CHIKUNGUNYA IgM MICROLISA

Microwell ELISA Test for the Qualitative Detection of Chikungunya Specific IgM antibody in Human Serum/Plasma

1. INTRODUCTION

Chikungunya virus (CHIKV) is an insect-borne virus, of the genus *Alphavirus*, that is transmitted to humans by virus-carrying *Aedes* mosquitoes. There have been recent breakouts of CHIKV associated with severe illness. CHIKV causes an illness with symptoms similar to dengue fever. CHIKV manifests itself with an acute febrile phase of the illness lasting only two to five days, followed by a prolonged arthralgic disease that affects the joints of the extremities. The pain associated with CHIKV infection of the joints persists for weeks or months, or in some cases years. Serological diagnosis requires a larger amount of blood than the other methods and uses an ELISA assay to measure Chikungunya-specific IgM levels.

2. INTENDED USE

Chikungunya IgM Microlisa is designed for in vitro qualitative detection of Chikungunya IgM in human serum or plasma and is used as a screening test for testing of collected blood samples suspected for Chikungunya.

3. PRINCIPLE

Chikungunya IgM Microlisa test is an enzyme immunoassay based on "MAC Capture ELISA".

Anti-human IgM antibodies are coated onto microtiter wells. Specimens, controls and calibrator are added to the microtiter wells and incubated.

IgM antibodies to Chikungunya 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. Chikungunya antigen is added to each well. This antigen will bind to human antibodies against chikungunya anti-human IgM complex present. The plate is washed to remove unbound material. Enzyme conjugate (anti-chikungunya antibodies conjugated Horseradish peroxidase (HRPO)) is added to each well. This conjugate will bind Chikungunya antigen present. 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 Chikungunya 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 Chikungunya 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	IVD	*In vitro* diagnostic medical device
Σ	No. of tests		See Instruction for use
LOT	Lot Number Batch Number	2°C 8°C	Temperature Limitation
	Manufacturing Date		Caution See instruction for use
	Expiry Date	REF	Catalogue Number
	Do not use if package is damaged		Keep away from sunlight
BIO	Contains biological Material of Animal Origin	BIO	Contains biological Material of Human Origin
	Keep Dry	IN	Country of Manufacture

5. KIT PRESENTATION

- 96 Test Pack

6. KIT & ITS COMPONENTS

Microwells	Breakaway microwells coated with anti-human IgM antibodies packed in a pouch provided with desiccant.
Sample Diluent	Buffer containing protein stablizers and antimicrobial agents as preservative.
Chikungunya Antigen (Lyophilized)	Lyophilized Chikungunya antigen, to be reconsituted with 1.5 ml of Antigen Diluent.
Enzyme Conjugate	Anti-chikungunya antibody labelled with horseradish peroxidase with preservative. Ready to use
Antigen Diluent	Buffer containing stabilizers.
Wash Buffer Concentrate (25X)	PBS with surfactant. Dilute 1:25 with distilled water before use.
TMB Substrate	To be diluted with TMB Diluent before use.
TMB Diluent	Buffer solution containing H_2O_2 with preservative.
Control —	Ready to use, Normal human serum negative for Chikungunya Antibodies and contains preservative.
Control +	Ready to use, Positive for Chikungunya IgM antibodies and contains preservative.
Calibrator	Ready to use, Positive for Chikungunya IgM antibodies and contains preservative.
Stop Solution	Ready to use, 1N sulfuric acid.
Plate Sealers	Adhesive sheets to cover the microwells during incubation.

7. STORAGE AND STABILITY

The kit should be stored at 2-8°C in the cool and driest area available. Expiry date on the kit indicates the date beyond which kit and its components should not be used. Chikungunya IgM Microlisa should not be frozen and must be protected from exposure to humidity.

8. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

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

9. SPECIMEN COLLECTION & HANDLING

1. Human serum or plasma samples should be used for the test. While preparing serum samples, remove the serum form 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 2-8°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 given false results. Hemolyzed and Icteric hyperlipemic samples may give erroneous results.

10. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

Chikungunya 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 15 minutes)

(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. WARNING & PRECAUTION

CAUTION: THIS KIT CONTAINS MATERIALS OF HUMAN ORIGIN. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION. NEGATIVE CONTROL, POSITIVE CONTROL & ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION.

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 and should be run by competent person only.
5. Do not pipette by mouth.
6. 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 at 15psi for 60 min. Do not autoclave materials or solution containing 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 accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. Controls and Sample diluent 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. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to remove Azide salts" (Center 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.

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. Take care while preparing working substrate solution as vials of TMB Substrate & TMB Diluent are of same size.
5. Prepare working substrate solution just 10 minutes prior to adding in the wells.
6. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
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^{o}C) before use.
14. Do not combine reagents form 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 negative control, positive control and calibrator in each assay.
17. Use freshly collected, clean serum/ plasma 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^{o}C) before beginning the assay and can remain at room temperature during testing. Return reagents to 2-8^{o}C after use. All containers used for preparation of reagents must be cleaned throughly and rinsed with distilled or deionized water. Prewarm the incubator to 37^{o}C.

i) Microwells (Anti-human IgM antibodies coated strips)
Bring foil pack to room temperature (20-30^{o}C) before opening to prevent condensation on the microwells.

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^{o}C, with dessicant in an aluminium pouch with clamp & rod. Open one pouch at a time as per no. of samples to be tested.

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 (0.5 ml. sample diluent + 5 μl serum sample). Use a separate tip for each sample and then discard as biohazardous waste.

iii) Preparation of Working Wash Buffer:
a) Check the wash buffer concentrate for the presence of salt crystals. If crystals are present in the solution, resolubilize by warming at 37^{o}C until all crystals dissolve.

b) Prepare at least 50 ml. (2ml. concentrated buffer with 48 ml. distilled or deionized 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. Wash buffer is stable for 2 months when stored at 2-8°C.

iv) Preparation of Working antigen:
Gently tap the vial of Chikungunya Antigen (Lyophilized) on work bench to remove any substance from rubber cap, carefully remove the cap and add 1.5 ml antigen diluent into lyophilized antigen vial. Put the cap and let it stand for 10 minutes. Mix solution throughly before use. The working antigen is stable for 7 days at 2-8^{o}C and 2 months at -20^{o}C (only 2 freeze thaw of liquid antigen are allowed at -20^{o}C).

v) Preparation of working substrate solution :
Mix TMB substrate and TMB Diluent in 1:1 ratio to prepare working substrate.

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.

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 Susbstrate (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

15. 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^{o}C) before testing begins. Return the reagents to 2-8^{o}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.

16. TEST PROCEDURE

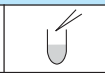
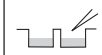
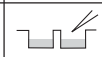
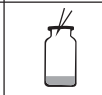
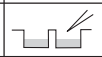
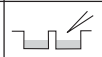
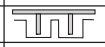
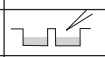
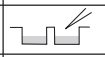
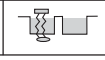
Once the assay has started, complete the procedure without interruption. All the reagents should be dispensed in the centre of the well and the tip of the pipette should not touch the wall of the microwell.

Fit the stripholder 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 50 μl Negative Control in A-1well.
2. Add 50 μl calibrator in B-1, C-1 & D-1 wells.
3. Add 50 μl Positive Control in E-1 well.
4. Add 50 μl of each sample diluted in sample diluent (1:100), in respective well starting from F-1 well. (Refer **TUBE DILUTION**).
5. Apply cover seal.
6. Incubate at 37^{o}C $\pm$ 1^{o}C for 30 min. $\pm$ 1min.
7. While the samples are incubating, prepare working Wash Solution and working antigen 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 50 μl of working antigen Solution in each well.
10. Apply cover seal.
11. Incubate at 37^{o}C $\pm$ 1^{o}C for 30 min $\pm$ 1min.
12. Aspirate and wash as described in step no. 8.
13. Add 50 μl of enzyme conjugate (Ready to use) solution in each well.
14. Apply cover seal.
15. Incubate at 37^{o}C $\pm$ 1^{o}C for 30 min $\pm$ 1min.
16. Aspirate and wash as described in step no. 8.

