Quality Control:

To assure the validity of the results, each assay must include both Positive and Negative Controls. The average optical density (OD450) of each control must fall within the values listed in the following table. If OD450 values of controls do not meet the requirements in the following table, the test is invalid and must be repeated. Items OD450 value Control Result for Valid Assay Quality Control >0.6 Negative Control <0.3 Positive Control

Items	OD450 value	Control Result for Valid Assay
Quality Control	>0.6	Negative Control
	<0.3	Positive Control

Interpretation of results:

The 50% signal inhibition cut off for COVID - 19 neutralizing antibody detection can be used for interpretation of the COVID - 19 Neutralization Antibody Detection Kit signal inhibition rate. The operator can determine the result of the sample by comparing the inhibition rate to the following table.

Percent Signal Inhibition = 1 - $\frac{\text{OD value of Sample}}{\text{OD value of Negative Control}}$ × 100%

Example: $\frac{0.954\text{(OD of sample)}}{1.004\text{(D of negative control)}} = 0.95$

1-0.95 = 0.005×100

= 5%

	Result	Test Result Interpretation
≥ 50% Signal Inhibition	Positive	Neutralizing antibodies for COVID - 19 are detected.
< 50% Signal Inhibition	Negative	Neutralizing antibodies for COVID - 19 are not detected.

REFERENCE

1. Chinese Center for Disease Control and Prevention (2020) Public protection guidelines for Novel coronavirus pneumonia, People's Medical Publishing House (PMPH).
2. ZHOU Peng, YANG Xinglou. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature, 2020.
3. XUE Xiongyan, ZHU Changlin, HUANG Shaozhen, Inactivation of 2019 new coronary virus before antibodies detection by different methods. Journal of Southern Medical University, 2020.
4. SHI Heshui, HAN Xiaoyu, FAN Yanqing. Radiologic Features of Patients with 2019-n Co V Infection. Journal of Clinical Radiology, 2020.

IFU/CNAELI01/00
Date: 25/02/2021

Symbols used on Meril Diagnostics labels:

REF Catalogue No.	LOT Batch No.	Consult instruction for use
Manufacturer	Expiry date	Keep away from direct sunlight
Manufacturing date	Keep dry	Do not use if box open or damaged
Storage temperature	Sufficient for	
IVD In Vitro Diagnostics	Caution	

Merilisa COVID-19 Neutralizing Antibody ELISA Test

Meril Diagnostics

IVD For in vitro diagnostic use
Read this pack insert thoroughly before use

REF CNAELI-01
Kit Pack Size : 96 Tests

Intended Use:

Merilisa COVID - 19 Neutralizing Antibody Detection Kit is a blocking enzyme-linked immunosorbent assay intended for qualitative direct detection of total neutralizing antibodies to COVID-19 in human serum and plasma.

Introduction :

Severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2, or 2019-nCoV) is an 3 enveloped non-segmented positive-sense RNA virus. It is the causative agent of Coronavirus disease 2019 (COVID-19), which is contagious in humans. COVID - 19 has several structural proteins including spike (S), envelope (E), membrane (M) and nucleocapsid (N). The spike protein (S) contains a receptor-binding domain (RBD), which is responsible for recognizing the cell surface receptor, angiotensin converting enzyme-2 (ACE2). It is found that the RBD of the COVID - 19 S protein strongly interacts with the human ACE2 receptor leading to endocytosis into the host cells of the deep lung and viral replication. Infection with COVID - 19initiates an immune response, which includes the production of antibodies, or binding antibodies, in the blood. Not all binding antibodies can block cellular infiltration and replication of the COVID - 19virus. The subpopulation of the binding antibodies that can block cellular infiltration and replication of the virus are named neutralizing antibodies. It is unknown how long it takes for neutralizing antibodies to be produced, and if they are always produced after COVID - 19infection. While individuals infected with SARS-CoV-2 develop binding antibodies to the virus, not all of them develop neutralizing antibodies to SARS-CoV-2. The COVID - 19 Neutralization Antibody Detection Kit is specific to COVID - 19 neutralizing antibodies.

