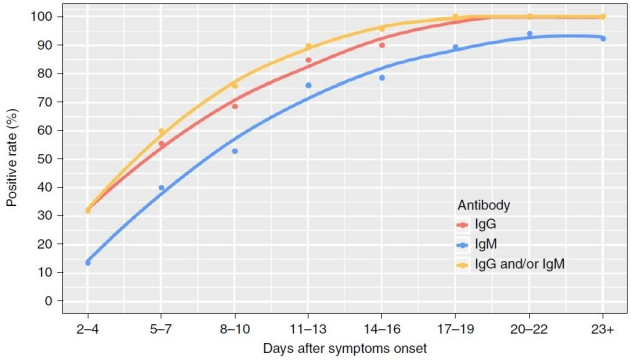
IgA, IgM and IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

The sensitivity of Rapid Test 2019-nCoV Total Ig early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results for Rapid Test 2019-nCoV Total Ig may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

3. General Information

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. The S protein is very immunogenic with the Receptor Binding Domain (RBD) being the target of many neutralizing antibodies[1]. The 2019 Novel Coronavirus, formerly known as 2019-nCoV and now known as SARS-CoV-2, emerged in the Chinese province of Hubei (Wuhan) in December 2019 and has been declared a pandemic on 11 March 2020. This outbreak has spread rapidly, with millions of reported cases and thousands of deaths worldwide. Comparisons of the genetic sequences of this virus have shown similarities to SARS-CoV and bat coronaviruses. The 2019-nCoV disease (COVID-19) is a respiratory disease caused by infection with the SARS-CoV-2 virus and the incubation period of COVID-19 is generally 7 days, with a minimum of 3 and a maximum of 14 days. Fever, cough, shortness of breath or difficulties in breathing, pain in the muscle and tiredness are the most common signs and symptoms of the virus. In severe cases, the infection



Days *	2–4 (N = 22)	5–7 (N = 45)	8–10 (N = 70)	11–13 (N = 79)	14–16 (N = 70)	17–19 (N = 47)	20–22 (N = 17)	23+ (N = 13)
IgG	7	25	48	67	63	47	17	13
IgM	3	18	37	60	55	42	16	12
IgG and/or IgM	7	27	53	71	67	47	17	13

* Number of serum samples with positive results

Antibody responses against SARS-CoV-2: Graph of positive rates of virus-specific IgG and IgM versus days after symptom onset in 363 serum samples from 262 patients. *(Long et al., 2020)* (Published 29 April 2020).

can further lead to pneumonia, severe acute respiratory syndrome, kidney failure and death. Currently, there is no medication or vaccine available against infection with this new virus. Antibodies are produced by the immune system after an average of 7 days after infection. In general, Immunoglobulins M and A are the first immunoglobulins to be produced and IgG is the most abundantly found immunoglobulin that can be detected in the body for a long period. Within 19 days after symptom onset, all patients tested are positive for IgG against SARS-CoV-2. Seroconversion for IgG and IgM occurs simultaneously or sequentially. Both IgG and IgM titers reach a plateau within 6 days after seroconversion. Serological testing may be helpful for the diagnosis of suspected patients with negative RT–PCR results (determining the number of cases of COVID-19) and for the identification of asymptomatic infections[2].

4. Principle of the Method

Rapid 2019-nCoV Total Ig test is an antibody capture immunochromatographic assay for the detection of total immunoglobulins (IgA, IgM and IgG) in human serum, plasma or whole blood specimens. SARS-CoV-2 S-protein is conjugated to colloidal gold and deposited on the conjugate pad. The same protein is immobilized on the test line of the nitrocellulose membrane. After the dilution of the specimen, in the test tube, using the provided Dilution Buffer, the test strip is immersed into the solution. The sample mixes with the SARS-CoV-2 antigen-colloidal cold conjugate and flows across the pre-coated membrane by capillary action. While the sample solution migrates through the sample pad, it dissolves the test detection system particles that were dried on it. In case SARS-CoV-2 antibodies are present in the sample, they bound to the S-protein in the test line, forming a visible pink line, indicating positive results. If SARS -CoV-2 antibodies are absent in the sample, no pink line will appear in the test line, indicating a negative result.

