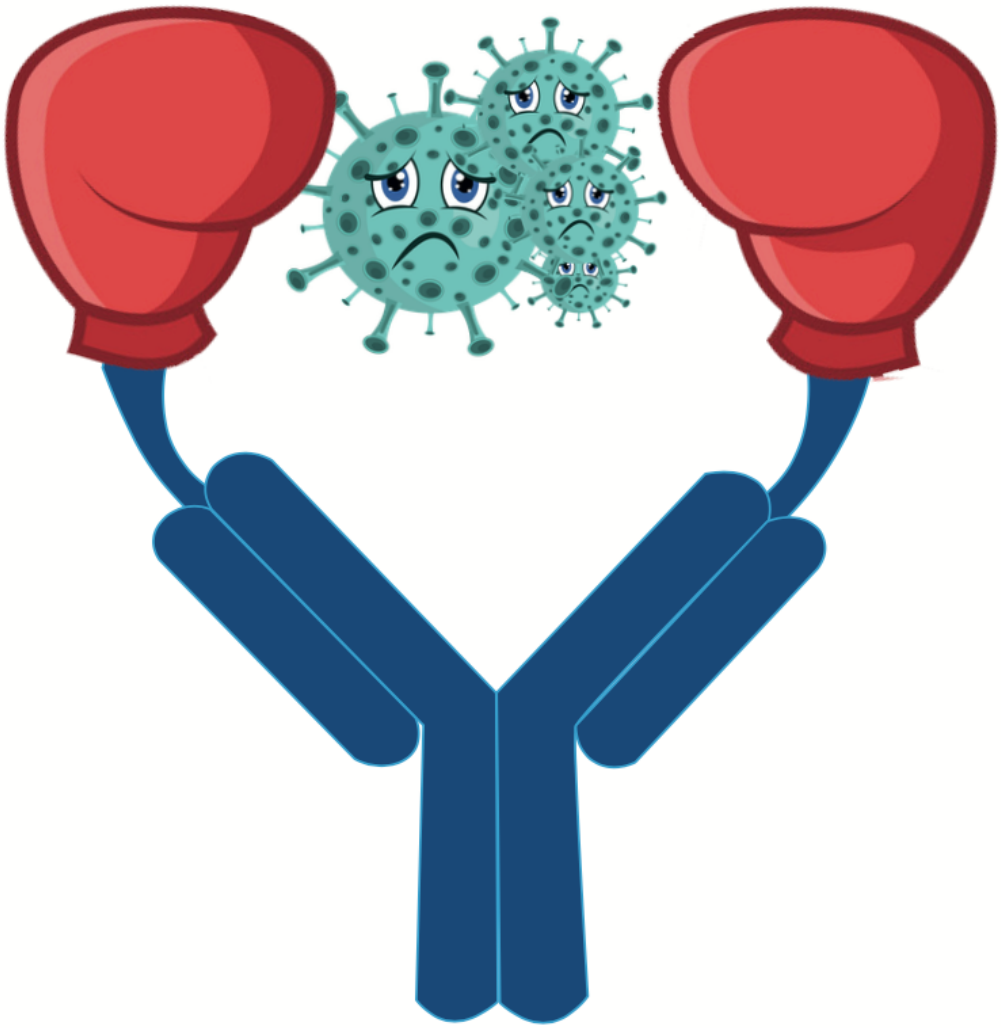
CHIMERA® COVI-ELISA

Neutralising IgG Antibody Detection Assay



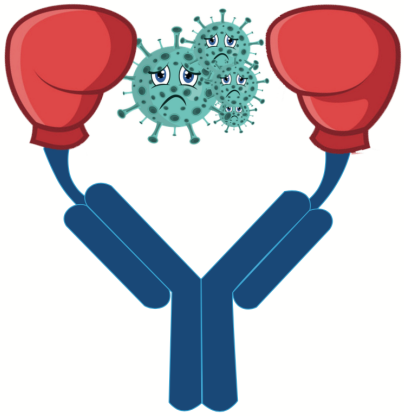
Catalog No. CE10

DISTINGUISHING FEATURES

- Developed in accordance with **ISO:13485:2016 regulations**
- Fabricated to specifically diagnose the presence of **COVID-19 fighting IgG antibodies**
- Determined **efficacy and stability** of all the reagents
- **No compromise** in the quality of consumables
- Assured supply of **sufficient reagent volumes**, with consideration to the wastage possibility
- Ensured **no non-specific binding** to IgM or IgA antibodies
- Designed to ensure **high specificity and sensitivity** with low sample volume