Principle :

COVID - 19 Neutralization Antibody Detection Kit is a capture ELISA. Using purified receptor binding domain (RBD), protein from the viral spike (S) protein and the host cell receptor ACE2, this test is designed to mimic the virus-host interaction by direct protein-protein interaction in a test tube or a well of an ELISA plate. The highly specific interaction can then be neutralized, the same manner as in a conventional Virus Neutralization Test (VNT). Recent studies demonstrated that the test does not detect binding antibodies such as those typically detected in conventional indirect ELISA tests, but rather the presence of neutralizing antibodies.

Kit/And Its Components :

Reagents and Materials	Description	Qty / Vol 96 Tests
Microwell Plate	Microwells coated with streptavidin	1 Plate (96 test)
Positive Control	Buffer containing protein stabilizers and antimicrobial agents as preservatives	1 vial (0.250mL)
Negative Control	Normal human serum negative for Anti HIV 1-2, HCV and HBsAg with preservative	1 vial (0.250 mL)
Biotin Conjugate (RTU)	Buffer containing biotin and protein stabilizers.	1 bottle (15.0 mL)
Conjugate Diluent	Buffer Containing Protein Stabilizers	1 bottle (15.0 mL)
51X Enzyme Conjugate	COVID-19 specific Protein labelled with Horseradish peroxidase (HRPO) with protein stabilizers .	1 Vial (0.3 mL)
20X Wash Solution	Buffer containing inactivated and Concentrated Phosphate buffer with surfactant	1 bottle (30.0mL)
Substrate solution(RTU)	TMB with hydrogen peroxide and stabilizers.	1 bottle (12.0 mL)
Stop Solution	1N Sulfuric acid solution	1 bottle (6.0 mL)
Adhesive Sheets	Adhesive sheets (each sheet contains 12 adhesive films) to cover the microwells during incubation	2 Nos.

Storage And Stability:

Store the kit and its components at +2 to +8°C. Expiry date on the kit indicates the date beyond which kit should not be

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used. In case, the desiccant pouch changes colour from blue to light pink or colourless, the strips should not be used.

Additional Materials And Instruments Required But Not Provided Along With The Kit:

- ❖ Micropipettes and microtips
- ❖ ELISA Reader
- ❖ Distilled or deionized water
- ❖ Graduated Cylinders
- ❖ Sodium hypochlorite solution
- ❖ Paper towels or absorbent tissues
- ❖ Timer
- ❖ ELISA washer
- ❖ Incubator
- ❖ Disposable gloves
- ❖ Bio hazard waste container

Specimen Collection And Handling:

1. Human serum, EDTA plasma, Citrate plasma or Heparin plasma samples should be used for the test. While preparing serum samples, remove the serum from the clot as soon as possible to avoid hemolysis. Fresh serum/plasma samples are preferred.
2. Specimens should be free of microbial contamination and may be stored at +2 to +8°C for one week or frozen at -20°C or lower. Avoid repeated freezing and thawing.
3. DO NOT use heat inactivated samples as their use may give false results.Hemolyzed, Icteric and hyperlipemic samples may give erroneous results.
4. DO NOT use Sodium Azide as preservative because it inactivates Horseradish Peroxidase.

Warning And Precautions:

Caution: Kit contain materials of human origin. No test method can offer complete assurance that human blood components will not transmit infection. Controls and samples to be tested should be handled as potentially hazardous though capable of transmitting infection.

1. The use of disposable gloves and proper bio hazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case, there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. DO NOT smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for in vitro diagnostic use only and should be run by qualified person only.
5. DO NOT pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in 1:10 dilution of 5% sodium hypochlorite solution for 30-60 minutes before disposal or by autoclaving at +121°C at 15psi for 60 minutes. DO NOT autoclave materials or solution that contain sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of an accident, contact with eyes or if the contaminated materials are ingested or came in contact with skin puncture / wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. Controls contain Sodium Azide as a preservative. If these materials are to be disposed off through a sink or other common plumbing systems, flush with generous amount of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No.CDC-22", Decontamination of Laboratory Sink Drains to remove Azide salts" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976.)
10. Stop solution contains sulfuric acid. If sulfuric acid comes in contact with the skin, wash thoroughly with water. In case of contact with eyes, flush with excess of water.
11. All materials used in the assay and samples should be disposed off in the manner that will inactivate the virus.

