ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY VIBRANT COVID-19 AB ASSAY ((VIBRANT AMERICA CLINICAL LABS)

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

(The Vibrant COVID-19 Ab Assay will be performed at Vibrant America Clinical Labs, San Carlos, CA, a laboratory certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a to perform high complexity tests as per Laboratory Instructions for Use that was reviewed by the FDA under this EUA.)

INTENDED USE

The Vibrant COVID-19 Ab assay is a chemiluminescence immunoassay (CLIA) intended for the qualitative detection and differentiation of IgM and IgG antibodies to SARS-CoV-2 in human serum or Dry Blood Spot (DBS) using fingerstick blood specimen collected by their health care provider.

The Vibrant COVID-19 Ab assay is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. Testing is limited to the Vibrant America Lab, San Carlos, CA 94070, that is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the detection of SARS CoV-2 antibodies. IgM and IgG 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 should report all positive results to the appropriate public health authorities as required.

The sensitivity of the Vibrant COVID-19 Ab assay 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 the Vibrant COVID-19 Ab assay may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

The Vibrant COVID-19 Ab assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Product Overview and Testing Principle:

The Vibrant COVID-19 Ab assay is a chemiluminescence immunoassay (CLIA) developed for the qualitative detection and differentiation of IgG and IgM antibodies to SARS-CoV-2 antigens from patients who are suspected of COVID-19. The antigens included in the test are listed in Table 1.

Table 1: Antigens Included in the Vibrant COID-19 Ab Assay

Antigens included in the test	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.

Description of Test:

The assay is performed in two 96 well pillar plates with SARS-CoV-2 antigens, one for detection of IgM antibodies and one for detection of IgG antibodies following the steps described in the laboratory SOP. The purified SARS-CoV-2 antigens listed in Table 1 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 (1 chip for each SARS-CoV-2 antigens and 4 reference chips used in software analysis) using automated semiconductor assembly techniques.

Diluted patient sera or DBS eluates and controls (including positive and negative control) are added to individual wells of each of the two plates allowing the SARS-CoV-2 specific antibodies, if present, to bind to the immobilized antigens. Unbound sample is washed away, and an enzyme labeled anti-human IgG 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. All test steps are performed automatically by the Hamilton Microlab STAR liquid handling workstation that is programmed to follow all the assay steps according to the laboratories SOP.

Dried Blood Spot Process Workflow Overview

1. Once DBS samples are received in the lab, the DBS cards are punched with a 6mm diameter head using Hamilton easyPunch STAR. For each sample, one accepted DBS spot is punched into a 96-well fritted deep well plate as described in the laboratory SOP.
2. COVID-19 assay sample diluent is pipetted (Volume = 380 µL) into the deep well plate.
3. The fritted deep well plate is stacked with a regular 96-well deep well plate. The stacked composition is processed in a standard plate shaker at 650 rpm for 30 minutes followed by centrifugation at 3000xg for 15 minutes.
4. The eluted samples are immediately loaded on the Hamilton Microlab STAR for the regular assay process; 100 µL is used for the IgG assay and 100 µL is used for the IgM assay.

In this process, DBS elution and sample dilution are performed together in the same step. From internal studies, this process provided increased recovery and improved precision compared to DBS elution separately and dilution during assay. The elution/dilution buffer contains a proprietary composition of BSA, PEG 6000, 6-Aminocaproic acid, Ethylenediaminetetraacetic acid and Bovine gamma globulin in phosphate buffered saline. The diluted samples are assayed within 1 hour of DBS elution. If assay is not performed within 1 hour of elution, the samples are discarded and the process from punching is repeated.

COMPONENTS SPECIFIC TO THE TEST

The components of the Vibrant COVID-19 Ab Assay are listed in Table 2.

