SCoV-2 Detect™ IgM ELISA

Instructions for Use
For Emergency Use Authorization (EUA) only

For *In Vitro* Diagnostic (IVD) Use
Rx Only
Samples should be collected from individuals within 7 days to 64 days post symptom onset. Samples should not be tested less than 7 days post symptom onset. Negative samples collected before 12 days post symptom onset should be reflexed to direct detection of the virus. Negative samples collected 12 days or more post-symptom onset should be reflexed to a test that detects and reports SARS-CoV-2 IgG.

INTENDED USE

The SCoV-2 *Detect*™ IgM ELISA is an *in vitro* diagnostic test for the qualitative detection of IgM antibodies to SARS-CoV-2 in human serum.

The SCoV-2 *Detect*™ IgM ELISA 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 SCoV-2 *Detect*™ IgM ELISA should 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, that meet the requirements to perform high complexity tests.

Results are for the detection of IgM SARS-CoV-2 antibodies. IgM antibodies 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 SCoV-2 *Detect*™ IgM ELISA 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 for SCoV-2 *Detect*™ IgM ELISA may occur due to cross-reactivity from pre-existing antibodies or other possible causes.
The SCoV-2 *Detect*™ IgM ELISA is only for use under the Food and Drug Administration Emergency Use Authorization (EUA).

SUMMARY AND EXPLANATION OF THE TEST

The novel coronavirus, SARS-CoV-2 (the causative agent of COVID-19), has been responsible for the pandemic of pneumonia-like symptoms and associated deaths from late 2019 and into 2020. The detection of the initial outbreak in the Hubei Province of China and the subsequent need for an effective diagnosis were quickly described (Li et al., 2020; Wu et al., 2020; Zhou et al., 2020).

It has been reported that PCR-confirmed SARS-CoV-2 positive patient may seroconvert and develop antibodies against SARS-CoV-2 antigens anywhere from 6-21 days after the onset of clinical symptoms (Okba et al., 2020). The specific and reliable detection of human IgM antibodies to SARS-CoV-2 may provide indication of recent infection.

The SCoV-2 *Detect*™ IgM ELISA is a qualitative immunoassay for the detection of IgM antibodies targeting SCoV-2 related antigens.

PRINCIPLE OF THE TEST

The SCoV-2 *Detect*™ IgM ELISA is a qualitative indirect ELISA for the detection of IgM antibodies targeting epitopes derived from SARS-CoV-2. Diluted serum specimens are added to antigen-coated wells and incubated. After incubation and washing, human antibodies targeting SARS-CoV-2 antigens remain bound to the plate surface. Secondary antibody conjugated to horseradish peroxidase (HRP) targeting human IgM is then added to each well. After incubation, the ELISA wells are washed once again before a tetramethylbenzidine (TMB) substrate is added. An acidic stopping solution is finally used to stop the reaction and the degree of enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometers.

Positive, Negative and Cut-Off Controls are provided to ensure the integrity of the test and to determine the assay-specific threshold. Up to 90 specimens may be evaluated with each kit (as the controls are run in duplicate). The entire procedure takes approximately 1 hour and 50 minutes.

KIT CONTENTS

Warning: Do not use any reagents where damage to the packaging has occurred.

The kit contains the following reagents:

