Platelia SARS-CoV-2 Total Ab

1 plate - ▽ 96 REF 72710
5 plates - ▽ 480 REF 12013798

For use under an Emergency Use Authorization (EUA) Only
Prescription Use only.
For In Vitro Diagnostic Use Only.

The Platelia SARS-CoV-2 Total Ab assay is a qualitative *in vitro* diagnostic test, in a one-step antigen capture format, for the detection of Total antibodies to SARS-CoV-2 in human serum and plasma (EDTA) specimens.

IVD

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1 INTENDED USE

The Platelia SARS-CoV-2 Total Ab assay is a one-step antigen capture format, Enzyme-Linked Immunosorbent Assay (ELISA), intended for the qualitative detection of total antibodies (including IgM/ IgA/IgG) to SARS-CoV-2 in human serum and plasma EDTA. The Platelia SARS-CoV-2 Total Ab assay is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The Platelia SARS-CoV-2 Total Ab assay should not be used to diagnose acute SARS-CoV-2 infection. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a, to perform high complexity tests.

Results are for the detection of SARS CoV-2 total antibodies. Total antibodies (including IgM/IgA/IgG) 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.

Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

The sensitivity of the Plateila SARS-CoV-2 Total Ab assay 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 with the Platelia SARS-CoV-2 Total Ab assay may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

The Platelia SARS-CoV-2 Total Ab assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

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 and 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, including the 2003 outbreak mainly in Asia (SARS-CoV) and the 2014 outbreak in the Middle East (MERS-CoV). 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 individuals requiring intensive care urged authorities around the world to set up containment measures. The incubation period ranges from 1 to 14 days. Immune response is expected to build at > 7 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 has been shown to attach to pulmonary cells via the ACE-2 receptors followed by endocytosis.

Diagnosis mainly relies on real-time reverse transcription polymerase chain reaction (RT-PCR) testing of respiratory specimens. Individuals RT-PCR 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.

RT-PCR can lead to false negative results due to low viral loads or unsuitable collection, handling, and storage of swabs (oropharyngeal or nosopharyngeal), or failure during the reaction process. Imagery techniques such as computed tomography (CT) can also be performed and show bilateral multilobar ground-class 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 an individual 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 (EDTA) specimens.

The assay uses a recombinant SARS nucleocapsid protein in a one-step antigen capture format assay. Serum or plasma (EDTA) 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 after the addition 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 serum.

4 REAGENTS

4.1 Description

Component	Contents	Preparation
R1 • Microplate 1 plate or 5 plates	• 12 strips of 8-wells each, coated with recombinant nucleocapsid protein of SARS • Specific ID number = 19	Use as supplied. Return unused strips to the pouch and reseal. Do not remove desiccant.
R2 • Wash Solution Concentrate (20X) 1 bottle (70mL) or 1 bottle (235 mL)	• TRIS-NaCl buffer • ProClin 300 (0.04%)	Dilute to working dilution with deionized water. Clinical laboratory reagent water is acceptable.
R3 • Negative Control 1 vial (1 mL)	• TRIS-NaCl buffer • Bovine serum albumin • Glycerol • ProClin 300 (0.1%)	Use as supplied.

Component	Contents	Preparation
R4 • Cutoff Control 1 vial (1 mL)	• TRIS-NaCl buffer • Bovine serum albumin • Glycerol • Rabbit polyconal anti-SARS nucleocapsid antibodies • ProClin 300 (0.1%)	Use as supplied.
R5 • Positive Control 1 vial (1 mL)	• TRIS-NaCl buffer • Bovine serum albumin • Glycerol • Rabbit polyclonal anti-SARS nucleocapsid antibodies • ProClin 300 (0.1%)	Use as supplied.
R6 • Conjugate 1 bottle (9 mL) or 2 bottles (23 mL)	• Recombinant SARS nucleocapsid protein coupled with horseradish peroxidase • TRIS-NaCl buffer • Phenol red • ProClin 300 (0.5%)	Use as supplied.
R7 • Sample Diluent 1 bottle (12 mL) or 2 bottles (23 mL)	• TRIS-NaCl buffer • Phenol red • ProClin 300 (0.5%)	Use as supplied
R8 • TMB Substrate Solution 1 bottle (60 mL)	• Hydrogen Peroxide • Citric Acid/Sodium Acetate buffer • Dimethylsulfoxide (DMSO)	Use as supplied
R9 • Chromogen 1 vial (5 mL) or 2 vials (5 mL)	Solution containing Tetramethylbenzidine (TMB)*	Dilute with Substrate Buffer as described.
R10 • Stopping Solution 1 vial (28 mL) or 3 vials (28 mL)	1N Sulfuric acid solution (H_2SO_4)	Use as supplied.

