Diagnostics Biochem Canada Inc.
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Since 1973

Anti-SARS-CoV-2 IgG ELISA Kit

REF	CAN-IGG-19	Version: 18.0/CANADA (DRAFT) Effective: June 13, 2022

INTENDED USE

The DBC Anti-SARS-CoV-2 IgG kit is a qualitative ELISA test intended for the detection of IgG antibodies to SARS-CoV-2 in human serum or K2/K3 EDTA plasma from the adult population. The DBC Anti-SARS-CoV-2 IgG kit is intended for use as an aid in identifying individuals with an adaptive immune response due to SARS-CoV-2 infection (indicating recent or prior infection) and to vaccination with a vaccine that targets the full-length spike protein or RBD region of the spike protein.
The Anti-SARS-CoV-2 IgG ELISA kit is intended for use by trained laboratory personnel and is for laboratory use only.

LIMITATIONS RELATED TO THE INTENDED USE

1. This assay is not intended to be used for screening patients or as an aid for diagnosis of patients with suspected COVID-19 infection.
2. This assay is not intended for home testing (or self-testing).
3. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions.
4. Negative results must be combined with clinical observations, patient history, and epidemiological information.
5. False negative results can occur in elderly and immunocompromised patients.
6. False positive results for IgM and IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes.
7. The performance of this device to detect an antibody response following COVID-19 vaccination was assessed on individuals who had received the Moderna or Pfizer-BioNTech mRNA COVID-19 vaccine, which targets the full-length spike protein. The performance towards other vaccines that target the full-length spike protein or RBD region of the spike protein is expected to be similar, however, it has not been evaluated.
8. When testing samples for an antibody response to vaccination, it is recommended to wait for at least 2 weeks following the administration of the final vaccine dose.
9. The detection of antibodies with this device following vaccination does not confirm that an individual is protected with neutralizing antibodies or any other means of immunity towards SARS-CoV-2.
10. This test identifies antibodies to the spike protein of the SARS-CoV-2 virus and is therefore unable to distinguish between previously infected individuals and vaccinated individuals.
11. The performance of the device has not been assessed on specimens from individuals who have been infected with variants of SARS-CoV-2, including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (P.1.617.2) and Omicron (B.1.1.529).
12. The performance of this device was not assessed on immunocompromised patients who had received Moderna or Pfizer COVID-19 vaccines.
13. This device should not be used to evaluate the magnitude of the immune response developed in response to vaccination.

PRINCIPLE OF THE TEST

Diluted serum or plasma specimens are added to wells coated with SARS-CoV-2-derived antigens. If IgG antibodies specific for SARS-CoV-2 are present in the specimen, they bind to the SARS-CoV-2 antigens attached to the well, forming stable antigen/antibody complexes. The microplate wells are then washed and an anti-human IgG antibody-horseradish peroxidase (HRP) conjugate is added. If the antigen/antibody complex is present, the HRP conjugate binds to the complex and remains in the well.
After a second wash step, the TMB substrate is added, which reacts with the HRP conjugate to produce a blue colour that is more intense than the colour produced in samples that do not contain any anti-SARS-CoV-2 IgG antibodies.
The stopping solution is added to each well to terminate the reaction between the HRP-conjugate and the TMB substrate. The optical density (OD) in the wells is measured with an absorbance micro-plate reader at 450 nm.

CLINICAL BACKGROUND

Serological assays (antibody tests) for SARS-CoV-2 play a significant role in evaluating the virus epidemiology in the general population regardless of the individuals' history of COVID-19 symptoms. They are an important tool in monitoring and responding to the COVID-19 pandemic and may be helpful for the identification of suspected patients with negative RT–PCR results and asymptomatic infections. However, at this moment it is unclear if the presence of anti-SARS-CoV-2 antibodies in blood can determine if an individual is immune and for how long those antibodies remain in blood [1]. Immunoglobulins against other viruses have been found in blood years after the infection [2].
The present ELISA kit enables for the determination of anti-SARS-CoV-2 IgG antibodies in serum and plasma. IgG antibodies are the most abundant class of antibodies. They typically appear in blood between one and three weeks after the infection [3] and remain in blood for a longer period of time than other immunoglobulin classes. In a study involving 285 patients with COVID-19, 100% of the individuals tested positive for anti-viral IgG within 19 days after the infection and the titer plateau was within 6 days after seroconversion [4]. Another study involving 80 patients with PCR-confirmed COVID-19, reports that the seroconversion rate for IgG was 93.8% with a median seroconversion time of 18 days post exposure or ten days post onset [5].
The antigen used in the DBC Anti-SARS-CoV-2 ELISA kit comprises the RBD region of the spike protein which is currently a target of several vaccines against SARS-CoV-2 [6].

