

# DENGUE IgM MICROLISA

## MAC-ELISA Test for the Detection of Dengue IgM Antibodies in Human Serum/Plasma

### 1. INTRODUCTION

The mosquito-borne dengue viruses (serotype 1-4) cause dengue fever, a severe flu-like illness. The disease is prevalent in third world tropical regions and spreading to sub-tropical developed countries - including the United States. WHO estimates that 50-80 million cases of dengue fever occur worldwide each year, including a potentially deadly form of the disease called dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Primary infection with dengue virus results in a self-limiting disease characterized by mild to high fever lasting 3 to 7 days, severe headache with pain behind the eyes, muscle and joint pain, rash and vomiting. Secondary infection is the more common form of the disease in many parts of Southeast Asia and South America. This form of the disease is more serious and can result in DHF and DSS. The major clinical symptoms can include high fever, haemorrhagic events, and circulatory failure, and the fatality rate can be as high as 40%. Early diagnosis of DSS is particularly important, as patients may die within 12 to 24 hours if appropriate treatment is not administered. Primary dengue virus infection is characterized by elevations in specific IgM antibody levels 3 to 5 days after the onset of symptoms; this generally persists for 30 to 60 days. IgG levels also become elevated after 10 to 14 days and may persist for years depending on host immunity. During secondary infection, IgM levels generally rise more slowly and reach lower levels than in primary infection, while IgG levels rise rapidly from 1 to 2 days after the onset of symptoms.

### 2. INTENDED USE

**DENGUE IgM MICROLISA** is designed for in-vitro qualitative detection of Dengue IgM Antibodies in human serum or plasma and is used as a screening test for testing of collected blood samples suspected for DENGUE. The kit detects all four subtypes; DEN1, DEN2, DEN3 & DEN4 of Dengue Virus.

### 3. 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 absorbed onto the surface of the wells. The plate is then washed to remove unbound material. Horseradish peroxidase (HRPO) conjugated Dengue antigen (DEN 1-4) 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. 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. DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO 15223-1:2021.

|  |   |
|--|---|
|  Manufactured By                              |  <i>In vitro</i> diagnostic medical device     |
|  No. of tests                                 |  See Instruction for use                       |
|  Lot Number<br>Batch Number                   |  Temperature Limitation                        |
|  Manufacturing Date                           |  Caution<br>See instruction for use            |
|  Expiry Date                                  |  Catalogue Number                              |
|  Do not use if package is damaged             |  Keep away from sunlight                       |
|  Contains biological Material of Human Origin |  Contains biological Material of Animal Origin |
|  Country of Manufacture                       |  Keep Dry                                      |

### 5. KIT PRESENTATION

- 96 Test Pack

### 6. KIT & ITS COMPONENTS

| COMPONENT  | DESCRIPTION   |
|------------|---|
| Microwells | Breakway microwells coated with Anti-human IgM antibodies packed in a pouch with dessicant. |

|                               |  |
|-------------------------------|--|
| Sample Diluent                | Buffer containing protein stabilizers and antimicrobial agents as preservative.            |
| Enzyme Conjugate              | Dengue antigen labelled with horseradish peroxidase with protein stabilizers. Ready to use |
| Wash Buffer Concentrate (25X) | PBS with surfactant. Dilute 1:25 with distilled water before use.                          |
| TMB Diluent                   | Buffer solution containing H <sub>2</sub> O <sub>2</sub> with preservative.                |
| TMB Substrate                 | To be diluted with TMB Diluent before use.   |
| Control<br>—                  | Ready to use, Normal human serum negative for Dengue antibodies with preservative.         |
| Control<br>+                  | Ready to use, positive for Dengue IgM antibodies with preservative.                        |
| Calibrator                    | Ready to use, positive for Dengue IgM antibodies with preservative.                        |
| Stop Solution                 | Ready to use, 1N sulfuric acid.  |
| Plate Sealers                 | Adhesive packed sheets for sealing microtiter plate/strips.                                |

### 7. STORAGE AND STABILITY

Store the kit & its components at 2-8°C. Expiry date on the kit indicates the date beyond which kit should not be used.

### 8. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

- Micropipettes and microtips
- Elisa reader
- Distilled or deionized water
- Graduated Cylinders, for reagent dilution
- Paper towels or absorbent tissue
- Timer
- Elisa washer
- Incubator 37°C
- Disposable gloves
- Disinfectant Solution

### 9. SPECIMEN COLLECTION & HANDLING

1. 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 haemolysis. Fresh serum/plasma samples are preferred.
2. Specimens should be free of microbial contamination and may be stored at 2-8°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. Haemolyzed and Icteric hyperlipemic samples may give erroneous results.

