PictArray™ SARS-CoV-2 ELISA

ELISA Test Device for the detection of IgG Antibodies specific to SARS-CoV-2 Spike and Nucleocapsid proteins

Investigational Use Only

The PictArray™ SARS-CoV-2 ELISA Kit is a miniaturized, duplexed immunoassay for the qualitative detection of IgG antibodies in human sera to SARS-CoV-2 using the SCIREADER CL2 colorimetric reader with Pictorial for CL2 software. This *in vitro* test is intended as an aid in detecting reactive antibodies in human sera towards SARS-CoV-2

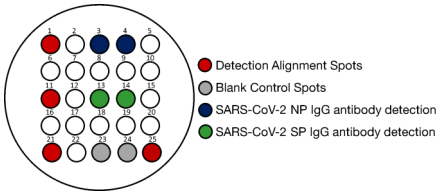

Figure 1: Layout of PictArray™ SARS-CoV-2 ELISA

SUMMARY

COVID-19 is caused by the β-coronavirus, SARS-CoV-2, an enveloped RNA virus comprising four main structural proteins, nucleocapsid protein (NP), spike protein (SP), membrane protein (MP), and the envelope protein (EP). It is a member of the coronaviridae family, which includes viruses that cause the common cold (HCoV-OC43 and HCoV-229E), SARS (SARS-CoV), and MERS (MERS-CoV). Infection with the latter two viruses can result in severe acute respiratory infections. The SARS-CoV-2 SP facilitates infection via interactions with the ACE2 receptor in the lower respiratory tract. Infection can lead to various clinical conditions depending on the immune status of the patient, age, and chronic health conditions.

Transmission of SARS-CoV-2 occurs through droplets in the air and surface contact. Initial symptoms experienced include upper respiratory tract infection, fatigue, cough, fever, and dyspnoea. Phase II of the infection shows progression to bilateral viral pneumonia with hypoxemia, while phase III involves respiratory failure and mortality. The World Health Organisation (WHO) declared COVID-19 a global pandemic on 11th March 2020.

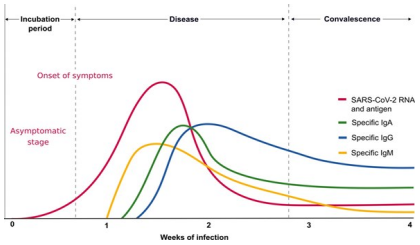

Figure 2: Production of IgA, IgG and IgM antibodies over the course of a typical SARS-CoV-2 infection.

SARS-CoV-2 IgG antibody screening will be associated with COVID-19 vaccine programs which are being rolled out worldwide. The SARS-CoV-2 IgG ELISA results indicate the presence of IgG antibodies towards SARS-CoV-2 SP and SARS-CoV-2 NP proteins, which will differentiate between a recent infection with the SARS-CoV-2 virus (SP and NP reactivity) or an immune response following vaccination (SP reactivity only).

A positive test result indicates IgG antibodies have been found during screening. A negative test result means no antibodies have been detected, in which case there has been no exposure to the virus and there is no current or past infection (or no memory immune response to vaccination). However, as indicated in Figure 1, immediately after contact with the infectious agent and during the incubation phase IgG tests results will be negative. In this case, if contact with infection is confirmed, it is recommended to retest at a later date.

PRINCIPLE

The duplexed detection of neutralising antibodies to SARS-CoV-2 SP or NP antigens by the PictArray™ SARS-CoV-2 IgG ELISA Kit is based on the ELISA principle.

1. Diluted serum samples are incubated in the wells of a PictArray™ SARS-CoV-2 IgG ELISA plate with each well containing duplicate spots of two different SARS-CoV-2 antigens, a negative control and four test control spots.
2. After washing off non-bound serum components, duplexed detection of bound IgG antibodies to each of the SARS-CoV-2 antigens within the well are tagged by incubation with HRP-labelled anti-human IgG.
3. The free conjugates are washed off and a substrate is added which produces a blue/purple precipitate.
4. The reaction is stopped by removing excess substrate and the plate is then imaged. The colour intensity of each spot is proportional to the amount of antibody bound to the antigen.