4.2 Storage and handling requirements

This kit should be stored at +2-8°C. Opened reagents must be stored as follows.

Identification	Preservation
R1	After opening the plate pouch, store the microwell strips at +2-8°C for up to 4 weeks in their original pouch with desiccant and resealed.
R2	The diluted washing solution can be stored at +2-30°C for 2 weeks. The concentrated washing solution (R2) can be stored unopened at +2-30°C or after opening +2-8°C until the expiration date on the label.
R3, R4, R5, R6, R7, R8, R9	After opening, these reagents are stable for 4 weeks stored at +2-8°C.
R8 + R9	Once diluted, the solution is stable for up to 6 hours in the dark at +18-30°C.
R10	After this reagent is opened, it is stable stored at +2-8°C until the expiration date shown on the label.

Do not use any kit components if contamination is observed.

5 WARNING AND PRECAUTIONS

For *in vitro* diagnostic use only by a professional user in a laboratory environment.
For use under an Emergency Use Authorization (EUA) Only.
Prescription Use only.

5.1 Health and safety precautions

1. 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.
2. 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.
3. Biological spills: Human source material spills should be treated as potentially infectious.
4. 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.
5. 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.

   Caution: Do not place solutions containing bleach into the autoclave!
6. 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.
7. For hazard and precaution recommendations related to some chemical components in this test kit, please refer to the symbol(s) shown on the labels and described below. The Safety Data Sheet is available on www.bio-rad.com.
8. This product contains human or animal components. Handle with care.

H317: May cause allergic skin reaction.
H412: Harmful to aquatic life with long lasting effects.
P273: Avoid release to the environment.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352: IF ON SKIN: Wash with plenty of soap and water.
P333 + P313: If skin irritation or rash occurs: Get medical advice/attention.
P501: Dispose of contents and container in accordance with local, regional, national, and international regulations.

H314: Causes severe skin burns and eye damage.
H290: May be corrosive to metals.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P301 + P330 + IF SWALLOWED: Rinse mouth. Do NOT
P331: induce vomiting.
P305 + P351 + IF IN EYES: Rinse cautiously with water for
P338: several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P501: Dispose of contents and container in accordance with local, regional, national, and international regulations.

5.2. Procedural precautions

1. DO NOT USE the kit if the packaging of components is damaged.
2. DO NOT USE expired reagents.
3. DO NOT USE microwell plates if there is no desiccant inside microplate pouch.
4. Bring all reagents to room temperature (18-30°C) before use.
5. Carefully prepare working reagents, avoiding any contamination.
6. The use of disposable material is recommended for preparation of reagents. If using glassware, wash thoroughly and rinse with deionized water.
7. Do not allow the microplate to dry between the end of a washing step and the addition of reagents.
8. 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 also stated on each strip.

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

   Verify the specific identification number before use. If it is missing, or is not 19, the strip should not be used.
9. Do not mix reagents from different lots within a test run.
10. Do not mix reagents from other kits that have different lot numbers, with the exception of the Washing Solution (R2, 20x), the peroxidase Substrate Buffer (R8), the Chromogen (R9) and the Stopping Solution (R10); within a given test run the same lot number of these reagents must be used.

    NOTE: The Bio-Rad Washing Solution R2, that is identified in green on the label as 20X, may not be mixed with the Bio-Rad Washing Solution R2 identified in blue on the label as 10X.
11. 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 containers, they can be washed with 1N HCl and rinsed thoroughly with distilled water and dried.
12. The development solution must be stored in the dark.
13. The development solution (substrate buffer + chromogen) must be pink. If it is any other color it cannot be used and must be replaced. This solution must be stored in the dark.
14. The time between the addition of the conjugate and the specimens to the microplate wells should not exceed 30 minutes.
15. The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the conjugate or substrate solutions.
16. Never use the same container for the conjugate and the development solution.
17. Do not change the assay procedure.
18. Each run of this assay must proceed to completion without interruption after it has been started. A delay of less than 5 minutes between steps is acceptable.
19. Check the pipettes and other equipment for accuracy and correct operation.
20. 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.
21. Use a new pipette tip for each specimen.
22. 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.

