Platelia SARS-CoV-2 Total Ab

1 plate - ∑ 96 REF 72710

5 plates - ∑ 480 REF 12013798

Platelia SARS-CoV-2 Total Ab assay is a qualitative one-step antigen capture ELISA test for the *in vitro* detection of IgM/IgA/IgG antibodies to SARS-CoV-2 in human serum and plasma (EDTA, heparin, ACD or citrate) specimens.

IVD

16008267CA-EN – 2021/03

BIO-RAD

Table of Content

1 INTENDED USE

Platelia SARS-CoV-2 Total Ab assay is a qualitative one-step antigen capture ELISA test for the *in vitro* detection of IgM/IgA/IgG antibodies to SARS-CoV-2 in human serum and plasma (EDTA, heparin, ACD or citrate) specimens.

The assay can be used as a screening tool for the detection of anti-SARS-CoV-2 total antibodies in order to determine seroprevalence in the general population and/or the immune status of individuals regarding exposure to SARS-CoV-2. See Section 8, Limitations.

Platelia SARS-CoV-2 Total Ab is intended for use by trained laboratory personnel. It can be used manually or on automated systems.

This test should not be used for screening of donated blood. Results from Platelia SARS-CoV-2 Total Ab assay should not be used for diagnosis. Canadian testing facilities are required to report all positive results to the appropriate public health authorities.

For Laboratory Use Only.

2 SUMMARY AND EXPLANATION OF THE TEST

Coronavirus (CoV) is an enveloped virus that contains a single-stranded positive-sense RNA. SARS-CoV-2, formerly known as 2019-nCoV, is a newly emerging coronavirus that mainly affects the respiratory tract that can lead to Severe Acute Respiratory Syndrome (SARS). The underlying disease caused by this virus is named COVID-19. Coronaviruses have been responsible for several outbreaks in the world during the two last decades. In 2003 and 2014, coronaviruses caused outbreaks mainly in Asia (SARS-CoV) and in the Middle East (MERS-CoV), respectively. Before the new SARS-CoV-2 emergence, six coronaviruses were known to affect humans (SARS-CoV, MERS-CoV and four other coronaviruses that cause mild upper and lower respiratory syndromes).

SARS-CoV-2 was first identified in December 2019, in Wuhan City, Hubei Province, China, after several patients developed severe pneumonia similar to that caused by SARS-CoV. The virus has since rapidly spread around the globe and in March 2020, WHO officially announced COVID-19 as a pandemic. Person-to-person transmission of the virus lead to quick spreading of COVID-19 and a high number of patients requiring intensive care urged authorities around the world to set up containment measures. The incubation period ranges from 2 to 14 days.

The virus has been detected in respiratory secretions, considered as the primary means of transmission. Once viral particles enter the respiratory tract, the virus attaches to pulmonary cells via the ACE-2 receptors followed by endocytosis. SARS-CoV-2 can also be transmitted via the fecal route.

Patients positive for SARS-CoV-2 and that are symptomatic are diagnosed with COVID-19. Symptoms can vary drastically and notably include fever, dry cough, anosmia, sputum production, headaches, dyspnea, fatigue, nausea, and diarrhea. While some cases can be asymptomatic, others can lead to acute respiratory distress syndrome (ARDS) and even death.

Diagnosis mainly relies on real-time reverse transcription polymerase chain reaction (RT-PCR) testing of respiratory specimens. However, RT-PCR can lead to false negative results due to low viral loads or unsuitable collection, handling, and storage of swabs (oropharyngeal or nasopharyngeal), or failure during the reaction process. Imagery techniques such as computed tomography (CT) can also be performed and show bilateral multilobar ground-glass opacities to aid in diagnosis.

Platelia SARS-CoV-2 Total Ab detects IgM, IgA, and IgG antibodies to SARS-CoV-2. In conjunction with other diagnostic tests it can be used to determine if a patient has been exposed to SARS-CoV-2.

3 PRINCIPLE OF THE PROCEDURE

Platelia SARS-CoV-2 Total Ab is a one-step antigen capture format Enzyme-Linked Immunosorbent Assay (ELISA) for qualitative detection of total anti-SARS-CoV-2 nucleocapsid antibodies (IgM/IgA/IgG) in human serum or plasma specimens.

