Vibrant COVID-19 Ab Assay Kit

Package Insert (Instructions For Use)

REF C901100

Rx Only

For the detection of IgG, IgA and IgM antibody to SARS-CoV-2 in serum.

Vibrant America Clinical Labs

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This package insert must be read in its entirety before using this product.

INTENDED USE

Vibrant COVID-19 Ab assay is an in-vitro diagnostic test intended for the qualitative detection of IgG, IgA and IgM antibodies to SARS-CoV-2 in human serum collected from individuals who are suspected of COVID-19.

This kit (Lab Developed Test) is manufactured and developed for use by Vibrant America Clinical Labs CLIA ID: 05D2078809, CAP Number: 8970308.

TEST BACKGROUND

The novel coronavirus (SARS-CoV-2) that is causing an epidemic of acute respiratory syndrome in humans belongs to the family coronaviridae and the genus Betacoronavirus. Comparisons of the genetic sequences of this virus have shown similarities to SARS-CoV and bat coronaviruses. In humans, coronaviruses cause respiratory infections. Coronaviruses are composed of several proteins including the spike (S), envelope (E), membrane (M), and nucleocapsid (N). Results suggest that the spike protein retains sufficient affinity to the Angiotensin converting enzyme 2 (ACE2) receptor to use it as a mechanism of cell entry. Human to human transmission of coronaviruses is primarily thought to occur among close contacts via respiratory droplets generated by sneezing and coughing. The sources of infection seen mainly consist of patients with pneumonia infected by the novel coronavirus.

IgG is the most abundantly found immunoglobulin to be produced in response to an antigen and will be maintained in the body after initial exposure for long term response. Research has shown that IgM and IgG antiviral antibodies can be detected in the serum samples of infected patients. After infection with SARS-CoV-2, the virus antigen stimulates the immune system to produce antibodies that can be detected in the blood. Among these antibodies, SARS-CoV-2 IgM antibodies appears early and are mostly positive after 3-5 days of onset. The SARS-CoV-2 IgM titers then decrease while the SARS-CoV-2 IgG antibody potency starts to rise rapidly. During the recovery phase, the titer of the SARS-CoV-2 IgG antibody may increase four times or more compared to the acute phase.

TEST PRINCIPLE

Purified COVID-19 antigens are bound to the functionalized silicon wafers under conditions that will preserve the antigen in its native state. The wafers are then diced into silicon chips which are then assembled onto a 96-pillar plate with a layout of 8 chips on each pillar (4 chips with COVID-19 antigens and 4 reference chips used in software analysis) using automated semiconductor assembly techniques.

Diluted patient sera and controls including positive and negative control are added to individual wells allowing the COVID-19 specific antibodies, if present, to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG conjugate (anti-human IgA conjugate and anti-human IgM conjugate in separate plates) is added to each well. After washing away the unbound enzyme labeled conjugate, the remaining enzyme activity is measured by adding a chemiluminescent substrate and measuring the intensity of the signal from each chip scanned. Three 96 pillar plates are used for each assay (1 for IgG antibody detection, 1 for IgA antibody detection and 1 for IgM antibody detection). The sample results are interpreted and quantitated by comparison with controls and cut-off values. The sample is considered to be negative if the sample intensity is equal to or less than the cut-off value chosen and positive if it is greater than the cut-off value chosen.

The antigens tested include –

Antigen Tested	Description
S1 subunit of Spike Protein (S1 SP)	The S1 subunit of the ectodomain mediates binding of the virion to host cell-surface receptors through its receptor-binding domain (RBD)
Receptor Binding Domain (RBD)	Part of the S1 Spike subunit that actually binds to the ACE2 receptor of human epithelial cell
S2 subunit of Spike Protein (S2 SP)	The S2 subunit fuses with both host and viral membranes, by undergoing dramatic structural changes
Nucleoprotein (NP)	Packages the positive strand viral genome RNA into a helical ribonucleocapsid (RNP) and plays a fundamental role during virion assembly through its interactions with the viral genome and membrane protein M. Plays an important role in enhancing the efficiency of subgenomic viral RNA transcription as well as viral replication.