PROCEDURAL CAUTIONS AND WARNINGS

1. This assay is for use by trained laboratory personnel. For laboratory in vitro use only.
2. Practice good laboratory practices (GLP) when handling kit reagents and specimens. This includes:
   - Do not pipette by mouth.
   - Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
   - Wear protective clothing and disposable gloves.
   - Wash hands thoroughly after performing the test.
   - Avoid contact with eyes; use safety glasses. In case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Avoid microbial contamination of reagents.
5. Do not use the kit beyond the expiry date stated on the label.
6. If the kit reagents are visibly damaged, do not use the test kit.
7. Do not reuse the microplate wells, they are for SINGLE USE only.
8. The controls (included in kit) must be included in every run and their results must fall within the specified ranges as stated in the QC certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing or im- proper reagent storage.
9. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
10. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
11. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
12. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
13. The TMB substrate is sensitive to light and must remain colourless if properly stored. Instability or contamination may be indicated if it is a blue colour prior to being used, in which case it must not be used.
14. When dispensing the TMB substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
15. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample and control.
16. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
17. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
18. This kit contains 1 M sulfuric acid in the stopping solution com-ponent. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
19. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or biocontaminated solutions.
20. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.

LIMITATIONS

1. This assay should be used in conjunction with the testing strategy outlined by public health authorities in your area.
2. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. IgM antibodies may not be detected in the first few days of infection; the sensitivity of the test early after infection is unknown.
3. Results are for the detection of SARS-CoV-2 antibodies. IgM antibodies to SARS-CoV-2 are generally the first type of anti-bodies detectable in blood several days after initial infection, although levels over the course of infection are not well characterized. IgG antibodies to SARS-CoV-2 become detectable later following infection. **At this time, it is unknown how long IgM or IgG antibodies may persist following infection**.
4. Positive results for both IgG and IgM could occur after infection and can be indicative of acute or recent infection and successful immune response to a vaccine, once developed.
5. False positive results for IgM and IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes.
6. The presence of specific antibodies are a sign of previous or current infection and can also be used to determine the efficacy of treatment.
7. Laboratories are required to report all positive results to the appropriate public health authorities.
8. This assay has been validated for the use of human serum and EDTA plasma. The use of any other types of specimens of human or animal origin has not been validated.
9. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored specimens.
10. Samples or control materials containing azide or thimerosal are not compatible with this kit as they may lead to false results.
11. IgG antibodies may not be detected in the first few days of infection; the sensitivity of the test early after infection is unknown.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDS

The kit reagents should be considered a potential biohazard and handled with the same precautions applied to blood specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.
The Negative Control contains human plasma that has been tested by approved methods and found to be negative for the presence of HBsAg and antibodies to HCV, HIV 1/2 and HIV NAT. However, no test method can offer complete assurance that any viable pathogens are absent. Therefore, this component should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen, following good laboratory practices.

CHEMICAL HAZARDS

Avoid contact with any of the kit reagents and specimens. Specific-ally avoid contact with the TMB substrate (contains tetramethyl-benzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water. Tetramethylbenzidine is a suspected carcinogen.

Sulfuric Acid Warning
The Stopping Solution contains 1M sulfuric acid. Contact with skin, eyes, and other mucous membranes should be avoided. In case of accidental contact, rinse with large amounts of water and seek medical attention.

Risk Phrases
R 36/38 Irritating to eyes and skin.

Safety Phrases
S 26 In case of contact with eyes rinse immediately with plenty of water and seek medical advice. Please refer to the SDS for further information.