1. **SCoV-2 Antigen Coated Microtiter Strips for IgM:** Strip holder in a resealable foil pouch, containing 96 polystyrene microtiter wells coated with SCoV-2 antigen in each well. Stable at 2-8°C until the expiration date.
2. **SCoV-2 IgM Negative Control:** One vial, 50 µL. Negative serum. The Negative Control will aid in monitoring the integrity of the kit. Stable at 2-8°C until the expiration date.
3. **SCoV-2 IgM Positive Control:** One vial, 50 µL. Positive Control sample. The Positive Control will aid in monitoring the integrity of the kit. Stable at 2-8°C until the expiration date.
4. **SCoV-2 IgM Cut-Off Control:** One vial, 50 µL. Cut-Off Control sample. The Cut-Off Control will aid in monitoring the integrity of the kit and estimating the proper threshold to determine test sample status. Stable at 2-8°C until the expiration date.
5. **Sample Dilution Buffer for SCoV-2:** Two bottles, 25 mL each, ready to use. Tris-HCl buffered solution (pH 7.2-7.6) with Tween 20 (0.05%), preservative (0.05% ProClin-300) and additives. The Sample Dilution Buffer will be used for the dilution of test samples and controls. Stable at 2-8°C until the expiration date.
6. **100X Conjugate for SCoV-2 IgM:** One vial, 100 µL, containing horseradish peroxidase-labeled antibody in a Tris-based buffer with 0.03% - 0.05% ProClin-300. Stable at 2-8°C until the expiration date.
7. **Conjugate Diluent for SCoV-2:** One bottle, 9 mL. This contains the diluent solution for the 100X Conjugate in a Tris-based buffer with 0.01% Thimerosal as a preservative. The 100X conjugate is diluted directly into this solution. The 100X conjugate should only be diluted into this solution immediately prior to running the assay. Unused diluted conjugate should be discarded. The conjugate diluent is stable at 2-8°C until the expiration date.
8. **10X Wash Buffer:** One bottle, 120 mL. 10X concentrated phosphate buffered saline with Tween 20 (pH 6.8-7.0). Stable at 2-8°C until the expiration date.
9. **Liquid TMB Substrate:** One bottle, 12mL, ready to use. Contains 3, 3', 5, 5'-tetramethylbenzidine (TMB) and hydrogen peroxide in a citric acid-citrate buffer (pH 3.3-3.8). Stable at 2-8°C until the expiration date. Note: The substrate should always be stored in the light-protected bottle provided.
10. **Stop Solution:** One bottle, 6mL, ready to use. 1N Sulfuric Acid. Used to stop the reaction. Stable at 2-8°C until the expiration date.

    Warning: Strong acid. Wear protective gloves, mask and safety glasses. Dispose all materials according to all applicable safety rules and regulations.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- ELISA spectrophotometer capable of absorbance measurement at 450 nm
- Biological or high-grade water
- Appropriately sized beakers and stir bars
- Vacuum pump
- Automatic plate washer
- 37°C incubator without CO_2 supply
- 1-10 µL single-channel pipettors, 50-200 µL single- and multichannel pipettors
- Polypropylene tubes or 96 well dilution plates
- Parafilm or plastic plate cover
- Timer
- Vortex

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use** under Emergency Use Authorization (EUA) only. A thorough understanding of this package insert is necessary for the successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert.
- 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 has been authorized only for the presence of IgM antibodies against SARS-CoV-2, not for any other viruses or pathogens.
- 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 Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled in accordance with good laboratory procedure.

SAFETY PRECAUTIONS

- All human source materials used in the preparation of the negative control have tested negative for antibodies to HIV 1&2, Hepatitis C and Hepatitis B surface antigen. However, no test method can ensure 100% efficiency. Therefore, all human controls and antigen should be handled as potentially infectious material. The Centers for Disease Control and Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.
- Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.
- Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
- Do not eat, drink, smoke, or apply cosmetics in the laboratory where immunodiagnostic materials are being handled.
- Do not pipette by mouth.

TECHNICAL PRECAUTIONS

- This test must be performed on human serum only. The use of whole blood, plasma or other specimen matrices has not been validated.
- Do not mix various lots of any kit component within an individual assay.
- All reagents must be equilibrated to room temperature (20-25°C) before commencing the assay. The assay will be affected by temperature changes.
- Avoid repeated freezing and thawing of the serum specimens to be evaluated.
- Dispense reagents directly from bottles using clean pipette tips. Transferring reagents may result in contamination.
- Unused microtiter wells must be resealed immediately in the ziplock foil pouch with the desiccant provided. Failure to do so may cause erroneous results with those unused microwells.
- Do not use any component beyond the expiration date shown on its label.
- Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.
- Some reagents may form a slight precipitate, mix gently before use.
- Incomplete washing will adversely affect the outcome and assay performance.
- To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stop solution into the wells in the same order and speed used to add the TMB solution.
- Avoid microbial contamination of reagents.
- Avoid contamination of the TMB Substrate Solution with the Conjugate Solution. The TMB Substrate Solution should be clear in color; a blue color change prior to use may indicate contamination has occurred.
- Use a clean disposable pipette tip for each reagent, standard, control or specimen.