REAGENTS

Part Number	Component	Quantity	Volume	Preservative
C901100	Vibrant COVID-19 Ab Assay Kit	1	N/A	N/A
C901101	Vibrant COVID-19 96 Pillar Plate	3	N/A	N/A
C901103	COVID-19 Blocking Buffer	1	60ml	Sodium azide

Part Number	Component	Quantity	Volume	Preservative
C901104	COVID-19 20X Wash Buffer	1	90ml	N/A
C901105	COVID-19 IgG Negative Control	1	250ul	Thimerosal
C901106	COVID-19 IgA Negative Control	1	250ul	Thimerosal
C901107	COVID-19 IgM Negative Control	1	250ul	Thimerosal
C901108	COVID-19 IgG Cut-off Control	1	250ul	Thimerosal
C901109	COVID-19 IgA Cut-off Control	1	250ul	Thimerosal
C901110	COVID-19 IgM Cut-off Control	1	250ul	Thimerosal
C901111	COVID-19 IgG Positive Control	1	250ul	Thimerosal
C901112	COVID-19 IgA Positive Control	1	250ul	Thimerosal
C901113	COVID-19 IgM Positive Control	1	250ul	Thimerosal
C901114	COVID-19 Sample Diluent	1	90ml	Thimerosal
C901115	COVID-19 IgG Conjugate	1	20ml	Thimerosal
C901116	COVID-19 IgA Conjugate	1	20ml	Thimerosal
C901117	COVID-19 IgM Conjugate	1	20ml	Thimerosal
C901118	COVID-19 Chemiluminescence Substrate A	1	25ml	N/A
C901119	COVID-19 Chemiluminescence Substrate B	1	25ml	N/A

SAFETY AND WARNINGS

1. All human source material used in the preparation of controls for this product has been tested and found negative for antibody to HIV, HBsAg, and HCV by FDA cleared methods. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, negative control, positive control and cut-off control should be handled in the same manner as potentially infectious material.
2. Universal precautions utilizing proper PPE physical safeguards (face and eye protection, gloves, lab coats) must be used at all times during the sample handling and testing process while performing this assay.
3. Thimerosal is hazardous in case of skin contact (irritant), of ingestion, of inhalation and slightly hazardous in case of eye contact (irritant). Follow the safety data sheet provided in case of exposure.
4. The chemiluminescent substrate solution contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
5. Adhere to the daily cleaning and decontamination maintenance procedure to avoid contamination with biohazard materials.
6. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

PRECAUTIONS

1. Substitution of components other than those provided in this system may lead to inconsistent results.
2. Incomplete or inefficient washing/drying and insufficient liquid removal from the 96 pillar plate will cause poor precision and/or high background.
3. A variety of factors influence the assay performance. These include the starting temperature of the reagents, the ambient temperature, the accuracy and reproducibility of the pipetting technique, the thoroughness of washing and liquid removal, the chemiluminescent imager used to scan the results, and the length of the incubation times during the assay. Careful attention to consistency is required to obtain accurate and reproducible results.
4. Strict adherence to the protocol is recommended.
5. The kit can only be used for one time. Once an assay is completed, the reagents and well plates must be discarded following proper procedures.
6. Chemical contamination of the HRP conjugate can result from improper cleaning or rinsing of equipment or instruments. Residues from common laboratory chemicals such as formalin, bleach, ethanol or detergent will cause degradation of the HRP conjugate over time. Thoroughly rinse all equipment or instruments after the use of chemical cleaners/disinfectants.

STORAGE CONDITIONS

1. Store all the kit reagents at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.

2. Do not use if reagent is not clear or if a precipitate is present. All reagents must be brought to room temperature prior to use.