- The assay uses a recombinant SARS nucleocapsid Protein in a one-step antigen capture format assay.
- Serum or plasma specimens and controls are pre-diluted. Conjugate (recombinant SARS nucleocapsid Protein coupled with peroxidase) is added to each specimen and then the mixture is incubated one hour at 37 °C in wells coated with the recombinant SARS nucleocapsid Protein. During this incubation, if IgM and/or IgG and/or IgA antibodies are present in the specimen, they form a complex between the recombinant SARS-nucleocapsid Protein on the wells and the recombinant SARS-nucleocapsid Protein coupled with peroxidase.
- After a washing step, the presence of immune complex (SARS-nucleocapsid Protein / anti-SARS nucleocapsid antibodies / SARS-nucleocapsid Protein labeled with peroxidase) is demonstrated by distribution of a chromogenic solution initiating a color development reaction.
- After 30 minutes of incubation at room temperature, the enzymatic reaction is stopped by addition of an acid solution. The optical density reading obtained with a spectrophotometer set at 450 / 620 nm is proportional to the amount of antibodies present in the specimen. The presence of anti-SARS-CoV-2 nucleocapsid antibodies in an individual specimen is determined by comparing the optical density reading of the specimen to the optical density of the Cut-off Control.

4 REAGENTS

4.1 Description

Identification on label		Description	Presentation/ Preparation	
R1	**Microplate**	**Microplate** - 96 wells (12 strips of 8 wells each) sensitized with recombinant nucleocapsid protein of SARS - **Specific ID number = 19**	1 plate **Ready for use**	5 plates **Ready for use**
R2	**Concentrated washing solution (20X)**	**Concentrated washing solution (20X)** - TRIS-NaCl buffer (pH 7,4) - Preservative: 0.04% ProClin 300	1 vial 70 mL **To be diluted**	1 vial 235 mL **To be diluted**
R3	**Negative Control**	**Negative Control** - TRIS-NaCl buffer (pH 8 ± 0.1), bovine serum albumin, glycerol - Preservative: 0.1% ProClin 300	1 vial 1.0 mL **Ready for use**	1 vial 1.0 mL **Ready for use**
R4	**Cut-off Control**	**Cut-off Control** - TRIS-NaCl buffer (pH 8 ± 0.1), bovine serum albumin, glycerol - Rabbit polyclonal antibodies anti-SARS nucleocapsid - Preservative: 0.1% ProClin 300	1 vial 1.0 mL **Ready for use**	1 vial 1.0 mL **Ready for use**
R5	**Positive Control**	**Positive Control** - TRIS-NaCl buffer (pH 8 ± 0.1), bovine serum albumin, glycerol - Rabbit polyclonal antibodies anti-SARS nucleocapsid - Preservative: 0.1% ProClin 300	1 vial 1.0 mL **Ready for use**	1 vial 1.0 mL **Ready for use**
R6	**Conjugate**	**Conjugate** - Recombinant SARS nucleocapsid protein coupled with horseradish peroxidase - TRIS-NaCl buffer (pH 8 ± 0.1), phenol red - Preservative: 0.5% ProClin 300	1 vial 9 mL **Ready for use**	2 vials 23 mL **Ready for use**

R7	**Sample Diluent**	**Sample Diluent** - TRIS-NaCl buffer (pH 8 ± 0.1), phenol red - Preservative: 0.5% ProClin 300	1 vial 12 mL **Ready for use**	2 vials 23 mL **Ready for use**
R8	**Substrate buffer**	**Substrate buffer** Citric acid and sodium acetate solution pH 4.0, containing hydrogen peroxide (H_2O_2 0.015%) and dimethyl sulfoxide (DMSO 4%)	1 vial 60 mL **To be reconstituted**	2 vials 60 mL **To be reconstituted**
R9	**Chromogen: TMB solution (11X)**	**Chromogen: TMB solution** Solution containing 3.3', 5.5' tetramethylbenzidine (TMB)	1 vial 5 mL **To be reconstituted**	2 vials 5 mL **To be reconstituted**
R10	**Stopping solution**	**Stopping Solution** Sulphuric acid solution (H_2SO_4 1N)	1 vial 28 mL **Ready for use**	3 vials 28 mL **Ready for use**

4.2 Storage and handling requirements

This kit should be stored at +2-8 °C. Open reagents must be stored according to the instructions below.

Identification	Preservation
R1	After opening the vacuum-sealed bag, store the microwell strips at +2-8 °C for up to 4 weeks, in their original bag with desiccant resealed with tape.
R2	The diluted washing solution can be stored at +2-30 °C for 2 weeks. The concentrated washing solution (R2) can be stored at +2-30 °C until the expiration date. If opened, the concentrated washing solution (R2) can be stored at +2-8 °C until the expiration date, in the absence of contamination.
R3, R4, R5, R6, R7, R8, R9	After opening, these reagents stored at +2-8 °C, are stable for 4 weeks, in the absence of contamination.
R8 + R9	Once diluted, the solution is stable for up to 6 hours in the dark at +18-30 °C.
R10	After this reagent stored at +2-8 °C is opened, it is stable until the validity date shown on the label if there is no contamination.

5 WARNING AND PRECAUTIONS

For *in vitro* use by a professional user in a laboratory environment only.

