PROBLEM	POSSIBLE CAUSE	SOLUTION
	f) Incorrect (low) incubator temperature, timing or pipetting	Check incubator temperature, procedure & repeat assay
	g) Improper preparation of wash buffer, error of dilution, improper mixing of reagents.	Check procedure & repeat assay
	h) 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 20 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/	If plates get scratches/
	binding of other reagent	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	Check the container and quality
	solution (1X). Poor quality of water used for diluting wash buffer conc.	of water used for dilution. Use of distilled water is preferred.
	c) Over incubation of	Follow the procedure
	substrate and delay in addition of stop solution.	meticulously.
	d) Insufficient washing. i) Washing not consistent due to blockage of probes	Check wash device and clean probes of manifold. fill the well close to the top.
	ii) Filling volume not	After washing, blot the
	sufficient.	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 Dengue NS1 Ag Microlisa wash solution.
	f) Working substrate not protected from light	Incubate the plate in dark after addition of substrate.
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.
5. False Positive	a) Beside 3a, b, c, d, e & f	Check the calculation
	incorrect interpretation and calculation of final results	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, procedure & 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

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R-00
VER-03
MN/EDA/007
Rev. Date: Apr.-26

REF IR031096

DENGUE NS1 Ag MICROLISA

Microwell ELISA Test for the Detection of Dengue NS1 Antigen in Human Serum/Plasma

1. INTRODUCTION

Dengue virus is a flavivirus found largely in areas of the tropic and sub-tropics. There are four distinct but antigenically related serotypes of dengue viruses, and transmission is by mosquito, principally Aedes aegypti and Aedes albopictus.

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. Secondry 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 evets, 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 NS1 antigen levels 0 to 9 days after the onset of symptoms; this generally persists up to 15 days. Consequently, testing for the dengue NS1 antigen via ELISA is a highly effective diagnostic tool because it enables early diagnosis prior to seroconversion. Earlier diagnosis of Dengue reduces risk of complication such as DHF or DSS, especially in countries where dengue is endemic. Unlike early antigens **IgM antibodies** do not become detectable until 3 to 5 days after symptom onset. Secondary dengue virus infections are characterized by rapidly rising **IgG levels**, which peak within a 6-to-15-day detection window and may be accompanied by elevated IgM. The J. Mitra Dengue IgG Microlisa is specifically engineered to detect these high, diagnostic thresholds of IgG antibodies. Therefore, to ensure maximum diagnostic accuracy across various clinical stages, a combined testing protocol using both the **J. Mitra Dengue IgM Microlisa** and the **Dengue IgG Microlisa** is highly recommended.

2. INTENDED USE

DENGUE NS1 Ag MICROLISA is an in vitro diagnostic medical device intended for the qualitative detection of Dengue Virus Non-Structural Protein 1 (NS1) antigen in human serum or plasma specimens. The assay is intended to aid in the early diagnosis of acute dengue virus infection caused by Dengue virus serotypes DEN-1, DEN-2, DEN-3, and DEN-4 in individuals suspected of dengue infection based on clinical symptoms and epidemiological risk factors.

The device is a manual microplate ELISA-based assay intended for professional laboratoy use by trained healthcare professionals. The assay provides qualitative results and is intended to be used in conjunction with clinical findings, patient history, and other laboratory diagnostic information. The test is not intended as a standalone confirmatory assay.

3. PRINCIPLE

DENGUE NS1 Ag MICROLISA is a solid phase enzyme linked immunosorbent assay (ELISA) based on the "Direct Sandwich" principle. The microwells are coated with Anti-dengue NS1antibodies with high reactivity for Dengue NS1 Ag. The samples are added in the wells followed by addition of enzyme conjugate (monoclonal anti-dengue NS1 antibodies linked to Horseradish Peroxidase (HRPO)). A sandwich complex is formed in the well wherein dengue NS1 (from serum sample) is "trapped" or "sandwiched" between the antibody and antibody HRPO conjugate. Unbound conjugate is then washed off with wash buffer. The amount of bound peroxidase is proportional to the concentration of dengue NS1 antigen present in the sample. Upon addition of the substrate buffer and chromogen, a blue colour develops. The intensity of developed blue colour is proportional to the concentration of dengue NS1 antigen in sample. To limit the enzyme-substrate reaction, stop solution is added and a yellow colour develops which is finally read at 450nm spectrophotometrically.

