COVID-PRESTO®

Rapid Diagnostic Orientation Test (RDT)
(Whole Blood/Serum/Plasma) – Cassette

Ref.: TR-COV-002

INDICATION FOR USE

COVID-PRESTO®, IgG/IgM (Whole Blood/Serum/Plasma) TROD – Cassette, is a solid-phase immunochromatographic assay for the rapid, qualitative and differential detection of IgG and IgM antibodies to the novel Coronavirus 2019 in human whole blood, serum or plasma. This test makes it possible to determine immunity to SARS-CoV-2, which makes it possible to affirm, even in the absence of symptoms, contact with the virus and an acquired immunity that is a priori protective.

INTRODUCTION

Coronaviruses are enveloped RNA viruses that are widely distributed in humans, other mammals, and birds and cause respiratory, enteric, liver, and neurological illnesses. Seven species of coronaviruses are known to cause disease in humans. Four viruses — 229E, OC43, NL63, and HKU1 — are prevalent and usually cause cold symptoms in immunocompetent individuals. The other three strains – severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and novel coronavirus (SARS-CoV-2) – are zoonotic in origin and have been linked to sometimes fatal diseases. IgG and IgM antibodies to the novel coronavirus can be detected 1 to 3 weeks after the onset of symptoms.

PRINCIPLE OF THE TEST

COVID-PRESTO® is a lateral flow immunochromatographic test. The test uses human antibodies against IgM (IgM test line), human antibodies against IgG (IgG test line) and rabbit IgG (control line (C)) immobilized on a nitrocellulose strip. The Conjugate (recombinant COVID-19 antigens labeled with colloidal gold) is also integrated into the strip.
When the blood sample is added to the sample well (S) and then buffer to the buffer well (B), IgM and/or IgG antibodies, if present, bind to COVID-19 conjugates, forming antibody-antigen complexes.
These complexes migrate through the nitrocellulose membrane by capillary action. When the complexes meet the line of the corresponding immobilized antibody (human IgM antibody and/or human IgG antibody), the complexes are trapped and form a burgundy band that confirms the reactivity of the test. The absence of a coloured band in the test region indicates a negative result.
To serve as a procedural control, the colored line of the control region will always change from blue to red, indicating that a sufficient sample volume has been used and that migration to the membrane has been performed.

EQUIPMENT PROVIDED

- 25 sealed pouches, each containing a test cassette and a moisture absorber
- 2 x Buffer Bottles
- 25 lancets
- 25 micropipettes of 10µL
- 1 user manual

MATERIALS REQUIRED BUT NOT PROVIDED

- Sample Collection Containers
- Centrifuge (for plasma only)
- Laboratory pipettes
- Gloves
- Timer

CONSERVATION AND STABILITY

The kit can be stored at room temperature or refrigerated (2-30°C). The test is stable until the expiration date printed on the sealed pouch. The cassette should remain in its sealed pouch until used. DO NOT FREEZE. Do not use beyond the expiration date.

WARNINGS AND PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after the expiration date.
- Read the entire procedure carefully before performing the test. Failure to follow the procedure may result in incorrect results.
- Do not use if the sealed pouch is damaged or the buffer tube is broken.
- The test cassette is for single use. Do not reuse under any circumstances.
- Handle all specimens as if they contained infectious agents. Follow the safety instructions for microbiological hazards and follow the procedures in place for the proper disposal of samples.
- Wear protective clothing such as lab coats, disposable gloves, and goggles during procedures.
- Humidity and temperature can affect the results.
- Do not perform the test in a room with high air circulation, such as a fan or a powerful air conditioner.

