BIOGENIX

STANDARD OPERATIONAL PROCEDURE FOR SARS-COV-2 VIRUS IGM ANTIBODY

	NAME	DESIGNATION	SIGNATURE	DATE
Prepared by	MS. PREETY RAHEJA	QUALITY MANAGER		30/06/2020
Reviewed by	Dr Bhagyashree Thakre	Deputy Lab Director		01/07/2020
Approved by	Dr Sally Abdulla Ibrahim	Lab Director		01/07/2020

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2. REVISION HISTORY

#	Version	Date	Changes Made by	Reason for Changes	Clause Changed
1	1.0				

3. REVIEW HISTORY

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4. PURPOSE

The SARS-CoV-2 IgM assay is an indirect method (ELISA) used for the qualitative detection of IgM antibodies to SARS-CoV-2 in human serum and plasma in vitro on the URANUS AE Automated ELISA system Assay Analyzer. The SARS-CoV-2 IgM assay is to be used as an aid in the diagnosis of SARS-CoV-2 infection in conjunction with clinical presentation and other laboratory tests. Antibody detection in serum can provide a convenient metric to help confirm SARS-Cov-2 exposure.

5. TEST PRINCIPLE

This kit uses an indirect method (ELISA) to detect IgM antibodies against SARS-CoV-2 virus in human serum or plasma.

The microwell plate is coated with anti-u chain monoclonal antibody. After the samples to be tested are added into the well, their constituent IgM antibodies are captured directly. The unbound material is washed away, and the horseradish peroxidase-labeled (HRP) SARS-CoV-2 antigen is added, which can recognize specific SAR-CoV-2 IgM antibodies. Thus, a complex consisting of anti-u chain monoclonal antibody, human anti-SAR-CoV-2 IgM antibody, and HRP-antigen conjugate will be formed on the surface of the microplate. After the plate is washed again, a substrate solution is added. In the presence of the anti-u chain monoclonal antibody, human anti-SARS-CoV-2 IgM antibody and HRP-antigen conjugate complex, the HRP linked to the complex will catalyze a chromogenic reaction to produce a blue chemical complex. Finally, after stopping reaction, the color will change to yellow, indicating specific SAR-COV-2 IgM antibodies were present in the original sample. The absence of color indicates that no specific SAR-CoV-2 IgM antibodies were detected in the original sample.

The OD value is measured on a microplate reader or an enzyme immunoassay system, and the presence of SAR-Cov-2 virus IgM antibody will be determined by the OD value.

6. PERFORMANCE CHARACTERISTICS

6.1 This test is verified with accuracy and precision

6.2 Please refer method verification file

7. TYPE OF SAMPLE/CONTAINER/ADDITIVE/PATIENT PREPARATION

7.1. SAMPLE TYPE

Specimen Type	Stability & Storage
Serum (yellow top, red top)	Room temperature (15 to 30°C) 2 days Stable for 3 Days at 2-8°C Stable for -20°C long term storage
Plasmas: Potassium EDTA (purple top)	Room temperature (15 to 30°C) 2 days Stable for 3 Days at 2-8°C Stable for -20°C long term storage

7.2. HANDLING OF SPECIMENS

7.2.1. Centrifuge serum samples after complete clot formation (3000rpm/10minutes)

7.2.2. Ensure the patients' samples are at ambient temperature (20-25°C) before measurement.

7.2.3. For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.

7.3. SAMPLE ACCEPTANCE AND REJECTION CRITERIA

7.3.1. Grossly hemolyzed (> 500 mg/dL hemoglobin)

7.3.2. Obvious microbial contamination

7.3.3. Fungal growth

7.3.4. Heat-inactivated specimens

7.3.5. Pooled specimens

8. PATIENT PREPARATION

8.1. NO SPECIAL PATIENT PREPARATION

8.2. REQUIRED EQUIPMENT AND REAGENT

8.2.1 Reagents

SARS-CoV-2 IgM coated plate	The plate coated with the anti-u chain monoclonal antibody
SARS-CoV-2 IgM Positive control	Human serum with SARS-CoV-2 IgM antibody with stabilizer and preservatives.
Negative Control	Human serum with SARS-CoV-2 IgM antibody with stabilizer and preservatives.
SARS-CoV-2 IgM Enzyme Solution	Horseradish peroxidase-labelled) HRP SARS-COV-2 antigen with preservatives.
Concentrated washing buffer	PBST with appropriate amount of preservatives
Sample Diluent Buffer	
Substrate A	Urea Peroxidase solution
Substrate B	TMB solution, stored avoiding light
Stop Buffer	Diluted H2SO4

8.2.2 Universal Plate washer
8.2.3 Purified water
8.2.4 Measuring cylinder
8.2.5 Storage and Stability:

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position.
Opened	2 to 8°C	Valid until 6 months	

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Wash Buffer	10-30°C	1 week	If crystal appear in the concentrated washing buffer heat the buffer to 37°C to fully dissolve the crystals and mix.