SPECIMEN COLLECTION

1. This procedure should be performed with a serum specimen. Addition of azide or other preservatives to the test samples may adversely affect the results. Microbially contaminated, heat-treated, or specimens containing visible particulate should not be used. Grossly hemolyzed or lipemic serum or specimens should be avoided.
2. Please pay attention to the risk of infection during sample collection and preparation. Please refer to the CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19): https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html as well as your local, state and federal government's mandated requirements.
3. Venous blood samples should be collected by venipuncture, then allowed to clot 30 minutes prior to centrifugation to collect serum samples.
4. Store samples at room temperature no longer than 8 hours. If the assay will not be completed within 8 hours, refrigerate the sample at 2-8°C.
5. If the assay will not be completed within 7 days, freeze at -20°C or lower. Frozen specimens must be mixed well after thawing and prior to testing.
6. Repeated thawing and freezing should be avoided.

SYMBOLS USED ON LABELS

Catalog number

Lot number

For In Vitro Diagnositc Use

Storage temperature


Use by


Consult instructions for use


Number of tests


Manufacturer

Rx Only Caution: Federal law restricts this device to sale by or on the order of a licensed healthcare provider

PROCEDURE

MATERIALS PROVIDED

Part Number	Component	Quantity
C901100	Vibrant COVID-19 Ab Assay Kit	1
C901101	Vibrant COVID-19 96 Pillar Plate	3
C901103	COVID-19 Blocking Buffer	1
C901104	COVID-19 20X Wash Buffer	1
C901105	COVID-19 IgG Negative Control	1
C901106	COVID-19 IgA Negative Control	1
C901107	COVID-19 IgM Negative Control	1
C901108	COVID-19 IgG Cut-off Control	1
C901109	COVID-19 IgA Cut-off Control	1
C901110	COVID-19 IgM Cut-off Control	1

Part Number	Component	Quantity
C901111	COVID-19 IgG Positive Control	1
C901112	COVID-19 IgA Positive Control	1
C901113	COVID-19 IgM Positive Control	1
C901114	COVID-19 Sample Diluent	1
C901115	COVID-19 IgG Conjugate	1
C901116	COVID-19 IgA Conjugate	1
C901117	COVID-19 IgM Conjugate	1
C901118	COVID-19 Chemiluminescence Substrate A	1
C901119	COVID-19 Chemiluminescence Substrate B	1

ADDITIONAL MATERIALS REQUIRED

1. Equipment: Quansys Q-View Imager Pro (Chemiluminescence Imager)
2. Equipment: Hamilton Microlab STAR (Automated liquid handler)
3. Microplate shaker
4. Deionized or distilled water
5. Consumables: 10 µL tips, 100 µL tips, 300 µL tips, 10-100 µL pipettor, 0.5-10 µL pipettor, 100-1000 µL pipettor, and 96 well plate(s) (A black 96 well plate is required for Chemiluminescence imaging)
6. Compressed air / Nitrogen supply
7. 1L container for diluted Wash Buffer
8. Clean test tubes and test tube rack for patient sample dilutions
9. Timer
10. Reagent reservoirs

ASSAY AND OPERATION PROCEDURE

Method before the start of the assay

1. Bring all reagents to room temperature (20-26°C) and mix well prior to use.
2. Dilute the wash buffer from 20x to 1x using DI Water.
3. Prepare a 1:20 dilution of each patient sample by adding 5µL of sample to 95µL of sample diluent. Diluted samples must be used within 8 hours of preparation.
4. DO NOT DILUTE the IgG Conjugate, Cut-off Controls, Positive Controls and Negative Controls.
5. Determination of the presence or absence of COVID-19 antigens requires one well for the cut-off control, one well for positive control, one well for negative control, and one well (or two wells if duplicates are run) for each patient sample.

Assay Procedure

I. Assay Steps

1. The assay steps are repeated across 3 plates (one for IgG, one for IgA and one for IgM). Only the conjugate incubation step will differentiate between the 3 plates. Please note the plate barcode for each individual sub-class tested.
2. In a new 96 well plate, dispense 100ul of blocking buffer into each well.
    a. Incubate with the COVID-19 Ab 96 Pillar Plate for 30 minutes.
3. In a new 96 well-plate, pipette 100ul of 1x wash buffer in the wells which contain the samples, cut-off control and the positive and negative controls.
    a. Wash the pillar plate with 1x wash buffer for 2 mins each. Shake the plate at 300rpm.
4. Aspirate out 100ul of wash buffer. Repeat dispense, incubation and aspirate process for a total of 2 times.
5. In a new 96 well plate, pipette 100ul of COVID-19 Ab calibrators, controls and diluted samples into each well.
    a. Incubate with the COVID-19 Ab 96 Pillar Plate for 30 minutes. Shake the plate at 300rpm.