CHIMERA® COVI-ELISA

Neutralising IgG Antibody Detection Assay



FEATURES DEFINING CONVENIENCE AND SAFETY

- **Breakable strips** for ease of testing Ready to use reagents
- **Controls** provided for satisfactory validation of the assay
- **Color-coded labelling patterns** for ease of identification

CHIMERA®

We promise to be there to help you

CONTACT US

Head Office

209C, IInd and IIIrd Floor, Masjid Moth,
South Extension-II
New Delhi - 110049

Contact Number

+91 9953660319
+91 7042193187

Email Address

nikita@translational.in

RESPONSE TIME < 24 Hours

CONTENTS

INTENDED USE

Chimera® COVI-ELISA is designed to detect the presence of COVID-19 neutralising IgG antibodies in the serum.

Alternatively, the assay can be used to diagnose **the exposure to the coronavirus.**

HERD IMMUNITY

Chimera® COVI-ELISA can be used to identify individuals with **SARS-Cov-2 neutralising antibodies.** This will help them answer the following questions -

- Am I safe to step out to work?
- Am I a threat to others?

VACCINE EFFICACY

The assay can be used as a reliable tool **for validating the efficacy of vaccines against SARS-Cov-2.** The assay provides a direct qualitative measure of neutralising antibodies, that are expected to be developed in the host, as a response to the vaccination.

CONVALESCENT PLASMA'S POTENCY

Convalescent plasma therapy is widely adopted as a curative measure for patients suffering through critical illness, due to coronavirus infection. Chimera® COVI-ELISA empowers the **clinician to monitor the titer of neutralising antibodies in the patient's serum before and after the therapy**. This would help the clinician decide the amount of convalescent plasma doses required by an individual patient.

The assay does **NOT** provide an accurate measure of the ongoing infection.

EXPLANATION OF THE TEST

Molecular diagnostic techniques (polymerase chain reaction(PCR) and next generation sequencing) are widely adopted for detecting the SARS-Cov-2 infection.However, there is an urgent need for an assay that determines an **individual's capability of fighting the infection too.**

As soon as the coronavirus infects the host, the immune system of the host gets activated and develops antibodies that have a strong potential to neutralise the virus and alleviate the disease symptoms. **This first-ever, Made in India neutralising IgG antibody detection assay offers an effective way to detect the presence of COVID-19 fighting antibodies.**

The studies highlight the primary role of **human ACE2 receptor for viral entry.** Antibodies against the coronavirus region (Spike 1 RBD), that binds to the human ACE2 are called as neutralising antibodies.

Neutralising antibodies competitively binds to SI-RBD region of coronavirus, blocks it's entry, and guard the cells.

Chimera® COVI-ELISA mimics this scenario with the help of recombinant Spike 1 RBD protein, that is plated on the wells of a 96-well plate. The assay provides an optimised environment for detecting the bond between neutralising antibodies and coronavirus protein in am in-vitro diagnostic setting.

PRINCIPLE OF THE ASSAY

The assay is used to detect the presence of COVID-19 neutralising antibodies in the serum.

Diluted serum samples are incubated with an antigen immobilised on microtiter wells.

After washing away unbound serum components, goat anti-human IgG conjugated to horseradish peroxidase is added to the wells, and this binds to surface-bound antibodies in the second incubation.

Unbound conjugate is removed by washing, and a solution containing 3,3',5,5'- tetramethylbenzidine (TMB) substrate solution is added. The solution develops a colored complex and facilitates the detection of specific antibody binding.

Addition of stop solution terminates the reaction and optical densities of the controls and samples are measured using a microplate reader at 450nm.

KIT CONTENTS

- 96 Well- Tray
- Sample Diluent
- Wash Buffer
- Conjugate
- Substrate
- Stop Solution
- PC
- NC

The kit contains consumables sufficient for **96 tests**

STORAGE AND HANDLING INSTRUCTIONS

- Do not use strips and solutions if the foil bag is damaged or liquids have leaked. Request for a replacement in such cases.
- Store the contents at **2-8 degree Celsius.**

The product is not classified as hazardous. However, good clinical diagnostic procedures should be followed for obtaining the best results.