To serve as an internal process control, a control line should always appear at Control Zone (C) after the test is completed. Absence of a pink control line in the Control Zone is an indication of an invalid result.

5. Reagents Provided

	V1210	V1230
Vial containing 10 Lateral Flow test strips and silica gel	1	3
Dilution Buffer dropper bottle	5 mL	5 mL
Disposable microsafe tube (5µl)	10	30
Disposable safety Lancets (for fingerstick whole blood specimens)	10	30
Alcohol pads	10	30
Clear plastic test tubes	10	30

6. Materials required but not provided

- Adjustable single channel micropipette of 10 µL with disposable tips (for other than fingerstick whole blood specimens)
- Gloves and container for biohazardous waste
- Clock or timer
- Centrifuge for serum or plasma specimens

7. Storage Instructions

Store kit components between 4 and 30°C (39.2 - 96°F). Do not freeze any components provided. Reseal the unused strips in the storing tube 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, familiar with the potential hazards, in a clinical or research laboratory.
- Dispose of all specimens and materials used to perform the test as though they contain an infectious agent. Laboratory, chemical or biohazardous wastes must be handled and discarded in accordance with the local, regional and national regulations. 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 (commonly 1:10 dilution of household bleach or 70-80% Ethanol) and should be wiped dry.

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. The manufacturer is not responsible for any results obtained by visual analysis of patient samples.
- 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 strips.
- All reagents should be warmed in room temperature before use. Use a clean disposable plastic pipette tip for each reagent, to avoid cross-contamination.
- 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. Specimens preparation

9.1 Whole blood specimen

- **Fingerstick:** Using the alcohol swab, clean the area of finger to be lanced and allow to dry. Twist the cap of the lancet, place the lancet against the area and press the trigger button. After you wipe away the first drop of blood collect the blood droplet using a capillary tube.

NOTE 1: Massage and/or rub the hand to stimulate blood flow towards the collection area.

NOTE 2: Place the capillary towards the collection area allowing the blood to flow till the indicated line. Press the bulb only to expel the drawn up blood into the test tube containing the dilution buffer and mix thoroughly by pipet 4-5 times.

- **Venipuncture:** Collect venous whole blood in a tube with anticoagulant. In general, whole blood samples should be tested immediately after sample collection. Whole blood specimens must be stored at 2-8˚C if not tested immediately and tested within 24 hours of collection. **Do not freeze whole blood specimens.**

9.2 Serum and Plasma specimens Collection

Blood specimens should be collected aseptically using venipuncture techniques by qualified personnel. The Correct performance of specimen collection and storage is crucial for the test result. Keep tubes sealed at all times. The use of sterile or aseptic techniques will preserve the integrity of the specimens.

- **Serum:** Use a serum separator tube (SST) and allow specimens to clot for 2 hours at room temperature or overnight at 4°C. Centrifuge the specimen for 10min at 4000xg at room temperature. Remove serum and assay immediately or aliquot and store specimens at –20°C or –80°C.
- **Plasma:** Collect plasma using Heparin, EDTA or Citrate as an anticoagulant. Centrifuge for 15 minutes at 4000xg within 30 minutes of collection. Assay immediately or aliquot and store specimens at –20°C or –80°C.

NOTE: Serum or plasma specimens can be subjected to a maximum of 1 freezing/ thawing cycle. **Do not heat the specimens**. Avoid highly lipemic, icteric or hemolytic specimens because they could affect the results of the assay. Specimens with visible microbial contamination should not be used. After centrifugation, serum or plasma can be stored at 2-8°C if the test is performed within 4 days.

10. Method Procedure

10.1 Add 4 drops of the Dilution Buffer to the tube from the dropper bottle.

10.2 Introduce 5μL of the sample (or the indicated volume of the capillary tube) into the test tube using a micropipette of 10 μL. Mix by priming pipetting at least 5 times.

10.3 Immerse a test strip into the test tube following the direction shown by the arrows, so the uncovered area of the strips gets soaked.

Note: In case the test strip 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 strip.