17. Add 75 µl of working substrate solution in each well.
18. Incubate at room temperature (20-30°C) for 15 min. in dark.
19. Add 100 µl of stop solution.
20. Read absorbance at 450 nm. within 30 minutes in ELISA READER. (Bichromatic absorbance measurement with a reference wavelength 600-650 nm is recommended when available).

SUMMARY OF PROCEDURE

Dilute Serum samples (1:100)		Sample 5 µl / Sample Diluent 0.5 ml
Add diluted samples, Controls * & Calibrator*		50 µl
Cover the plate & incubate		30 mins. at 37°C
Wash		5 Cycles
Prepare Working antigen		Add 1.5 ml of Antigen Diluent in 1 vial of Lyophilized Chikungunya Antigen
Add working antigen		50 µl
Cover the plate & incubate		30 mins. at 37°C
Wash		5 Cycles
Add Enzyme Conjugate (RTU)		50 µl
Cover the plate & incubate		30 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 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
Add Substrate		75 µl
Incubate in dark		15 mins. at Room Temp.
Add Stop Solution		100 µl
Read Results		450 nm./630 nm.

* Controls and calibrator are ready to use

TEST VALIDITY:

Ensure the following is within specified acceptance criteria

i) NC or NCx O.D. must be < 0.3. If it is not so, the run is invalid and must be repeated. In case differential filter is not available in the reader the NC value may go higher.

ii) PC O.D. must be > 1.1. If it is not so, the run is invalid and must be repeated.

iii) Mean Calibrator O.D. must be ≥ 0.30. If it is not so, the run is invalid and must be repeated.

17. 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 chikungunya IgM units : Calculate by multiplying the sample O.D. ratio by 10.

Chikungunya IgM units = sample O.D. ratio x 10.

e.g.: Mean O.D. of calibrator = 0.7

Calibration factor = 0.5

Cut off value = 0.7 x 0.5 = 0.35

e.g.: sample absorbance (O.D.) = 0.80

Cut off value = 0.35

Sample O.D. ratio = 0.80 / 0.35 = 2.28

Chikungunya IgM units = 2.28 x 10 = 22.8

18. INTERPRETATION OF RESULTS

a. If the Chikungunya IgM units is < 9 then interpret the sample as Negative for Chikungunya IgM antibodies.

b. If the Chikungunya IgM units is between 9 - 11 then interpret the sample as Equivocal for Chikungunya IgM antibodies.

c. If the Chikungunya IgM units is > 11 then interpret the sample as Positive for Chikungunya IgM antibodies.

19. PERFORMANCE CHARACTERISTICS

a) In-House Evaluation

An elaborated study has been done on Chikungunya IgM Microlisa to determine its performance. The performance of the test was evaluated with fresh as well as frozen samples and compared with a licensed commercially available test in house by using a panel of 3160 Nos. of known Negative & Positive Serum / Plasma samples. The panel included cross-reacting samples: HIV, HCV and Dengue Virus . The results obtained are as follows:

No. of Samples	Status	Chikungunya IgM Microlisa		
		Positive	Equivocal	Negative
155	Chikungunya Positive	154	0	1
3005	Chikungunya Negative	0	2	3003

Sensitivity : 99.35% **Specificity : 99.93%**

(ii) External Evaluation :

The Kit has been evaluated with known Chikungunya Positive (26 nos.), Negative (110 nos.) and Cross-reating samples (14 nos.); Leptospira, Rubella, Dengue and Hepatitis-A from B J Medical College, Pune and Apollo Hospitals, Bangalore and the results of the evaluations are:

Sensitivity: 100% **Specificity:** 100%

This information is provided for the Scientific Community Enquiring for an independent evaluation other than company's in house evaluation. It is not for commercial or promotional purpose.