6 SPECIMENS

1. The test is performed on serum or plasma (EDTA) specimens.
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 all the time to prevent contamination.
   - After centrifugation, collect 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 4 days.
   - If the test cannot be completed within 4 days, freeze the specimens at -20°C or colder.
   - Serum or plasma specimens can be subjected to a maximum of 1 freezing/ thawing cycle. 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 hemolysed specimens containing up to 10 g/l of hemoglobin.
4. Do not heat the specimens.

7 PROCEDURE

7.1 Materials required but not provided

1. Sterile distilled or deionized water to dilute the concentrated washing solution
2. Sodium hypochlorite (household bleach) and sodium bicarbonate
3. Absorbent paper
4. Adhesive film
5. Gloves and eye / face protection
6. Disposable tubes
7. Precision pipettes or a multipipettor 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
9. Microplate washing system
10. Dry-heat incubator, capable of maintaining 37°C ± 2°C
11. Microplate reader equipped with 450 and 620 nm filters
12. Container for biohazardous waste

7.2 Reagents preparation

7.2.1 Ready-for-use reagents

Reagent 1 (R1): Microplate
The microplates each contain 12 strips and are in a sealed pouch. Cut the pouch 0.5 to 1 cm above the seal. Open the pouch and remove the frame. Place any unused strips back and the desiccant back into the pouch. Close the pouch and store it 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

7.2.2 Reagents to prepare

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 full microplate (12 strips), excluding dead volume required for the equipment that is used.

Reagent 8 (R8) + Reagent 9 (R9): Enzyme development solution
Prepare a 1:11 dilution of the Chromogen (R9) in the Substrate Buffer (R8) (e.g. 2 mL of Reagent R9 + 20 mL of Reagent R8). Twenty (20) mL are required for 12 strips. Mix thoroughly.

7.3 Assay Procedure

1. **Bring reagents to room temperature (+18-25°C) for at least 30 minutes before use.**
2. Use the Negative and Positive Controls with each run to validate the results.
3. Identify the individual wells for addition of controls and patient specimens, using the template below as a guide. Use 1 well for the Negative Control, 1 well for the Positive Control, and 3 wells for the Cut-off Control.

	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 Working Washing Solution (R2) *(Refer to section 7.2)*
5. In a clean pre-dilution microplate, dilute Controls R3, R4, R5 and test specimens E1, E2, etc., in R7, to give a **1:5 dilution,** by adding **60 µL** of R7 followed by **15 µL** of specimen to each well.
6. Add **75 µL** of Conjugate solution (R6) to all the wells of the pre-dilution microplate.
7. Mix by aspirating and dispensing once, and then **transfer immediately 100 µL** of the pre-diluted controls and specimens to the wells of the reaction microplate (R1).
8. Cover the microplate with an **adhesive plate sealer**, or use other means to minimize evaporation. Incubate the microplate in a controlled **37°C +/- 2C** water bath or microplate incubator for **60 minutes** (+/- 5 min)**.**
9. Prepare the enzyme development solution (R8+R9) *(Refer to section 7.2)*
10. At the end of incubation period, carefully remove the plate cover. Aspirate the contents of all wells into a biohazard waste container (containing sodium hypochloride). Wash the plate **5 times with a microplate washer** (using 800 µL of Working Washing Solution per well). Invert microplate and gently tap on absorbent paper to remove remaining liquid.
11. Quickly add **200 µL** of the development solution (R8+R9) into each well. Incubate plates in the dark for 30 minutes (+/- 4 min) at room temperature (+18-30°C). **Do not use adhesive plate sealer during this incubation step**.
12. Add **100 µL** of Stopping Solution (R10) to each well, using in the same sequence and rate of addition as for the development solution. Mix thoroughly.
13. Carefully wipe the plate bottom.
14. 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 be protected from light before reading.

7.4 Quality Control

Use the 1 replicate of the Positive Control, 1 replicate of the Negative Control, and 3 replicates of the Cut-off Control on each microplate every time the test is performed.

7.5 Test Validation criteria

Calculate the mean absorbance value (OD) for cut-off control R4 (OD_M **R4**).

	Validation criteria
R4	The OD_M **R4** must be greater than 0.5 and less than 1.4 ($0.5 < OD_M$ **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_MR4 is the mean value of the optical densities of the Cut-off Control R4.
Specimen results are calculated using the S/CO ratio: Specimen ratio = Specimen OD / OD_MR4.