### 10. SPECIMEN PROCESSING

#### (A) FROZEN SAMPLE

Dengue IgM MICROLISA test is best used with fresh samples that have not been frozen and thawed. However most frozen samples will perform well if the procedure suggested below is followed.

Allow the frozen sample to thaw in a vertical position in the rack. Do not shake the sample. This allows particles to settle to the bottom. If a centrifuge is available, the sample should be centrifuged. (5000 rpm for 5 min.)

#### (B) TRANSPORTATION

If the specimen is to be transported, it should be packed in compliance with the current Government regulations regarding transport of aetiologic agents.

### 11. CAUTION

1. The use of Disposable Gloves and proper Biohazardous 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.
5. All the samples to be tested should be handled as though capable of transmitting infection.

6. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. In case of needle prick or other skin puncture or wounds, wash the hands with excess of water and soap.
7. Controls contain Sodium Azide as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds.
8. All materials used in the assay and samples should be disposed off in the manner that will inactivate virus.
9. ELISA Reader & micropipettes used in testing should be calibrated at regular interval to ensure accurate results.

## 12. 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. 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.
4. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
5. Take care while preparing working substrate solution as vials of TMB Substrate & TMB Diluent are of same size.
6. Prepare working substrate solution just 10 minutes prior to adding in the wells.
7. If blue colour or white particles appears in working substrate solution then do not use it. Take fresh containers and tips and prepare it again.
8. Use separate tips for TMB substrate and TMB diluent.
9. Do not allow microwells to dry once the assay has started.
10. 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.
11. 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 minimises 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.
12. Distilled or deionised water must be used for wash buffer preparation.
13. Bring all the reagents to room temperature (20-30°C) before use.
14. Do not combine reagents from different batches, as they are optimized for individual batch to give best results.
15. Due to interchange of caps the reagents may get contaminated. Care should be taken while handling the reagents to avoid contamination of any sort.
16. Run calibrator, negative and positive controls in each assay.
17. Use freshly collected, clean serum samples for assay. Try to avoid Haemolyzed turbid, lipemic serum or plasma samples.
18. Use a separate tip for each sample and then discard it as biohazardous waste.
19. Thorough washing of the wells is critical to the performance of the assay.
20. Avoid strong light exposure during the assay.

## 13. PREPARATION OF REAGENTS

Prepare the following reagents before or during assay procedures. Reagents and samples should be at room temperature (20-30°C) before beginning the assay and can remain at room temperature during testing. Return reagents to 2-8°C after use. All containers used for preparation of reagents must be cleaned thoroughly and rinsed with distilled or deionized water. Prewarm the incubator to 37°C.

### i) Anti human IgM antibodies coated strips

Bring foil pack to room temperature (20-30°C) before opening to prevent condensation on the microwell strips.

- a. Break-off the required number of strips needed for the assay and place in the well holder. Take the strip holder with the required number of strips, taking into account that one each of negative & positive control & three calibrator should be included in the run while opening the fresh kit. However for one or two strips, one each of negative, positive control & two calibrator and for more strips one each of negative and positive control & three calibrator should be included in each subsequent runs.
- b. **Unused wells should be stored at 2-8°C, with dessicant in aluminium pouch with clamp & rod. Microwells are stable for 30 days at 2-8°C from the date of opening of sealed pouch, when stored with desiccant along with clamp & rod.**

**Caution :** Handle microwell strip with care. Do not touch the bottom exterior surface of the wells.

### ii) Sample Preparation:

**TUBE DILUTION:** Mark the tubes carefully for the proper identification of the samples. Dilute the serum samples to be tested, with sample diluent 1:100 in separate tubes (1 ml. sample diluent + 10 µl serum samples). Use a separate tip for each sample and then discard as biohazardous waste.

### iii) Preparation of Working Wash Buffer:

- a) 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.
- b) Prepare at least 25ml. (1ml. concentrated buffer with 24 ml. water) of buffer for each strip used. Mix well before use.
- c) Mix 20 ml. of 25X wash buffer concentrate with 480 ml. of distilled or deionized water. **Working wash buffer is stable for 2 months when stored at 2-8°C.**

### iv) Preparation of working substrate solution :

Mix TMB substrate and TMB Diluent in 1:1 to prepare working substrate.

|                    |     |     |     |     |     |     |     |     |     |     |     |     |
|--------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 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 (ml) | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 4.5 | 5.0 | 5.5 | 6.0 |
| TMB Diluent (ml)   | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 4.5 | 5.0 | 5.5 | 6.0 |

**Do not store working substrate.** Prepare a fresh dilution for each assay in a clean plastic/glass vessel. Determine the quantity of working substrate solution to be prepared from table. Mix solution thoroughly before use. Discard unused solution. A deep blue color present in the substrate solution indicates that the solution has been contaminated and must be discarded.

