DIPAS-VDx COVID-19 IgG Antibody
Microwell ELISA Detection Kit

Intended Use

DIPAS-VDx Covid 19 IgG ELISA test is intended to be used for the in-vitro, qualitative detection of IgG antibodies to SARS-CoV-2 in human serum or plasma.

Introduction

The novel coronavirus, SARS-CoV-2 (the causative agent of Covid -19), has been responsible for the pandemic of pneumonia-like symptoms and associated deaths from December 2019 and into 2020. The detection of the initial outbreak in the Hubei Province of China and the subsequent need for an effective diagnosis were described (Li et al., 2020; Wu et al., 2020; Zhou et al., 2020).

It has been reported that RTPCR-confirmed SARS-CoV2 positive patients may seroconvert and develop antibodies against SARS-CoV-2 antigens, anywhere from 6 to 21 days after the onset of clinical symptoms (Okba et al., 2020). The specific and reliable detection of human IgG antibodies to SARS-CoV-2 remains a key method to monitor infections, to effect contact tracing, and for sero-surveillance (Okba et al., 2020).

IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time for which antibodies are present post-infection, is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

The current methodology used for detection and diagnosis of SCOV-2 is based on RT-PCR using nasal and throat swabs of infected patents to identify and isolate the individuals for prevention and control of disease.

These patients, however, need to be monitored for the production of the corresponding antibody and therefore, DIPAS-VDx Covid 19 IgG ELISA test may be suitable for use for sero-surveillance purposes and for monitoring the progression of recovery of patients and further medical decision making.

Principle of the Test

DIPAS-VDx Covid 19 IgG ELISA Test is a solid phase Enzyme Linked Immunosorbent Assay, based on the principle of indirect immunoassay. In this assay, the polystyrenes microwell strips are coated with a cocktail of highly purified Covid -19 recombinant protein and peptide antigen selected from the immunodoment spike and nucleocapsid regions of SARS-COVI D-2 virus.

The sample specimens (serum or plasma), along with positive and negative controls are added into the coated microwells, followed by the addition of the Enzyme Conjugate (Goat anti- human IgG) linked to horseradish peroxidase (HRPO) and incubated.

The wells are then washed using the Wash Buffer to remove unbound/non-reactive components. The bound enzyme is detected by addition of the Substrate Buffer and Chromogen- 3,3',5',5'-Tetramethyl benzidine (TMB), through the development of a blue color, in case of a sample positive for SCOV-2 IgG antibody.

The reaction is stopped by addition of the Stop Solution.

A yellow color develops, which is finally read at 450nm using an ELISA reader.

Kit Contents

I **Microwells ELISA plates:** One plate of 96 microwells coated with a cocktail of highly purified Covid-19 recombinant protein and peptide antigen selected from the immunodoment spike and nucleocapsid regions of SARS-COVID-2 virus, packed in a sealed aluminum pouch with desiccant.

Allow the strips to reach room temperature (15 to 30°C) before removal from the pouch. Place unused strips in the sealable storage pouch provided and return to 2 to 8°C. Once opened, microwells should be used within one month

ii **Sample Diluent:** One bottle containing 15ml of Sample Diluent buffer, containing proteins, stabilizers and preservatives to be used for sample dilution as per the protocol of the test.

iii **Conjugate Diluent:** One bottle containing 15ml of Conjugate Diluent buffer, containing proteins, stabilizers and preservatives with heterophilic blocking agents, to be used for conjugate dilution.

iv **Negative Control:** One vial containing 0.3ml of inactivated and stabilized normal human serum with preservatives. Negative Control has been tested and found to be negative for SCOV-2 antibody, anti-HIV 1+2 antibodies, HBsAg, AntiHCV antibodies and Syphilis.

v **Positive Control:** One vial containing 0.2ml of inactivated and stabilized SCOV-2 human serum with preservatives. Positive Control has been tested and found to be positive for SCOV-2 IgG antibody and negative for anti-HIV 1+2 antibodies, HBsAg, Anti-HCV antibodies and Syphilis.

vi **Enzyme Conjugate (101x):** One vial containing 0.3ml of Enzyme Conjugate (101x) containing Goat anti-human IgG linked to horseradish peroxidase (HRPO) with stabilizers. For preparation of the Working Enzyme Conjugate solution, refer to preparation of Working Reagents.

vii **TMB Substrate (101x):** One vial containing 0.3ml of 3,3',5',5'-Tetramethyl benzidine - TMB, (101x) containing Dimethyl Sulfoxide (DMSO) as solvent.

viii **Substrate Buffer:** One bottle containing 15ml of Substrate Buffer containing Hydrogen Peroxide (H_2O_2) and Stabilizers. For preparation of Working TMB Substrate solution, refer to the preparation of Working Reagents.

ix **Wash Buffer (25X):** One bottle containing 25ml of concentrated

Wash Buffer (25x) containing phosphate buffer saline with detergents and preservatives. For preparation of Working Wash Solution, refer to preparation of Working Reagents.

x   **Stop Solution:** One bottle containing 10ml of ready to use Stop Solution containing 2N H_2SO_4 and stabilizers.

xi **Micro strip Cover sealer:** Adhesive sheets to cover microwells strips during incubation.

xii **Product Insert/ IFU:** Containing detailed information about the kit.