Table 2: Components Included in the Vibrant COVID-19 Ab Assay

Part Number	Component	Composition
C901200	Vibrant COVID-19 Ab Assay Kit	N/A
C901201	Vibrant COVID-19 96 Pillar Plate IgG	N/A
C901202	Vibrant COVID-19 96 Pillar Plate IgM	N/A
C901203	COVID-19 Blocking Buffer	PBS with protein additive and 0.1% Sodium Azide
C901204	COVID-19 20X Wash Buffer	TBS containing Tween-20
C901205	COVID-19 IgG Negative Control	Pooled negative plasma with preservative and stabilizer
C901206	COVID-19 IgM Negative Control	Pooled negative plasma with preservative and stabilizer
C901207	COVID-19 IgG Calibrator 1[a]	Contains antibodies to all four analytes diluted with pooled negative serum at fixed titer
C901208	COVID-19 IgG Calibrator 2	Contains IgG antibodies to all four analytes diluted with pooled negative serum at fixed titer
C901209	COVID-19 IgG Calibrator 3	Contains IgG antibodies to all four analytes diluted with pooled negative serum at fixed titer

C901210	COVID-19 IgG Calibrator 4	Contains IgG antibodies to all four analytes diluted with pooled negative serum at fixed titer
C901211	COVID-19 IgG Calibrator 5	Contains IgG antibodies to all four analytes diluted with pooled negative serum at fixed titer
C901212	COVID-19 IgM Calibrator 1	Contains IgM antibodies to all four analytes diluted with pooled negative serum at fixed titer
C901213	COVID-19 IgM Calibrator 2	Contains IgM antibodies to all four analytes diluted with pooled negative serum at fixed titer
C901214	COVID-19 IgM Calibrator 3	Contains IgM antibodies to all four analytes diluted with pooled negative serum at fixed titer
C901215	COVID-19 IgM Calibrator 4	Contains IgM antibodies to all four analytes diluted with pooled negative serum at fixed titer
C901216	COVID-19 IgM Calibrator 5	Contains IgM antibodies to all four analytes diluted with pooled negative serum at fixed titer
C901217	COVID-19 IgG Low Positive Control[b]	Contains IgG antibodies to all four analytes diluted with pooled negative serum at fixed titer
C901218	COVID-19 IgM Low Positive Control[b]	Contains IgM antibodies to all four analytes diluted with pooled negative serum at fixed titer
C901219	COVID-19 IgG High Positive Control[b]	Contains IgG antibodies to all four analytes diluted with pooled negative serum at fixed titer
C901220	COVID-19 IgM High Positive Control[b]	Contains IgM antibodies to all four analytes diluted with pooled negative serum at fixed titer
C901221	COVID-19 Sample Diluent	Contains BSA, PEG 600, 6-aminocaproic acid, EDTA and bovine gamma globulin in PBS
C901222	COVID-19 IgG Conjugate	Enzyme labeled Anti-human-IgG conjugate in buffer
C901223	COVID-19 IgM Conjugate	Enzyme labeled Anti-human-IgM conjugate in buffer
C901224	COVID-19 Chemiluminescence Substrate A	N/A
C901225	COVID-19 Chemiluminescence Substrate B	N/A

[a] Calibrator 1 – 5 contain varying levels of antibodies to each analyte to generate a calibration curve for

each assay.
[b] The High- and Low- level IgG and IgM controls have assigned value as described in Table 3 below.

COMPONENTS REQUIRED BUT NOT PROVIDED WITH THE TEST

- Equipment: Quansys Q-View Imager Pro (Chemiluminescence Imager)
- Equipment: Hamilton Microlab STAR (Automated liquid handler)
- Hamilton easyPunch STAR for assays with DBS
- Microplate shaker
- Deionized or distilled water
- 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)
- Compressed air / Nitrogen supply
- 1L container for diluted Wash Buffer
- Clean test tubes and test tube rack for patient sample dilutions
- Timer
- Reagent reservoirs

The Vibrant COVID-19 Ab assay is performed using an automated liquid handling workstation (Hamilton Microlab STAR). The automated liquid handling workstation is programmed by Vibrant COVID-19 Ab Assay Method V1.00.1 to follow all the steps of the assay.

Vibrant COVID-19 Ab Assay Method V1.0.0.1 – The Vibrant COVID-19 Ab Assay Method, a software developed by Vibrant America clinical lab, is programmed and executed only on Hamilton MicroLab STAR liquid handling system for automated processing of the Vibrant COVID-19 Ab Assay. The program loads and scans the pillar plates (Total of 2 plates – One each for IgG and IgM), serum or DBS eluate samples and assay reagents (including assay controls) and saves the barcoded information to the database for traceability. After the assay is completed, the plates are scanned in the Quansys Q-View Imager Pro.