## 14. PROCEDURAL NOTES:

1. Material should not be used after the expiry date shown on the labels. Components and test specimen should be at room temperature (20-30°C) before testing begins. Return the reagents to 2-8°C after use.
2. All reagents must be mixed well before use.
3. To avoid contamination, do not touch the top or bottom of strips or edge of wells.
4. All pipetting steps should be performed with utmost care and accuracy. Cross contamination between reagents and samples will invalidate results.
5. Prevent evaporation during sample incubation by covering the strips with sealer; remove sealer before washing.
6. Routine maintenance of wash system is strongly recommended to prevent carry over from highly reactive specimens to non reactive specimens.













## 15. TEST PROCEDURE

Once the assay has started, complete the procedure without interruption. All the reagents should be dispensed in the centre of the well and the tip of the pipette should not touch the wall of the microwell.

Fit the strip holder with the required number of Anti-human IgM coated strips. The sequence of the procedure must be carefully followed. Arrange the assay control wells in a horizontal or vertical configuration. Configuration is dependent upon reader software.

1. Add 100 µl Negative Control in A-1 well.
2. Add 100 µl calibrator in B-1, C-1 & D-1 wells.
3. Add 100 µl Positive Control in E-1 well.
4. Add 100 µl of each sample diluted in sample diluent (1:100), in each well starting from F-1 well. (Refer **TUBE DILUTION**).
5. Apply cover seal.
6. Incubate at 37°C ± 1°C for 60 min. ± 1 min.
7. While the samples are incubating, prepare working Wash Solution as specified in preparation of reagents.
8. Take out the plate from the incubator after the incubation time is over and, wash the wells 5 times with working Wash Solution.
9. Add 100 µl of Enzyme Conjugate in each well.
10. Apply cover seal.
11. Incubate at 37°C ± 1°C for 60 min ± 1 min.
12. Aspirate and wash as described in step no. 8.
13. Add 100 µl of working substrate solution in each well.
14. Incubate at room temperature (20-30°C) for 30 min. in dark.

15. Add 50  $\mu$ l of stop solution.
16. Read absorbance at 450 nm and 630 nm (reference filter) within 30 minutes in ELISA READER.

| SUMMARY OF PROCEDURE                       |   |  |                     |
|--|---|--|---------------------|
| Dilute Serum samples                       |    | Sample 10 $\mu$ l  | Sample Diluent 1 ml |
| Add diluted samples & *Calibrator/ Control |    | 100 $\mu$ l  |                     |
| Cover the plate & incubate                 |    | 60 mins. at 37°C   |                     |
| Wash                                       |    | 5 Cycles   |                     |
| Add Enzyme Conjugate*                      |    | 100 $\mu$ l  |                     |
| Cover the plate & incubate                 |    | 60 mins. at 37°C   |                     |
| Wash                                       |    | 5 Cycles   |                     |
| Prepare Working Substrate                  |    | No of Strips 1 2 3 4 5 6 7 8 9 10 11 12<br>TMB 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0<br>Substrate (ml) 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0<br>TMB Diluent (ml) 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 |                     |
| Add Substrate                              |    | 100 $\mu$ l  |                     |
| Incubate in dark                           |  | 30 mins. at Room Temp.   |                     |
| Add Stop Solution                          |  | 50 $\mu$ l   |                     |
| Read Results                               |  | 450 nm./630 nm.  |                     |

\* Calibrator/Controls and Enzyme Conjugate are Ready to use

#### TEST VALIDITY:

##### Ensure the following is within specified acceptance criteria

- i) NC O.D. must be < 0.3. If it is not so, the run is invalid and must be repeated.
- ii) PC O.D. must be > 1.0. If it is not so, the run is invalid and must be repeated.
- iii) Mean Calibrator O.D. must be  $\geq$  0.35. If it is not so, the run is invalid and must be repeated.
- iv) Cut off value must be  $\geq$  1.5 x NC O.D. If it is not so, the run is invalid and must be repeated.
- v) Ratio of PC O.D. / cut off must be > 1.1. If it is not so, the run is invalid and must be repeated.

