SARS-CoV2 IgM ELISA (µ-Capture) IVD

AL-1002

INTENDED USE

The Ansh Labs SARS-CoV2 IgM ELISA, µ-Capture system is intended for the qualitative and semi-quantitative detection of IgM antibodies in serum and plasma collected from the individuals suspected with signs and symptoms of COVID-19 infection by their healthcare provider.

Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate and high complexity tests.

This test is intended for In Vitro Diagnostic Use Only and is being distributed under Section D of Policy for Diagnostic Tests for Coronavirus Disease-2019 during the Public Health Emergency.

SUMMARY AND EXPLANATION

Coronavirus disease (COVID-19) is an infectious disease caused by a newly discovered coronavirus known as severe acute respiratory syndrome coronavirus 2, SARS-CoV2. SARS-CoV2 is genetically like other human respiratory coronaviruses, including SARS-CoV and MERS-CoV. Due to the rapid rise in number of cases and uncontrolled and vast worldwide spread, the WHO has declared SARS-CoV2 a pandemic. SARS-CoV2 virus belongs to the family of coronavirus, which owns the name due to crown-like spikes on their surface. Most described coronaviruses are found in birds or mammals, particularly bats[1-2]. The virus can cause mild symptoms to severe respiratory disease (i.e. pneumonia) and death. SARS-CoV2 appeared in Wuhan, China in December 2019. Although health officials are still tracing the exact source of this new coronavirus, early hypotheses thought it may be linked to a seafood market in Wuhan, China. Some people who visited the market developed viral pneumonia caused by the new coronavirus. COVID-19 is now a fast-growing global pandemic.

SARS-CoV2 infection occurs mainly in the respiratory tract. Currently, RT-PCR tests are being used to diagnose the patients worldwide because of its availability. However**,** RT-PCR based diagnosis has its limitation. Detecting SARS-CoV2 by RT-PCR requires high-quality nasopharyngeal specimens that contain an enough amount of intact viral RNA. The challenges associated with collection of this specimen type has led to many reported high false-negative rates[3-4].

Antibody production (immune response) is the primary defense mechanism against any virus or bacterial infections. IgM antibody is the first to be produced in response to viral proteins (antigens) and will be primarily detectable during the early onset of the disease. IgG antibody is produced late in response to an antigen and will be maintained in the body for long-term response. The advantage of immunoassays is their ability to detect recent and past infections. IgG antibodies are long-lasting and can persist in the bloodstream for many years after infection. This test has the advantage of detecting not only individuals with active infection, but also those who were previously exposed to the virus and have subsequently developed immunity. In addition, such immunoassays only require well established serum or plasma as sample types, thereby greatly reducing challenges associated with collection of nasopharyngeal specimens[5].

PRINCIPLE OF THE TEST

The SARS-CoV2 IgM ELISA kit is based on the ELISA technique, with the µ-capture principle. In the assay, calibrators and unknowns are incubated in microtitration wells coated with an anti-human IgM µ-capture antibody. After incubation and washing, the wells are treated with the conjugate, composed of purified SARS-CoV2 recombinant antigens labeled with peroxidase. After a second incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by wavelength absorbance measurement 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of human IgM antibodies present in specimen.

MATERIALS SUPPLIED

CAL-1002 A - CAL-1002 C Calibrators

Three vials, 1.2 mL each, with SARS-CoV2 IgM in phosphate buffer saline with BSA containing sodium azide as a preservative. Refer to the calibration card for exact calibrator concentrations. Store at 2-8°C until expiration date.

Note: ***Calibrators are provided ready to use and should not be diluted 1:101 in sample diluent.***

PLT-1002 Antibody-Coated Microtitration Strips

One strip holder containing 12x8 (96) microtitration wells coated with anti-human IgM monoclonal antibody. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.

SPD-1002 IgM Sample Diluent

One bottle, 100 mL, containing a BSA solution with sodium azide as a preservative. Store at 2-8°C until expiration date.

ECC-1002 SARS-CoV2 IgM Enzyme Conjugate Concentrate (10x)

One vial, 1.5 mL, containing antigen labelled with peroxidase, in a buffer solution with Proclean-400. Store at 2-8°C until expiration date.

CND-1002 Enzyme Conjugate Diluent

One bottle, 12 mL, Protein based buffer solution with Proclean-400. Store at 2-8°C until expiration date.

TMB-100 TMB Chromogen Solution

One bottle, 12 mL, containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2 to 8°C until expiration date.

WSH-100 Wash Concentrate A

One bottle, 60 mL, containing buffered saline with a nonionic detergent. Store at 2 to 30°C until expiration date. Dilute 25-fold with deionized water prior to use.

STP-100 Stopping Solution

One bottle, 12 mL, containing 0.2 M sulfuric acid. Store at 2 to 30°C until expiration date.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microtitration plate reader capable of absorbance measurement at 450 nm, 405nm and 630 nm.
2. Deionized/Distilled water
3. Precision pipette to deliver 10 µL, 100 µL and 1 mL
4. 1.5 mL culture tubes
5. Automatic microtitration plate washer
6. Incubator for microplate incubation at 37°C temperature
7. Vortex mixer

WARNINGS AND PRECAUTIONS

This test is for In Vitro Use Only. The following universal Good Laboratory Practices should be observed: Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic materials. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose all reagents and materials in compliance with applicable regulations.