Interpretation of results

- A specimen ratio less than 0.8 is considered to be **negative for the presence of anti-SARS-CoV-2 antibodies**.
- A spec*i*men ratio between 0.8 and 1.0 is considered to be **equivocal for the presence of anti-SARS-CoV-2 antibodies**. Samples with equivocal results should be repeat tested in duplicate. If one or more results are positive the final interpretation of the specimen is positive; if the repeat results are equivocal or negative the final interpretation is equivocal and another specimen should be collected.
- A specimen ratio equal to or greater than 1.0 is considered to be **positive for the presence of anti-SARS-CoV-2 antibodies**.

Initial Result **Platelia SARS-CoV-2 Total Ab assay**		
Initial Result (Specimen Ratio)	**Initial Results**	**Retest Procedure**
< 0.8	Negative	No retest is required.
0.8 ≤ x < 1.0	Equivocal	Retest in duplicate with the Platelia SARS-CoV-2 Total Ab assay.
≥ 1.0	Positive	No retest is required.

Repeat Results – Specimens Initially Equivocal **Platelia SARS-CoV-2 Total Ab assay**		
Result (Specimen Ratio)	**Repeat Results**	**Final Result**
< 0.8	1 or 2 repeat results are Negative	Equivocal
0.8 ≤ x < 1.0	1 or 2 repeat results are Equivocal	Equivocal
≥ 1.0	1 or 2 repeat results are Positive	Positive

8 LIMITATIONS OF THE PROCEDURE

1. 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.
2. Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.
3. 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. Thus, a negative result does not preclude or rule out COVID-19 infection.
4. The results obtained with this test should only be interpreted in conjunction with clinical findings, and the results from other laboratory tests and evaluations.
5. 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.
6. Platelia SARS-CoV-2 Total Ab assay can detect total antibodies specific to SARS-CoV-1 and to SARS-CoV-2, and cross-reaction is possible with MERS-CoV.
7. This test should not be used for screening of donated blood.

Conditions of Authorization for the Laboratory

The Platelia SARS-CoV-2 Total Ab assay Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd. However, to assist clinical laboratories using the Platelia SARS-CoV-2 Total Ab test ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

- Authorized laboratories* using your product will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using your product will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
- Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/ CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Bio-Rad Laboratories, Inc. (TechSupportUSSD-Redmond@bio-rad.com) any suspected occurrence of false reactive or false non-reactive results and significant deviations from the established performance characteristics of your product of which they become aware.
- All laboratory personnel using your product must be appropriately trained in immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling. All laboratory personnel using the assay must also be trained in and be familiar with the interpretation of results of the product
- Bio-Rad Laboratories, Inc., authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

*The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as "authorized laboratories."

9 PERFORMANCES CHARACTERISTICS

9.1 Analytical Performance Characteristics

9.1.1 Precision Measurement

Repeatability (Within Run Precision)
The within-run precision (Repeatability) of the Platelia SARS-CoV-2 Total Ab assay was evaluated by testing three (3) positive specimens and one (1) negative specimen 30 times in the same run. The repeatability results show %CV below 10% for the negative specimen and below 5% for all the positive specimens. The results are summarized in the table below.

Repeatability

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 (Within Laboratory Precision)

The inter-assay precision of the Platelia SARS-CoV-2 Total Ab assay was evaluated by testing three (3) positive specimens and one (1) negative specimen in 10 separate runs over 6 days. Nested ANOVA was used to estimate within run, between run, between days and total precision. The within laboratory precision results show %CV below 30% for the negative specimen and below 10% for all the positive specimens. The results are summarized in the table below.

Within Laboratory Precision

Specimen ID	N	Mean Ratio	Repeatability		Between run		Between day		Within Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative	20	**0.05**	0.005	**10.9%**	0.005	**11.7%**	0.009	**20.0%**	0.012	**25.6%**
Positive 1	20	**1.26**	0.046	**3.6%**	0.026	**2.0%**	0.040	**3.2%**	0.066	**5.3%**
Positive 2	20	**1.77**	0.053	**3.0%**	0*	**NA**	0.122	**6.9%**	0.133	**7.5%**
Positive 3	20	**2.52**	0.065	**2.6%**	0*	**NA**	0.089	**3.5%**	0.110	**4.4%**

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

9.1.2 Analytical Specificity/Cross Reactivity

The cross-reactivity of the Platelia SARS-CoV-2 Total Ab assay was evaluated by testing SARS-CoV-2 seronegative specimens from patients with antibodies to 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. The results are summarized in the table below.