#### 16. CALCULATION OF RESULTS

**Imp. Note:** The calibration factor detail is batch specific and stamped on back page of Instruction manual.

- a. Cut off value = mean O.D. of calibrator x calibration factor
- b. Calculation of sample O.D. ratio : Calculate sample O.D. ratio as follows:

$$\text{Sample O.D. ratio} = \frac{\text{Sample O.D.}}{\text{Cut off Value}}$$

- c. Calculation of Dengue IgM units : Calculate by multiplying the sample O.D. ratio by 10.  
Dengue IgM units = sample O.D. ratio x 10.  
e.g.: Mean O.D. of calibrator = 0.75  
Calibration factor = 0.7  
Cut off value = 0.75 x 0.7 = 0.525  
e.g.: sample absorbance (O.D.) = 0.925  
Cut off value = 0.525  
Sample O.D. ratio = 0.925 / 0.525 = 1.761  
Dengue IgM units = 1.761 x 10 = 17.61

#### 17. INTERPRETATION OF RESULTS

- a. If the Dengue IgM Units is < 9 then interpret the sample as Negative for Dengue IgM antibodies.
- b. If the Dengue IgM Units is between 9 - 11 then interpret the sample as Equivocal for Dengue IgM antibodies.
- c. If the Dengue IgM Units is > 11 then interpret the sample as Positive for Dengue IgM antibodies.

#### 18. PERFORMANCE CHARACTERISTICS

The kit has been evaluated with the known panel of Dengue IgM positive and Negative samples. The samples included cross-reacting samples; Epstein-BARR virus, Leptospira, Malaria, Hepatitis-A, Influenza A & B, S. typhi. Following is the in-house evaluation.

| No. of Samples | Status          | Dengue IgM Microlisa |           |          |
|----------------|-----------------|----------------------|-----------|----------|
|                |                 | Positive             | Equivocal | Negative |
| 175            | Dengue Positive | 175                  | 0         | 0        |
| 5306           | Dengue Negative | 3                    | 0         | 5303     |

**Sensitivity** : 100%

**Specificity** : 99.88%

**Precision** : Within-run and between-run precisions have been determined by testing 10 replicates of five samples: 1 negative and 4 dengue IgM positive; 2 weak positive, 1 medium positive & 1 strong positive. The C.V.(%) of negative, weak positive, medium positive & strong positive values were within 10%.

#### 19. LIMITATION OF THE TEST

1. The test should be used for detection of IgM antibodies of Dengue in human serum / plasma.
2. **This is only a screening test** and will only indicate the presence or absence of Dengue antibodies in the specimen. All reactive samples should be confirmed by confirmatory test. Therefore for a definitive diagnosis, the patients clinical history, symptomatology as well as serological data should be considered. The results should be reported only after complying with the above procedure.
3. False positive results can be obtained due to cross reaction with Epstein-BARR virus, RA, Leptospira, Malaria, Hepatitis-A, Influenza A & B, S. typhi Japanese encephalites, west Nile virus disease. This occurs in less than 1% of the sample tested.
4. Immuno-depressive treatments presumably after the immune response to infection, inducing negative results in IgM in Dengue patients.

#### 20. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an *in vitro* diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacture's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed.

The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application thereof.

#### 21. REFERENCES

1. Pinheiro FP, Corber SJ: Global situation of dengue and dengue haemorrhagic fever and its emergence in the Americas. World Health Stat 1 50(3/4):161-169, 1997.
2. Gubler DJ, Trent DW: Emergence of epidemic dengue/dengue hemorrhagic fever as a public health problem in the Americas. Infect Agents Dis 2:383-393, 1993.
3. Wu SJ, Hanson B, Paxton H, Nisalak A, Vaughn DW, Rossi C, Henchal EA, Porter KR, Watts DM, Hayes CG. Evaluation of a dipstick test for detection of antibodies to dengue virus. Clin Diagn Lab Immunol 1997; 4(4):452-7.

#### 22. TROUBLE SHOOTING CHART

| PROBLEM                                    | POSSIBLE CAUSE  | SOLUTION   |
|--|---|--|
| 1. No colour developed at the end of assay | a) Any one reagent has been added in wrong sequence.      | Follow the procedure meticulously & repeat assay.  |
|  | b) Inactivated enzyme conjugate due to improper storage   | Check storage of enzyme conjugate at 2-8°C and it should be free of any contamination.                               |
|  | c) Microplate inactivated, due to improper storage        | Keep unused strips in aluminium poly pouch with the dessicant pouch inside and properly closed with clamp & rod.     |
|  | d) Inactivated substrate, improper storage or preparation | It should be free of any contamination. Use freshly prepared substrate solution. Recheck procedure and repeat assay. |