MATERIALS

MATERIALS PROVIDED

Table 1: Table showing components of a PictArray™ SARS-CoV-2 IgG ELISA kit

Kit Components			
Component	**Abbreviation**	**Description**	**Kit Size**
PictArray™ SARS-CoV -2 Plate	-	PictArray™ Plate containing 12 x 8 (96 well) plate printed with SARS-CoV-2 test spots	1 plate (96 tests)
10X Wash Buffer	WASH	Buffer containing phosphate buffered saline with Tween 20	55mL
10X Sample Diluent	DIL	Buffer containing blocking agent	6mL
20X Conjugate G	CONJ-G	Anti-human IgG antibody conjugated to HRP	0.75mL
1X Substrate	SUBS	3,3',5,5'-Tetramethylbenzidine (TMB) based detection Substrate	15mL
PictArray™ Instructions	-	Protocol for use	1

MATERIALS REQUIRED BUT NOT PROVIDED

1. Deionized or distilled water.
2. Quality control reagents
3. Precision pipettes to deliver volumes from 2µL to 200 µL.
4. Adjustable 1000µL pipettes for reagent and sample preparation.
5. Serological pipette to deliver volumes above 2mL.
6. Adhesive plate covers.
7. Sample transfer plate
8. Laboratory timer
9. Incubator at 37°C.
10. SciREADER CL2 colorimetric reader manufactured by Scienion for image capture.
11. Pictorial© for CL2 Image Analysis software from Pictor.

STORAGE AND STABILITY

Store the kit at 2-8 °C.

DO NOT FREEZE.

PictArray™ SARS-CoV-2 IgG ELISA Kits have a shelf-life of one year from date of manufacture.

PRECAUTIONS

SAFETY PRECAUTIONS

1. All reagents in this kit are for *in vitro* diagnostic use only.
2. Handle all human material as if potentially infectious. Users should wear gloves and protective clothing when handling any patient samples.
3. Avoid any contact of the reagents in the kit with the skin and mucosa (toxicity, irritation and burn hazard). Material safety data sheets for kit components are available upon request.
4. Handle and dispose of samples and reagents in accordance with local legislation and established laboratory protocols.

TECHNICAL PRECAUTIONS

1. Follow complete instructions for proper performance of the kit and to obtain reliable results.
2. Only freshly drawn and properly refrigerated samples should be used in this assay. Samples may be stored at 2-8°C for no longer than 48 hours. If a delay in testing is anticipated, samples should be stored at -20°C.
3. Repeated freeze-thaw cycles of the sample should be avoided.
4. Do not allow the wells on the plate to dry between any steps during sample processing, to the final step (substrate addition) has been performed.
5. Ensure that **all** reagents are equilibrated to room temperature before use.
6. It is important to add samples and reagents into wells without any delay after the removal of solution at the end of the wash steps. Please ensure that all the samples and reagents are ready to be dispensed.
7. Ensure there is no residual wash buffer remaining in wells following wash steps.
7. Use different barrier pipette tips for each sample and change tips between the addition of different reagents. Cross contamination of reagents and/or samples can lead to incorrect results.

PREPARATION OF REAGENTS

NOTE: Volumes below are sufficient to process 96 wells.
Bring all reagents to room temperature before running the assay.
Vortex all tubes in the kit thoroughly before use.

1X WASH BUFFER (WASH) PREPARATION

NOTE: If crystals are visible in the solution, warm the tube at 37°C for 10 minutes and check if the crystals have dissolved. If crystals persist, vortex to dissolve prior to use.

1. Add 30mL of 10X Wash Buffer (WASH) into a labelled 500mL bottle using a serological pipette.
1. Add 270mL of distilled water to make the volume up to 300mL.
2. Mix well.
3. Store at 2-8°C.

