1. Intended Use :
Maxlisa Dengue IgM ELISA is an in vitro enzyme immunoassay for the qualitative determination of human anti-DENGUE IgM antibody in human serum and plasma.

2. Summary and Explanation:
Dengue is a large positive-sense single-stranded ribonucleic acid (RNA) virus. It has a diameter of 50–200 nm and possesses spikes on its surface (up to 20 nm in length) that provide it the crown-like appearance, a characteristic of coronaviruses (CoVs). Coronaviruses are comprising of four structural proteins, i.e., nucleocapsid protein (NP) that holds the viral RNA, spike protein (SP), envelope protein (EP), and membrane protein (MP), that create the viral envelope. In humans, coronaviruses cause respiratory infections. Human to human transmission of coronaviruses is primarily spread during close contact, most often via small droplets produced by coughing, sneezing and talking. IgG is the most abundantly found immunoglobulin to be produced in response to an antigen and will be maintained in the body after initial exposure for long term response.

3. Test Principle:
Dengue IgM Microlisa test is an enzyme immunoassay based on "MAC Capture ELISA". Anti human IgM antibodies are coated onto microtiter wells. Specimens and controls are added to the microtiter wells and incubated. Antibodies to Dengue if present in the specimen, will bind to the Anti human IgM antibodies adsorbed onto the surface of the wells. The plate is then washed to remove unbound material. Horseradish peroxidase (HRPO) conjugated Dengue antigen is added to each well. This Dengue antigen conjugate will bind to Dengue specific IgM antibodies which is complex with anti human IgM antibodies. Finally substrate solution containing chromogen and hydrogen peroxide is added to the wells and incubated. A blue colour will develop in proportion to the amount of Dengue antibodies present in the specimen. The colour reaction is stopped by a stop solution. The enzyme substrate reaction is read by EIA reader for absorbance at a wavelength of 450 nm/620 nm. If the sample does not contain Dengue IgM antibodies then enzyme conjugate will not bind and the solution in the wells will be either colourless or only a faint background colour develops.

4. Key Contents:
Store all components at 2-8°C when not in use.

Material	96 Tests
Dengue-antigen coated microplate (1 x 96 well microplate)	Microplate coated with anti human IgM, packed in a pouch with desiccant
Positive Control (1 x 0.5 ml)	Ready to use
Negative Contol (1 x 1 ml)	Ready to use
Sample Diluent (1 x 30 ml)	Buffer solution containing stabilizing proteins and preservatives
Conjugate diluent (1 x 12 ml)	Buffer solution containing stabilizing proteins and preservatives
Conjugate concentrate (100X) (1 x 0.2 ml)	HRP conjugate to be diluted with conjugate diluent.
Wash Buffer (25X) (1 x 30 ml)	Buffer containing surfactants
TMB Diluent (1 x 15 ml)	Buffer solution containing H_2O_2 with preservative.
TMB Substrate (1 x 0.2 ml)	To be diluted in TMB diluent before use.
Stop solution (1 x 15 ml)	Ready to use, 0.1N Sulfuric acid
Pack insert	

5. Materials required but not provided:
- Distilled or Deionized water.
- Micropipettes and Micro tips.
- Graduated cylinders for reagent bottles.
- Paper towels or Absorbent tissue.
- 70% Isopropanol solution.
- Vortex mixer.
- Incubator (37°C).
- ELISA Washer.
- ELISA Reader.
- Timer.
- Biohazard waste container with sodium hypochlorite solution.
- Disposable gloves.

6. Sample collection, Preparation and Storage:
- Only human serum or 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.
- Serum and plasma (EDTA) samples may be stored for up to 7 days at 2-8°C or at least 6 months as frozen (-20 to -70°C).
- Avoid repeated freezing and thawing.
- Do not use sodium azide as preservative because it inactivates horseradish peroxidase.
- Microbial contaminated and hemolyzed samples may give erroneous results.

7. Precautions:
- For in vitro diagnostic use only
- Bring all reagents and specimen to room temperature before use.
- The use of disposable gloves and proper biohazards clothing is strongly recommended while running the test.
- Do not eat, drink or smoke in the area where testing is done
- In case there is a cut/wound in hand, do not perform the test.
- Do not pipette any material by mouth.
- Do not mix components of one kit with another.
- Do not allow liquid from one well to mix with other wells.
- Do not let the strips dry in between the steps.
- All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121°C for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
- Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
- 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.

8. Preparation of Reagents:
Note: Before use, allow reagents and samples at room temperature (20-30°C).
8.1. Ready for use reagents:
8.1.1. Microplate:
Each frame support containing 12 strips is wrapped in a sealed foil bag. Cut the bag using scissors or a scalpel above the sealing. Open the bag and take out the frame. Put the unused strips back into the bag. Close the bag carefully and put it back into storage at ±2-8°C.