9. ENVIRONMENT & SAFETY CONTROL

9.1. Humidity / Temperature

9.2. In vitro diagnostic use

9.3. Exercise the standard precautions required for handling all Laboratory reagents.

9.4. Disposal of all waste material in accordance with Procedure for Waste Management

9.5. Avoid the formation of foam with all reagents and sample types (specimens, Calibrators, and controls).

9.6. For environmental and safety precautions in using SARS-COV-2 IgM reagent go to MSDS sheet file

10. CALIBRATION

10.1. CALIBRATION FREQUENCY

- QUALITY CONTROL Every 30 days.
- A reagent kit with a new lot number is used.
- • Daily quality control results are outside of quality control limits used to monitor and control system performance.
- This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

10.2. TRACEABILITY

10.3. CONTENTS

10.4. CALIBRATOR STORAGE & STABILITY

11.

11.1. QC MATERIALS

11.1.1 2 levels of QC supplied by the manufacturer will be run per batch of samples.

- Negative
- Positive

11.1. FREQUENCY OF RUNNING INTERNAL QC

With Every batch of sample

11.2. EXTERNAL QC

We are enrolled in CAP PT program

12. PROCEDURE

12.1 Follow standard Personnel Protective equipment precautions.

12.2 Check the request for Routine or urgent, Confirm the label on the tube and bar-coded sticker with accompanying requisition form.

12.3 Prepare the sample as per our Procedure for Sample Handling, Preparation and Storage

12.4 Take the Request form and check all the Demographic details and Test order in LIS;

12.5 Check for all consumables and reagent needed before processing the sample.

12.6 Click on the “Initialize” Button to Initialize the system.

12.7 After Initialization, Click the Washer application program to maintenance the washed.

12.8 Connect the system liquid tube in the tank buffer island and click “Rinse” Button to flush the fluidic tubing for 60 seconds, check if there is any blockage on the manifold.

12.9 Clean up the waste liquid container and waste tip bucket.

12.10 Refill the disposable tips.

12.11 Place the required reagents and controls according to position, check the volume and make sure there are no bubbles.

12.12 Prepare washing buffer: Dilute concentrated washing buffer with purified water (dilution ratio 1:9)

12.13 Position the pre-dilution plates and wells.

12.14 Position the samples on the sample racks, barcode label facing the scanner.

12.15 Click “Add SMPs” to go to test program page, select test program and enter the number of samples.

12.16 Click “Next” to go to barcode scanning page, and pushing the rack slowly forward.

12.17 Click “Next” to turn the page presenting the details of samples and corresponding program

12.18 Click “Next” to turn the page to show the position of assay plate that display in red.

12.19 Click ‘Complete” and check the preparation and click “OK” button to confirm, then Click “Run” icon to start the process.

12.20 Schedule monitoring page will be shown to check the status of sample processing.

12.21 Machine will alarm when test is done and to view the result by clicking the “Reader” button.

13. INTERFERENCE

13.1. For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.

13.2. Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

13.3. To ensure consistency in results, recentrifuged specimens prior to testing if they contain fibrin, red blood cells, or other particulate matter.

14. CALCULATION

14.1. If the OD value of the tested sample is greater that the cut off value, the result is considered positive for IgM antibody against SAR-CoV-2. If the OD value of a tested

sample is less than the cut off value, the result is considered negative for IgM antibody against SARS-CoV-2.

15. BIOLOGICAL REFERENCE INTERVALS

15.1. The cut off value 0.10 mean of negative control (calculated as 0.05 if mean OD value of negative control is less than 0.05).

15.2. Negative test results indicate that an individual has not mounted a sufficient immune response to SARS-CoV-2.

15.3. Positive test results indicate that an individual may have been exposed to SARS-CoV-2 and should be combined with clinical symptoms and other diagnostic results for further confirmation.

16. DILUTIONS

16.1. Dilution: N/A

17. CRITICAL VALUE

17.1. Critical value: N/A

18. LABORATORY CLINICAL INTERPRETATION

18.1. POINTS TO BE NOTED BEFORE RELEASING RESULTS:

18.2. Result evaluation:

18.2.1 Result of the patient evaluated according to provisional diagnosis of the patient and the type of the sample.

18.2.2 Result confirmation:

18.2.3 Result is confirmed by revising the patient identification from the request with the sticker on the sample

18.2.4 The laboratory technical staff confirms the acceptability of quality control results prior to reporting patient results.

18.2.5 Patients with previous Laboratory records, check first the latest results before releasing the current results.

18.2.6 Confirm and repeat any High / low result.

18.2.7 Review the results for any Flags on the system.

19. POTENTIAL SOURCES OF VARIATION

19.1. PERSONAL:

19.1.1Competent of staff.

19.2. MACHINE /EQUIPMENT:

19.2.1 Centrifuge

19.2.2 Maintenance

19.2.3 PPM

19.2.4 Pipette

19.3. REAGENT:

19.3.1 Stability of reagent and expiry date

19.3.2 Temperature of refrigerator

20. REFERENCES

20.1. User Manual for Uranus AE Automated ELISA Assay Analyzer

20.2. Kit insert of SARS-CoV-2 IgM.

BIOGENIX

Abu Dhabi, UAE

Call: 0466 XXX XX
Fax: 046XXX XXX

name@biogenix.com

Thank You