Precautions For Use:

Optimal assay performance requires strict adherence to the assay procedure described in the manual.

1. DO NOT use kit components beyond the expiration date, which is printed on the kit.
2. Avoid microbial contamination of reagents.The use of sterile disposable tips is recommended while removing aliquots from reagent bottles.
3. If blue colour or white particles appears in working substrate solution then DO NOT use it.
4. DO NOT allow microwells to dry once the assay has started.
5. Ensure that the microwell strips are levelled in the strip holder. Before reading, wipe the bottom of the microwell strips carefully with soft, absorbent tissue to remove any moisture.
6. If available, a microwell reader which contains a reference filter with settings at 620 or 630 nm should be used. Use of a reference filter minimizes interference due to microwells that are opaque, scratched or irregular. However, if a reference filter is unavailable, the absorbance may be read at 450 nm without a reference filter.
7. Distilled or deionized water must be used for wash buffer preparation.
8. Bring all the reagents to room temperature before use.
   Note: As per USP room temperature is +15°C to +30°C.
9. DO NOT combine reagents from different batches, as they are optimized for individual batch to give best results.
10. Due to interchange of caps the reagents may get contaminated. Care should be taken while handling the reagents to avoid any sort of contamination.
11. Run negative control and positive control in each assay.
12. Use freshly collected samples for assay.Try to avoid hemolyzed turbid, lipemic serum or plasma samples.
13. Use a separate tip for each sample and then discard it as bio-hazardous waste.
14. Thorough washing of the wells is critical for the best performance of the assay.
15. Avoid strong light exposure during the assay.

Preparation Of Working Conjugate Solution

Dilute Conjugate (51X), 1:51 with Conjugate Diluent as per Table 1.

Table 1 :

No.of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Conjugate Diluent, mL	1	2	3	4	5	6	7	8	9	10	11	12
Conjugate (51X), µl	20	40	60	80	100	120	140	160	180	200	220	240

Preparation Of Working Wash Buffer :

a) Check the buffer concentrate for the presence of salt crystals. If crystals are present in the solution, re-solubilize by warming at +37°C until all crystals dissolve.

b) Prepare at least 20ml.(1ml. concentrated buffer with 19 ml.of distilled or deionized water) of buffer for each strip used. Mix well before use.

c) Mix 30 ml. of 20X wash buffer concentrate with 570ml. of distilled or deionized water. Wash buffer is stable for 1 month when stored at +2 to +8°C.

Test Procedure :

Step 1:

Use only number of wells required for the test. Avoid touching the tops or bottoms of the wells. Replace the remaining wells in the provided zip lock pouch and seal it immediately.

Step 2:

Add 100 µl of ready to use Biotin conjugate to each well except blank well (A1) (Blank is optional)

Step 3:

Add 50 µl of Negative Control In to B1 well,add 50 µl of the Positive Control in to C1 well and add 50 µl of sample from D1 onwards.

Step 4:

Add 100 µl working Enzyme conjugate in to each well except blank.

Step 5:

Cover the wells with adhesive sheet(s) and incubate for 90 minutes at +37°C ± 1°C.

Step 6:

After incubation Fill the wells with working washing solution (350µl).Thereafter decant the buffer and blot the plate on absorbent paper. Repeat this step for four (04) additional times.

Step 7 :

Add 100 µl of the ready to use substrate solution to each well.

Step 8 :

Cover the wells with adhesive strip and incubate for 30 minutes in dark at room temperature. A blue or bluish green color should develop in well containing non-reactive and colorless well indicate reactive sample.

Step 9 :

Add 50 µl of stop solution to each well.

Step 10 :

Within 15 minutes read the absorbance at 450 nm using 630 nm as the reference wavelength.

Procedural Flow Chart