Vibrant COVID-19 Ab Reporter V1.0.0.1 - The Vibrant COVID-19 Ab Reporter is a software for automatically implementing Region of Interest (ROI) processing of scanned biochip image, providing easy-to use GUI for users to analyze image and access test results for each sample. The scanned plate from the Quansys Imager is saved as a TIFF image with the plate barcode saved as the image name. The software analyzes the images and connects with the database to retrieve the sample information associated with the plate barcode and calculates the final results for each sample based on the calibrators and controls for each specific assay. The Vibrant COVID-19 Ab assay software (Vibrant COVID-19 Ab Reporter V1.0.0.1) performs the analysis of the Vibrant COVID-19 Ab assay and determines the final result.

CONTROLS TO BE USED WITH THE VIBRANT COVID-19 Ab Assay

The assay includes the following controls:

a) IgG Low Positive Control
b) IgG High Positive Control
c) IgG Negative Control

d) IgM Low Positive Control
e) IgM High Positive Control
f) IgM Negative Control

All controls must be run with each assay and all controls must pass the acceptance criteria to interpret the patient result**s.** Target values for controls are in arbitrary Chemiluminescence Units (CU). Target values and acceptance criteria for controls are tabulated in Table 3 below.

Table 3: Control Interpretation - Acceptance Criteria

Analyte	Control Type	Target (CU)	Acceptance Range
S1 Spike IgG	Negative	0.28	0.23-0.33
S1 Spike IgG	Low Positive	1.13	1.01-1.25
S1 Spike IgG	High Positive	1.9	1.66-2.14
S1 Spike IgM	Negative	0.54	0.44-0.63
S1 Spike IgM	Low Positive	1.25	1.11-1.39
S1 Spike IgM	High Positive	2	1.78-2.23
RBD IgG	Negative	0.48	0.39-0.57
RBD IgG	Low Positive	1.27	1.12-1.41
RBD IgG	High Positive	1.94	1.72-2.17
RBD IgM	Negative	0.5	0.41-0.58
RBD IgM	Low Positive	1.15	1.02-1.29
RBD IgM	High Positive	1.75	1.56-1.94
S2 Spike IgG	Negative	0.43	0.35-0.5
S2 Spike IgG	Low Positive	1.27	1.12-1.41
S2 Spike IgG	High Positive	2.13	1.87-2.38
S2 Spike IgM	Negative	0.47	0.38-0.55
S2 Spike IgM	Low Positive	1.18	1.05-1.32
S2 Spike IgM	High Positive	1.93	1.7-2.15
NP IgG	Negative	0.46	0.38-0.53
NP IgG	Low Positive	1.3	1.14-1.47
NP IgG	High Positive	1.92	1.68-2.17
NP IgM	Negative	0.55	0.45-0.64
NP IgM	Low Positive	1.17	1.04-1.31
NP IgM	High Positive	1.83	1.62-2.04

Controls are manufactured by diluting pooled human serum containing high titers of COVID-19 antibodies (antibodies to S1 spike protein, Nucleoprotein, RBD and S2 spike protein) with pooled human negative serum. Negative control is pooled human serum negative for COVID-19 antibodies.

RESULTS INTERPRETATION

The Vibrant COVID-19 Ab assay 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 result 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.

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) for all four antigens in the assay, and positive (if the calculated unit value is > 1) for one or more antigens in the assay. The Vibrant COVID-19 Ab assay software (Vibrant COVID-19 Ab Reporter V1.0.0.1) performs the analysis of the Vibrant COVID-19 Ab assay and determines the final result.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

There is no standard reference SARS-CoV-2 antigen material available; accordingly, absolute analytical sensitivity cannot be calculated.

2) Analytical Specificity:

Reactivity/lnclusivity

Although mutations in the SARS-CoV-2 genome have been identified as the virus has spread, no serologically unique strains have been described relative to the originally isolated virus (this research is limited at present).

Analytical Specificity and Microbial Interference:

Class Specificity

The enzyme labeled anti-human IgM and IgG conjugates were shown to be specific for IgM and IgG, respectively, by immunoelectrophoresis.