WARNING: Potential Biohazardous Material

This kit may contain some reagents made with human and animal sources material (e.g. serum, plasma or bovine albumin) or used in conjunction with human and animal source material. The material in this kit has been tested by CE recommended methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HbsAg; the material has no record of any animal infection. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 5th Edition, 2007[6].

WARNING: Potential Chemical Hazard

Some of the reagents in this kit contain sodium azide[7] as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system. Sample diluent and calibrators contain diluted BSA.

For further information regarding hazardous substances in the kit, please refer to the MSDS, either at AnshLabs.com or by request.

SAMPLE COLLECTION AND PREPARATION

a) Serum is the preferred sample type. Serum, lithium heparin plasma or K_2EDTA plasma can be used. Usual precautions for venipuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at –20°C. Avoid repeated freezing and thawing of samples.

b) For shipping, place specimens in leak proof containers in biohazard specimen bags with appropriate specimen identification and test requisition information in the outside pocket of the biohazard specimen bag. Follow DOT and IATA requirements when shipping specimens[8].

PROCEDURAL NOTES

A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affect the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed to add the TMB Chromogen Solution. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB Chromogen Solution with the Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

PREPARATION OF REAGENTS

Wash Solution: Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature when stored in a tightly sealed bottle.

Microtitration Wells: Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

SARS-CoV2 Antigen-Enzyme Conjugate Solution: The SARS-CoV2 Antigen-Enzyme Conjugate Concentrate (ECC-1002) should be diluted at a ratio of 1 part into 10 parts of the Enzyme Conjugate diluent, 1:11 (CND-1002), according to the number of wells used. **For an entire plate, pipet exactly 1.1 mL of the ECC-1002 into 10.9 mL of the CND-1002.**

NOTE: *The SARS-CoV2 Antigen-Enzyme Conjugate concentrate should be freshly diluted 10–15 minutes prior to use.*

A typical example of Plate Configuration when testing 96 samples:

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL A	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
B	CAL B	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
C	CAL C	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
D	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
E	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
F	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
G	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
H	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93

ASSAY PROCEDURE

Allow all samples and reagents to reach room temperature. Mix reagents thoroughly by gentle inversion before use.

1. Mark the microtitration strips to be used.
2. **Dilute** serum samples **1:101** by distributing **10 µL of serum into 1 mL** of IgM Sample Diluent **(SPD-1002)** in a **culture tube.** Vortex to homogenize the diluted samples and use after **10 minutes**.
3. Pipette **100 µL** of calibrators and **diluted serum samples** to the appropriate wells.

4. Incubate for **30 minutes** at **37°C**. ***No shaking is required.***
5. Aspirate and wash each well **5 times** with the wash solution using an automatic microplate washer.
6. Prepare the SARS-CoV2 IgM Antigen-Enzyme Conjugate Solution by diluting the ECC-1002 conjugate concentrate in the CND-1002 conjugate diluent as described under the "Preparation of Reagents" section of this instructions for use.
7. Add **100 µL** of SARS-CoV2 IgM Antigen-Enzyme Conjugate Solution into each well.
8. Incubate for **30 minutes** at **37°C**. ***No shaking is required***.
9. Aspirate and wash each well **5 times** with the wash solution using an automatic microplate washer.
10. Add **100 µL** of the TMB chromogen solution to each well using a precision pipette. ***Avoid direct exposure to heat and sunlight.*** Incubate the wells, for **8-12 min** at **room temperature**. ***Do not Incubate at 37°C***
    NOTE*: Visually monitor the color development to optimize the incubation time.*
11. Add **100 µL** of the stopping solution to each well using a precision pipette.
12. Read the absorbance of the solution in the wells within **5 minutes**, using a microplate reader set to 450 nm.
    NOTE*: Set the instrument to dual wavelength measurement at 450 nm with background wavelength correction at 630 nm.*

RESULTS

NOTE: *The results in this package insert were calculated by plotting the data on a log vs. log scale using a linear regression curve-fit. Other data reduction methods may give slightly different results.*

1. Calculate the mean absorbance for each calibrator and unknown samples.
2. Plot the mean absorbance readings for each of the Calibrators along the y-axis versus the calibrator concentrations in AU/mL (Arbitrary Unit/mL) along the x-axis, using a linear regression curve fit.
3. Determine the SARS-CoV2 IgM concentrations of the samples from the calibration curve by matching their mean absorbance readings with the corresponding SARS-CoV2 IgM concentrations.

Result Interpretation:

Semi-Quantitative results*:*

Results are expressed in AU/mL as follows:

Negative or Non-Reactive Results: Sample concentration **< 10 AU/mL.**
Individuals with nonreactive results are presumed to be not infected with SARS-CoV2 and susceptible to primary infection.