5.1 Health and safety precautions

This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately with the requisite Good Laboratory Practices.

No known test method can offer complete assurance that infectious agents are absent. Therefore, all human blood derivatives, reagents and human specimens should be handled as if capable of transmitting infectious disease, following recommended Universal Precautions for bloodborne pathogens as defined by local, regional and national regulations.

Biological spills: Human source material spills should be treated as potentially infectious.
Spills not containing acid should be immediately decontaminated, including the spill area, materials and any contaminated surfaces or equipment, with an appropriate chemical disinfectant that is effective for the potential

biohazards relative to the specimens involved (commonly a 1:10 dilution of household bleach, 70-80% Ethanol or Isopropanol, an iodophor such as 0.5% Wescodyne™ Plus, etc.), and wiped dry.

Spills containing acid should be appropriately absorbed (wiped up) or neutralized, the area flushed with water and wiped dry; materials used to absorb the spill may require biohazardous waste disposal. Then the area should be decontaminated with one of the chemical disinfectants.

REMARK: Do not place solutions containing bleach into the autoclave!

Dispose of all specimens and material 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 all local, regional and national regulations.

For hazard and precaution recommendations related to some chemical components in this test kit, please refer to the pictogram(s) mentioned on the labels and the information supplied at the end of instruction for use. The Safety Data Sheet is available on www.bio-rad.com.

5.2. Precautions related to the procedure

5.2.1. Preparing

- DO NOT USE the kit if the packaging of components is damaged.
- DO NOT USE expired reagents.
- DO NOT USE if desiccant is absent inside microplate bag.
- Before use, wait for 30 minutes for the reagents to stabilize at room temperature (18-30 °C).
- Carefully reconstitute the reagents avoiding any contamination.
- The use of disposable material is preferred for preparation of reagents. If using glassware, wash thoroughly and rinse with deionized water.
- Do not mix or associate reagents from different lots within a test run.
- Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
- The name of the test, as well as a specific identification number for the test, are written on the frame of each microplate. This specific identification number is stated on each strip too.

  Platelia SARS-CoV-2 Total Ab: Specific ID number = 19

  Verify the specific identification number before use. If the identification number is missing, or different from the stated number corresponding to the assay to be tested, the strip should not be used.
- Do not mix reagents from other kits that have different lot numbers, with the exception of the Washing Solution (R2, identification*: 20x coloured green), the peroxidase Substrate Buffer (R8, identification*: TMB buf., coloured blue), the Chromogen (R9, identification*: TMB 11X coloured purple) and the Stopping Solution (R10, identification*:1N coloured red), provided that these reagents are strictly equivalent and that the same lot number is used within a given test run.

  REMARK: The Washing Solution (R2, identified* in green as 20X) may not be mixed with the Washing Solution (R2 identified* in blue as 10X) provided in Bio-Rad reagent kits.
  ****on the vial label.***
- Preparation of the development solution or the conjugate working solution must be made in a clean plastic tray or glass container. Single use plastic containers are recommended. When using reusable plastic container, they can be cleaned by overnight soaking with distilled water or washing solution. When using glass container, they can be washed with 1N HCl and rinsed thoroughly with distilled water and dried.
- The development solution must be stored in the dark.
- The development solution (substrate buffer + chromogen) must be coloured pink. The modification of this pink colour within a few minutes of reconstitution indicates that the reagent cannot be used and must be replaced. Preparation of the development solution can be made in a clean disposable single use plastic tray or glass container that has first been pre-washed with 1N HCl and rinsed thoroughly with distilled water and dried. This reagent must be stored in the dark.
- The specimen distribution must begin immediately after the conjugate distribution. Waiting time between the dispensing of the conjugate and the specimens should not exceed 30 minutes.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various conjugate or substrate solutions.

- Never use the same container to distribute conjugate and development solution.

5.2.2. Processing

- Do not change the assay procedure.
- Each run of this assay must proceed to completion without interruption after it has been started. A delay shorter than 5 min between two steps is acceptable.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not carry out the test in the presence of reactive vapours (acid, alkaline, aldehyde vapours) or dust that could alter the enzymatic activity of the conjugate.
- Use a new distribution tip for each specimen.
- Microplate washing is a critical step in this procedure: follow the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
- Carefully follow the washing procedures described to obtain maximum test performance. With some instruments, it could be necessary to optimize the washing procedure (increase of number of cycles of washing step and/or volume of wash buffer for each cycle) to obtain an acceptable level of OD background for the negative specimens.
- Contact your local commercial contact for the adaptations and special procedures.