Analyte	Total Number Tested	Number Reactive	Number Non-reactive
anti-229E (alpha coronavirus)	6	0	6
anti-NL63 (alpha coronavirus)	3	0	3
anti-OC43 (beta coronavirus)	13	0	13
anti-HKU1 (beta coronavirus)	7[1]	0	7[1]
Anti-influenza vaccine	15	0	15
anti-*Mycoplasma pneumoniae*	4	0	4
anti-VZV	5	0	5
anti-HSV	9	0	9
anti-EBV	4	0	4
Rheumatoid factor	5	0	5
Influenza A infection	1[1]	0	1[1]
RSV infection	1[1]	0	1[1]

[1] One patient was co-infected with CovHKU1 + INF A and one patient was co-infected with CoVHKU1 + RSV

9.1.3 Interfering Substances

A study was performed with potentially interfering substances to determine the effect on results with the Platelia SARS-CoV-2 Total Ab assay. No interference was seen with the following substances in negative, weakly positive, or moderately positive samples at the concentrations listed.

Substance	Concentration
Albumin	90g/L
Bilirubin	100mg/L
Hemoglobin	10g/L
Triglycerides	36g/L

9.2 Clinical Performance Characteristics

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

9.2.1 Specificity

A total of 687 specimens (620 from blood donors and 67 from hospitalized asymptomatic patients) collected prior to the outbreak of the COVID-19 pandemic were tested. For the blood donor samples, 545 serum and 75 plasma specimens were evaluated. The specificity of serum was 99.6% (543/545) and the specificity of plasma (EDTA) was 100% (75/75). All samples from hospitalized patients were serum, with a specificity of 98.5% (66/67). The overall specificity for all 687 specimens is 99.56% (95% confidence interval of 98.72- 99.85%). The results are presented in the table below.

	Matrix	Number Reactive	Number Non-reactive	Total Number Tested
Blood donors	Plasma	0	75	75
	Serum	2	543	545
Hospitalized patients	Serum	1	66	67
Total		3	684	687

Specificity in Serum Specimens: 609/612 (99.51%); 95% confidence interval: 98.58 - 99.83%
Specificity in Plasma Specimens: 75/75 (100%); 95% confidence interval: 95.13 - 100%

9.2.2 Sensitivity

A longitudinal study was performed on 51 patients in the intensive care unit at 3 hospitals with clinical symptoms of COVID-19 and with a SARS-CoV-2 PCR positive result. Between 2 and 5 specimens per patient were collected from 2 to 42 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. Therefore, a total of 51 results from the 51 patients are presented below.

Of the 51 patients tested, 27/27 (100%) were reactive with serum specimens and 20/24 (83%) were reactive with plasma specimens. Of the 4 patients that remained non-reactive at all time points, 3 did not have samples tested more than 8 days post onset of clinical symptoms, and 1 was not tested after 18 days post onset of symptoms. When specimens were collected at >8 days after the onset of symptoms, 39/40 patients (97.5%) were reactive with the Platelia SARS-CoV-2 Total Ab assay for one or more specimens that were tested. For each patient that had a reactive result, the tables below summarize when the first reactive result was observed.

Serum Specimens:

Days between onset of symptoms and sample collection	Number of Patients with First Reactive Draw	Number of Non-reactive Patients	Total Number	%
≤8 days	3	0	3	100%
9-10 days	5	0	5	100%
11-15 days	16	0	16	100%
16-20 days	3	0	3	100%
21-42 days	0	0	0	NA
Total	27	0	27	100%

Note: 3 patients had a combination of serum and plasma samples collected at different time points in this study. For the data analysis, the patients are listed according to the matrix at which the samples first became reactive (1 serum at day 10, 1 at day 11, 1 at day 14).

Sensitivity in Serum Specimens: 27/27 (100%); 95% Confidence Interval: (98.72 - 99.85%)

Plasma Specimens:

Days between onset of symptoms and sample collection	Number of Patients with First Reactive Draw	Number of Non-reactive Patients	Total Number	%
≤8 days	5	3[1]	8	63%
9-10 days	1	0	1	100%
11-15 days	5	0	5	100%
16-20 days	6	1[2]	7	86%
21-42 days	3	0	3	100%
Total	20	4	24	83%

[1] No additional specimens after 8 days were available to follow immune response for these patients.
[2] No additional specimens after 18 days were available to follow immune response for this patient.

Note: 2 patients had a combination of serum and plasma samples collected at different time points in this study. For the data analysis, the patients are listed according to the matrix at which the samples first became reactive (1 plasma at day 29), or the matrix at the final time point if they remained nonreactive (1 plasma at day 18).