6. In a new 96 well-plate, pipette 100ul of 1x wash buffer in the wells which contain the samples, cut-off control and the positive and negative controls.
    a. Wash the pillar plate with 1x wash buffer for 2 mins each. Shake the plate at 300rpm.
7. Aspirate out 100ul of wash buffer. Repeat dispense, incubation and aspirate process for a total of 3 times.
8. In a new 96 well plate, add 100ul **COVID-19 Ab IgG** conjugate to each well.
    a. Incubate with the COVID-19 Ab 96 Pillar Plate for 30 minutes. Shake the plate at 300rpm.
9. In a new 96 well plate, add 100ul **COVID-19 Ab IgA** conjugate to each well.
    a. Incubate with the COVID-19 Ab 96 Pillar Plate for 30 minutes. Shake the plate at 300rpm.
10. In a new 96 well plate, add 100ul **COVID-19 Ab IgM** conjugate to each well.
    a. Incubate with the COVID-19 Ab 96 Pillar Plate for 30 minutes. Shake the plate at 300rpm.
11. In a new 96 well-plate, pipette 100ul of 1x wash buffer in the wells.
    a. Wash the pillar plate with 1x wash buffer for 2 mins each. Shake the plate at 300rpm.
12. Aspirate out 100ul of wash buffer. Repeat dispense, incubation and aspirate process for a total of 3 times.
13. In a new 96 well plate, dispense 100ul of DI water into each well.
    a. Incubate with Vibrant COVID-19 Ab 96 Pillar Plate for 1 minute. Shake the plate at 300rpm.
14. Aspirate out 100ul of DI water. Repeat dispense, incubation and aspirate process for a total of 2 times.
15. Dry the COVID-19 Ab 96 pillar plate using compressed air dryer (Nitrogen or argon gas may also be used in this step).
16. Mix COVID-19 Ab Chemiluminescence substrates A and B.
17. In a new 96 black well plate, pipette 100ul of the mix prepared in step 12, in each well.
18. Load the pillar plate into the black well plate and scan the COVID-19 Ab 96 Pillar Plate in the Chemiluminescence imager for 300 seconds.

II. Automated Liquid Handler (Hamilton)

1. The Vibrant COVID-19 Ab assay is performed using an automated liquid handling workstation (Hamilton Microlab STAR).
2. The automated liquid handling workstation is programmed to follow all the steps mentioned above in the assay steps section. A descriptive summary is provided below for using the automated process.
3. All materials including COVID-19 Ab pillar plates, blocking buffer, wash buffer, cut-off controls, controls, patient serum samples, sample diluent, IgG, IgA and IgM conjugates, and Chemiluminescence substrates should be loaded in designated racks and reservoirs as per Hamilton program layout.
4. The workstation consumables including pipette tips, 96-well plates and a black well plate must be loaded before beginning the assay.
5. The software method for the automated instrument is programmed to execute all steps from Step 1 – Step 13.
6. Load the pillar plate into the black well plate and scan the COVID-19 Ab 96 Pillar Plate in the Chemiluminescence imager for 300 seconds.

QUALITY CONTROL

1. Each time the assay is run, the cut-off control, positive and negative controls must be run.
2. The positive and negative controls are intended to monitor for substantial reagent failure.
3. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing at < -20°C.

4. In order for the test results to be considered valid, all of the criteria listed below must be met. If any of these are not met, the test should be considered invalid and the assay repeated.
5. All cut-off controls, positive controls and negative controls must be within the specified range before reporting patient results. For failed controls, the run should be repeated. The value of the prediluted positive control must be greater than the value of the corresponding negative control.