Precision : Within-run and between-run precisions have been determined by testing 10 replicates of six specimens: two negative, two weak positive and two strong positive. The C.V.(%) of low positive and strong positive samples were within 15%.

20. LIMITATION OF THE TEST

1. The test should be used for detection of IgM antibodies of Chikungunya in human serum / plasma.
2. This is only a screening test and will only indicate the presence or absence of Chikungunya IgM 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, Influenza A & B, Brucella, Dengue Virus. This occurs in less then 1% of the sample tested.

21. 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 there of.

22. REFERENCES

1. Grivard P, Le Roux K. Laurent P, et al, (2007) Molecular and serological diagnosis of Chikungunya virus infection, pathol Biol (Paris) 55:490-494.
2. Diallo M, Thonnon J, Traore-Lamizana M & Fontenille D (1999) Vectors of Chikungunya virus in Senegal: Current data and transmission cycles. Am J Trop Med Hyg 60: 281-286.
3. Kashyap et. al. Diagnosis of chikungunya fever in an Indian population by an indirect enzyme linked immunosorbent assay protocol based on antigen detection assay: A prospective cohort stury, Feb. 2010, clinical and vaccine immunology p291-297.

4. Detection of viral antigen, IgM and IgG antibodies in cerebrospinal fluid of chikungunya patient with neurological complications (2010) cerebrospinal fluid reserach 2010, 7:12.

5. Barbara W. Johnson, laboratory diagnosis of chikungunya virus infections and commercial sources of diagnostic assays. J. Infect. Dis. (2016), 214(5), S471-S474.

23. 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 conc. at 2-8ºC 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 proerly closed with clamp & rod.
		d) Deteriotion of working antigen or improper preparation of working antigen	Check reconstitutin procedure and storage. It should be used within 7 days at 2-8º and 2 months at -20ºC.
		e) Inactivated substrate, improper storage or preparation	Use freshly prepared substrate solution. Recheck procedure, repeat assay
		f) Omission of any step in test procedure	Follow the procedure meticulously & repeat assay.
		g) Incorrect incubator temperature, timing or pipetting	Check incubator temperature, procedure & repeat assay
		h) Improper preparation of wash buffer, error of dilution, improper mixing of reagents.	Check procedure & repeat assay
		i) Kit deterioration	Check storage of the kit and should be store at 2-8ºC.
2.	High O.D. value of Negative control	a) Plate not stopped after 15 minutes of additing stop solution	Follow the procedure meticulously & repeat assay.
		b) Same microtip used for calibrator, positive and negative controls	Change micropipette tips while addition of negative/ positive control or calibrator
		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. i) Washing not consistent	Check wash device, fill the well close to the top.
		ii) Filling volume not sufficient.	After washing, blot the microwells on absorbent
		iii) Insufficient no. of wash cycles.	tissue. Follow wash protocol meticulously
		iv) Contaminated wash device	
		e) Use of wash solution from other manufacturer.	Use only Chikungunya 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, clen 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. and repeat the readings.	Clean or dry the bottom of microwells, check for bubbles
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 2-8ºC.
		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 2-8ºC.
		j) Sample deterioration due to improper storage and / or microbial contamination.	Store the sample at 2-8ºC / -20ºC as recommended in the specimen collection & handling.

in vitro diagnostic Reagent, not for medicinal use

VER-01 R-05

J. Mitra & Co. Pvt. Ltd.
A 180-181, Okhla Ind. Area, Phase-1, New Delhi-110 020, INDIA
Ph.: +91-11-47130300, 47130500
e-mail: jmitra@jmitra.co.in Internet: www.jmitra.co.in

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