SPECIMEN COLLECTION, STORAGE & STABILITY

Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored specimens.

Serum
Approximately 0.05 mL of serum is required per test. Collect 4–5 mL of venous blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

Plasma (K2/K3 EDTA)
Approximately 0.05 mL of plasma is required per test. Collect 4–5 mL of venous blood into an appropriately labelled K2/K3 EDTA tube. Centrifuge and carefully remove the plasma layer. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

Specimen Stability
Samples may be stored at room temperature for up to 2 days, at 2–8°C for up to 8 days and at -20°C or lower for up to 1 month. Specimens may be more stable than indicated. The specimen stability was assessed using previously frozen specimens, and the stability of fresh specimens is unknown.

Diluted specimens (1:101 dilution used in the test) are stable for up to 8 hours after preparation if stored at 2–8°C. Do not store diluted specimens beyond this time limit or at different temperatures.

SPECIMEN PRE-TREATMENT

All serum and plasma specimens must be diluted 1:101 in the provided Sample Dilution Buffer before being used in the test. Follow the specimen pre-treatment procedure as stated below for each specimen that is to be tested:

1. Pipette 1 mL of the Sample Dilution Buffer into a new polypropylene microcentrifuge tube or HDPE tube.
2. Pipette 10 µL of the serum or plasma specimen into the tube from step 1 that contains 1 mL of sample dilution buffer.
3. Close the tube and label with specimen identification information.
4. Mix the contents of the tube by vortexing.

Diluted specimen samples must be used within 8 hours after preparation. Do not store diluted specimens for use at a later time.

Do not dilute or pre-treat the provided positive and negative controls; they are provided in a ready to use format and do not require dilution.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Calibrated single-channel pipettes to dispense 10 µL, 80 µL, and 1 mL.
2. Calibrated multi-channel pipettes to dispense 50 µL, 80 µL, 150 µL.
3. Calibrated multi-channel pipettes to dispense 350 µL (for manual washing only).
4. Disposable pipette tips.
5. Polypropylene or HDPE tubes for sample dilution (e.g. polypropylene microcentrifuge tubes).
6. Distilled or deionized water.
7. Absorbance microplate reader with a 450 nm filter and an upper OD limit of 3.0 or greater.
8. Automatic microplate washer (recommended).

REAGENTS PROVIDED

1. SARS-CoV-2 Antigen-Coated Microplate – Ready To Use

Contents:	One SARS-CoV-2 recombinant antigen-coated 96-well (12x8) microplate in a resealable pouch with desiccant.
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

2. Anti-Human IgG-Horseradish Peroxidase (HRP) Conjugate – Ready To Use

Contents:	One bottle containing Anti-Human IgG-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume:	12 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

3. Positive Control – Ready To Use

Contents:	One bottle containing a human anti-SARS-CoV-2 IgG antibody diluted in a protein-based buffer with a non-mercury preservative.
Format:	Ready to Use
Volume:	1.0 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

4. Negative Control – Ready To Use

Contents:	One bottle containing negative human plasma diluted in a protein-based buffer with a non-mercury preservative.
Format:	Ready to Use
Volume:	1.0 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

5. Sample Dilution Buffer – Ready To Use

Contents:	One bottle containing a protein-based buffer with a non-mercury preservative.
Volume:	120 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

6. Wash Buffer Concentrate – Requires Preparation **X20**

Contents:	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume:	50 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.
Preparation of Working Wash Buffer:	To prepare the working wash buffer that is used for washing the microplate, dilute the wash buffer concentrate 1:20 in distilled or deionized water before use. The wash buffer is provided as a 20x concentrate, if crystallization occurs in the concentrated buffer, place the bottle in a warm water bath (no more than 40°C) until all crystals dissolve. Mix bottle well before use. If the whole plate is to be used, add 50 mL of the wash buffer concentrate to 950 mL of deionized or distilled water.