6 SPECIMENS

1. The test is performed on serum or plasma specimens collected in EDTA, lithium heparin, ACD or sodium citrate anticoagulants.
2. Comply with the following guidelines for handling, processing and storing of blood specimens:
   - Collect a blood specimen according to standard laboratory procedures. For serum, allow specimens to clot completely before centrifugation.
   - Keep tubes sealed at all times.
   - After centrifugation, extract the serum or plasma and keep it in a sealed tube.
   - The specimens can be stored at +2-8 °C if the test is performed within 7 days.
   - If the test cannot be completed within 7 days, freeze the specimens at -20 °C (or -80 °C).
   - Serum or plasma specimens can be subjected to a maximum of 5 freeze / thaw cycles. Previously frozen specimens should be thoroughly mixed after thawing prior to testing.
3. The results are not affected by proteinemic specimens containing 90 g/l albumin, icteric specimens containing 100 mg/l bilirubin, lipemic specimen containing the equivalent of 36 g/l triolein (triglyceride), and hemolyzed specimens containing up to 10 g/l of haemoglobin.
4. Do not heat the specimens.

7 PROCEDURE

7.1 Materials required but not provided

1. Sterile distilled or demineralized water to dilute the concentrated washing solution.
2. Sodium hypochlorite (household bleach) and sodium bicarbonate.
3. Absorbent paper.
4. Adhesive film.
5. Protective goggles.
6. Disposable tubes.
7. Automatic or semiautomatic, adjustable or preset pipettes or multipipettes to measure and dispense 10 µL to 1000 µL, 1 mL, 2 mL and 10 mL.
8. Graduated cylinders of 25 mL, 50 mL, 100 mL and 1000 mL capacity. Vortex mixer.
9. Manual microplate washing system, water-bath, or equivalent microplate incubator, thermostatically set at 37 °C ± 1 °C (*).
10. Microplate reader or full automated system equipped with 450 and 620 nm filters (*).
11. Container for biohazardous waste.

(*) Consult us for detailed information about the equipment recommended by our technical department.

7.2 Reagents preparation

7.2.1 Ready for use reagents

Reagent 1 (R1): Microplate
Each frame support containing 12 strips is wrapped in a sealed bag. Cut the bag using scissors 0.5 to 1 cm above the sealing. Open the bag and take out the frame. Put the unused strips back with desiccant into the bag. Close the bag carefully and put it back into storage at +2-8 °C.

Reagent 3 (R3): Negative Control, Reagent 4 (R4): Cut-off Control, Reagent 5 (R5): Positive Control, Reagent 6 (R6): Conjugate, Reagent 7 (R7): Sample Diluent
These reagents are ready for use.

7.2.2 Reagents to reconstitute

Reagent 2 (R2): Concentrated washing solution (20X)
Prepare the working Washing Solution by diluting the Concentrated washing solution 1:20 in distilled water: 50 mL of R2 in 950 mL of distilled water. Use 800 mL of working Washing Solution for one complete 12 strip microplate, excluding dead volume due to the equipment used.

Reagent 8 (R8) + Reagent 9 (R9): Enzyme development solution
Dilute 1:11 the Chromogen (R9) in the Substrate Buffer (R8) (e.g. 2 mL reagent R9 + 20 mL of R8 reagent) given that 20 mL are necessary and sufficient for 12 strips. Homogenize.

7.3 Assay Procedure

i. Strictly follow the procedure and Good Laboratory Practice.

● EIA Procedure

1. **Bring reagents to room temperature (+18-30 °C) for at least 30 minutes before use.**
2. Use the Negative and Positive Controls with each run to validate the results.
3. Carefully set up the plan for distributing and identifying the controls and the patient specimens.

	1	2	3	4	5	6	7	8	9	10	11	12
A	R3	E4										
B	R4	E5										
C	R4	E6										
D	R4	E7										
E	R5	E8										
F	E1	E9										
G	E2	E10										
H	E3	E11										

4. Prepare the dilution of the Washing Solution (R2) *(Refer to section 7.2)*.
5. In an inert pre-dilution microplate, dilute Controls R3, R4, R5 and test specimens E1, E2, in R7 to give a **1:5 dilution** :
   - Distribute **60 µL** of R7, then add **15 µL** of specimen in each well.
   - Add **75 µL** of Conjugate solution (R6) to all the wells of the pre-dilution microplate.
   - Mix by aspirating and rejecting once, then **transfer immediately 100 µL** of the pre-diluted controls and specimens to the wells of the reaction microplate (R1).