Sensitivity in Plasma Specimens: 20/24 (83.33%); 95% Confidence Interval: (64.15 - 93.32%)

9.2.3 Clinical Agreement in Serum and Plasma

Serum: The Platelia SARS-CoV-2 Total Ab assay showed 100% (27/27) positive percent agreement (95% CI: 98.72- 99.85%) in 27 PCR positive subjects and 99.51% (609/612) negative percent agreement (95% CI: 98.58 - 99.83%) in 612 negative subjects.

Plasma: The Platelia SARS-CoV-2 Total Ab assay showed 83.3% (20/24) positive percent agreement (95% CI: 64.15 - 93.32%) in 24 PCR positive subjects and 100% (75/75) negative percent agreement (95% CI: 95.13 - 100%) in 75 negative subjects.

The agreement results for serum and plasma samples tested with the Platelia SARS-CoV-2 Total Ab are presented in tables below.

Clinical Agreement in Serum

		Comparator method/PCR Positive	
Platelia SARS-CoV-2 Total Ab		**Positive**	**Negative**
	Reactive	27	3
	Non-reactive	0	609

Positive Percent Agreement: 100% (27/27); 95% CI: (98.72% - 99.85%)
Negative Percent Agreement: 99.51% (609/612); 95% CI: (98.58- 99.83%)

Clinical Agreement in Plasma

		Comparator Method/PCR Positive	
Platelia SARS-CoV-2 Total Ab		**Positive**	**Negative**
	Reactive	20	0
	Non-reactive	4	75

Positive Percent Agreement: 83.3% (20/24); 95% CI: (64.15% - 93.32%)
Negative Percent Agreement: 100% (75/75); 95% CI: (95.13 - 100%)

Overall Clinical Agreement

		Comparator Method/PCR Positive	
Platelia SARS-CoV-2 Total Ab		**Positive**	**Negative**
	Reactive	47	3
	Non-reactive	4	684

Positive Percent Agreement: 92.16% (47/51); 95% CI: (81.5% - 96.91%)
Negative Percent Agreement: 99.56% (684/687); 95% CI: (98.72 – 99.85%)

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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

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FACT SHEET FOR HEALTHCARE PROVIDERS

Platelia SARS-CoV-2 Total Ab assay - Bio-Rad Laboratories, Inc. **April 29, 2020**

Coronavirus Disease 2019 (COVID-19)

This Fact Sheet informs you of the significant known and potential risks and benefits of the emergency use of the Platelia SARS-CoV-2 Total Ab assay.

Platelia SARS-CoV-2 Total Ab assay is authorized for the detection of total antibodies (including IgM/ IgA/IgG) to SARS-CoV-2 in human serum and plasma EDTA.

All individuals whose specimens are tested with this assay will receive the Fact Sheet for Recipients: Platelia SARS-CoV-2 Total Ab.

What are the symptoms of COVID-19?
Many individuals with confirmed COVID-19 have developed fever and/or symptoms of acute respiratory illness (e.g., cough, difficulty breathing). However, limited information is currently available to characterize the full spectrum of clinical illness associated with COVID-19. Based on what is known about the virus that causes COVID-19, signs and symptoms may appear any time from 2 to 14 days after exposure to the virus. Based on preliminary data, the median incubation period is approximately 5 days, but may range 2-14 days.

Public health officials have identified cases of COVID-19 infection throughout the world, including the United States, which poses risks to public health. Please check the CDC webpage for the most up to date information.

What do I need to know about COVID-19 testing?
Current information on COVID-19 for healthcare providers is available at CDC's webpage, *Information for Healthcare Professionals* (see links provided in "Where can I go for updates and more information" section).

- The Platelia SARS-CoV-2 Total Ab assay can be ordered by healthcare providers to test human serum and plasma EDTA specimens to detect if there has been an adaptive immune response to COVID-19, indicating a recent or prior infection.
- The Platelia SARS-CoV-2 Total Ab assay should not be used to diagnose or exclude acute infection and should not be used as the sole basis for treatment or patient management decisions. Direct testing for SARS-CoV-2 should be performed if acute infection is suspected.
- The Platelia SARS-CoV-2 Total Ab assay is only authorized for use in laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

This test measures total human SARS-CoV-2 antibodies that are generated as part of the adaptive immune response to the virus and is to be performed only using human serum and plasma EDTA.

Specimens should be collected with appropriate infection control precautions. Current guidance for COVID-19 infection control precautions are available at the CDC's website (see links provided in "Where can I go for updates and more information" section).