CAUTION:. All human source material used in the preparation of standards and control for this product have been tested and found negative by ELISA for antibodies to HIV, HbsAg and HCV. No test method, however, can offer complete assurance that infectious agents are absent. **Therefore, all reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.**

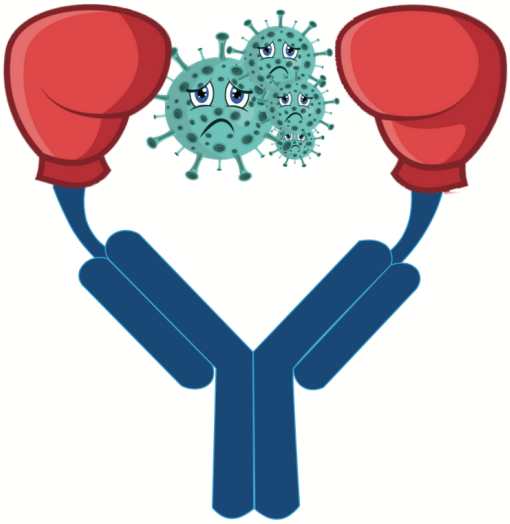
KIT EXPIRY

Unopened - up to **12 months** from date of manufacturing
Opened - up to **6 months** from date of opening

A STEP BY STEP GUIDE FOR OPTIMAL RESULTS

CHIMERA® COVI-ELISA

Neutralising IgG Antibody Detection Assay



GETTING STARTED

Collect the following items (not provided in the assay) for getting started -

- Microplate Reader (Kindly note that it is a vendor neutral assay and can be performed using any microplate reader with the capability to measure absorbance at 450 nm).
- Microplate Washer (Optional)
- Shaker
- Adjustable pipettes; multichannel or repeating pipettor recommended
- Tips, tissues and related consumables
- Reagent toughs (Options)
- 100 ul Vials for preparing dilutions
- Labels
- Aluminium plate sealer

TRAINED PERSONNEL

Contact us for an online or onsite demo. Refer to the contact information given above.

SPECIMEN COLLECTION

- **Plasma** collected in EDTA or Heparin vials and **serum** collected in silica or plain vials can be used.
- The samples should be stored at **2-8°C for short term use** (upto 14 days) and at **-20°C for long term preservation**.
- Repeated freezing and thawing **should be avoided**, as it can affect the results.
- Addition of preservatives to the serum sample **should be avoided**, as it may adversely affect the results.
- Microbial contaminated, heat-treated or specimens containing particulate matter **should not be used.**
- Grossly hemolyzed, icteric or lipaemia specimens **should be avoided**.

TEST PREPARATION

- **Vortex** all samples before use.
- **Dilute** the wash buffer to **1X** (Provided concentration 10X), before use.
- **Dilute** controls with sample diluent in the ratio of **1:100**, before use.
- Use rest of the regents **as is**.
- Allow the reagents to **come to room temperature** before use (20° to 25°C).
- **Chart out** the plate design, based on the number of samples to be tested. It is highly recommended to test the controls in **duplicates**.
- For cancelling the effect of background noise, put up a **blank test using only sample diluent** with every run.

ASSAY PROCEDURE

1. Dilute the serum sample, in separate vials, with the provided **Sample Diluent** in the ratio of 1:100. Assemble the number of strips required for the assay.
2. Add 100 µl of diluted sample to each well and incubate for 2 hours at room temperature, on a shaker. Make sure to cover the plate.
3. Decant or aspirate the well contents, and wash the wells three times with the provided **Wash Buffer** (Check wash procedure). Use 300 µl of Wash Buffer each time for each well. **Kindly note that the Wash Buffer is 10X concentrated, dilute it to 1X before use. [Wash procedure:** Use automated washer or empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with a wash buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process 2 more times.]
4. Add 100µl of the provided **Conjugate Solution** and incubate it at room temperature for 1 hour, on a shaker.
5. Repeat washing as mentioned in step3.
6. Add 100µl of the **Substrate Solution** and incubate for 5-10 minutes in dark.
7. Add 50 µl of provided **Stop Solution**
8. Read the optical density (OD) of each well at 450nm in a microplate reader within 10 minutes.