COLLECTION OF SERUM OR PLASMA SAMPLES

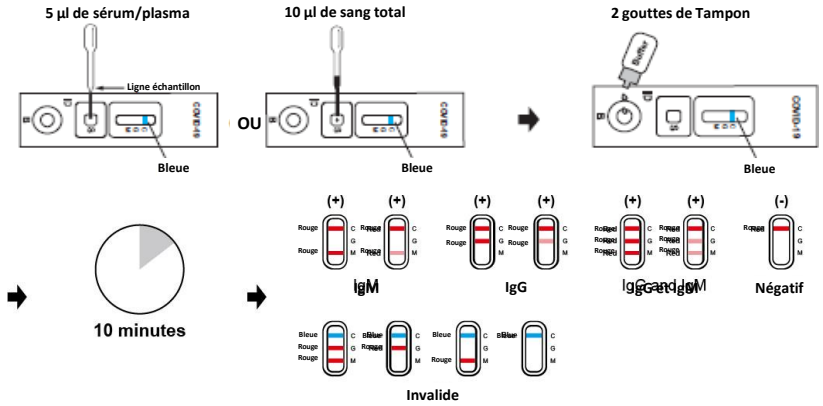
COVID-PRESTO® can be performed from whole blood, serum, or plasma. If serum or plasma:

1. Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed samples.
2. Perform the test immediately after sample collection. Do not leave samples at room temperature for long periods of time. Serum and plasma samples can be stored at a temperature between 2 and 8°C for up to 3 days. For long-term storage, samples should be stored at a temperature below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be performed within 2 days of collection. Do not freeze whole blood samples. Whole blood taken from the fingertip should be tested immediately.
3. Allow samples to reach room temperature before testing. Frozen samples must be completely thawed and thoroughly mixed before performing the test. Samples should not be frozen and thawed repeatedly.
4. If samples are to be shipped, they must be packaged in accordance with local regulations regarding the transport of infectious agents.

CARRYING OUT THE TEST

Wait until the sample and components are at room temperature (15-30°C) before performing the test. Remove the test cassette from the sealed bag. Test within one hour of opening the pouch.

1. Place the test cassette on a flat, clean surface.
2. Perform the test as follows, depending on the type of sample:
   - For **fingerstick whole blood samples (using the 10µL micropipette):**
     - Using the lancet, prick the side with the fingertip. Form a large drop of blood suspended.
     - Hold the micropipette horizontally and bring the tip of the pipette into contact with the blood. The micropipette fills automatically (about 2 to 3 seconds).
     - Place the micropipette vertically over the well (S) of the cassette and gently lower until the end of the micropipette touches the well (S). The micropipette empties automatically.
       Note: If the micropipette does not empty, gently tap the micropipette with your index finger, keeping it in contact with the well (S) to expel the blood.
     - Immediately add 2 drops of buffer to the well (B) of the cassette. Avoid air bubbles
   - For **venous whole blood samples** : take **10µl** of blood sample using a laboratory pipette and place it in the well (S) of the cassette. Immediately add 2 drops of buffer to the well (B) of the cassette. Avoid air bubbles.
   - For **serum or plasma samples** : collect **5µl** of serum/plasma sample using a laboratory pipette and place them in the well (S) of the cassette. Immediately add 2 drops of buffer to the well (B) of the cassette. Avoid air bubbles.
3. Wait for the coloured stripe(s) to appear. After 2 minutes, if the red color has not crossed the test window, add 1 additional drop of buffer to the well (B).
   Read the results at 10 minutes. Positive results can be visible as early as 2 minutes. Do not interpret the result after 15 minutes.



INTERPRETATION OF THE RESULTS

Negative : The colored line in the control line region (C) changes from blue to red. No lines appear in the regions of the M or G test lines. The result is negative.

Positive IgM : The colored line in the control line region (C) changes from blue to red, and a colored line appears in the test line region M. The result is positive for the anti-COVID-19 IgM.

Positive IgG : The colored line in the control line region (C) changes from blue to red, and a colored line appears in the test line region G. The result is positive for the anti-COVID-19 IgG.

Positive IgG and IgM : The colored line in the control line region (C) changes from blue to red, and two colored lines appear in the test line regions M and G. The result is positive for IgM and IgG anti-COVID-19.

Invalid : The control line is always blue (even partially) and does not completely change from blue to red. Insufficient sample volume or incorrect execution of the procedure are the most likely reasons for the control line to fail. Review the procedure and repeat the test with a new test cassette. If the problem persists, stop using the kit and contact your local distributor.

QUALITY CONTROL

Internal quality control is included in the test. A red line appearing in the control region (C) is the internal procedure control. It confirms that a sufficient sample volume was used and that the test procedure was correctly followed.
External quality checks are not provided in this kit; However, it is recommended to test positive and negative controls as good laboratory practice to confirm the procedure and verify the performance of the test.