CALCULATION OF RESULTS

The Vibrant COVID-19 Ab assay kit is analyzed using the Vibrant COVID-19 Ab Reporter software. The intensity of each chip in every pillar of the 96 pillar plate is first determined. The reactivity for each sample can then be calculated by dividing the intensity of the sample by the intensity of the corresponding cut-off control. If the reactivity results is <= 1, the result is NEGATIVE and if the reactivity is > 1, the result is POSITIVE. The software automatically interprets the result and is connected to a lab LIS for sample review and approval.

RESULTS INTERPRETATION

The COVID-19 assay is very sensitive to technique and is capable of detecting even small differences in patient populations. The values shown below are suggested values only. Vibrant America has established its cut-off and normal range based on techniques, controls, equipment and patient population according to conventional procedures.

The sample can then be classified as negative (if the calculated value is <=1), and positive (if the calculated unit value is >1). The analysis of the Vibrant COVID-19 Ab assay is performed using the Vibrant COVID-19 Ab software which automatically determines the final result for each antigen tested.

ASSAY LIMITATIONS

1. The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and produce false positives in this assay.
2. Results of this assay should be used in conjunction with clinical findings and other serological tests.
3. Not all disease positive patients are necessarily positive for the COVID-19 antigens.
4. Assay results should not be used as the sole basis for the diagnosis and exclusion of novel coronavirus pneumonia, but only as a supplement to existing viral nucleic acid detection reagents and imaging features.
5. The assay performance characteristics have not been established for matrices other than serum.
6. This test has not been reviewed by the FDA.
7. 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.
8. Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
9. Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
10. Not for the screening of donated blood

CLINICAL SENSITIVITY AND SPECIFICITY

The clinical study tested a panel containing retrospectively collected patient serum samples that were previously confirmed infected / not infected by SARS-CoV-2 RT PCR along with healthy controls (samples collected prior to SARS-CoV-2 outbreak) and other disease controls including Lyme disease, CMV, Hepatitis C, Syphilis, and Celiac disease SLE, and Rheumatoid arthritis.

Vibrant COVID-19 Ab		**Clinical Diagnosis – NP Swab Positive**		**Total**	**Analysis**
		Positive	Controls		**(95% Confidence)**
Overall IgG/IgA/IgM	Positive	34	5	39	Sensitivity = 97.14% (85.47% - 99.50%)
	Negative	1	300	301	Specificity = 98.36% (96.22% - 99.30%)
Total		35	305	340	

Vibrant COVID-19 Ab		Clinical Diagnosis – NP Swab Positive		Total	Analysis (95% Confidence)
		Positive	Controls		
S1 SP IgG	Positive	24	3	27	Sensitivity = 68.57% (52.02% - 81.45%)
	Negative	11	302	313	Specificity = 99.02% (97.15% - 99.67%)
Total		35	305	340	

Vibrant COVID-19 Ab		Clinical Diagnosis – NP Swab Positive		Total	Analysis (95% Confidence)
		Positive	Controls		
S1 SP IgA	Positive	15	1	16	Sensitivity = 42.86% (27.98% - 59.14%)
	Negative	20	304	324	Specificity = 99.67% (98.17% - 99.94%)
Total		35	305	340	

Vibrant COVID-19 Ab		Clinical Diagnosis – NP Swab Positive		Total	Analysis (95% Confidence)
		Positive	Controls		
S1 SP IgM	Positive	28	2	30	Sensitivity = 80.00% (64.11% - 89.96%)
	Negative	7	303	310	Specificity = 99.34% (97.64% - 99.82%)
Total		35	305	340	

Vibrant COVID-19 Ab		Clinical Diagnosis – NP Swab Positive		Total	Analysis (95% Confidence)
		Positive	Controls		
RBD IgG	Positive	23	3	26	Sensitivity = 65.71% (49.15% - 79.17%)
	Negative	12	302	314	Specificity = 99.02% (97.15% - 99.67%)
Total		35	305	340	

Vibrant COVID-19 Ab		Clinical Diagnosis – NP Swab Positive		Total	Analysis (95% Confidence)
		Positive	Controls		
RBD IgA	Positive	18	2	20	Sensitivity = 51.43% (35.57% - 67.01%)
	Negative	17	303	320	Specificity = 99.34% (97.64% - 99.82%)
Total		35	305	340	