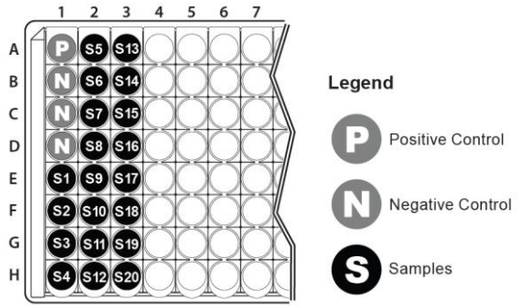
7. TMB Substrate – Ready To Use

Contents:	One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Volume:	18 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

8. Stopping Solution – Ready To Use

Contents:	One bottle containing 1M sulfuric acid. **Warning**
Volume:	10 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

ASSAY LAYOUT



ASSAY PROCEDURE

All human serum and plasma specimens that will be tested must be diluted 1:101 prior to use (refer to Specimen Pre-Treatment section). Do not dilute the positive and negative controls.

All kit components, controls and specimen samples must reach room temperature prior to use. Once the procedure has been started, all steps should be completed without interruption.

1. After all kit components have reached room temperature, mix gently by inversion.
2. Prepare the working wash buffer. See the *Reagents Provided* section, *6. Wash Buffer Concentrate*.
3. Prepare all specimen samples that will be tested (1:101 dilution). Refer to the *Specimen Pre-Treatment* section.
4. Plan the microplate wells to be used for the positive control, negative control and specimen samples. See *Assay Layout* section.

   Remove the strips that will not be used from the microplate frame and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator**.**
5. Pipette 80 µL of the Positive Control into well A1 and pipette 80 µL of the Negative Control into each of 3 wells (B1,C1, D1).
6. Pipette 80 µL of each 1:101 diluted specimen samples into individual wells (E1, F1, G1, H1, etc.), using a new pipette tip for each sample.
7. Incubate the microplate at room temperature for **30 minutes**. Do not shake, leave uncovered and avoid intense light and air currents.
8. Wash the wells either with an automatic microplate washer (preferred) or manually as stated below.

   Automatic: Using an automatic microplate washer, wash the wells 5 times with working wash buffer, using 350 µL/well for each wash. Following washing, tap the plate firmly against absorbent paper to ensure that wells are dry.

   Manually: Briskly empty the contents of the wells over a waste container. Using a multi-channel pipette, add 350 µL of working wash buffer into each well. Briskly empty the contents of the wells over a waste container. Repeat the same above pipetting and emptying steps 4 more times. After the final time, tap the plate firmly against absorbent paper to ensure that wells are dry.
9. Pipette 80 µL of the Anti-Human IgG-Horseradish Peroxidase (HRP) Conjugate into each well (the use of a multi-channel pipette is recommended).
10. Incubate the microplate at room temperature for **30 minutes**. Do not shake, leave uncovered and avoid intense light and air currents.
11. Wash the wells again as stated in step 8.
12. Pipette 150 µL of TMB Substrate into each well (the use of a multi-channel pipette is recommended).
13. Incubate the microplate at room temperature for **20 minutes**. Do not shake, leave uncovered and avoid intense light and air currents.
14. Pipette 50 µL of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB substrate. Gently tap the microplate frame to mix the contents of the wells.
15. Measure the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the stopping solution.

INTERPRETATION OF RESULTS

1. Calculate the Negative Control Mean Optical Density Value (NC)

The negative control mean optical density value (NC) is determined by calculating the mean OD value based on the three individual OD values obtained for the Negative Control wells, according to the formula stated below:

$$NC = \frac{\text{Sum of the three Negative Control OD Values}}{3}$$

Example:

Well	OD (450 nm)
B1	0.135
C1	0.130
D1	0.143

Sum	
	0.135
	0.130
+	0.143
	0.408

$$NC = \frac{0.408}{3} = 0.136$$

2. Calculation of the Cut-Off (CO)

The Cut-Off (CO) is calculated by multiplying the NC value by a factor of 1.5, according to the formula stated below.
Cut-Off (CO) = NC x **1.5**

Example:

NC = 0.136

Cut-Off (CO) = 0.136 x 1.5 = 0.204

3. Determine the Validity of the Assay

One replicate of the Positive Control and three (3) replicates of the Negative Control must be included in each run. The control results must be examined before the sample results can be interpreted.