Materials required but not provided

Distilled or deionized water, Graduated Cylinder for reagents dilutions, Micropipettes and Microtips, Paper towels or absorbent paper, Timer, Incubator, maintained at 37±1°C, ELISA Reader, ELISA Washer, Sodium Hypochlorite solution (free available chlorine 50-500mg/dl) and Disposable gloves.

Storage & Stability

-   Store the kit and its contents at 2 - 8°C, up to the expiry date printed on the labels.

Sample/Specimen collection & Handling

-   Only human serum or plasma should be used for the test.

-   Specimen should be free of particulate matter and microbial contamination.

-   It is preferable to use fresh samples.

-   Specimens can be stored at 2-8°C for a short duration of one week.

-   For longer storage, freeze the sample specimen at -20 °C or below.

-   Avoid repeat freezing and thawing of the samples.

-   Do not heat inactivate the sample before use.

-   Hemolyzed and hyperlipemic samples may give erroneous results.

-   After thawing, ensure samples are thoroughly mixed before testing.

Precautions

a. Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated area swabbed with 1.0% Sodium Hypochlorite solution before work is continued. Sodium Hypochlorite should not be used on acid-containing spills unless the spill area is first wiped dry. Materials used to clean spills, including gloves, should be disposed off as potentially bio-hazardous waste. Do not autoclave materials containing Sodium Hypochlorite.

b. Do not pipette by mouth. Wear disposable gloves and eye protection while handlings specimens and performing the assay. Wash hand thoroughly there after.

c. Sulphuric acid used in Stop Solution is corrosive and should be handled with appropriate care. If it comes into contact with skin or eyes, wash thoroughly with water. If any of the reagents come in contact with the skin or eyes, wash the area extensively with water.

d. Do not mix the reagents from different batches and do not use the reagents beyond the stated expiry date.

e. Do not modify the test procedure or substitute reagents from other manufactures or other lots.

f. Do not reduce any of the recommended incubation times.

g. Allow the reagents and samples to come to 15 to 30°C before use. Immediately after use, return reagents to the recommended storage temperature.

h. Do not allow wells to become dry during the assay procedure.

i. Do not interchange the caps and cross-contaminate reagents. It is recommended to use dedicated separate pipettes for dispensing the Substrate Solution and Conjugate.

J. Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate.

Preparation for the test

1. Bring all the reagents to room temperature, 30 minutes before use ensure for homogeneous mixing of these reagents.

2. Take out the required number of strips from the sealed ELISA plate pouch. The remaining strips must be kept at 2-8°C with the desiccant after proper sealing of the pouch.

Test Procedure

(a) Take the required number of strips and fix them on to the plate frame.

(b) Pipette 100 µl of Sample Diluent into each microwell.

(c) Add 10 µl of Negative Control into B1,C1 & D1 wells and 10 µl of Positive Control into E1 & F1 wells and then, add 10 µl each of the samples to be tested into the remaining wells. Do not add anything in Blank well (A1).

(d) After covering the plate with adhesive sealer, Incubate at 37±1° C for 30±1° minutes.

(e) Take out the plate from the incubator after incubation time is over and then wash each well 5 times with 300 µl of Working Wash Solution. Invert the plate and tap it on absorbent paper to remove the remaining Wash Solution.

(f) Add 100 µl of Working Conjugate Solution in each well. Do not add anything in Blank well A1.

(g) After covering the plate with adhesive sealer, Incubate at 37±1° C for 30±1° minutes.

(h) Take out the plate form the incubator after incubation time is over and then wash each well 5 times with 300 III of Working Wash Solution.

(I) Invert the plate and tap it on absorbent paper to remove the remaining Wash Solution and then, pipette 100 µl of Working Substrate solution into each well including the Blank well (A1).

(j) Incubate the plate at room temperature (20-30 °C) for 15 minutes, in dark after covering with the adhesive strip cover sealers, provided in the kit. Avoid exposure to light.