  Cover working area with disposable absorbent paper.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

This kit contains reagents made with human serum or plasma. The serum or plasma used has been heat-inactivated unless otherwise stated. Handle all sera and kits used as if they contain infectious agents. Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of specimens.

CHEMICAL HAZARD

Safety Data Sheets (SDSs) are available for all components of this kit. Review all appropriate SDSs before performing this assay. Avoid all contact between hands and eyes or mucous membranes during testing. If contact does occur, consult the applicable SDS for appropriate treatment.

SPECIMEN COLLECTION AND PREPARATION

- Only human serum should be used for this assay, and the usual precautions for venipuncture should be observed. Blood obtained by venipuncture should be allowed to clot at room temperature (20-25°C) for 30 to 60 minutes and then centrifuged according to the Clinical and Laboratory Standards Institute (CLSI Approved Guideline – Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; GP44).
- Testing should be performed as soon as possible after collection. Do not leave sera at room temperature for prolonged periods. Separated serum should remain at 20-25°C for no longer than 8 hours. If assays are not completed within 8 hours, serum should be refrigerated at 2-8°C. If assays are not completed within 48 hours, or the separated serum is to be stored beyond 48 hours, serum should be frozen at or below -20°C.
- Avoid repeated freezing and thawing of samples as this can cause analyte deterioration. Frost-free freezers are not suitable for sample storage.
  Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use. Always quick spin before use.
- If sera are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.
- Do not use sera if any indication of microbial growth is observed.

TEST PROCEDURE

CAUTION: *The test procedure must be strictly followed. Any deviations from the procedure may produce erroneous results. Bring all reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. NOTE: For long-term storage, serum samples should not be repeatedly thawed and frozen more than four times. Sera should be further divided into small aliquots and stored at -20°C or below.*

This assay is intended to be performed manually. Plate washing must be performed using a properly calibrated automated plate washer. This kit has not been optimized by InBios for use with a specific automated ELISA processing system. Use with an automated ELISA processing system will require proper validation to ensure results are equivalent to the expectations described in this package insert.

Preparation of Reagents:

- Preparation of 1X Wash Buffer

  Dilute the 10X Wash Buffer to 1X using Biological or High-Grade Water. To prepare a 1X Wash Buffer solution, mix 120 mL 10X Wash Buffer with 1080 mL distilled (or deionized) water. Mix thoroughly to ensure that any precipitate is dissolved and that the solution is uniform. Once diluted to 1X, the solution can be stored at room temperature for up to 6 months. Properly label the 1X Wash Buffer solution and carefully note the expiration date on the label. Check for contamination prior to use. Discard if contamination is suspected.
- Microtiter Strip Wells

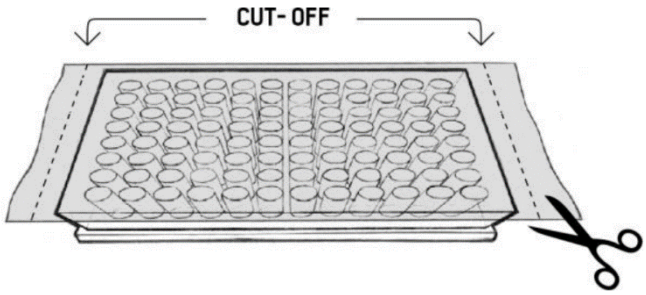
  Select the number of coated wells required for the assay. The remaining unused wells should be repackaged immediately with the supplied desiccant and stored at 2-8°C until ready to use or expiration.
- Preparation of Conjugate Solution