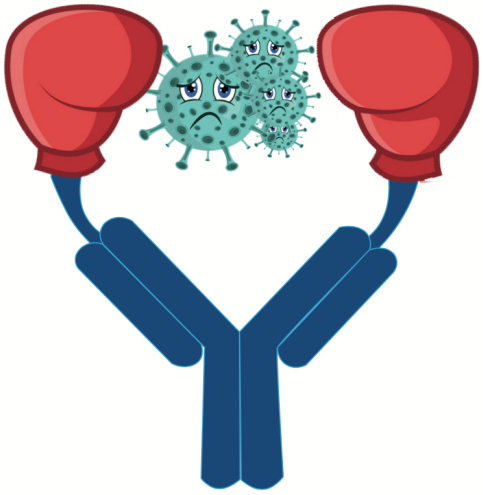
TECHNICAL PRECAUTIONS

- Pour out only the required volume of reagents. **Do not pour** the reagents back into the provided bottles.
- **Do not mix** components from different batches. Do not mix with components from other manufacturers.
- Before opening the Positive and Negative Controls, **tap the vials** on the benchtop to ensure that all liquid is at the bottom of the vial.
- Ensure that all glassware being used is **clean**.
- **Cover the plate** with aluminium plate sealer during incubation.
- Use only **Milli-Q water**
- Strips and solutions **should not be used** if the foil bag is damaged or liquids have leaked.
- Allow all reagents and the microplate to reach **room temperature before use**.
- Ensure that the microplate foil bag containing any unused strips is well sealed and contains the desiccant to avoid moisture. **Store at 2-8 °C after use.**
- Include the Positive Control in **every test run** to monitor for reagent stability and correct assay performance.
- **Do not allow** microwells to dry between incubation steps.
- **Strictly follow** the described wash procedure.
- **Insufficient washing** may cause high background signals.
- **Avoid direct sunlight** and exposure to heat sources during all incubation steps.

RESULT EVALUATION AND INTERPRETATION

CHIMERA® COVI-ELISA

Neutralising IgG Antibody Detection Assay



QUALITY CONTROL

- Controls are intended to monitor for **substantial reagent failure.**
- Any well **positive** by microplate reader but **without visible colour** should be cleaned on the underside and re-read.
- If **OD-values below zero** are observed, the wavelengths used should be verified, the reader re- blanked to air and the measurements repeated.
- The value of blank should be **less than 0.15**

RESULT EVALUATION

Calculate the final OD, by adjusting the sample OD with blank sample.

Final OD = Obtained OD - Blank

RESULT INTERPREATION

Analyse the final OD, as per the below given criteria

Final Sample OD < 0.5	Negative
0.5 = < Final Sample OD <= 0.6	Borderline Positive
0.6 < Final Sample OD < = 1	Positive
Final Sample OD > 1	Strong Positive

**Blank sample - Assay performed with only sample diluent*

CUT-OFF VALUES

Users may calculate their own cut-off values based on the following protocol -

1. Run negative control or know negative samples. A minimum of five samples should be considered.
2. Use the following formula for calculating the cutt-off -

Cutt off = $\overline{X}$ **+ 3 *** δ

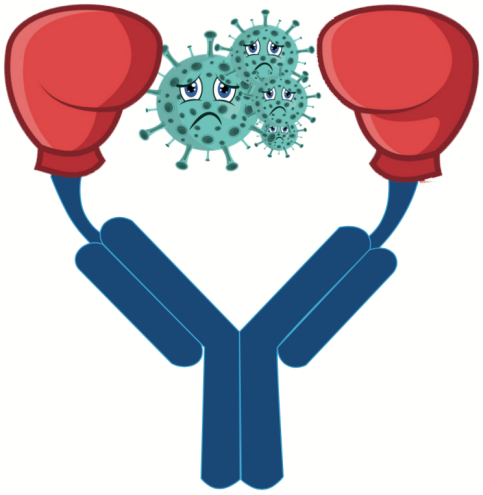
$\overline{X}$ stands for mean (confirmed negative samples)

δ stands for standard deviation

PERFORMANCE CHARACTERISTICS

CHIMERA® COVI-ELISA

Neutralising IgG Antibody Detection Assay



VALIDATION OF CONTROLS

For all the batches, the negative and positive controls are prepared using the same source. The stability of these controls was assessed by evaluating the **intra-batch and inter-batch performance of the prepared controls**.