(k) Pipette 50 µl of Stop Solution to each well including the Blank well (A1).

(l) Read the absorbance at 450 nm (reference wavelength at 450-630 nm) within 10 minutes after pipetting the stop solution.

Preparation of Working Reagents

A. Preparation of Working Wash Solution:

Bring the concentrated Wash Buffer (25x) to room temperature and mix well till the appearance becomes clear. If any crystals are observed in the Wash Buffer, keep it at 37°C until the crystals dissolve. Dilute the Wash Buffer with distilled or deionized water to make the Working Wash Solution as below:

B.

Wash buffer (25x) in mL	1	2	3	4	5	6	7	8
Distilled/DI water in mL	24	48	72	96	120	144	168	192
Total volume in mL	25	50	75	100	125	150	175	200

The Working Wash Solution is stable at room temperature for up to one week, if stored in a clean and closed container.

C. Preparation of Working Conjugate solution:

Take out the Enzyme Conjugate (101x) from the kit and mix gently. Dilute the Enzyme Conjugate with Conjugate Diluent to make the Working Conjugate solution as below:

D.

No. of strips	1	2	3	4	6	8	10	12
Conjugate Diluent in mL	1	2	3	4	6	8	10	12
Enzyme conjugate (101x) in µl	10	20	30	40	60	80	100	120

Prepare the Working Conjugate solution in clean and dried container just before use for better performance.

E. Preparation of Working Substrate solution:

No. of strips	1	2	3	4	6	8	10	12
Substrate Buffer in mL	1	2	3	4	6	8	10	12
TMB Substrate (101x) in µl	10	20	30	40	60	80	100	120

Prepare the Working Substrate solution in clean and dried container just before use for better performance.

Quality Control

Results of an assay are considered to be valid, if the following criteria for the Controls are met.

a. Absorbance of Blank should be < 0.050.

b. Mean Absorbance of Negative Controls should be < 0.100. If absorbance of the Negative Control reads in the negative, e.g.-0.003, it should be considered as zero.

c. Mean Absorbance of Positive Controls should be > 0.500.

d. If the absorbances obtained do not comply with the ranges mentioned above, the test should be conducted again while following the protocol, meticulously.

Interpretation of results

A. Interpretation of results by the Qualitative Method:

1. Calculation of the Cut-off-Value (COV)

- Calculate the Negative Control Mean (NCX) e.g. Negative Control 1 absorbance = 0.009, Negative Control 2 absorbance = 0.010, Negative Control 3 absorbance = 0.011

- Negative Control Mean (NCX) = (0.009+0.010+0.011)/3 = 0.010

- Cut-off-Value (COV) = NCX +0.200 = 0.010+0.200 = 0.210

2. Interpretation of Results

Reactive Results: - Samples with absorbance values greater than or equal to the Cut-off-Value are considered Reactive by the assay.

Non-Reactive Results: - Samples with absorbance values less than the Cut-off-Value are considered Non-Reactive by the assay.

Gray Zone Results:- Samples whose absorbance values fall within ±10% of the Cut-off Value, should be again considered to be in the Gray Zone and should be tested within 2-3 days from the date of first bleed.

- If a sample shows a non-reactive result, the test should be repeated two more times.

- In case the re-tests show non-reactive results, the sample should be considered as non -reactive.

- The samples found to be reactive should be tested again, by a similar or a higher version of the test, followed by clinical correlation for the final interpretation.

B. Interpretation of results by the Semi-Quantitative method:

1. Calculation of Index Units (IU) for determination of the level of immune response

$$\text{Index Units IU} = \frac{\text{OD of Unknown Sample}}{\text{COV}} \times \text{Dilution Factor} (= 10)$$

2. Interpretation of Results

Results in IU	Interpretation
≤ 10	No Immune Response
>10 to 20	Low Immune Response
>20 to 40	Medium Immune Response
>40	High Immune Response

Disclaimer:

- The determination of the immune response by the Index Units method has been derived after testing of known negative and known positive samples and validating against a reference assay.

- These Index Units provide a broad estimate of the level of immunity present in a patient.

- These Index Units should not be correlated with the values obtained by the Quantitative Methods. For quantitative results, the sample should be tested using a higher version of the immunoassay such as a chemiluminescence based test followed

by clinical correlation for final interpretation.

Note:

- Semi-Quantitative interpretation can be used as reference for comparing the level of immune response among the individuals previously infected and or exposed to SARS-COV-2.

- Semi-Quantitative interpretation can also be used for comparing the immune response among the individuals vaccinated by any vaccine for SARS-COV-2.

Limitation of the Test

- DIPAS-VDx Covid 19 IgG ELISA Test, should be used for detection of Covid-19 IgG antibody in serum or plasma only and not in other body fluids.

