

BIO-RAD

Platelia SARS-CoV-2 Total Ab

1 plate - ∇ 96

REF 12014591

5 plates - ∇ 480

REF 12014044

For Prescription Use only

For In Vitro Diagnostic Use Only

For Emergency Use Authorization Only

The Platelia SARS-CoV-2 Total Ab 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 (dipotassium EDTA, tripotassium EDTA, lithium heparin, ACD, or sodium citrate) specimens.

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1 INTENDED USE

The Platelia SARS-CoV-2 Total Ab is a one-step antigen capture format, enzyme-linked immunosorbent assay (ELISA), intended for the qualitative detection of total antibodies (IgM/IgG/IgA) to SARS-CoV-2 in human serum and plasma (dipotassium EDTA, tripotassium EDTA, lithium heparin, ACD, or sodium citrate). The Platelia SARS-CoV-2 Total Ab 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. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a, that meet requirements to perform high complexity tests.

Results are for the detection of SARS CoV-2 total antibodies. Total antibodies (IgM/IgG/IgA) 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 Platelia SARS-CoV-2 Total Ab 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 may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

The Platelia SARS-CoV-2 Total Ab 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 last 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 specimens, and respiratory droplets are 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 of acute SARS-CoV-2 infection 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

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diarrhea. While some cases can be asymptomatic, others can lead to acute respiratory distress syndrome (ARDS) and even death.

Platelia SARS-CoV-2 Total Ab detects IgM, IgG, and IgA 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/IgG/IgA) in human serum or plasma (dipotassium EDTA, tripotassium EDTA, lithium heparin, ACD, or sodium citrate) 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 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.

4 REAGENTS

4.1 Description

Component	Contents	Preparation
R1 • Microplate 1 plate or 5 plates	<ul style="list-style-type: none">• 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 • Concentrated Washing Solution (20X) 1 or 2 bottles (120 mL)	<ul style="list-style-type: none">• TRIS-NaCl buffer• ProClin 300 (0.04%)	Dilute to working dilution with deionized or distilled water. Clinical laboratory reagent water is acceptable.
R3 • Negative Control 1 vial (1.7 mL)	<ul style="list-style-type: none">• Human serum; negative for antibodies to SARS-CoV-2, HIV and HCV; negative for HBsAg• ProClin 300 (0.3%)	Use as supplied.
R4 • Cut-off Control 1 vial (1.7 mL)	<ul style="list-style-type: none">• TRIS-NaCl buffer• Bovine serum albumin• Glycerol• Rabbit polyclonal anti-SARS nucleocapsid antibodies• ProClin 300 (0.1%)	Use as supplied.
R5 • Positive Control 1 vial (1.7 mL)	<ul style="list-style-type: none">• Human serum; negative for antibodies to SARS-CoV-2, HIV and HCV; negative for HBsAg• Rabbit polyclonal anti-SARS nucleocapsid antibodies• ProClin 300 (0.3%)	Use as supplied.

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Component	Contents	Preparation
R6 • Conjugate 1 bottle (46 mL)	<ul style="list-style-type: none"> Recombinant SARS nucleocapsid protein coupled with horseradish peroxidase TRIS-NaCl buffer Phenol red ProClin 300 (0.5%) 	Use as supplied.
R7 • Sample Diluent 1 bottles (46 mL)	<ul style="list-style-type: none"> TRIS-NaCl buffer Phenol red ProClin 300 (0.5%) 	Use as supplied
R8 • TMB Substrate Buffer 1 bottle (120 mL)	<ul style="list-style-type: none"> Hydrogen Peroxide Citric Acid/Sodium Acetate buffer Dimethylsulfoxide (DMSO) 	Use as supplied
R9 • Chromogen 1 bottle (12 mL)	Solution containing Tetramethylbenzidine (TMB)	Dilute with Substrate Buffer as described.
R10 • Stopping Solution 1 or 3 bottles (28 mL)	1N Sulfuric acid solution (H ₂ SO 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 resealed with desiccant in their original pouch.
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, R10	After opening, these reagents are stable until the expiration date shown on the label when 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.