   Depending on the system used, it is possible to modify the position of controls or the order of distribution, provided the modification has been first validated.
6. Cover the reaction microplate with an **adhesive plate sealer**, pressing firmly onto the plate to ensure a tight seal. Incubate the microplate in a thermostat controlled water bath or microplate incubator for **60 minutes (+/- 5 min) at 37 °C (+/- 2 °C).**
7. Prepare the enzyme development solution (R8+R9) *(Refer to section 7.2)*.
8. At the end of incubation period, remove the adhesive plate sealer. Aspirate the contents of all wells into a container for biohazard waste (containing sodium hypochloride). Wash the plate **5 times with a microplate washer** (using 800 µL of Working Washing Solution). Invert microplate and gently tap on absorbent paper to remove remaining liquid.
9. Quickly distribute into each well **200 µL** of the development solution (R8+R9). Allow the reaction to develop in the dark for 30 minutes **(+/-** 4 min) at room temperature (+18-30°C). **Do not use adhesive plate sealer during this incubation step**.
10. Add **100 µL** of Stopping Solution (R10) to each well, using in the same order and at the same rate as for the addition of the development solution. Mix thoroughly.
11. Carefully wipe the plate bottom.
12. Read the optical density of each well at 450 nm (reference filter at 620 nm) **within 30 minutes** after addition of the Stopping Solution (the strips must always be kept away from light before reading).
13. Before reporting results, check for agreement between the reading and the distribution plan of the plates.

7.4 Quality Control

Use the controls on each microplate every time the test is performed.

7.5 Test Validation criteria

Calculate the mean value of the optical densities of the cut-off control R4: OD_M.

If one of the cut-off control R4 individual values differs by more than 30% from the mean value, disregard the value and carry out the calculation again with the two remaining cut-off control values.

	Validation criteria
R4	The OD_M **R4** must be: 0.5 < OD_M **R4** < 1.4
R3 / R4	The **ratio (OD R3 /** OD_M **R4)** must be ≤ 0.25
R5 / R4	The **ratio (OD R5 /** OD_M **R4)** must be ≥ 1.1

7.6 Calculation / Interpretation of the results

The cut-off value $OD_M R4$ corresponds to the mean value of the optical densities of the cut-off control R4.
Specimen results are expressed by ratio using the following formula: Specimen ratio = Specimen OD / $OD_M R4$.

Interpretation of results

- Specimen ratio less than 0.8 is considered to be **«negative» for the presence of anti-SARS-CoV-2 antibodies**.
- Specimen ratio greater than or equal to 0.8 but less than 1.0 is considered to be **«equivocal» for the presence of anti-SARS-CoV-2 antibodies**. Another specimen should be collected and tested a few days later.
- Specimen ratio equal or greater than 1.0 is considered to be **«positive» for the presence of anti-SARS-CoV-2 antibodies**.

Specimen Ratio	Result
R < 0.8	Negative
0.8 ≤ R < 1.0	Equivocal
R ≥ 1.0	Positive

8 TEST LIMITATIONS

1. This assay is not intended to be used for screening patients or as an aid for diagnosis of patients with suspected COVID-19 infection. This assay is to be used in conjunction with the testing strategy outlined by public health authorities in your area.
2. This assay is not intended for home testing (or self-testing).
3. Clinical diagnosis of COVID-19 should not be established based on a single test result. Follow-up and supplemental testing as well as other clinical and laboratory data should be considered.
4. False positive results for IgM and IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes.
5. The detection of anti-SARS-CoV-2 antibodies in serum or plasma is linked to the frequency of the tests performed on the patients. In order to increase the sensitivity and the earliness of the test positivity, a regular monitoring of patients suspected to be infected by SARS-CoV-2 should be performed.
6. The detection of anti-SARS-CoV-2 antibodies is dependent on the presence of the analyte in the specimen. A negative or non-reactive result can occur if the quantity of antibodies for the SARS-CoV-2 virus present in the specimen is below the detection limit 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; the sensitivity of the test early after infection is unknown. Thus, a negative result is not an evidence for the absence of COVID-19 infection.
7. False negative results can occur in elderly and immunocompromised patients.
8. Performance characteristics of Platelia SARS-CoV-2 Total Ab have not been evaluated with specimens of serum or plasma originating from newborns or pediatric patients.
9. Results are for the detection of SARS-CoV-2 antibodies. IgM antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although levels over the course of infection are not well characterized. IgG antibodies to SARS-CoV-2 become detectable later following infection. **At this time, it is unknown how long IgM or IgG antibodies may persist following infection.**
10. Positive results for IgG, IgM and/or IgA could occur after infection and can be indicative of acute or recent infection, and successful immune response to a vaccine, although the performance of Platelia SARS CoV-2 AB has not been assessed in a population vaccinated against COVID-19.
11. The presence of specific antibodies is a sign of previous or current infection and can also be used to determine the efficacy of treatment.
12. Platelia SARS-CoV-2 Total Ab assay can detect total antibodies specific to SARS-CoV-1 and to SARS-CoV-2 without any differentiation. Besides, cross-reactions are possible with MERS-CoV.

13. "The performance of Platelia SARS CoV-2 Total Ab has not been assessed on specimens from individuals who have been infected with emerging variants of SARS-CoV-2 of public health concern."
14. Laboratories are required to report all positive results to the appropriate public health authorities.