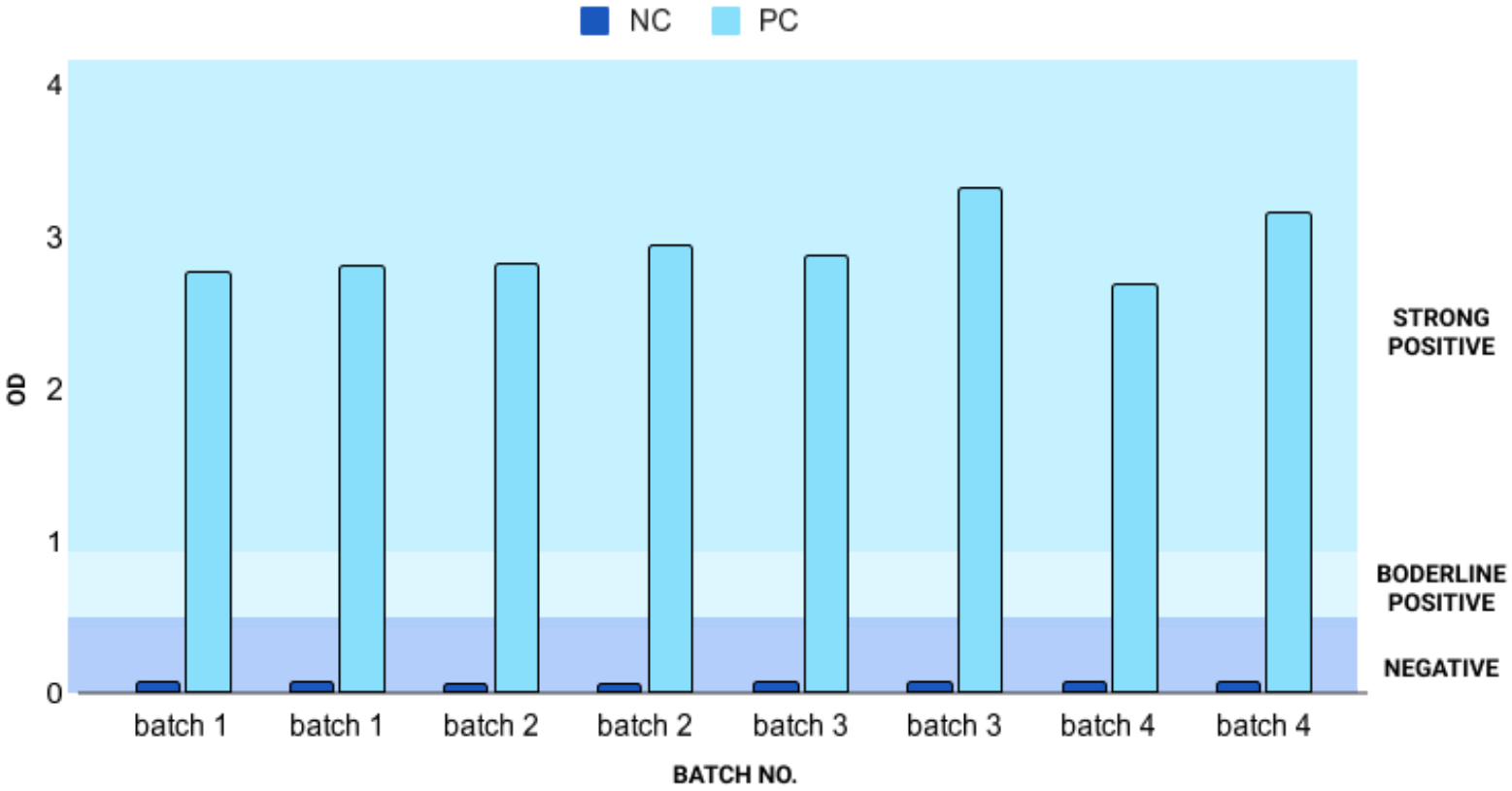


Fig. - depicts the blank adjusted OD values of negative and positive controls across various batches of Chimera Covi-ELISA

Positive Control		Negative Control	
Intra Batch Variability	**1.7%**	Intra Batch Variability	**3.3%**
Inter Batch Variability	**6.8%**	Inter Batch Variability	**4.34%**

ASSAY VALIDATION

The samples procured from different centres were marked as confirmed positive/negative based on the results obtained from atleast one of the similar platforms available for commercial use.

CENTRE 1		
	Confirmed Positive / Borderline Positive	**Confirmed Negative**
Positive	20	0
Negative	0	0

CENTRE 2		
	Confirmed Positive / Borderline Positive	**Confirmed Negative**
Positive	16	0
Negative	0	0