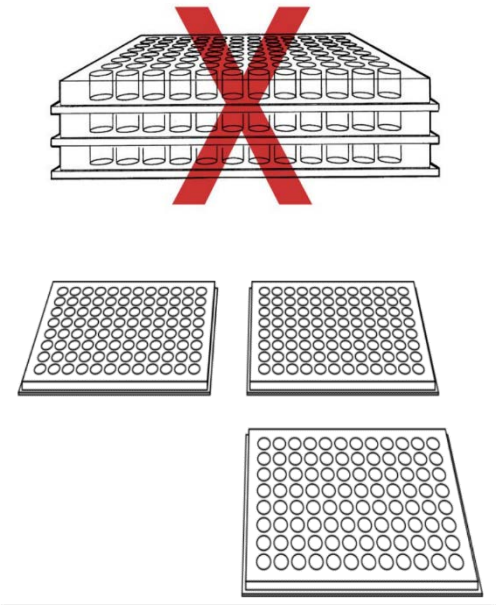
  Add 90 µL of 100X Conjugate for SCoV-2 IgM directly to the 9 mL bottle of Conjugate Diluent for SCoV-2 (1 part : 100 parts). Alternatively, use a clean pipette to remove the required volume of Conjugate Diluent and add the necessary volume of 100X Conjugate for SCoV-2 ELISA into a clean polypropylene test tube in order to maintain the 1:100 ratio. Mix by inverting the solution several times. This conjugate solution should be prepared immediately prior to running the assay and discarded immediately after use in the assay.

Assay Procedure:

1. Positive, negative and cut-off controls should be assayed in duplicate (and run on every plate, each time an assay is performed). Unknown serum samples may be tested in singlicate. Up to ninety test specimens can be tested in singlicate with an entire plate. Immediately place any unused ELISA plate wells back into the original foil packaging with the provided desiccant, properly seal and store at 2-8°C.
2. Dilute each control and each test specimen 1:100 by adding 4 µL of sample to 396 µL of Sample Dilution Buffer for SCoV-2. Dilute samples into a dedicated sample dilution block or an appropriately sized tube.
3. Add 50 µL of the 1:100 diluted controls and test specimens onto the appropriate locations in the SCoV-2 Antigen Coated Microtiter Strip plate (ELISA plate). Note and record the locations of all controls and test samples in the ELISA plate wells.
4. Cover the top of the plate with parafilm (or a plastic plate cover) and remove any excess parafilm from the edges of the plate.

   Note: *This is to make sure the temperature distribution is evenly spread out in all wells from bottom and sides; any extra parafilm can be cut off once the top is sealed to block evaporation.*





CORRECT METHOD

Note: *Do not stack plates on top of each other. They should be spread out as a single layer. This is very important for even temperature distribution. Do not use CO_2 or other gases. Do not place plates in contact with any wet substances such as wet paper towels etc.*

5. Cover the plate with parafilm or a plastic plate cover and incubate the plate(s) at 37°C for 1 hour in an incubator.
6. After the incubation, wash the plate 6 times with an automatic plate washer using 1X Wash Buffer. Use 300 µL per well in each wash cycle.
7. Prepare the Conjugate Solution (90 µL of 100X Conjugate : 9 mL of Conjugate Diluent) and add 50 µL per well of this Conjugate Solution into all wells using a multi-channel pipettor. Discard the remaining Conjugate Solution.
8. Cover the plate with parafilm or a plastic plate cover and incubate the plate(s) at 37°C for 30 minutes in an incubator.
9. After the incubation, wash the plate 6 times with the automatic plate washer using 1X Wash Buffer.
10. Add 75 µL per well of Liquid TMB substrate into all wells using a multi-channel pipettor.
11. Incubate the plate, uncovered at room temperature **in the dark**, for 20 minutes.
12. Add 50 µL per well of Stop Solution into all appropriate wells using a multi-channel pipetter. Make sure to add the Stop Solution in the same order and at approximately the same speed at which the TMB was applied. (Note: As the TMB substrate produces an enzymatic reaction with the HRP-conjugate, it is critical this incubation time point is followed as closely as possible). Let the plate stand, uncovered at room temperature, for 1 minute.
13. Read the optical density at 450 nm (OD_{450}) with a microplate reader. DO NOT SUBTRACT OR NORMALIZE ANY BLANK VALUES OR WELLS.
14. Record the raw OD_{450} and evaluate the sample status as indicated in the Quality Control and Interpretations of Results sections.