Cross Reactivity Study

A panel of samples was tested to determine the cross-reactivity/analytical specificity of the Vibrant COVID-19 Ab assay. Results are summarized in Table 4 and indicate that no cross reactivity was observed with antibodies directed towards the tested viruses.

Table 4: Summary of Cross-Reactivity Testing

Antibody	Number of samples	Source/Type	# False Positive
Anti-influenza A (IgG and IgM)	42	Serum	0
Anti- influenza B (IgG and IgM)	26	Serum	0
Anti-HCV (IgG and IgM)	14	Serum	0
Anti-HBV (IgG and IgM)	18	Serum	0
ANA	79	Serum	0

Antibody	Number of samples	Source/Type	# False Positive
Anti-respiratory syncytial virus	10	Serum	0
Anti-Haemophilus influenzae	5	Spiked antibody	0
Anti-229E (alpha coronavirus)	5	Serum	0
Anti-NL63 (alpha coronavirus)	6	Serum	0
Anti-OC43 (beta coronavirus)	6	Serum	0
Anti-HKU1 (beta coronavirus)	5	Serum	0
Adenovirus	4	Serum	0
Coxsackie virus	31	Serum	0
Echovirus	28	Serum	0
Poliovirus	11	Serum	0
Rhinovirus	4	Serum	0
Anti-HIV	10	Serum	0

Interference Study

Samples across the assay range were spiked with certain levels of interference agents and the percent agreement was calculated. Each interference substance is spiked in panel of 5 samples which include 2 positive samples, 1 negative sample, a sample with concentration +20% above cut-off and a sample with concentration -20% below cut-off with 3 replicates. The spiking was performed in a ratio of 5% interfering substance to 95% serum to achieve the required concentration of the corresponding interfering substance. Both serum and DBS were evaluated. Recovery of the sample when spiked with the interfering substance was between 90% to 110% for all analytes tested. No significant interference was observed for the tested concentrations of interfering agents as given in Table 5.

Table 5: List of Potentially Interfering Agents

Interference Agent	Concentration
Bilirubin	40 mg/dL
Triglycerides	1000 mg/mL
Hemoglobin	1000 mg/ mL
Rheumatoid Factor	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

3) Analytical Performance Precision/Reproducibility Study:

The precision studies were performed according to CLSI EP12-A2 User Protocol for Evaluation of Qualitative Test Performance, second edition. Two test operators tested a panel of 6 samples, 4 replicates daily over a period of 5 days. The 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 total % Coefficient of Variation for precision as well as lot to lot reproducibility was < 15%. The results met the acceptance criteria for Lot-to-Lot and Operator-to-Operator studies. Since the testing will be done only at Vibrant America Clinical Lab no site to site variable was evaluated.

4) Matrix Equivalency:

The only matrices used for the Vibrant COVID-19 Ab Assay are serum and Dry Blood Spot (DBS). The clinical evaluation was performed using these two matrices. No additional matrix equivalency study was therefore performed.

5) High-dose Hook Effect

The sponsor stated that potential hook effect has been designed out by the assay format. Excess analyte is washed out of the reaction prior to the addition of detection conjugate. There is no potential for a high dose hook due to assay design.

6) Clinical Evaluation:

<u>Serum Testing</u>

The clinical study tested a panel of retrospectively collected serum samples from patients who were previously confirmed as SARS-CoV-2 infected and not infected, respectively, by SARS-CoV-2 RT PCR (FDA authorized) on a nasopharyngeal swab specimen of the patient. Serum specimens for the antibody test were collected 4 – 26 days (median = 14 days) after the NP swab test. In addition, SARS-CoV-2 negative samples from healthy controls collected prior to SARS-CoV-2 outbreak in the US population were tested.

The overall performance of the Vibrant COVID-19 Ab Assay is reported in Table 6 below based on the combined IgM and IgG result of the test. In addition, results for the individual antibody isotypes detected by Vibrant COVID-19 assay, i.e., IgG and IgM, are described in Tables 7 and 8 below demonstrating the ability of the assay to detect both antibody isotypes with similar performance.