BOUNDS

- Use fresh samples whenever possible. Frozen and thawed samples (especially repeatedly) contain particles that can block the membrane. This slows down the flow of reagents and can lead to membrane staining, making it difficult to interpret the results.
- Optimal test performance requires strict application of the test procedure described in this user manual. Deviations can lead to aberrant results.
- This test is not intended to be used for the purpose of diagnosing COVID-19 infection, but for the diagnosis of acquired immunization against the COVID-19 virus.
- A negative result indicates the absence of detectable antibodies against COVID-19. However, a negative result does not exclude the possibility of exposure or infection with COVID-19.
- A negative result may occur if the antibodies detected are not present at the stage of the disease at which the sample is collected or if the level is below the detection limit of the test.
- An abnormally high concentration of heterophilic antibodies or rheumatoid factor may affect the test results.
- Some preliminary data seem to indicate that these antibodies can appear late (D30), especially in the case of pauci/asymptomatic infection.
- COVID-PRESTO® is a TROD; The definitive diagnosis of immunization should be made in a medical laboratory.

Reference method	Negative COVID-19 PCR
Number of samples	72
Results	**100%**

PERFORMANCE

The performance of the COVID-PRESTO® test was evaluated at the Orléans CHR (Infectious Diseases Department – publication of results in progress) based on:

- Sensitivity: 148 capillary blood samples obtained from 134 patients who had a positive COVID-19 PCR due to suggestive symptoms (fever and/or cough and/or dyspnea and/or influenza-like syndrome)
- Specificity: 72 patients who had a negative COVID-19 PCR sampled due to symptoms that led to the performance of the PCR which was negative without signs of severity

Sensitivity

Reference method	Positive COVID-19 PCR			
Period range after 1st symptoms (number of patients)	D2-D6 (n=24)	D7-D10 (n=39)	D11-D15 (n=39)	J16-J31 (n=46)
Number of negative samples	20	16	12	0
Number of IgM positive samples alone	2	8	3	1
Number of positive IgM and IgG samples	2	10	24	40
Number of IgG positive samples alone	0	5	0	5
Results	**16.7%**	**58.9%**	**69.2%**	**100%**

Specificity

Interference
The substances listed below were tested: rheumatoid factors (80IU/ml), antinuclear antibody (1:160), anti-mitochondrial antibody (1:20), human anti-mouse antibody (HAMA) (2464ng/ml), ascorbic acid (20mg/dL), hemoglobin (1000mg/L), bilirubin (10mg/dL), human serum albumin (2000mg/dL), triglycerides (500mg/dL).
The results showed no interference at the concentrations indicated.

Precision
Within: 3 samples (one negative, one IgG positive, and one IgM positive) were tested 10 times each. The results were all correct.
Inter-batch: 3 samples (one negative, one IgG positive and one IgM positive) were tested 10 times in 3 batches. The results were all correct.

Cross-reactions
Samples containing antibodies against the following pathogens were tested: Influenza A virus, Influenza B virus, Respiratory syncytial virus, Adenovirus, Rhinovirus, Human metapneumovirus, Mycoplasma pneumoniae, Chlamydia pneumoniae, Bacterial pneumonia, Endemic coronavirus HKU1, Endemic coronavirus OC43, Endemic coronavirus NL63, Endemic coronavirus 229E.
The results showed no cross-reaction.

REFERENCES

- Weiss SR, Leibowitz JL. Coronavirus pathogenesis. Adv Virus Res 2011; 81: 85-164.
- Masters PS, Perlman S. Coronaviridae. In: Knipe DM, Howley PM, eds. Fields virology. 6th ed. Lippincott Williams & Wilkins, 2013: 825-58.
- Su S, Wong G, Shi W, et al. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol 2016; 24: 490-502.
- Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019; 17: 181-192.

SYMBOL LEGEND

	Read the user manual	Σ	Number of tests per kit		Maker
IVD	For use in *in vitro diagnostics* only		Expiry date		Do not reuse
2°C 30°C	Store between 2 and 30°C	LOT	Lot number	REF	Reference
CE	CE marking				

AAZ Votre santé sous contrôle
43 rue de Bellevue
92100 Boulogne-Billancourt France
contact@aazlab.fr