QUALITY CONTROL

Each kit contains positive, negative and cut-off controls. The negative and positive controls are intended to monitor for substantial reagent failure. The test is invalid and must be repeated if the control samples do not meet the specifications. If the test is invalid, the results cannot be used. Quality Control (QC) requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to CLSI C24 and 42 CFR 493.1256 for guidance on appropriate QC practices. The results below are given strictly for guidance purposes only and applicable for spectrophotometric readings only.

First, calculate the mean (average) negative, positive and cut-off control raw OD_{450} values as shown in the following examples.

Example 1: SCoV-2 Negative Control

	OD_{450}
Replicate 1	**0.145**
Replicate 2	**0.133**
Sum	**0.278**

Average Negative Control = 0.278 ÷ 2 = 0.139

Example 2: SCoV-2 Positive Control

	OD_{450}
Replicate 1	**1.876**
Replicate 2	**1.685**
Sum	**3.561**

Average Positive Control = 3.561 ÷ 2 = 1.7805

Example 3: SCoV-2 Cut-Off Control

	OD_{450}
Replicate 1	**0.645**
Replicate 2	**0.571**
Sum	**1.216**

Average Cut-Off Control = 1.216 ÷ 2 = 0.608

Finally, verify that the quality control requirements, listed in the table below, are fulfilled.

Quality Control Requirements

Control	Requirement
Positive Control	OD ≥ 0.85
Negative Control	OD < 0.25
Cut-Off Control	0.25 < OD < 0.85

Summary: The results on the table above must be obtained for the assay to be considered valid. Non-fulfillment of these criteria is an indication of deterioration of reagents or an error in the test procedure and the assay must be repeated.

INTERPRETATION OF RESULTS

The assay cut-off value was determined by screening a large number (>100) of normal human serum (NHS) samples that were collected prior to the COVID-19 outbreak (~November, 2019). The cut-off selection was performed by estimating the mean of the negative specimens plus three (3) standard deviations.

The status of the unknown sample is determined by first calculating the cut-off of the assay (shown above in Example 3), followed by calculating the ratio of the optical density (OD_{450}) divided by the cut-off.

Calculate Immunological Status Ratio (ISR): The immunological status ratio (ISR) is calculated from the ratio of the optical density (OD) obtained with the test sample divided by the calculated Cut-Off Value. Calculate the ISR for each test sample. If unknown samples were tested in duplicate, then calculate the average optical density (OD_{450}) before dividing by cut-off to determine ISR.

Example 4: Calculate the ISR for a Sample

Sample ID	Raw OD_{450}
Unknown Sample #1	0.952

ISR Value = Raw OD ÷ Cut-Off Value

ISR Value = 0.952 ÷ 0.608 = 1.566

ISR Value	**Results**	**Interpretation**
0.9 - 1.1	Retest	If tested in singlicate, those sera with OD values close to the cut-off (0.9 < ISR < 1.1) must be repeated in duplicate along with controls to verify the sample status. If the average ISR value from the repeat duplicate testing is ≥ 1, the sample is considered positive for IgM antibodies to SCoV-2. If the average ISR value from the duplicate testing is < 1, the sample is considered negative for IgM antibodies targeting SCoV-2.
≥ 1.1	Positive	Presence of detectable IgM antibodies targeting SCoV-2 antigen.
≤ 0.9	Negative	No detectable IgM antibodies targeting SCoV-2 antigen were found. The result does not rule out the possibility of SARS-CoV-2 infection.