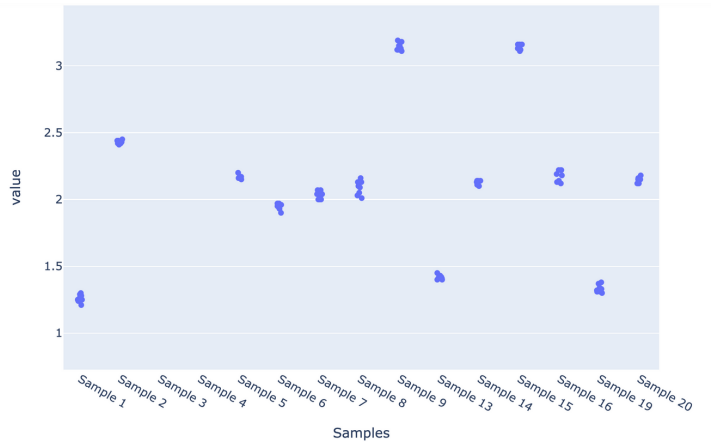
CENTRE 3		
	Confirmed Positive / Borderline Positive	**Confirmed Negative**
Positive	23	1
Negative	0	29

Sensitivity	100%
Specificity	97%
Negative Predictive Value	100%
Positive Predictive Value	98%

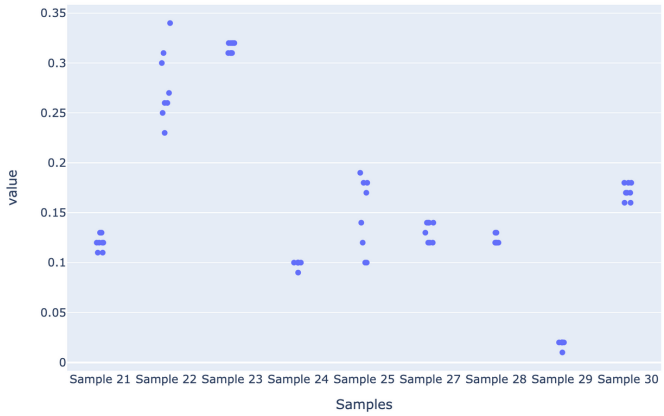
REPEATABILITY AND REPRODUCIBILITY

Intra and inter batch reproducibility was tested with a batch of 30 samples.

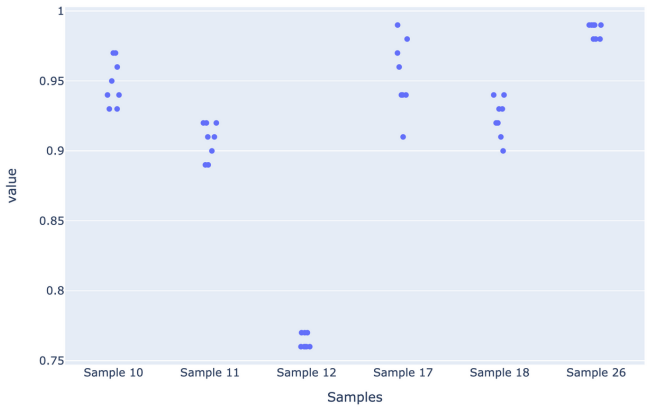
Positive Samples



Negative Samples



Borderline Positive Samples



Average Sample Variability

4.54%

ACKNOWLEDGEMENT

Incubator Partner

The assay has been developed with consistent support from our **incubator partner, KIIT, and under the esteemed guidance of Mr. Mrityunjay Suar.**

The research and development activities undertaken to develop this assay has been funded by the **Department of Science and Technology**, under their program **CAWACH**.

REFERENCES

- Rosanna W Peeling, Catherine J Wedderburn, Patricia J Garcia, Debrah Boeras, Noah Fongwen, John Nkengasong, Amadou Sall, Amilcar Tanuri, David L Heymann, Serology testing in the COVID-19 pandemic response,The Lancet Infectious Diseases; 20, Issue 9,2020; 245-249.

- World Health Organization, Regional Office for the Eastern Mediterranean. (2005). Good clinical diagnostic practice: a guide for clinicians in developing countries to the clinical diagnosis of disease and to making proper use of clinical diagnostic services. https://apps.who.int/iris/handle/10665/119735

- Crowther J.R. (1995) Basic Principles of ELISA. In: Crowther J.R. (eds) ELISA. Methods in Molecular Biology™, vol 42. Humana Press. https://doi.org/10.1385/0-89603-279-5:35

We are product to offer India's first COVID-19 antibody neutralising assay.

Kindly support is in reaching out to potential users and we promise that we will NOT let your recommendation down.

Lets unite to fight COVID-19

Chimera Translational Research Fraternity Pvt. Ltd.
209C, IInd & IIIrd Floor, Masjid Moth,
South Extension-II, New Delhi-110049
website - www.translational.in