Caution: Handle Microwell strips with care. Do not touch the bottom exterior surface of the walls.
8.1.2. Negative control
8.1.3. Positive control
8.1.5. Sample diluent
8.1.6. Stop solution
8.2. Reagents to reconstitute:

8.2.1. Wash buffer (25X):

- Check the buffer concentrate for the presence of salt crystals. If crystals are present in the solution, resolubilize by warming at 37°C until all crystals dissolve.
- Dilute 1:25 in distilled water to obtain the ready to use washing solution. Mix 20 ml of 25X wash buffer concentrate with 480ml of distilled or deionized water. Working wash buffer is stable for 2 months when stored at 2-8°C.

8.2.2. Preparation of working conjugate:

Make a 1:100 dilution of conjugate concentrate with conjugate diluent. Do not store working conjugate. Prepare conjugate 10 minutes before use.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
Enzyme Conjugate Concentrate (µl)	10	20	30	40	50	60	70	80	90	100	110	120
Conjugate Diluent in (ml)	1	2	3	4	5	6	7	8	9	10	11	12

8.2.4. Preparation of Substrate:

Mix TMB Substrate and TMB Diluent in1:1 ratio to prepare working substrate buffer 5 to 10 minutes before use. Avoid exposure to light. Substrate should be used only after thawing at 37°C, if crystallized.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
TMB Substrate (µl)	10	20	30	40	50	60	70	80	90	100	110	120
TMB Diluent (ml)	1	2	3	4	5	6	7	8	9	10	11	12

9. Test procedure:

- Bring all the reagents and specimen to room temperature before use.
- Take the required number of strips and fix them to frame and immediately close the pouch.
- Prepare template in data sheet indicating the location of controls and specimens.
- Add 100µl of sample diluent to required number of wells
- Add 10µl of negative control A1 & B1 wells respectively.
- Add 10µl of positive control in C1
- Add 10µl of test samples in each well, starting from E1.
- Mix gently and cover plate with plate sealer and incubate for 45 minutes at 37°C.
- Before 5 to 10 minutes of incubation, make a 1:100 dilution of conjugate with conjugate diluent.
- After incubation, aspirate the contents from all the wells and wash each well 6 times with by filling approximately 300µl diluted wash buffer.
- Invert the plate and tap it on absorbent paper to remove the remaining washing solution, and then pipette 100µl of prepared diluted conjugate into each well.
- Incubate the plate at 37°C for 30 minutes.
- Before 5 to 10 minutes of incubation, make a 1:100 dilution of substrate with substrate buffer.
- Aspirate and wash as described in step no 10.
- Invert the plate and tap it on absorbent paper to remove the remaining washing solution, and then pipette 100µl of prepared diluted substrate into each well and incubate at room temperature for 30 minutes.
- Add 100µl of stop solution each well.
- Read absorbance at 450nm/620 nm within 30 minutes in ELISA READER

10. Flow chart for Test Run criteria:

Add 100µl of samples diluent in each wells
↓
Add 10µl of negative, positive controls and samples
↓
Apply plate sealer and incubate for 45 mins at 37°C
↓
Wash each well 6 times with 300µl of wash buffer and dry with absorbent paper
↓
Add 100µl of conjugate in each well and incubate at 37°C for 30mins
↓
Wash each well 6 times with 300µl of wash buffer and dry with absorbent paper
↓
Add 100µl of substrate solution in each well and incubate at RT for 30mins
↓
Add 100µl of stop solution to stop reaction
↓
Read results at 450/620nm

11. Calculation of the cut-off value
Negative control means (Ncx)

Absorbance of Negative control (NC)	
Negative Control 1	0.095
Negative Control 2	0.089
Negative Control Mean (NCx)	**(0.095+0.089)/2 = 0.092**

Cut off value: NCx+0.2 = 0.092 + 0.2 = 0.292
Positive control acceptance criteria:
PC must be ≥0.50.
Abbrevations:
NC - Absorbance of Negative control
NCx - Mean Negative control
PC - Absorbance of the Positive control

13. Interpretation of Results:

- For the assay to be valid, the positive control must be greater than 0.5.
- For the assay to be valid, the negative controls mean should be less than 0.2.

15. References:

- Centers for Disease Control, Update on Acquired Immune Deficiency Syndrome (AIDS) MMWR 1982; 31: 507-508.
- Chenjia Yuan, Shi Jinsong , Qiudong An , Liu Chang , Li Xin , Qiang , Ruanji Shou , mountains . Wuhan 2019 Bioinformatics coronavirus genome analysis [J / OL]. Bioinformatics : 1-10 [2020-02-10].

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MAXLISA Dengue IgM ELISA

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