LIMITATIONS

- The assay performance characteristics have not been established for visual result determination.
- The assay performance characteristics have not been established for matrices other than serum.
- The assay should not be used to diagnose or exclude acute infection. Results are not intended to be used as the sole basis for patient management decisions.
- A positive result may not indicate previous SARS-CoV-2 infection. Consider other information, including clinical history and local disease prevalence, in assessing the need for a second but different serology test to confirm an immune response.
- A negative result for an individual subject indicates absence of detectable anti-SARS-CoV-2 antibodies. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. A negative result can occur if the quantity of the anti-SARS-CoV-2 antibodies present in the specimen is below the detection limit of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
- It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to re-infection.
- False positive results due to cross-reactivity with antibodies to other coronaviruses can occur.
- Assay performance characteristics have not been established for testing cord blood, for testing neonates, for prenatal screening.
- Samples that are hemolyzed should be avoided for analysis with this assay.
- Results from immunosuppressed patients must be interpreted with caution.
- Assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The SCoV-2 *Detect*™ IgM ELISA 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 Authorized laboratories using the SCoV-2 *Detect*™ IgM ELISA, must adhere to the Conditions of Authorization indicated in the Letter of Authorization as listed below:

- Authorized laboratories[a] using the SCoV-2 *Detect*™ IgM ELISA will include with test result reports, 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 the SCoV-2 *Detect*™ IgM ELISA will us it as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the SCoV-2 *Detect*™ IgM ELISA are not permitted.
- Authorized laboratories that receive the SCoV-2 *Detect*™ IgM ELISA will notify the relevant public health authorities of their intent to run the assay prior to initiating testing.
- Authorized laboratories using the SCoV-2 *Detect*™ IgM ELISA 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 the SCoV-2 *Detect*™ IgM ELISA and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH EUA Reporting@fda.hhs.gov) and InBios Technical Support (https://inbios.com/technical-support/) any suspected occurrence of false reactive or false non-reactive results and significant deviations from the established performance characteristics.
- All laboratory personnel using the SCoV-2 *Detect*™ IgM ELISA must be appropriately trained in immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the SCoV-2 *Detect*™ IgM ELISA 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 the SCoV-2 *Detect*™ IgM ELISA.

- InBios International Inc., authorized distributors, and authorized laboratories using the SCoV-2 *Detect*™ IgM ELISA 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.

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

PERFORMANCE CHARACTERISTICS

Positive Agreement

The positive percent agreement (PPA) of the SCoV-2 *Detect*™ IgM ELISA was estimated by testing a panel of serum specimens collected from 85 individuals who tested positive with a SCoV-2 PCR assay at an earlier time point. Of the 120 specimens provided by the 85 subjects, 111 specimens tested reactive (positive) with the SCoV-2 *Detect*™ IgM ELISA. Reactivity was correlated with elapsed days after symptoms onset. Results are shown below.

Summary of results in relation to days post onset of symptoms

Days post symptom onset	# PCR Positive at any time	SCoV-2 *Detect*™ IgM ELISA		
		# Positive results	PPA	95%CI*
≤7	9	6	66.67%	35.42% - 87.94%
8-14	38	35	92.11%	79.20% - 97.28%
≥15	51	48	94.12%	84.08% - 97.98%
unknown	22	22	100%	85.13% - 100%

Overall positive percent agreement for the SCoV-2 *Detect*™ IgM ELISA

Positive Percent Agreement (PPA)	**92.50% (111/120)** 95% Confidence Interval*: 86.36% - 96.00%

*95% confidence interval calculated by Wilson method.

Negative Agreement

The negative percent agreement (NPA) of the SCoV-2 *Detect*™ IgM ELISA was estimated by testing a panel of 95 normal human serum specimens. 94 of the 95 specimens tested non-reactive (negative) with the SCoV-2 *Detect*™ IgM ELISA.

Negative Percent Agreement (NPA)	**98.95% (94/95)** 95% Confidence Interval*: 94.28% - 99.81%

*95% confidence interval calculated by Wilson method.