9 PERFORMANCES CHARACTERISTICS

Performances presented here below have been obtained during Platelia SARS-CoV-2 Total Ab assay evaluations. Results obtained in laboratories can be different from these.

9.1 Analytical Performance Characteristics

Analytical studies were carried out at the Bio-Rad R&D laboratory.

9.1.1 Precision measurement

Intra-assay precision (Repeatability)

Three (3) positive specimens and 1 negative specimen were assayed 30 times in the same run. Within-run CV are below 10% for the negative specimen and below 5% for all the positive specimens.

Specimen ID	N	Mean Ratio	SD	CV%
Negative	30	**0.05**	0.004	**7.1%**
Positive 1	30	**1.15**	0.038	**3.3%**
Positive 2	30	**1.54**	0.055	**3.6%**
Positive 3	30	**2.36**	0.095	**4.0%**

Intermediate precision (Inter-assay)

3 positive specimens and 1 negative specimen were assayed in duplicates by 2 different operators per day during 5 days. Nested ANOVA was used to estimate within run, between run, between days and total precision. The CVs obtained on the positive specimens are less than or equal to 10% for repeatability and less than or equal to 15% for intermediate precision.

Specimen ID	N	Mean Ratio	Repeatability		Between run		Between day		Within Laboratory	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%
Negative	20	**0.08**	0.002	**2.3%**	0.021	**25.9%**	0.006	**6.9%**	0.021	**26.9%**
Positive 1	20	**1.33**	0.074	**5.5%**	0.020	**1.5%**	0.019	**1.4%**	0.079	**5.9%**
Positive 2	20	**2.59**	0.050	**1.9%**	0.089	**3.4%**	0.068	**2.6%**	0.122	**4.7%**
Positive 3	20	**3.34**	0.065	**2.0%**	0.108	**3.2%**	0*	**NA**	0.127	**3.8%**

Note: (*) The negative variance value is estimated at 0.

9.1.2 Analytical Specificity / Cross Reactivity

Cross-reactivity has been evaluated by testing 168 SARS-CoV-2 seronegative specimens from patients positive for other coronaviruses or medical conditions. There was no cross-reactivity (false positive results) seen with the Platelia SARS-CoV-2 Total Ab assay in any of the specimens that were tested.

Analyte	Sample(s) tested	Non Reactive	Reactive
CoV 229E (alpha-coronavirus)	6	6	0
CoV NL63 (alpha-coronavirus)	5	5	0
CoV HKU1 (beta-coronavirus)	5	5	0
CoV OC43 (beta-coronavirus)	13	13	0
Adenovirus	2	2	0
INF A H1 N1	1[1]	1[1]	0
INF A H3N2	2	2	0
Influenza	10	10	0
Flu Vaccine	15	15	0
Metapneumovirus	3	3	0
Metapneumovirus Ab	5	5	0
ParaInfluenza 1	2	2	0
ParaInfluenza 2	1	1	0
ParaInfluenza 3	2	2	0
ParaInfluenza 4	1	1	0
Parainfluenza Virus Ab	5	5	0
Rhinovirus/Enterovirus	2	2	0
RSV (Respiratory syncytial virus)	3[1]	3[1]	0
RSV Ab	5	5	0
HIV Ab	5	5	0
HCV Ab	5	5	0
HBV	5	5	0
CMV IgG	5	5	0
CMV IgM	5	5	0
EBV IgG	5	5	0
EBV IgM	5	5	0
Malaria IgG	5	5	0
Dengue Ab	5	5	0
Rheumatoid Factor	5	5	0
HAMA	5	5	0
ANA	5	5	0
Pregnant Women	5	5	0
Anti-*E. Coli*	5	5	0
Mycoplasma pneumoniae IgG	5	5	0
Candida albicans IgG	5	5	0

1 One patient was co-infected with CovHKU1 + INF A and one patient was co-infected with CoVHKU1 + RSV.
The specificity on this target population is 100% (168/168) with a 95% Confidence Interval of [97.8%-100%].

9.1.3 Hook Effect

Three (3) high positive specimens were serially diluted and were tested neat and diluted with the Platelia SARS-CoV-2 Total Ab assay.

Whatever the specimens, no negative results were observed on non-diluted specimens and no hook effect was observed with the three serially diluted specimens.

No hook effect is observed on the Platelia SARS-COV-2 Total Ab assay with the test of 3 high positive specimens.

9.2 Interferences

The results are not affected by proteinemic specimens containing 90 g/L albumin, icteric specimens containing 100 mg/L bilirubin, lipemic specimen containing the equivalent of 36 g/L triolein (triglyceride), and hemolyzed specimens containing up to 10 g/L of haemoglobin.