Vibrant COVID-19 Ab		Clinical Diagnosis – NP Swab Positive		Total	Analysis (95% Confidence)
		Positive	Controls		
RBD IgM	Positive	21	2	23	Sensitivity = 60.00% (43.57% - 74.45%)
	Negative	14	303	317	Specificity = 99.34% (97.64% - 99.82%)
Total		35	305	340	

Vibrant COVID-19 Ab		Clinical Diagnosis – NP Swab Positive		Total	Analysis (95% Confidence)
		Positive	Controls		
S2 SP IgG	Positive	28	1	29	Sensitivity = 80.00% (64.11% - 89.96%)
	Negative	7	304	311	Specificity = 99.67% (98.17% - 99.94%)
Total		35	305	340	

Vibrant COVID-19 Ab		Clinical Diagnosis – NP Swab Positive		Total	Analysis (95% Confidence)
		Positive	Controls		
S2 SP IgA	Positive	17	0	17	Sensitivity = 48.57% (32.99% - 64.43%)
	Negative	18	305	323	Specificity = 100.00% (98.76% - 100.00%)
Total		35	305	340	

Vibrant COVID-19 Ab		Clinical Diagnosis – NP Swab Positive		Total	Analysis (95% Confidence)
		Positive	Controls		
S2 SP IgM	Positive	31	1	32	Sensitivity = 88.57% (74.05% - 95.47%)
	Negative	4	304	308	Specificity = 99.67% (98.17% - 99.94%)
Total		35	305	340	

Vibrant COVID-19 Ab		Clinical Diagnosis – NP Swab Positive		Total	Analysis (95% Confidence)
		Positive	Controls		
NP IgG	Positive	25	4	29	Sensitivity = 71.43% (54.95% - 83.67%)
	Negative	10	301	311	Specificity = 98.69% (96.68% - 99.49%)
Total		35	305	340	

Vibrant COVID-19 Ab		Clinical Diagnosis – NP Swab Positive		Total	Analysis (95% Confidence)
		Positive	Controls		
NP IgA	Positive	13	1	14	Sensitivity = 37.14% (23.16% - 53.66%)
	Negative	22	304	326	Specificity = 99.67% (98.17% - 99.94%)
Total		35	305	340	

Vibrant COVID-19 Ab		Clinical Diagnosis – NP Swab Positive		Total	Analysis (95% Confidence)
		Positive	Controls		
NP IgM	Positive	24	1	25	Sensitivity = 68.57% (52.02% - 81.45%)
	Negative	11	304	315	Specificity = 99.67% (98.17% - 99.94%)
Total		35	305	340	

Combining all antigens and classes for each antibody, the total clinical sensitivity and specificity are determined as below.

Vibrant COVID-19 Ab		Clinical Diagnosis – NP Swab Positive		Total	Analysis (95% Confidence)
		Positive	Controls		
Overall IgG	Positive	33	5	38	Sensitivity = 94.29% (81.39% - 98.42%)
	Negative	2	300	302	Specificity = 98.36% (96.22% - 99.30%)
Total		35	305	340	

Vibrant COVID-19 Ab		Clinical Diagnosis – NP Swab Positive		Total	Analysis (95% Confidence)
		Positive	Controls		
Overall IgA	Positive	23	3	26	Sensitivity = 65.71% (49.15% - 79.17%)
	Negative	12	302	314	Specificity = 99.02% (97.15% - 99.67%)
Total		35	305	340	

Vibrant COVID-19 Ab		Clinical Diagnosis – NP Swab Positive		Total	Analysis (95% Confidence)
		Positive	Controls		
Overall IgM	Positive	32	4	36	Sensitivity = 91.43% (77.62% - 97.04%)
	Negative	3	301	304	Specificity = 98.69% (96.68% - 99.49%)
Total		35	305	340	

ANALYTICAL PERFORMANCE CHARACTERISTICS

Precision/Reproducibility Study: Two test operators tested a panel of 6 samples, 4 replicates daily over a period of 5 days for a total of 40 data points. Panel consisted of positive control, negative control, positive sample, negative sample, a sample with concentration +20% above cut-off, and a sample with concentration -20% below cut-off. The results are summarized below.