Cross-Reactivity (Analytical Specificity)

Cross-reactivity of the SCoV-2 *Detect*™ IgM ELISA Kit was be evaluated by testing SARS-CoV-2 seronegative specimens from patients with antibodies to other viral infections and autoantibodies which could potentially cause false positive results. One hundred eighty normal human serum (NHS) samples that were collected in the US prior to the COVID-19 outbreak (i.e. known negatives) were also tested. SCoV-2 *Detect*™ IgM ELISA demonstrates no cross-reactivity against IgM antibodies for influenza A, influenza B, hepatitis B, hepatitis C, human immunodeficiency, respiratory syncytial viruses or anti-nuclear antibodies or rheumatoid factor or human anti-mouse antibody. 178 of 180 NHS tested negative.

Category	Number of samples tested	Number reactive
Anti-Influenza A/B	7	0
Anti-Hepatitis B	5	0
Anti-Hepatitis C	5	0
Anti-Nuclear Antibody	5	0
Rheumatoid Factor	18	0
Human Anti-Mouse Antibody	3	0
Anti-HIV	8	0
Anti-Respiratory Syncytial Virus	4	0
Normal Human sera	180	2

Reproducibility

Reproducibility of the SCoV-2 *Detect*™ IgM ELISA was evaluated by having three operators test the SCoV-2 *Detect*™ IgM ELISA Kit on three different days (total of nine runs). All runs were performed by trained personnel at InBios International as per the kit's instructions for use and the same kit lot was used in all runs. Each run included kit controls (positive, negative, and cut-off) and a seven-member serum panel comprised of positive, negative, and borderline samples. All kit controls and each panel member were tested in triplicate.

Each sample was tested a total of 27 times. Positive samples tested positive 27 times, negative samples tested negative 27 times.

Within-run, between-run, between-operator, and overall variability of immunological status ratios (ISRs) are summarized below. Because average values tended to be lower for the negative samples, the %CV tended to be higher, but % agreement with expected result remained high.

			Within-Run (Repeatability)		**Between-Run**		**Between-Operator**		**Overall Reproducibility (Within-Laboratory)**	
Sample Description	**Average Value**	**N**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Panel 1	0.410	27	0.075	18.4%	0.057	13.8%	0.033	8.1%	0.100	24.3%
Panel 2	4.226	27	0.166	3.9%	0.293	6.9%	0.449	10.6%	0.561	13.3%
Panel 3	1.060	27	0.049	4.6%	0.090	8.5%	0.112	10.6%	0.152	14.3%
Panel 4	0.336	27	0.056	16.7%	0.039	11.7%	0.000	0.0%	0.068	20.3%
Panel 5	3.325	27	0.139	4.2%	0.174	5.2%	0.435	13.1%	0.488	14.7%
Panel 6	0.316	27	0.036	11.3%	0.041	12.9%	0.033	10.5%	0.064	20.1%
Panel 7	1.028	27	0.031	3.0%	0.088	8.5%	0.209	20.3%	0.228	22.2%

Interference

Potential interferents in human serum were tested at or above physiologically relevant levels to determine whether they could cause false positives or false negatives on SCoV-2 *Detect*™ IgM ELISA Kit. Samples at different anti-SARS-CoV-2 IgM antibody concentrations were spiked with potential interfering substances, then tested in duplicates. No interference was observed for concentrations up to 10 mg/mL hemoglobin, 0.4 mg/mL bilirubin (conjugated or unconjugated), 15 mg/mL triglycerides, and 4 mg/mL cholesterol.

Blood-derived potential interferents, their normal concentrations in human blood and serum, and the concentrations tested in this study are shown below.