9.3 Specimen Matrix

Different types of matrices – serum, K3 EDTA, Sodium Citrate, Lithium Heparin and ACD – were validated on a panel of 5 negative specimens and 5 positive specimens for each matrix.
No impact was observed whatever the matrix for the negative and positive specimens.

9.4 Clinical Performance Characteristics

The clinical performance of the Platelia SARS-CoV-2 Total Ab assay was assessed during a multi-evaluation on specimens obtained from a general asymptomatic population of pre-epidemic individuals (blood donors, hospitalized patients) and on symptomatic patients from Intensive Care Units with clinical symptoms of coronavirus COVID-19 tested positive with RT-PCR assay. Both prospective and retrospective studies of asymptomatic populations and infected patients were conducted.

9.4.1 Specificity

A total of 600 specimens (500 from blood donors and 100 from hospitalized asymptomatic patients) collected prior to the outbreak of the COVID-19 pandemic were tested. The percent negative agreement was **99.3% (596/600) with a 95% Confidence Interval of [98.3% – 99.8%].**

9.4.2 Clinical Agreement in Serum and Plasma

A longitudinal study was performed on 50 patients (127 specimens) hospitalized in intensive care units in 3 French hospitals with clinical symptoms of COVID-19 and a PCR positive result.

One to five consecutive specimens were collected per patient from 2 to 92 days post onset of clinical symptoms. Results were analyzed for each patient to determine the first sample that was SARS-CoV-2 total antibody positive.

The table below summarizes when the first positive result was observed for each patient relative to day between the onset of symptoms and specimen collection.

Table 1: Overall agreement expressed as Percent Positive Agreement according to the day between onset of symptoms and sample collection

Days between onset of symptoms and sample collection	First Positive draw for Patient	Patient Negative	Total	PPA (%) according to PCR results [95% Confidence Interval]
2-8 days	**11**	**1**[1]	**12**	**92% [61.5%-99.8%]**
9-15 days	**30**	**0**	**30**	**93%**[3] **[77.9%-99.2%]**
16-22 days[2]	**8**	**0**	**8**	**100% [63.0%-100%]**

[1] No additional specimens after 8 days were available to follow immune response

[2] All patients turned positive within 22 days and their positive status was confirmed when the subsequent specimens were drawn beyond 22 days.

[3] For 2 patients, the first sample drawn on day 8 or on day 10 was found positive with Platelia SARS-CoV-2 Total Ab whereas, it was found negative with RT-PCR. The second specimen drawn on day 11 (for both patients) was positive with both Platelia SARS-CoV-2 Total Ab and RT-PCR.

Results obtained from this longitudinal analysis indicated that the Platelia SARS-CoV-2 Total Ab test was able to detect antibodies against SARS –CoV-2 in 92% (11/12) patients tested before or at 8 days and in 100% (38/38), 95%CI [90.7%- 100%] patients tested >8 days past onset of symptoms and who were found positive

for Covid-19 disease by PCR. The patient who was negative at ≤8 days was also negative with another serological predicate assay and was not tested further to assess an immune response.

Of the 50 patients that were followed in this study, 45 had all samples collected in only one matrix and 5 had a combination of both serum and plasma. For the data analysis below, the patients are listed according to the matrix at which the patient first became reactive.

Table 2: Agreement in serum specimens expressed as Percent Positive Agreement according to the day between onset of symptoms and sample collection

Days between onset of symptoms and sample collection	First Positive draw for Patient	Patient Negative	Total	PPA (%) according to PCR results [95% Confidence Interval]
2-8 days	**3**	**0**	**3**	**100% [29.2%-100%]**
9-15 days	**22**	**0**	**22**	**95.5%[2] [77.2%-99.0%]**
16-22 days[1]	**2**	**0**	**2**	**100% [15.8%-100%]**

[1] All patients turned positive within 22 days and their positive status was confirmed when the subsequent specimens were drawn beyond 22 days.
[2] For 1 patient, the first serum sample drawn on day 10 was found positive with Platelia SARS-CoV-2 Total Ab whereas, it was found negative with RT-PCR. The second specimen drawn on day 11 was positive with both Platelia SARS-CoV-2 Total Ab and RT-PCR.

Results obtained from this longitudinal analysis on serum specimens indicated that the Platelia SARS-CoV-2 Total Ab test was able to detect antibodies against SARS –CoV-2 in 100% (3/3) patients tested before or at 8 days and in 100% (24/24), 95%CI [85.8%- 100%] patients tested >8 days past onset of symptoms and who were found positive for Covid-19 disease by PCR.