- The Negative Control mean absorbance (NC) must meet the criteria as stated in the QC certificate. If the criteria is not met, the run should be repeated.
- The Positive Control absorbance value must meet the criteria as stated in the QC certificate. If the criteria is not met, the run should be repeated.
- The Positive Control ratio must meet the criteria as stated in the QC certificate. If the criteria is not met, the run should be repeated.

The Positive Control Ratio is calculated as follows:

$$\text{Positive Control Ratio} = \frac{\text{Positive Control OD}}{\text{Cut-Off (CO)}}$$

4. Calculate the Ratio for Specimen Samples

For each specimen sample, a ratio is calculated based on the absorbance value of the sample in relation to the Cut-Off (CO). Calculate the ratio for each specimen sample according to the formula below:

$$\text{Ratio} = \frac{\text{OD of specimen sample}}{\text{Cut-Off (CO)}}$$

5. Interpretation of the Specimen Results

DBC recommends that the specimen results be interpreted as follows based on the calculated ratio:

Ratio ≤ 1.0 : **Negative** for Anti-SARS-CoV-2 IgG antibodies
Ratio ≥ 1.2 : **Positive** for Anti-SARS-CoV-2 IgG antibodies
Ratio > 1.0 to < 1.2 : **Borderline Result**

To further evaluate borderline results, it is recommended to draw a new serum or plasma sample from the patient after one or two weeks from the initial test date and then retest the new sample with this kit.

PERFORMANCE CHARACTERISTICS

INTERFERENCE

No significant interference was observed for concentrations up to 2g/L for haemoglobin, 20 mg/dL for Bilirubin (conjugated and unconjugated), 5.0 mg/mL for triglycerides, 1.2 µg/mL for HAMAS, 2.4 µg/ mL for Biotin, 1500 IU/mL for Rheumatoid Factor, 4.0 g/dL for protein, 18 mg/mL for total IgG, 2.5 mg/mL for total IgM and 4.5 mg/mL for total IgA.
Interference was observed when samples were spiked with an additional 8 g/dL of protein.

SPECIFICITY

Cross-Reactivity

The cross-reactivity of the Anti-SARS- CoV-2 IgG ELISA was evaluated against samples that contained antibodies towards the specific pathogens listed in the table below. The overall cross-reactivity showed the following results:

Type of Pathogen Antibody	Anti-SARS-CoV-2 ELISA (IgG)		
	Number Tested	Negative	Percent Negatives
Adenovirus IgM	1	1	100
Adenovirus IgG	80	80	100
Anti-Hanta-Viren-Pool 1 "Eurasia" IgG	2	2	100
Antinuclear Antibodies (ANA)	13	12	92
Borrelia IgG	10	10	100
Chlamydia pneumoniae IgA	9	9	100
Chlamydia pneumoniae IgG	10	10	100
Chlamydophila pneumoniae IgG	2	2	100
Chlamydophila pneumoniae IgM	2	2	100
Coronavirus 229E Nucleocapsid protein IgG	5	5	100
Coronavirus OC43 Nucleocapsid protein IgG	5	5	100
Coronavirus HKU1 Nucleocapsid protein IgG	5	5	100
Coronavirus NL63 Nucleocapsid protein IgG	5	4	80
Cytomegalovirus (CMV) IgG	84	84	100
Enterovirus IgG	17	17	100
Enterovirus IgM	1	1	100
Eppstein-Barr-Virus-Capsid-Antigen IgG	86	86	100
Epstein-Barr Virus (infectious mononucleosis) IgG	1	1	100
Epstein-Barr Virus Epstein-Barr Nuclear Antigen (EBV: EBNA) IgG	17	17	100
Haemophilus influenzae B IgG	82	82	100
Haemophilus influenzae IgG	44	44	100
Hepatitis B surface Antigen (HBs)	5	5	100
Hepatitis C Virus (HCV)	12	11	92
Herpes Simplex Virus (HSV) IgG	82	78	95
Human Metapneumovirus (hMPV) IgG	5	4	80
Influenza A IgM	5	5	100
Influenza A IgG	94	94	100
Influenza B IgG	90	90	100
Influenza due to other identified influenza virus with pneumonia	3	3	100
Influenza due to unidentified influenza virus with pneumonia	7	7	100
Measles IgG	71	70	99
Mumps IgG	80	80	100
Mycoplasma pneumoniae IgA	10	9	90
Mycoplasma pneumoniae IgG	31	30	97
Parainfluenza 1 – 4 IgG	5	5	100
Parainfluenza 1/2/3 IgG	89	89	100
Parvovirus B19 IgG	94	94	100
Respiratory Syncytial Virus (RSV) IgG	90	90	100
Rheumatoid Factor (RF) IgG	10	10	100
Rhinovirus IgG	5	5	100
Varicella Zoster Virus (VZV / HHV-3) IgG	7	7	100
Varicella zoster virus IgG	49	49	100