Antigen	Sample	Sample Type	# of Accurate Results	Total # of Results	Reproducibility %
S1 SP IgG	Sample 1	Positive control	120	120	100%
S1 SP IgG	Sample 2	Negative control	120	120	100%
S1 SP IgG	Sample 3	Positive sample	120	120	100%
S1 SP IgG	Sample 4	Negative sample	120	120	100%
S1 SP IgG	Sample 5	20% above cut-off	118	120	98.3%
S1 SP IgG	Sample 6	20% below cut-off	119	120	99.2%
S1 SP IgA	Sample 1	Positive control	120	120	100%
S1 SP IgA	Sample 2	Negative control	120	120	100%
S1 SP IgA	Sample 3	Positive sample	120	120	100%
S1 SP IgA	Sample 4	Negative sample	120	120	100%
S1 SP IgA	Sample 5	20% above cut-off	120	120	100%
S1 SP IgA	Sample 6	20% below cut-off	118	120	98.3%
S1 SP IgM	Sample 1	Positive control	120	120	100%
S1 SP IgM	Sample 2	Negative control	120	120	100%
S1 SP IgM	Sample 3	Positive sample	120	120	100%
S1 SP IgM	Sample 4	Negative sample	120	120	100%
S1 SP IgM	Sample 5	20% above cut-off	117	120	97.5%
S1 SP IgM	Sample 6	20% below cut-off	116	120	96.7%

Antigen	Sample	Sample Type	# of Accurate Results	Total # of Results	Reproducibility %
RBD IgG	Sample 1	Positive control	120	120	100%
RBD IgG	Sample 2	Negative control	120	120	100%
RBD IgG	Sample 3	Positive sample	120	120	100%
RBD IgG	Sample 4	Negative sample	120	120	100%
RBD IgG	Sample 5	20% above cut-off	120	120	100%
RBD IgG	Sample 6	20% below cut-off	118	120	98.3%
RBD IgA	Sample 1	Positive control	120	120	100%
RBD IgA	Sample 2	Negative control	120	120	100%
RBD IgA	Sample 3	Positive sample	120	120	100%
RBD IgA	Sample 4	Negative sample	120	120	100%
RBD IgA	Sample 5	20% above cut-off	120	120	100%
RBD IgA	Sample 6	20% below cut-off	120	120	100%
RBD IgM	Sample 1	Positive control	120	120	100%
RBD IgM	Sample 2	Negative control	120	120	100%
RBD IgM	Sample 3	Positive sample	120	120	100%
RBD IgM	Sample 4	Negative sample	120	120	100%
RBD IgM	Sample 5	20% above cut-off	119	120	99.2%
RBD IgM	Sample 6	20% below cut-off	116	120	96.7%

Antigen	Sample	Sample Type	# of Accurate Results	Total # of Results	Reproducibility %
S2 SP IgG	Sample 1	Positive control	120	120	100%
S2 SP IgG	Sample 2	Negative control	120	120	100%
S2 SP IgG	Sample 3	Positive sample	120	120	100%
S2 SP IgG	Sample 4	Negative sample	120	120	100%
S2 SP IgG	Sample 5	20% above cut-off	120	120	100%
S2 SP IgG	Sample 6	20% below cut-off	120	120	100%
S2 SP IgA	Sample 1	Positive control	120	120	100%
S2 SP IgA	Sample 2	Negative control	120	120	100%
S2 SP IgA	Sample 3	Positive sample	120	120	100%
S2 SP IgA	Sample 4	Negative sample	120	120	100%
S2 SP IgA	Sample 5	20% above cut-off	119	120	99.2%
S2 SP IgA	Sample 6	20% below cut-off	118	120	98.3%
S2 SP IgM	Sample 1	Positive control	120	120	100%
S2 SP IgM	Sample 2	Negative control	120	120	100%
S2 SP IgM	Sample 3	Positive sample	120	120	100%
S2 SP IgM	Sample 4	Negative sample	120	120	100%
S2 SP IgM	Sample 5	20% above cut-off	119	120	99.2%
S2 SP IgM	Sample 6	20% below cut-off	119	120	99.2%