Table 3: Agreement in plasma specimens expressed as Percent Positive Agreement according to the day between onset of symptoms and sample collection

Days between onset of symptoms and sample collection	First Positive draw for Patient	Patient Negative	Total	PPA (%) according to PCR results [95% Confidence Interval]
2-8 days	**8**	**1[1]**	**9**	**89% [51.8%-99.7%]**
9-15 days	**8**	**0**	**8**	**87.5%[3] [47.4%-99.7%]**
16-22 days[2]	**6**	**0**	**6**	**100% [54.1%-100%]**

[1] No additional specimens after 8 days were available to follow immune response
[2] All patients turned positive within 22 days and their positive status was confirmed when the subsequent specimens were drawn beyond 22 days.
[3] For 1 patient, the first plasma sample drawn on day 8 was found positive with Platelia SARS-CoV-2 Total Ab whereas, it was found negative with RT-PCR. The second specimen drawn on day 11 was positive with both Platelia SARS-CoV-2 Total Ab and RT-PCR.

Results obtained from this longitudinal analysis on plasma specimens indicated that the Platelia SARS-CoV-2 Total Ab test was able to detect antibodies against SARS –CoV-2 in 89% (8/9) patients tested before or at 8 days and in 100% (14/14), 95%CI [76.8%- 100%] patients tested >8 days past onset of symptoms and who were found positive for Covid-19 disease by PCR.

According to current publications, immune response is expected to build at > 7 days (Zhao et al., 2020)7

10 BIBLIOGRAPHY

1. Cheng ZJ and Shan J. 2019 Novel coronavirus: where we are and what we know. I*nfection*. 2020, Apr 48(2): 155-163. doi: 10.1007/s15010-020-01401-y
2. Cui J, Li F, and Shi ZL. Origin and evolution of pathogenic coronaviruses. *Nature Reviews*. 2020, Mar 17: 181-192. doi: 10.1038/s41579-018-0118-9
3. He F, Deng Y, Li W. Coronavirus disease 2019: What we know? *Journal of Medical Virology*. 2020, Mar 14. doi: 10.1002/jmv.25766
4. Guo L, Ren L, Yang S et al. Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19). *Clinical Infectious Disease*. 2020, Mar 21, pii: ciaa310. doi: 10.1093/cid/ciaa310
5. Weiss SR and Navas-Martin S. Coronavirus Pathogenesis and the Emerging Pathogen Severe Acute Respiratory Syndrome Coronavirus. *Microbiology and molecular biology reviews*. 2005, Dec 69 (4): 635-664. doi:10.1128/MMBR.69.4.635–664.2005
6. Zhang W, Du RH, Li B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerging Microbes & Infections*. 2020, 9:1, 386-389. doi: 10.1080/22221751.2020.1729071
7. Zhao J, Yuan Q, Wang H, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. *Clinical Infectious Disease*. 2020, Mar 28, pii: ciaa344. doi: 10.1093/cid/ciaa344
8. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020, Mar 579 (7798): 270-273. doi: 10.1038/s41586-020-2012-7

(EN) • This product contains human or animal components. Handle with care.
(FR) • Ce produit contient des composants d'origine humaine ou animale. Manipuler avec précaution.

H314 - H317 - H412

P273 - P280 P305+P351+P338
P301+P330+P331
P303+P361+P353
P333+P313 - P501

(EN)
Danger
Causes severe skin burns and eye damage. May cause an allergic skin reaction. Harmful to aquatic life with long lasting effects.
Avoid release to the environment. Wear protective gloves/protective clothing/eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF SWALLOWED: rinse mouth. Do NOT induce vomiting. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. If skin irritation or rash occurs: Get medical advice/attention. Dispose of contents/container in accordance with local/regional/national/international regulations.

(FR)
Danger
Provoque des brûlures de la peau et des lésions oculaires graves. Peut provoquer une allergie cutanée. Nocif pour les organismes aquatiques, entraîne des effets néfastes à long terme.
Éviter le rejet dans l'environnement. Porter des gants de protection/des vêtements de protection/un équipement de protection des yeux/du visage. EN CAS DE CONTACT AVEC LES YEUX: rincer avec précaution à l'eau pendant plusieurs minutes. Enlever les lentilles de contact si la victime en porte et si elles peuvent être facilement enlevées. Continuer à rincer. EN CAS D'INGESTION: rincer la bouche. NE PAS faire vomir. EN CAS DE CONTACT AVEC LA PEAU (ou les cheveux): enlever immédiatement les vêtements contaminés. Rincer la peau à l'eau/se doucher. En cas d'irritation ou d'éruption cutanée: consulter un médecin. Éliminer le contenu/récipient conformément à la réglementation locale/régionale/nationale/internationale.

Bio-Rad
3, boulevard Raymond Poincaré
92430 Marnes-la-Coquette - France
Tel. : +33 (0) 1 47 95 60 00
Fax : +33 (0) 1 47 41 91 33
www.bio-rad.com

16008267CA-EN
2021/03