Table 6: Clinical Performance of the Vibrant COVID-19 Ab Assay in Serum - Results Based on the Combined Results of the IgG and the IgM Assay Component of the Test

SERUM		**Clinical Diagnosis – NP Swab (RT-PCR) or Prior to Outbreak**		**Total**	**Analysis (95% Confidence)**
		Positive	Negative		
Vibrant COVID-19 Ab assay **Combined IgG/IgM**	Positive	52	7	59	Positive Percent Agreement (PPA) = **98.11%** (90.06% - 99.67%)
	Negative	1	494	495	Negative Percent Agreement (NPA) = **98.60%** (97.14% - 99.32%)
Total		53	501	554	

Table 7: Clinical Performance of the Vibrant COVID-19 Ab Assay in Serum - Results of the IgG Assay Component of the Test

SERUM		**Clinical Diagnosis – NP Swab (RT-PCR) or Prior to Outbreak**		**Total**	**Analysis (95% Confidence)**
		Positive	Negative		
Vibrant COVID-19 Ab assay **IgG**	Positive	51	7	58	PPA = **96.96%** (87.25% - 98.96%)
	Negative	2	494	496	NPA = **98.60%** (97.14% - 99.32%)
Total		53	501	554	

Table 8: Clinical Performance of the Vibrant COVID-19 Ab Assay in Serum - Results of the IgM Assay Component of the Test

SERUM		**Clinical Diagnosis – NP Swab (RT-PCR) or Prior to Outbreak**		**Total**	**Analysis (95% Confidence)**
		Positive	Negative		
Vibrant COVID-19 Ab assay **IgM**	Positive	49	6	55	PPA = **92.45%** (82.14% - 97.03%)
	Negative	4	495	499	NPA = **98.80%** (97.41% - 99.45%)
Total		53	501	554	

The antibody results for the SARS-CoV-2 PCR positive specimens stratified based on the time in days between NP swab RT-PCR test results and the time serum was collected for the antibodies to SARS-CoV-2 antigens are presented in Table 9.

Table 9: Clinical Performance of the Vibrant COVID-19 Ab Assay in Serum Stratified by the Time between Nasopharyngeal Swab and IgM/IgG Seropositivity

Days after NP swab RT-PCR test	**# of samples**	**Combined (IgG + IgM) PPA**	**IgG PPA**	**IgM PPA**
< 7 days	7	85.71%	71.43%	85.71%
7 –14 days	20	100%	100%	100%
> 14 days	26	100%	100%	88.46%

Dried Blood Spot Testing

Patient matched DBS/serum pairs were prospectively collected from 53 RT-PCR (FDA authorized) positive and 105 RT-PCR negative individuals as described above for the serum study. Serum samples were collected by a licensed phlebotomist at the draw site. The dried blood spots by fingerstick method were collected within 2 days of the serum draw by a health care provider. The samples were received at the testing site and tested to evaluate the comparability of the serum and the DBS sample.

Clinical performance of the dried blood spot specimen is summarized in Table 10 below for the combined IgM/IgG result of the test and for the individual immunoglobulin isotypes in Tables 11 and 12. PPA and NPA of the Vibrant COVID-19 Ab assay was calculated as indicated in the Tables. Finally, Table 13 demonstrates that, similar to the performance in serum, most DBS specimens are positive for both IgG and IgM.

Table 10: Clinical Performance of the Vibrant COVID-19 Ab Assay in DBS - Results Based on the Combined Results of the IgG and the IgM Assay Component of the Test

DBS		**Clinical Diagnosis – NP Swab (RT-PCR)**		**Total**	**Analysis (95% Confidence)**
		Positive	Negative		
Vibrant COVID-19 Ab assay **Combined IgG/IgM**	Positive	52	1	53	PPA = **98.11%** (90.06% - 99.67%)
	Negative	1	104	105	NPA = **99.05%** (94.80% - 99.83%)
Total		53	105	158	

Table 11: Clinical Performance of the Vibrant COVID-19 Ab Assay in DBS - Results Based on the IgG Component of the Test

DBS		**Clinical Diagnosis – NP Swab (RT-PCR)**		**Total**	**Analysis (95% Confidence)**
		Positive	Negative		
Vibrant COVID-19 Ab assay **IgG**	Positive	51	1	52	PPA = **96.23%** (87.25% - 98.96%)
	Negative	2	104	106	NPA = **99.05%** (94.80% - 99.83%)
Total		53	105	158	