Use appropriate personal protective equipment when collecting and handling specimens from individuals suspected of having COVID-19 as outlined in the CDC *Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19)*. For additional information, refer to CDC *Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) (*see links provided in "Where can I go for updates and more information" section).

What does it mean if the specimen tests positive for total human SARS-CoV-2 antibodies?
A positive test result with the Platelia SARS-CoV-2 Total Antibody assay indicates that antibodies to SARS-CoV-2 were detected, and the individual has potentially been exposed to COVID-19.

The Platelia SARS-CoV-2 Total Antibody assay detects total antibody as indicative of an adaptive immune response to SARS-CoV-2 infection in individuals suspected of SARS-CoV-2 infection. The Platelia SARS-CoV-2 Total Ab assay detects the presence of IgM, IgA, and/or IgG antibodies but does not identify each

separately. 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, although detection of IgG antibodies does not exclude recently infected patients who are still contagious. Positive results for IgM, IgA, and IgG could occur after infection and can be indicative of acute or recent infection. It is unknown how long IgM IgA, and/or IgG antibodies to SARS-CoV-2 will remain present in the body after infection and if they confer immunity to infection.

A positive result for Platelia SARS-CoV-2 Total Ab assay may not mean that an individual's current or past symptoms were due to COVID-19 infection. Laboratory test results should always be considered in the context of clinical observations and epidemiological data in making a final diagnosis and patient management decisions.

The Platelia SARS-CoV-2 Total Ab assay has been designed to minimize the likelihood of false positive test results. False positive results may occur due to cross-reactivity from pre-existing antibodies or other possible causes. However, in the event of a false positive result, risks to individuals could include the following: risk of infection by exposure to persons with active COVID-19. If a recent infection is suspected a false positive result may lead to a recommendation for isolation of the individual, monitoring of household or other close contacts for symptoms, isolation that might limit contact with family or friends and may increase contact with other potentially COVID-19-infected individuals, limits in the ability to work, or other unintended adverse effects.

All laboratories using this test must follow standard confirmatory testing and reporting guidelines according to their appropriate public health authorities.

What does it mean if the specimen tests negative for total human SARS-CoV-2 antibodies?
A negative test result with this test means that SARS-CoV-2 specific antibodies were not present in the specimen above the limit of detection. However, a negative result does not rule out COVID-19 and should not be used as the sole basis for treatment, patient management decisions, or to rule out active infection. Individuals tested early after infection may not have detectable IgG antibody despite active infection; in addition, not all individuals will develop a detectable IgM and/or IgG response to SARS-CoV-2 infection. The absolute sensitivity of the Platelia SARS-CoV-2 Total Ab assay is unknown.

When testing is negative, the possibility of a false negative result should be considered in the context of an individual's recent exposures and the presence of clinical signs and symptoms consistent with COVID-19. The possibility of a false negative result should especially be considered if the individual's recent exposure or clinical presentation indicate that COVID- 19 is likely and diagnostic tests for other causes of illness (e.g., other respiratory illness) are negative. Direct testing for virus (e.g., PCR testing) should always be performed in any individual suspected of COVID-19, regardless of the Platelia SARS-CoV-2 Total Ab assay result.

Risks to an individual of a false negative result include: delayed or lack of supportive treatment, lack of monitoring of infected individuals and their household or other close contacts for symptoms resulting in increased risk of spread of COVID-19 within the community, or other unintended adverse events.

What is an EUA?
The United States FDA has made this test available under an emergency access mechanism called an Emergency Use Authorization (EUA). The EUA is supported by the Secretary of Health and Human Service's (HHS's) declaration that circumstances exist to justify the emergency use of in vitro diagnostics (IVDs) for the detection and/or diagnosis of the virus that causes COVID-19.

An IVD made available under an EUA has not undergone the same type of review as an FDA-approved or cleared IVD. FDA may issue an EUA when certain criteria are met, which includes that there are no adequate, approved, available alternatives, and based on the totality of scientific evidence available, it is reasonable to believe that this IVD may be effective.

The EUA for this test is in effect for the duration of the COVID-19 declaration justifying emergency use of IVDs, unless terminated or revoked (after which the test may no longer be used).

Where can I go for updates and more information?