NOTE: Store undiluted stock reagent at 2-8°C immediately after use.
Diluted 1X Wash Buffer is stable for 3 months when stored at 2-8°C.

1X ASSAY DILUENT (DIL) PREPARATION

1. Add 5mL of 10X Assay Diluent (DIL) into a labelled 50mL tube.
2. Add 45mL of 1X wash buffer using a serological pipette to make the vol ume up to 50mL.
1. Mix well.
2. Store at 2-8°C.

NOTE: Store undiluted stock reagent at 2-8°C immediately after use.
Diluted 1X Assay Diluent is stable for 3 months when stored at 2-8°C.

1X CONJUGATE G (CONJ-G) PREPARATION

1. Add 600µL of 20X Conjugate G (CONJ-G) into a labelled 15mL tube.
2. Add 11.4mL of 1X Assay Diluent to make the volume up to 12mL.
3. Mix well.
4. Store in the dark at room temperature.

TEST PROTOCOL

NOTE: If all wells of a plate are not used, the plate should be kept refrigerated in the zip-lock bag provided, with desiccant.

SAMPLE LAYOUT

Please follow the instructions in the Pictorial© for CL2 Image Analysis Instruction Manual to generate a sample layout.

SAMPLE COLLECTION AND PREPARATION

Use only freshly drawn or frozen patient samples as described in technical precautions.
1. Allow the plate to reach room temperature prior to performing the SARS -CoV-2 IgG assay.

Dilute the patient test samples 1:101 in 1X Diluent
A. Add 2µL of patient sample into 200 µL of 1X Diluent in a transfer plate
B. Ensure samples are thoroughly mixed

2. Add 100 µL of the diluted samples into corresponding wells using a multi-channel pipette.
3. Cover the plate with a removable plate cover and incubate at 37°C for 30 minutes.
4. Carefully remove the plate cover and wash assay wells three times with 300µL of 1X Wash Buffer per well (this can be manual or using an automat ed plate washer).
5. Tap the plate on absorbent paper to remove residual wash buffer.

ADDITION OF CONJUGATE (CONJ-G)

1. Add 100µL of 1X Conjugate G to assay wells.
2. Cover the plate with a removable plate cover and incubate at 37°C for 15 minutes
3. Carefully remove the plate cover and wash assay wells three times with 300µL of 1X Wash Buffer per well (this can be manual or using an automat ed plate washer).
4. Tap the plate on absorbent paper to remove residual wash buffer.

ADDITION OF SUBSTRATE (SUBS)

Ensure the substrate solution (SUBS) has been equilibrated to room tem perature before use.

1. Add 100µL of the 1X Substrate Solution (SUBS) to all assay wells.
2. Cover the assay wells with a removable plate cover and incubate for 20 minutes in the dark at room temperature.
3. Remove the 1X Substrate Solution from assay wells – do this by flicking solution into a container, followed by inverting the plate and tapping it on absorbent paper to remove residual liquid. Discard waste substrate solution into an appropriate biohazard waste container.
4. Scan and analyse the wells using a sciREADER CL2 colorimetric reader imaging device

INTERPRETATION OF RESULTS

The Pictorial© for CL2 Image Analysis software measures the colour intensity values for each of the array spots.
The intensity values of test spots are corrected against background intensity values using proprietary algorithms.

QUALITY CONTROL

The control spots in each well are intended for process validation.
Additional controls may be tested according to guidelines or requirements of local regulatory agencies.
Measurement of the intensity values of control spots embedded in each PictArray™ well ensures that the test has been performed correctly.
If the intensities of the Detection Alignment Control Spots do not meet the QC criteria, test results are reported as an "Error" by Pictorial© for CL2 software.
For more information, refer to the Troubleshooting section in the Pictorial© for CL2 Image Analysis Manual.

LIMITATIONS

This is a qualitative test and the results will be shown as either positive or negative for SARS-CoV-2 IgG antibodies.
The diagnosis should not be made solely on the basis of PictArray™ SARS -CoV-2 IgG ELISA Kit test results.

REFERENCES

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