Table 12: Clinical Performance of the Vibrant COVID-19 Ab Assay in DBS - Results Based on the IgM Component of the Test

DBS		**Clinical Diagnosis – NP Swab (RT-PCR)**		**Total**	**Analysis (95% Confidence)**
		Positive	Negative		
Vibrant COVID-19 Ab assay **IgM**	Positive	49	1	50	PPA = **92.45%** (82.14% - 97.03%)
	Negative	4	104	108	NPA = **99.05%** (94.80% - 99.83%)
Total		53	105	158	

Table 13: Distribution of Seropositivity in DBS

Antibody Type	**Total**
IgG Only	3
IgM Only	1
IgG/IgM	48
Total Antibody Positive	52

Comparison of Serum and DBS

Since matched serum and DBS samples were collected, the agreement between serum and DBS was calculated as shown in Table 14 and 15. The agreement between serum and DBS was calculated as shown in Tables 14 and 15.

Table 14: Comparison of DBS and Serum Results

Combined IgG/IgM		**Vibrant COVID-19 Ab (Serum)**		**Total**	**Analysis (95% Confidence)**
		Positive	Negative		
Vibrant COVID-19 Ab (DBS)	Positive	52	0	52	PPA = 100.00% (93.12% - 100.00%)
	Negative	0	106	106	NPA = 100.00% (96.50% - 100.00%)
Total		52	106	158	

Table 15: Comparison of Serological Results between DBS and Serum based on the combined IgM/IgG data of the Vibrant COVID-19 Ab Kit

Analyte (Serum vs DBS)	Slope	Y-Intercept	R^2	Serum Pos	Serum Neg	DBS Pos	DBS Neg	Agreement (%)
S1 SP IgG	1.001	0.003	0.98	41	117	41	11	100%
S1 SP IgM	0.999	-0.007	0.98	44	114	44	11	100%
RBD IgG	1.004	-0.012	0.97	35	123	35	12	100%
RBD IgM	1.011	-0.015	0.97	43	115	43	11	100%
S2 SP IgG	1.008	-0.009	0.97	46	112	46	11	100%
S2 SP IgM	0.988	0.009	0.98	46	112	46	11	100%
NP IgG	0.991	0.009	0.98	39	119	39	11	100%
NP IgM	0.993	0.009	0.97	37	121	37	12	100%

Other studies to support DBS:

Selection of card of DBS collection: Three different filter paper were evaluated for the use as DBS collection card. They were compared using a panel of 50 DBS samples which includes 10 high positive samples, 25 samples with concentration ± 20% near cut-off, and 15 negative samples were tested for each card to evaluate the performance. Although the performance of all three cards were comparable, Whatman 903 card was selected for DBS collection due to better market availability.

DBS Drying time: The drying time was evaluated using 10 DBS cards for each time point from 30 min to 3 hours. Acceptance criteria was that the circular DBS spots are completely filled with blood saturating the area and no visible blood spot contamination on the flap of the DBS card. The spots are not wet. The drying of the DBS card at room temp. for 2 hours before shipping was chosen for drying the collected DBS specimens on the Whatman 903 card.

DBS Shipping Stability: A panel of 50 DBS samples which includes 10 high positive samples, 25 samples with concentration ± 20% near cut-off, and 15 negative samples were tested for stability study after cycling through the following conditions (Table 16) as per International Safe Transit Association (ISTA) Procedure 7E (*Testing Standard for Thermal Transport Packaging Used in Parcel Delivery System Shipment*) and Procedure 7D (*Temperature Test for Transport Packaging*).

Table 16: Shipping Results

Temperature	Cycle Period	Cycle Period (hrs)	Total Time (hrs)
40°C	1	8	8
22°C	2	4	12
40°C	3	2	14
30°C	4	36	50
40°C	5	6	56

This study demonstrated that the antibody recovery from the DBS cards cycled through the above simulated shipping conditions for IgM and IgG for all five antigens was acceptable. These data

demonstrate that the DBS specimens can withstand the simulated shipping condition stress.

Warnings:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA only for use by the authorized laboratory, Vibrant America Clinical Labs;
- This test has been authorized only for the presence of antibodies against SARS-CoV-2, not for any other viruses or pathogens; and 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.