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.
- This test has not been FDA cleared or approved; this test has been authorized by FDA under an EUA for use by laboratories certified under CLIA to perform high complexity tests.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- This test has been authorized only for the presence of total antibodies against SARS-CoV-2, not for any other viruses or pathogens.

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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 listed above.

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 + P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.

P301 + P330 + P331: induce vomiting.

P305 + P351 + P338: 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.

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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 (dipotassium EDTA, tripotassium EDTA, lithium heparin, ACD, or sodium citrate) 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 7 days.

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- If the test cannot be completed within 7 days, freeze the specimens at -20°C or colder.
 - Serum or plasma specimens can be subjected to a maximum of 5 freeze/thaw cycles. Previously frozen specimens should be thoroughly mixed after thawing and 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); hemolysed specimens containing up to 10 g/L of hemoglobin and specimens containing up to 50g/L of biotin.
 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 and the desiccant back into the pouch. Seal 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 deionized or distilled water: 50 mL of R2 in 950 mL of distilled water. Clinical laboratory reagent water is acceptable. Clinical laboratory reagent water is acceptable. 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 (+18-30°C) for at least 30 minutes before use.**
2. Use the Negative and Positive Controls with each run to validate the results.

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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										

- Prepare the Working Washing Solution (R2) (*Refer to section 7.2.2*)
- 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.
- Add **75 µL** of Conjugate solution (R6) to all the wells of the pre-dilution microplate.
- 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).
- 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).
- Prepare the enzyme development solution (R8+R9) (*Refer to section 7.2.2*)
- 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.
- 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.**
- 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.
- Carefully wipe the plate bottom.
- 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). The mean OD of the Cut-off Control (R4) must be greater than 0.5 and less than 1.4. If one of the Cut-off Control individual values differs by more than 30% from the mean value it should be discarded, and the cut-off mean calculated with the two remaining Cut-off Control values. There must be at least 2 valid Cut-off Control wells.

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Reagent	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

Assessment of Platelia SARS-CoV-2 Total Ab results should be performed after the positive and negative controls have been evaluated and determined to be valid. If the controls are not valid, the patient results cannot be interpreted.

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 specimen ratio greater than or equal to 0.8 and 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.
- A specimen ratio greater than or equal to 1.0 is considered to be **positive for the presence of anti-SARS-CoV-2 antibodies**.
- Specimens with absorbance values that are < 0.000 must be repeated.

Specimen Ratio	Result
$R < 0.8$	Negative
$0.8 \leq R < 1.0$	Equivocal
$R \geq 1.0$	Positive

8 LIMITATIONS OF THE PROCEDURE

1. This test should not be used to diagnose or exclude acute SARS-CoV-2 infection. Direct testing for SARS-CoV-2 should be performed if acute infection is suspected.
2. It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to re-infection.
3. 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.
4. 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.
5. The results obtained with this test should only be interpreted in conjunction with clinical findings, and the results from other laboratory tests and evaluations.
6. Performance characteristics of Platelia SARS-CoV-2 Total Ab have not been evaluated with specimens of serum or plasma originating from new-borns or pediatric patients.
7. Platelia SARS-CoV-2 Total Ab can detect total antibodies specific to SARS-CoV-1 and to SARS-CoV-2, and cross-reaction is possible with MERS-CoV.
8. This test should not be used for screening of donated blood.

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Conditions of Authorization for the Laboratory

The Platelia SARS-CoV-2 Total Ab 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/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas>. However, to assist clinical laboratories using the Platelia SARS-CoV-2 Total Ab (“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, that meet requirements to perform high complexity tests” as “authorized laboratories.”