Antigen	Sample	Sample Type	# of Accurate Results	Total # of Results	Reproducibility %
NP IgG	Sample 1	Positive control	120	120	100%
NP IgG	Sample 2	Negative control	120	120	100%
NP IgG	Sample 3	Positive sample	120	120	100%
NP IgG	Sample 4	Negative sample	120	120	100%
NP IgG	Sample 5	20% above cut-off	118	120	98.3%
NP IgG	Sample 6	20% below cut-off	117	120	97.5%
NP IgA	Sample 1	Positive control	120	120	100%
NP IgA	Sample 2	Negative control	120	120	100%
NP IgA	Sample 3	Positive sample	120	120	100%
NP IgA	Sample 4	Negative sample	120	120	100%
NP IgA	Sample 5	20% above cut-off	120	120	100%
NP IgA	Sample 6	20% below cut-off	118	120	98.3%
NP IgM	Sample 1	Positive control	120	120	100%
NP IgM	Sample 2	Negative control	120	120	100%
NP IgM	Sample 3	Positive sample	120	120	100%
NP IgM	Sample 4	Negative sample	120	120	100%
NP IgM	Sample 5	20% above cut-off	120	120	100%
NP IgM	Sample 6	20% below cut-off	120	120	100%

Cross Reactivity Study: The following panel of samples were tested to determine the cross-reactivity/analytical specificity of the Vibrant COVID-19 Ab assay.

Antibody	**Number of samples**	**# of false positives**
Anti-influenza A (IgG, IgA and IgM)	10	0
Anti- influenza B (IgG, IgA and IgM)	10	0
Anti-HCV (IgG, IgA and IgM)	10	0
Anti-HBV (IgG, Ig and IgM)	10	0
ANA	20	0
Anti-respiratory syncytial virus	10	0
Anti-Haemophilus influenzae	5	0

Interference Study: Samples across the assay range were spiked with certain levels of interference agents and the agreement % was calculated. No significant interference was observed for the tested concentrations of interfering agents as given below.

Interference Agent	Concentration Tested
Bilirubin	40 mg/dl
Triglycerides	1000 mg/ml
Hemoglobin	1000 mg/ml
Rheumatoid Factor (RF)	2000 IU/ml
Cholesterol	100 mg/ml
HAMA	12.5 ng/ml
Ribavirin	25 mg/dl
Levofloxacin	0.5 mg/dl
Azithromycin	0.5 mg/dl
Ceftriaxone sodium	25 mg/dl
Oxymetazoline	1.25 mg/dl
Sodium chloride	25 mg/dl
EDTA	12.5 mg/ml
Acetaminophen	50 mg/ml
Ibuprofen	50 mg/ml
Budesonide	1.25 mg/dl

REFERENCES

1. Center for Disease Control and Prevention. https://www.cdc.gov/
2. CDC (2020). Transmission of Novel Coronavirus (COVID-19).
3. Zhou, P., Yang, X., Wang, X. et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature (2020). https://doi.org/10.1038/s41586-020-2012-7.
4. Na Zhu, Ph.D., Dingyu Zhang, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019[J]. New England Journal of Medicine, 2020.
5. Chenjia Yuan , Shi Jinsong , Qiudong An , Liu Chang , Li Xin , Qiang , Ruanji Shou , mountains . Wuhan 2019 Bioinformatics coronavirus genome analysis [J / OL]. Bioinformatics: 1-10 [2020-02-10].
6. Wu, L.-P., Wang, N.-C., Chang, Y.-H., Tian, X.-Y., Na, D.-Y., Zhang, L. - Y., Liang, G.-D. (2007). Duration of Antibody Responses after Severe Acute Respiratory Syndrome. Emerging Infectious Diseases, 13(10), 1562–1564. doi: 10.3201/eid1310.070576
7. Xu, X., Chen, P., Wang, J., Feng, J., Zhou, H., Li, X., Hao, P. (2020). Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Science China Life Sciences*. doi: 10.1007/s11427-020-1637-5

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