Negative samples were spiked separately with two different human anti-HIV-1 IgG antibodies, up to 1000 µg/mL. There was no cross-reactivity observed with the antibodies tested.

Antibody Class Specificity

No cross-reactivity was observed with anti-SARS-CoV-2 IgM and IgA antibody classes when spiked to 0.1 mg/mL.

MATRIX EQUIVALENCE

Matrix equivalence was performed with 48 pairs of positive samples determined by PCR and/or serology tests and 30 pairs of negative samples obtained before November of 2019. Serum and plasma was drawn and prepared into EDTA plasma and serum simultaneously from each donor.
The correlation analysis between serum and plasma samples resulted in the following results:
Regression equation: y = 0.9654x – 0.0385, x = plasma, y = serum
Slope = 0.97, r = 0.995

HIGH-DOSE HOOK EFFECT

Positive serum samples were diluted from 1:101 (normal dilution) down to 1:2 and tested. The results are summarized in the table below.

Sample ID	SP1		SP2		SP3	
Sample Dilution	OD (450 nm)	Ratio	OD (450 nm)	Ratio	OD (450 nm)	Ratio
1:2	3.430	14.03	3.489	14.27	3.323	13.59
1:5	3.280	13.42	3.186	13.03	3.233	13.22
1:20	2.742	11.21	2.216	9.06	2.626	10.74
1:101	1.437	5.88	0.715	2.92	0.989	4.04

As each sample was diluted less (amount of antibody increased), there was no observable decrease in OD or plateau effect. Therefore, no observable high-dose hook effect was observed.

PRECISION

The precision study was performed according to EP5-A3. Three positive serum samples and three negative samples (collected before November 2019) were used and run each time in duplicate for each test.
The protocol used a nested components-of-variance design (5 x 8 x 2 design) with 5 testing days, 8 runs per day and two replicates per run. In this study were involved four scientists, two lots of the kit, and two microplate readers. In total, the study included eight runs per testing day (two runs per operator), and two replicate measurements per run for each sample (total of 80 results per sample).

The results were analysed with a two-way nested ANOVA and are summarized in the table below.

Sample	Ratio Mean	Overall Result	Between Test, CV%	Between Run, CV%	Between Day, CV%
1 (negative)	0.82	NEG	7.5%	0.0%	4.1%
2 (negative)	0.68	NEG	7.7%	4.1%	0.0%
3 (negative)	0.63	NEG	8.6%	2.9%	9.1%
4 (positive)	5.19	POS	8.3%	2.6%	3.6%
5 (positive)	1.93	POS	7.5%	2.9%	10.1%
6 (positive)	7.88	POS	8.8%	2.1%	10.1%

Sample	Total SD	Total CV%	% Positive	% Borderline	% Negative
1 (negative)	0.07	8.5%	0	0	100
2 (negative)	0.06	8.7%	0	0	100
3 (negative)	0.06	9.8%	0	0	100
4 (positive)	0.49	9.4%	100	0	0
5 (positive)	0.25	12.9%	100	0	0
6 (positive)	1.07	13.5%	100	0	0

CLINICAL PERFORMANCE

A clinical evaluation study was performed with a total of 782 samples. The 130 positive samples were positive by PCR and/or serology tests. The negative sample set consisted of 652 samples in total. The negative samples were considered negative by the fact that they were collected prior to November 2019. The results are tabulated below.