| PROBLEM  | POSSIBLE CAUSE   | SOLUTION  |
|--|--|---|
|  | e) Omission of any step in test procedure  | Follow the procedure meticulously & repeat assay.   |
|  | f) Incorrect (low) incubator temperature, timing or pipetting  | Check incubator temperature, procedure & repeat assay   |
|  | g) Improper preparation of working wash buffer (dilution error), improper mixing of reagents.                | Check procedure & repeat assay  |
|  | h) Kit deterioration   | Check storage of the kit and should be stored at 2-8°C.   |
| 2. High O.D. value of Negative control                         | a) Plate not stopped after 30 minutes of adding stop solution  | Follow the procedure meticulously & repeat assay.   |
|  | b) Same microtip used for Calibrator, Positive and negative controls.  | Change micropipette tips while addition of Calibrator, positive and negative control.                                     |
|  | c) Nonspecific attachment/ binding of other reagent  | If plates get scratches/ aberrations during washing, non specific proteins may bind while addition of next step.          |
| 3. Too much colour in all wells of the plate (high background) | a) Contaminated substrate  | Check substrate (TMB Diluent) it should be colourless. If blue in colour then discard and use clean disposable container. |
|  | b) Contaminated washing solution (1X). Poor quality of water used for diluting wash buffer conc.             | Check the container and quality of water used for dilution. Use of glass distilled water is preferred.                    |
|  | c) Over incubation of substrate and delay in addition of stop solution.                                      | Follow the procedure meticulously.  |
|  | d) Insufficient washing.   | Check wash device, fill the well close to the top.  |
|  | i) Washing not consistent  | After washing, blot the microwells on absorbent   |
|  | ii) Filling volume not sufficient.   | tissue. Follow wash protocol meticulously   |
|  | iii) Insufficient no. of wash cycles.  |   |
|  | iv) Contaminated wash device   |   |
|  | e) Use of wash solution from other manufacturer.   | Use only Dengue IgM Microlisa wash solution.  |
|  | f) Working substrate not protected from light  | Incubate the plate in dark after addition of substrate.   |
|  | g) Improper preparation of working wash buffer (dilution error), improper mixing of reagents.                | Check procedure & repeat assay  |
| 4. Poor reproducibility  | a) Washing problems.   | Check all 8 ports/ manifold for uniform flow of wash buffer. If there are blockage, clean the ports.                      |
|  | b) Uncalibrated pipettes or tips not well fitted, improper pipetting/ dispensing.                            | Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.                                  |
|  | c) Reagent & sera not at room temperature or not well mixed before use.                                      | Equilibrate reagents to room temperature and mix thoroughly before use  |
|  | d) Too long time for addition of calibrator, controls, samples or reagents, Inconsistency in time intervals. | Develop consistent and uniform technique.   |
|  | e) Interference in optical pathway due to Air bubbles.   | Clean or dry the bottom of microwells, check for bubbles and repeat the readings.   |

| PROBLEM   | POSSIBLE CAUSE   | SOLUTION   |
|---|--|--|
| 5. False Positive   | a) Beside 3a, b, c, d, e & f incorrect interpretation and calculation of final results | Check the calculation part given in the insert and correctly interpret.                  |
|   | b) High incubator temperature, incorrect timing or pipetting                           | Check incubator temperature, procedure & repeat assay.                                   |
|   | c) Use of turbid/ lipaemic or hemolyzed sample.  | Centrifuge the sample at 5000 rpm for 30 minutes and re-run the test with clear sample.  |
| 6. False Negative/ low O.D. of Calibrator, Positive control / positive sample | a) Inadequate addition of substrate/conjugate solution.                                | Follow the procedure meticulously & repeat assay.  |
|   | b) Kit expired, reagent of different kit used.   | Check the expiry of the kit before use.  |
|   | c) White particles in working substrate solution.                                      | Discard the substrate and prepare the working substrate again in fresh tube.             |
|   | d) Uncalibrated pipettes, improper pipetting.  | Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling. |
|   | e) Deterioration of Enzyme conjugate, TMB Substrate/ TMB Diluent.                      | Check storage of reagents. They shall be stored at 2-8°C.                                |
|   | f) Stop solution is added before 30 minutes. Reaction terminated before 30 minutes.    | Follow the test procedure meticulously.  |
|   | g) O.D. taken at incorrect wavelength.   | Read O.D. values at 450 nm and 630 nm.   |
|   | h) Incorrect incubator temperature, timing or pipetting                                | Check incubator temperature, pipetting & repeat assay.                                   |
|   | i) Kit deterioration   | Check storage of kit and it should be stored at 2-8°C.                                   |
|   | j) Sample deterioration due to improper storage and / or microbial contamination.      | Store the sample at 2-8°C / -20°C as recommended in the specimen collection & handling.  |

*in vitro* diagnostic Reagent, not for medicinal use

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