Positive or Reactive Sample Results: Sample concentration **> 12 AU/mL**.
A reactive result is potentially at risk of transmitting SARS-CoV2 virus infection and should be confirmed combined with clinical manifestations or other diagnostic methods.

Equivocal: Sample concentration ranges between 10 and 12 AU/mL.
If the result is equivocal, repeat the test. If it remains equivocal, collect a new specimen for analysis.

LIMITATIONS

The reagents supplied in this kit are optimized to measure SARS-CoV2 IgM in human serum and plasma. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the samples[9].

For a medical diagnosis, the serological test result should always be interpreted together with the clinical symptoms of the patient and other results.

Negative results do not rule out the SARS-CoV2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered as a rule out infection in these individuals.

Results from antibody testing should not be used as a sole basis to diagnose or exclude SARS-CoV2 infection or to inform infection status.

Positive results may be due to past or present infection with non-SARS-CoV2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.

QUALITY CONTROL

Each laboratory should establish acceptable criteria to assure proper performance. Labs controls should fall within established results.

ANALYTICAL CHARACTERISTICS

Typical Calibration Curve: *The curve specification has been generated using 45 independent runs with calibrators A-C run in singletons.*

Calibrator ID	Mean Absorbance (450 nm-630nm)	Standard Deviation (OD)	Concentration (AU/mL)
Calibrator A	0.142	0.018	2.3
Calibrator B	0.54	0.039	10.6
Calibrator C	1.737	0.080	35

CAUTION: *The data below must not be employed in lieu of data obtained by the user in the laboratory.*

Imprecision:

Precision was determined using SARS-CoV2 reagents (AL-1002) on specimens from non-reactive, low reactive and high reactive specimens according to guidance from CLSI EP5-A2. The table below summarizes the 45 run results.

Sample	Mean conc.	Within run		Between run		Total	
	(AU/mL)	SD	%CV	SD	%CV	SD	%CV
Sample-1	3.8	0.18	4.8%	0.26	6.8%	0.32	8.3%
Sample-2	8.2	0.27	3.3%	0.45	5.5%	0.52	6.4%
Sample-3	28.3	0.77	2.7%	1.03	3.6%	1.29	4.5%

Interference:

Interference was tested according to CLSI EP7-A2. Serum samples with SARS-CoV2 IgM concentrations in the table listed below were evaluated as controls (prior to dosing) and tests with the doses of interferents specified in the table below in replicates of five. Interference was considered significant if the analyte recovery is ± 10% of the value of SARS-CoV2 IgM measured. At the concentrations tested, none of the potential interferents tested showed significant difference in sample measurements.

Interferent Dose	Sample ID	Control Sample SARS-CoV2 IgM (AU/mL)	Test Sample SARS-CoV2 IgM (AU/mL)	% Difference to Control
Hemoglobin 1000 mg/dL	1	7.88	7.88	-7.5
	2	16.43	15.49	-5.7
	3	5.20	5.04	-2.9
Intralipids 20 mg/mL	4	7.67	7.93	3.4
	5	16.23	15.97	-1.6
	6	5.63	5.90	4.7
Bilirubin 0.66 mg/mL	7	7.53	7.16	-4.9
	8	16.15	15.65	-3.1

	9	4.73	4.77	0.7
Biotin 200 ng/mL	10	7.07	7.17	1.5
	11	15.97	15.77	-1.3
	12	5.31	5.47	3.1

Clinical Study:

Prevalence: SARS-CoV2 appeared in Wuhan, China in December 2019. The expected SARS-CoV2 IgM prevalence values for the Unites States population in early 2019 is zero percent. The table below summarizes the prevalence in healthy pediatric, adult donor serums collected between October 2018 to August 2019 and adult donor serum collected in March-April 2020. The positive results observed on these subjects were used to calculate the prevalence. The observed positive results correspond to a SARS-CoV2 IgM ELISA specificity of 99%.

Donors	n	POS	NEG	Equivocal	%Positive Results	% Specificity
Pediatric (2018-2019)	39	0	39	0	0	100
Adult (2019)	100	0	100	0	0	100
Adult (April 2020, No symptoms)	40	0	40	0	0	100
Adult (March-April 2020, symptomatic)	143	30	111	2	21.3	78.7

Sensitivity and Specificity:

141 human sera were analyzed by Ansh Labs SARS-CoV2 IgM ELISA and a commercially available IVD ELISA (Test B) as reference method. Out of 141 samples, 9 were positive and 132 were negative for the presence of IgM antibodies to SARS-CoV2 IgM by Ansh Labs ELISA and 7 were positive and 134 were negative by commercial ELISA. Ansh Labs SARS-CoV2 IgM ELISA had a sensitivity of 100% and specificity of 98.5%. The results are summarized below.

Assay Comparison	*ANSH LABS*		
TEST B	Positive	Negative	Equivocal
Positive	7	0	0
Negative	2	132	0
Equivocal	0	0	0

REFERENCES

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Important notes:

This test is intended for *in vitro* diagnostic use and has not been reviewed by the FDA. This test is being distributed under Section D of the FDA Policy for Diagnostic Tests for Coronavirus Disease-2019 during the Public Health Emergency.

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