10.4 After 10 minutes, the test strip can be visually read and interpreted according to the following table and corresponding figure.

Note: If the test is not read within 15 minutes, it is considered to be invalid and safe results cannot be obtained.

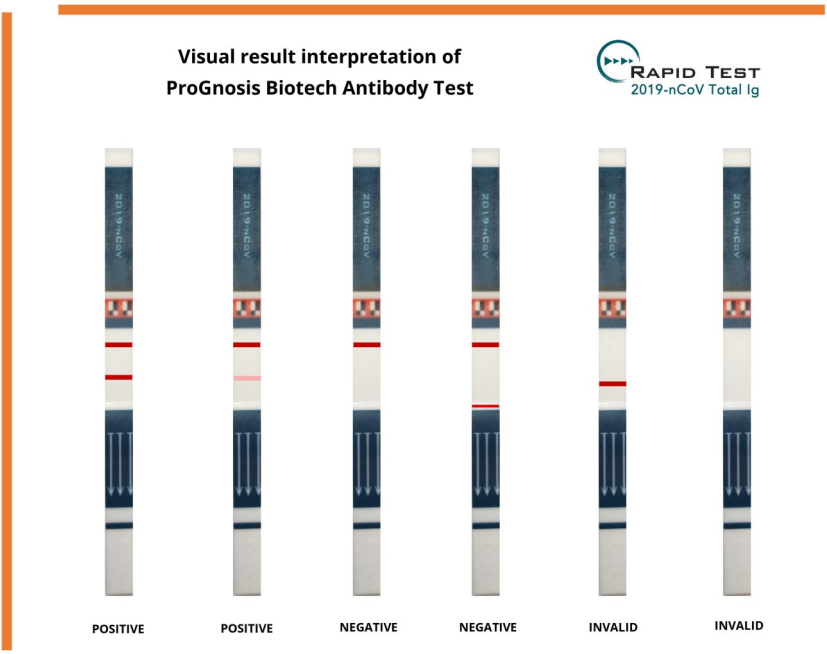
11. Interpretation of results

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

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

Note: In the case of whole blood specimens, a faded line may appear just above the pad of the strip. This line appears due to the blood composition and should not be taken under consideration.

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



12. Limitations of the immunoassay

- The detection of immunoglobulins against SARS-CoV-2 is dependent on the analyte concentration in the specimen. A negative or non-reactive result can occur if the quantity of anti-SARS-CoV-2 antibodies present in the specimen is below the LOD of the assay. During the acute infection phase and/or for immunosuppressed patients, anti-SARS-CoV-2 antibodies might not be detectable while the individual is infected by SARS-CoV-2. Thus, a negative result is not evidence for the absence of COVID-19 infection.
- The detection of antibodies against SARS-CoV-2 in serum or plasma is also linked to the frequency of the tests performed on the patients. In order to increase the sensitivity and the earliness of the test positivity, regular monitoring of patients suspected to be infected by SARS-CoV-2 should be performed.
- For a medical diagnosis, the serological test result should always be interpreted together with the clinical symptoms of the patient and other results, e.g. those of the direct pathogen detection. Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus.
- Specimens collected at the begging of infection or by some immunosuppressed individuals may not have detectable levels of antibodies. In cases, at the early stage of infection, it is recommended to obtain a second specimen between 14 and 21 days to be tested in parallel with the original specimen, in order to determine a seroconversion.
- This product is only used for testing of individual serum, plasma (Heparin, EDTA or Citrate) or whole blood. Other specimen types have not been evaluated and should not be used with this assay.

13. Immunoassay Performance

13.1 Cross-reactivity

Cross-reactivity has been evaluated by testing SARS-CoV-2 seronegative specimens from patients with antibodies to other coronaviruses or other pathogens. A total of 68 specimens from 11 different categories were tested. The number of samples tested for each category is listed below.