CDC webpages:

General: https://www.cdc.gov/COVID19
Healthcare Professionals: https://www.cdc.gov/coronavirus/2019-nCoV/guidance-hcp.html
Information for Laboratories: https://www.cdc.gov/coronavirus/2019-nCoV/guidance-laboratories.html
Laboratory Biosafety: https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html
Isolation Precautions in Healthcare Settings: https://www.cdc.gov/coronavirus/2019-ncov/infection-control/control-recommendations.html
Specimen Collection: https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
Infection Control: https://www.cdc.gov/coronavirus/2019-ncov/infection-control/index.html

FDA webpages:

General: www.fda.gov/novelcoronavirus
EUAs:(includes links to recipient fact sheet and manufacturer's instructions) https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations

Bio-Rad Laboratories, Inc.:

4000 Alfred Nobel Dr,
Hercules, CA 94547

Contact email: TechSupportUSSD-Redmond@bio-rad.com
Website: www.bio-rad.com

FACT SHEET FOR RECIPIENTS

Platelia SARS-CoV-2 Total Ab assay - Bio-Rad Laboratories, Inc. **April 29, 2020**

Coronavirus Disease 2019 (COVID-19)

You are being given this Fact Sheet because your sample(s) was tested for the Coronavirus Disease 2019 (COVID-19) using the Platelia SARS-CoV-2 Total Ab assay.

This Fact Sheet contains information to help you understand the risks and benefits of using this test for detecting antibodies to the virus that causes COVID-19. After reading this Fact Sheet, if you have questions or would like to discuss the information provided, please talk to your healthcare provider.

- **For the most up to date information on COVID-19 please visit the CDC Coronavirus Disease 2019 (COVID-19) webpage:**

- **https://www.cdc.gov/COVID19**

What is COVID-19?
COVID-19 is caused by the SARS-CoV-2 virus. The virus, which can cause mild to severe respiratory illness, was first identified in Wuhan, China, and has now spread globally, including the United States. There is limited information available to characterize the spectrum of clinical illness associated with COVID-19 but it likely spreads to others when a person shows signs or symptoms of being sick (e.g., fever, coughing, difficulty breathing, etc.).

What is the Platelia SARS-CoV-2 Total Ab assay?
The test is designed to detect antibodies in a blood sample that would indicate that you may have current or prior COVID-19 infection.

Why was my sample tested?
Testing of your sample(s) will help find out if you have antibodies to the virus that causes COVID-19.

What are the known and potential risks and benefits of the test?
Potential risks include:

- Possible discomfort or other complications that can happen during sample collection.
- Possible incorrect test result (see below for more information).

Potential benefits include:

- The results, along with other information, can help your healthcare provider make informed recommendations about your care.
- The results of this test may help limit the spread of COVID-19 to your family and others in your community.

What does it mean if I have a positive test result?
If you have a positive test result, it is likely that you have or previously had COVID-19 and that you have developed an antibody response to the virus. Your healthcare provider will work with you to determine how best to care for you based on the test results along with other factors of your medical history, including any previous symptoms, possible exposure to COVID-19, and the location of places you have recently traveled. There is also the chance that this test can give a positive result that is wrong (a false positive result).

- **Where can I go for updates and more information?** The most up-to-date information on COVID-19 is available at the CDC General webpage: **https://www.cdc.gov/COVID19.** In addition, please also contact your healthcare provider with any questions/concerns.

What does it mean if I have a negative test result?
A negative test result means that the antibodies to the virus that causes COVID-19 were not found in your sample. However, it is possible for this test to give a negative result that is incorrect (false negative) in some people with COVID-19 infection. A negative result may occur if you are tested early in your illness and your body hasn't had time to produce antibodies to infection. If this is the case, your healthcare provider will consider the test result together with all other aspects of your medical history (such as symptoms, possible exposures, and geographical location of places you have recently traveled) in deciding how to care for you.

It is important that you work with your healthcare provider to help you understand the next steps you should take.

Is this test FDA-approved or cleared?
No. This test is not yet approved or cleared by the United States FDA. When there are no FDA-approved or cleared tests available, and other criteria are met, FDA can make tests available under an emergency access mechanism called an Emergency Use Authorization (EUA). The EUA for this test is supported by the Secretary of Health and Human Service's (HHS's) declaration that circumstances exist to justify the emergency use of in vitro diagnostics for the detection and/or diagnosis of the virus that causes COVID-19. This EUA will remain in effect (meaning this test can be used) for the duration of the COVID-19 declaration justifying emergency of IVDs, unless it is terminated or revoked by FDA (after which the test may no longer be used).

- **Where can I go for updates and more information?** The most up-to-date information on COVID-19 is available at the CDC General webpage: **https://www.cdc.gov/COVID19.** In addition, please also contact your healthcare provider with any questions/concerns.