9 PERFORMANCE CHARACTERISTICS

9.1 Analytical Performance Characteristics

9.1.1 Precision Measurement

The precision of the Platelia SARS-CoV-2 Total Ab was evaluated by testing three (3) positive specimens and one (1) negative specimen in duplicate by 2 different operators per day over 5 days. Nested ANOVA was used to estimate within run, between run, between days and within laboratory precision. The results are summarized in the table below.

Specimen ID	N	Mean Ratio	Within run		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.

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9.1.2 Analytical Specificity / Cross Reactivity

The cross-reactivity of the Platelia SARS-CoV-2 Total Ab 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 in any of the specimens that were tested. The results are summarized in the table below.

Analytical Specificity / Cross Reactivity

Analyte	Number tested	Negative	Positive
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*	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*	3*	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- <i>E. Coli</i>	5	5	0
<i>Mycoplasma pneumoniae</i> IgG	5	5	0
<i>Candida albicans</i> IgG	5	5	0

* One patient was co-infected with CoV HKU1 + INF A and one patient was co-infected with CoV HKU1 + RSV

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 and 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.

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9.2.1 Clinical 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. For the blood donor samples, the specificity was 99.6% (498/500), and for the hospitalized patients the specificity was 98% (98/100). The overall specificity for all 600 specimens is 99.3% (95% confidence interval of 98.3- 99.8%). The results are presented in the table below.

	Positive Patients	Negative Patients	Total
Blood donors	2	498	500
Hospitalized patients	2	98	100
Total	4	596	600

9.2.2 Clinical Sensitivity

A longitudinal study was performed on 50 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 1 and 5 specimens per patient were collected from 2 to 92 days post onset of clinical symptoms, for a total of 127 samples that were tested. The first day of specimen collection for the 50 patients ranged from 2 to 22 days post onset of symptoms. All specimens that were collected >10 days post onset of symptoms were positive or equivocal.

Results were analyzed for each patient to determine the first sample that was SARS-CoV-2 total antibody positive. Of the 50 patients tested, 49 became positive. The patient that remained negative was not tested > 8 days post onset of clinical symptoms. The table below summarizes when the first positive result was observed for each patient.

Days between onset of symptoms and sample collection	No. of Patients with First Reactive Draw	No. of Non-reactive Patients (Negative)	Total	% Agreement with PCR
≤ 7 days	9	0	9	100%
8-14 days	26	1*	27	96%
≥15 days**	14	0	14	100%
Total	49	1	50	98%

*Specimens after 8 days were not available to follow immune response for this patient.

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

9.2.3 Summary of all Clinical Results:

- Serum:
 - Specificity: 346/350 (98.86%); 95% CI: 97.10% - 99.55%
 - Sensitivity: 27/27 (100%); 95% CI: 87.55% - 100.00%
- Plasma:
 - Specificity: 250/250 (100%); 95% CI: 98.50% - 100.00%
 - Sensitivity: 22/23 (95.6%); 95% CI: 79.01% - 99.23%
- Overall
 - Specificity: 596/600 (99.33%); 95% CI: 98.30% - 99.74%
 - Sensitivity: 49/50 (98%); 95% CI: 89.51% - 99.65%

9.3 Matrix Equivalency

Matrix equivalency studies with the test device (Platelia SARS-CoV-2 Total Ab) in the claimed matrices were conducted by lab professionals. Five (5) matched sets of samples from individual donors,

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comprised of serum, tripotassium EDTA plasma, and sodium citrate plasma were evaluated. Five (5) matched sets of samples from individual donor, comprised of serum, acid citrate dextrose (ACD) plasma, and lithium heparin plasma were also evaluated. Five (5) positive and 5 negative samples were evaluated using each matrix type. The mean and standard deviation of negative specimens were calculated for each condition. A comparison of mean was done between the serum matrix and the different plasma matrices. For positive specimens, the percentage (%) of difference in ratio between serum matrix and the different plasma matrices was calculated. Results of the study demonstrate that performance is equivalent for all matrices tested.


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