	Comparative Method		
DBC Method	Positive	Negative	Total
Positive	128	11	139
Negative	2	641	643
Total	130	652	782
	95% Confidence Intervals		
Summary Statistics	Percent	Lo Limit	Hi Limit
Positive Percent Agreement (PPA)	98.5%	94.6%	99.6%
Negative Percent Agreement (NPA)	98.3%	97.0%	99.1%
Overall Percent Agreement (OPA)	98.3%	97.2%	99.0%

Results for the 130 positive samples by days from symptom onset are tabulated below.

Days from Symptom Onset	Number of Samples Tested	IgG Positive Results	IgG PPA	95% CI
0–7 days	0	N/A	N/A	N/A
8–14 days	5	5	100%	56.6%–100%
≥ 15 days	76	75	98.7%	93.0%–99.8%
Unknown	49	48	98.0%	89.3%–99.6%
Total	130	128	98.5%	94.6%–99.6%

The clinical performance when only including positive samples that were confirmed positive by a PCR method, is shown in the two tables below.

	Comparative Method		
DBC Method	**Positive**	**Negative**	**Total**
Positive	111	11	122
Negative	1	641	642
Total	112	652	764
	95% Confidence Intervals		
Summary Statistics	**Percent**	**Lo Limit**	**Hi Limit**
Positive Percent Agreement (PPA)	99.1%	95.1%	99.8%
Negative Percent Agreement (NPA)	98.3%	97.0%	99.1%
Overall Percent Agreement (OPA)	98.4%	97.3%	99.1%

Days from Symptom Onset	Number of Samples Tested	IgG Positive Results	IgG PPA	95% CI
0–7 days	0	N/A	N/A	N/A
8–14 days	2	2	100.0%	34.2%–100%
≥ 15 days	70	69	98.6%	92.3%–99.7%
Unknown	40	40	100.0%	91.2%–100%
Total	112	111	99.1%	95.1%–99.8%

ANTIBODY IMMUNE RESPONSE TO VACCINATION

A study was conducted to assess the performance of this device in detecting an antibody immune response following vaccination with the Moderna (mRNA-1273) COVID-19 vaccine. The sample size consisted of 45 unique donors. For each donor, blood draws were performed prior to vaccination, 15-29 days (median of 26 days) after administering the first dose and 12-17 days (median of 14 days) after administering the second dose. The time interval between administering the first and second dose was as recommended by the manufacturer (average 27.9 days). Donors were verbally screened for a lack of COVID-19 symptoms, however there were asymptomatic cases within this group. The results for serum samples are shown in the table below.

Phase	#Days Between Dose & Blood Draw (Median)	IgG PPA	95% CI
Pre-Vaccine	-	20.5%	11.2%-34.6%
After First Dose	26	93.2%	81.8%-97.7%
After Second Dose	14	100%	92.1%-100%

A second study was conducted to assess the performance of this device in detecting an antibody immune response following vaccination with the Pfizer-BioNTech (mRNA-BNT162b2) COVID-19 vaccine. The sample size consisted of 15 unique donors. For each donor, blood draws were performed prior to vaccination, 17-18 days (median of 18 days) after administering the first dose and 12-15 days (median of 14 days) after administering the second dose. The time interval between administering the first and second dose was as recommended by the manufacturer (average 20.7 days). Donors were verbally screened for a lack of COVID-19 symptoms, however there were asymptomatic cases within this group. The results for serum samples are shown in the table below.

Phase	#Days Between Dose & Blood Draw (Median)	IgG PPA	95% CI
Pre-Vaccine	-	40%	19.8%-64.3%
After First Dose	18	100%	79.6%-100%
After Second Dose	14	100%	79.6%-100%

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SYMBOLS

REF	Catalogue number	IVD	In vitro diagnostic device	LOT	Lot number
Σ	Contains sufficient for <n> tests		Storage Temperature		Legal Manufacturer
	Use by		Consult instructions for use	X #	Dilute 1:# Before Use

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