Interfering Substance	Normal concentration	Test concentration	Solvent
Hemoglobin	<0.01-0.05 mg/mL for serum, 110-180 mg/mL for whole blood	10 mg/mL	Sample Dilution Buffer (SDB)
Bilirubin (conjugated and unconjugated)	0.002 – 0.01 mg/mL normal, >0.025 mg/mL jaundiced	0.4 mg/mL	0.1N NaOH
Triglycerides	<1.30-2.00 mg/mL	15 mg/mL	Sample Dilution Buffer (SDB)
Cholesterol	1.70-1.90 mg/mL normal, 2.80-3.20 mg/mL elevated	4 mg/mL	Isopropyl alcohol (IPA)

REFERENCES

Li, X., Geng, M., Peng, Y., Meng, L., & Lu, S. (2020). Molecular immune pathogenesis and diagnosis of COVID-19. *Journal of Pharmaceutical Analysis*. https://doi.org/10.1016/J.JPHA.2020.03.001

Okba, N. M. A., Muller, M. A., Li, W., Wang, C., GeurtsvanKessel, C. H., Corman, V. M., Lamers, M. M., Sikkema, R. S., de Bruin, E., Chandler, F. D., Yazdanpanah, Y., le Hingrat, Q., Descamps, D., Houhou-Fidouh, N., Reusken, C. B. E. M., Bosch, B.-J., Drosten, C., Koopmans, M. P. G., & Haagmans, B. L. (2020). SARS-CoV-2 specific antibody responses in COVID-19 patients. *MedRxiv*, 2020.03.18.20038059. https://doi.org/10.1101/2020.03.18.20038059

Okba, N. M. A., Muller, M. A., Li, W., Wang, C., GeurtsvanKessel, C. H., Corman, V. M., et al. (2020). Severe Acute Respiratory Syndrome Coronavirus 2−Specific Antibody Responses in Coronavirus Disease 2019 Patients. *Emerging Infectious Diseases,* 26(7).

Udugama, B., Kadhiresan, P., Kozlowski, H. N., Malekjahani, A., Osborne, M., Li, V. Y. C., Chen, H., Mubareka, S., Gubbay, J., & Chan, W. C. W. (2020). Diagnosing COVID-19: The Disease and Tools for Detection. *ACS Nano*. https://doi.org/10.1021/acsnano.0c02624

Wölfel, R., Corman, V.M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M.A., et al. (2020). Virological assessment of hospitalized patients with COVID-2019. *Nature*. https://doi.org/10.1038/s41586-020-2196-x

Wu, F., Zhao, S., Yu, B., Chen, Y.-M., Wang, W., Song, Z.-G., Hu, Y., Tao, Z.-W., Tian, J.-H., Pei, Y.-Y., Yuan, M.-L., Zhang, Y.-L., Dai, F.-H., Liu, Y., Wang, Q.-M., Zheng, J.-J., Xu, L., Holmes, E. C., & Zhang, Y.-Z. (2020). A new coronavirus associated with human respiratory disease in China. *Nature*, *579*(7798), 265–269. https://doi.org/10.1038/s41586-020-2008-3

Yong, S.E.F., Anderson, D.E., Wei, W.E., Pang, J., Chia, W.N., Tan, C.W., et al. (2020). Connecting clusters of COVID-19: an epidemiological and serological investigation. *The Lancet Infectious Diseases*. https://doi.org/10.1016/S1473-3099(20)30273-5

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., Chen, H.-D., Chen, J., Luo, Y., Guo, H., Jiang, R.-D., Liu, M.-Q., Chen, Y., Shen, X.-R., Wang, X., … Shi, Z.-L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, *579*(7798), 270–273. https://doi.org/10.1038/s41586-020-2012-7

InBios International, Inc.
307 Westlake Ave N, Suite 300
Seattle, WA 98109 USA
1-866-INBIOS1 (Toll-Free, USA)
1-206-344-5821 (International)
www.inbios.com

Part Number: 900256-00
Effective Date: 07/02/2020

REF Catalog No. COVE-M

Note: Additional copies of this SCoV-2 *Detect*™ IgM ELISA Kit Package Insert can obtained online at www.bit.ly/cove-m. Paper copies are available upon request at inquiries@inbios.com