- This test is designed for sero-surveillance of Covid-19 infected individuals and will only indicate the presence or absence of Covid-19 IgG antibody in the sample specimen.

- All reactive samples should be confirmed by a confirmatory test e.g. RT-PCR or other approved test kits. The definitive diagnosis including patient's clinical history, symptoms as well as serological evidence should be considered before reporting final results.

Performance Characteristics

Sensitivity and Specificity

Studies on DIPAS-VDX-COVID-19 IgG ELISA Kit were conducted at various sites using known positive and known negative samples. Results obtained were as under:

Testing Site	No. of samples	True positive	True Negative	Sensitivity	Specificity
Rajiv Gandhi Hospital, Delhi	90	45	45	97.7%	100%
DIPAS, Delhi	71	20	51	100%	100%
ICMR/NARI, Pune	200	100	100	97%	99%
In-house Evaluation	362	62	300	100%	100%
TOTAL	723	227	496	98.7%	99.8%

Bibliography

1. Li, X., Geng, M., Peng, Y., Meng, L., & Lu, S. (2020). Molecular immune pathogenesis and diagnosis of COVID-19. Journal of Pharmaceutical Analysis. https://doi.org/10.1016/J.JPHA.2020.03.001.

2. Okba, N. M. A., Muller, M. A., Li, W., Wang, C., GeurtsvanKessel, C. H., Corman, V. M., et al. (2020) . Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease 2019 Patients. Emerging Infectious Diseases, 26(7).

3. Okba, N. M. A., Muller, M. A., Li, W., Wang, C., GeurtsvanKessel, C. H., Corman, V. M., Lamers, M. M., Sikkema, R. S., de Bruin, E., Chandler, F. D., Yazdanpanah, Y., le Hingrat, Q., Descamps, D., Houhou-Fidouh, N., Reusken, C. B. E. M., Bosch, B.-J., Drosten, C., Koopmans, M.P.G., & Haagmans, B. L. (2020). SARS-CoV-2 specific antibody responses in COVID-19 patients. MedRxiv, 2020.03.18.20038059. https://doi.org/10.1101/2020.03.18.20038059

4. Okba, N. M. A., Muller, M. A., Li, W., Wang, C., GeurtsvanKessel, C. H., Corman, V. M., et al. (2020). Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease 2019 Patients. Emerging Infectious Diseases, 26(7).

5. Udugama, B., Kadhiresan, P., Kozlowski, H. N., Malekjahani, A., Osborne, M., Li, V. Y. C., Chen, H., Mubareka, S., Gubbay, J., & Chan, W. C. W. (2020). Diagnosing COVID-19: The Disease and Tools for Detection. ACS Nano. https://doi.org/10.1021/acsnano.0c02624

6. Wolfe', R., Corman, V.M., Guggemos, W., Seilmaier, M., Zange, S., Muller, M.A., et al. (2020). Virological assessment of hospitalized patients with COVID-2019. Nature. https://doi.org/10.1038/s41586-020-2196-x

7. Wu, F., Zhao, S., Yu, B., Chen, Y.-M., Wang, W., Song, Z.-G., Hu, Y., Tao, Z.-W., Tian, J.-H., Pei, Y.-Y., Yuan, M.-L., Zhang, Y.-L., Dai, F.-H., Liu, Y., Wang, Q.-M., Zheng, J.-J., Xu, L., Holmes, E. C., & Zhang, Y.-Z. (2020). A new coronavirus associated with human respiratory disease in China. Nature, 579(7798), 265-269. https://doi.org/10.1038/s41586-020-2008-3

8. Yong, S.E.F., Anderson, D.E., Wei, W.E., Pang, J., Chia, W.N., Tan, C.W., et al. (2020). Connecting clusters of COVID 19: an epidemiological and serological investigation. The Lancet Infectious Disease. https://doi.org/10.1016/S14733099(20)30273-5

9. Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., Chen, H.-D., Chen, J., Luo, Y., Guo, H., Jiang, R.-D., Liu, M.-Q., Chen, Y., Shen, X.-R., Wang, X., ... Shi, Z.-L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature, 579 (7798), 270 — 273 https://doi.org/10.1038/s41586-020-2012-7

Developed by:

Defence Institute of Physiology and Allied Sciences, DRDO, Lucknow Road, Timarpur Delhi - 110054

Manufactured By:

Vanguard Diagnostics Private Limited

C-123, Phase-1, Okhla Industrial Area, New Delhi-110020,

Email: info@vanguarddiagnostics.com

Web: www.vanguarddiagnostics.com