Pathogen	Tested sample number	False positive sample
Influenza A virus	6	0
Influenza B virus	5	0
Hepatitis C virus	6	0
Hepatitis B virus	7	0
Hemophilus influenzae	6	0
Alpha coronavirus 229E	7	0
Alpha coronavirus NL63	7	0
Beta coronavirus OC43	6	0
Beta coronavirus HKU1	5	0
Antinuclear antibodies	6	0
Respiratory syncytial virus	7	0

13.2 Internal validation characteristics

The internal validation of the Rapid Test 2019-nCoV Total Ig was assessed during a multi evaluation on specimens obtained from a general asymptomatic population of pre-epidemic individuals (blood donors, hospitalized patients) and on patients with clinical symptoms of coronavirus COVID-19 tested positive with RT-PCR assay.

13.2.1 Diagnostic Specificity

A total of 468 specimens (blood donors or hospitalized asymptomatic patients), collected prior to the outbreak of the COVID-19 pandemic, were tested. The specificity was 99.78%, 467/468 (CI: 0.988 to 0.9996).

13.2.2 Diagnostic Sensitivity

An internal longitudinal study was performed on 122 patients with clinical symptoms of COVID-19 and with a PCR positive result. Specimens per patient were collected from 2 to 58 days post onset of clinical symptoms. The sensitivity was 96.72%, 118/122 (CI: 0.9187 to 0.9872).

Days between onset of symptoms and sample collection	Positive Specimens	Nonreactive specimens	Total	%
2 - 7 days	21	4	25	84
8 - 14 days	22	0	22	100
15 - 21 days	25	0	25	100
22 - 28 days	28	0	28	100
29 - 58 days	22	0	22	100
Total	118	4	122	96.72

13.3 Clinical performance characteristics

The clinical performance of the Rapid Test 2019-nCoV Total Ig was conducted at AHEPA General University Hospital of Thessaloniki (referral hospital for SARS-CoV-2 in Greece). Specimens obtained from a general asymptomatic population of pre-epidemic individuals (blood donors, hospitalized patients) were used and patients with clinical symptoms of coronavirus COVID-19 tested positive with RT-PCR assay.

13.3.1 Clinical Diagnostic Specificity

A total of 114 specimens (blood donors or hospitalized asymptomatic patients), collected prior to the outbreak of the COVID-19 pandemic, was tested. The specificity was 100%, 114/114 (CI: 0.9674 to 1).

13.3.2 Clinical Diagnostic Sensitivity

An longitudinal study was performed on 80 patients with clinical symptoms of COVID-19 and with a PCR positive result. Specimens per patient were collected from 2 to 58 days post onset of clinical symptoms. The sensitivity was 98,75%, 79/80 (CI: 0.9325 to 0.9978).

Days between onset of symptoms and sample collection	Specimens	Nonreactive specimens	Total	%
2 - 7 days	5	1	6	83.33
8 - 14 days	37	0	37	100
15 - 21 days	12	0	12	100
22 - 28 days	9	0	9	100
29 - 58 days	16	0	16	100
Total	79	1	80	98.75

14. Method Summary

Total procedure time (after specimens preparation): 10 min.

Add 4 drops of the Dilution Buffer into the test tube

⇩

In the case of the capillary tube, pierce the finger with the lancet

⇩

Add 5 μL of sample into the test tube and mix thoroughly

⇩

Place test strip into the test tube with the arrows pointing down, so the uncovered area gets soaked

⇩

(Wait 10 mins)
Formation of 2 lines indicates presence of SARS-CoV-2 antibodies in the sample

15. References

1. Long, Q., Liu, B., Deng, H. et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med* (2020). https://doi.org/10.1038/s41591-020-0897-1.

2. Berry, J .D., e t al. Neutralizing epitopes of the SARS-CoV S-protein cluster independent of 446 repertoire, antigen structure or mAb technology. *MAbs* 2, 53-66 (2010).

3. Tai, W., He, L., Zhang, X. 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). https://doi.org/10.1038/s41423-020-0400-4.

IVD In Vitro Diognostics Medical Device

4°C 30° Storage Conditions

Σ n Content sufficient for <n> tests

Instructions for use

REF Catalog number

EXP Expiration Date

Manufacturer

www.prognosis-biotech.com
e: info@prognosis-biotech.com
t: +30 2410 623922 | f: +30 700 700 6262
Farsalon 153 | 41335 Larissa, Greece

CE