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 European Standard EN ISO 15223-1:2021.

	Manufactured By	IVD	In vitro diagnostic medical device
Σ	No. of tests		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
	Keep away from sunlight		Do not use if package is damaged
BIO	Contains biological Material of Human Origin	BIO	Contains biological Material of Animal Origin
IN	Country of Manufacture		Keep Dry

5. KIT PRESENTATION

- 96 Tests

6. KIT & ITS COMPONENTS

Microwells	Microwells coated with anti-Dengue NS1 antibodies packed in a sealed pouch with desiccant.
Diluent	Buffer containing protein stablizers & antimicrobial agents as preservative and to be used for Sample & Conjugate dilution.
Enzyme Conjugate Concentrate (50X)	Containing Monoclonal Anti-Dengue NS1 linked to horseradish peroxidase with protein stabilizers.
Wash Buffer Concentrate (25x)	Concentrated Phosphate buffer with surfactant.
TMB Substrate	To be diluted with TMB diluent before use.
TMB Diluent	Buffer solution containing H_2O_2 with preservative
Control —	Normal human serum negative for Dengue NS1 antigen with preservative.
Control +	Recombinant Dengue NS1 antigen, with preservative.
Calibrator	Recombinant Dengue NS1 antigen, with preservative.
Stop Solution	Ready to use, 1N H_2SO_4.
Plate Sealers	Adhesive sheet to cover the microwell during incubation.

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. The kit should not be frozen & 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
- Disinfectant Solution
- Disposable gloves

9. SPECIMEN COLLECTION & PREPARATION

1. 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.
2. Specimens should be free of microbial contamination and may be stored at 2-8ºC for one week, or frozen at -20ºC or lower. Avoid repeated freezing and thawing.
3. Do not use heat inactivated samples as their use may give false results. Hemolyzed and Icteric hyperlipemic samples may give erroneous results.
4. Do not use Sodium Azide as preservative because it inactivates Horseradish peroxidase.

10. 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 121ºC at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established biosafety guidelines for handling & disposal of potentially infective material.
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. 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.
10. ELISA Reader & micropipettes used in testing should be calibrated at regular interval to ensure accurate results.

11. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

Dengue NS1 Ag 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. (10,000 rpm for 15 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.

12. PRECAUTIONS FOR USE

Optimal assay performance requires strict adherence to the assay procedure described in the instruction for use.

1. Do not use kit components beyond the expiration date, which is printed on the kit.
2. Do not open the foil pouch until it attains room temperature.
3. Do not use the test if the packaging is damaged.
4. Do not use if the product has been exposed to excessive heat or humidity.
5. Follow the given test procedure and storage instructions strictly. Failure to follow the test procedure and instructions strictly can cause improper functioning of the test and inaccurate results
6. Avoid microbial contamination of reagents. The use of sterile disposable tips is recommended while removing aliquots from reagent bottles.
7. Prepare working substrate solution just 10 minutes prior to adding in the wells.
8. 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.
9. Use separate tips for TMB substrate and TMB diluent.
10. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
11. Do not allow microwells to dry once the assay has started.
12. 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.
13. 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.
14. Distilled or deionised water must be used for wash buffer preparation.
15. Bring all the reagents to room temperature (20-30ºC) before use.

16. Do not combine reagents from different batches, as they are optimized for individual batch to give best results.
17. Due to interchange of caps the reagents may get contaminated. Care should be taken while handling the reagents to avoid any sort of contamination.
18. Run negative control, positive control and calibrator in each assay.
19. Use freshly collected, clean serum/ plasma samples for assay. Try to avoid Haemolyzed/ turbid/ lipemic serum or plasma samples.
20. Use a separate tip for each sample and then discard it as biohazardous waste.
21. Thorough washing of the wells is critical to the performance of the assay.
22. Avoid strong light exposure during the assay.

13. PRELIMINARY PREPARATIONS

- Pre-warm the incubator to + 37°C.
- 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 strip holder. Take the strip holder with the required number of strips, taking into account that, one negative control, one positive control and three calibrator should be included in the run while opening the fresh kit. However for one or two strips one negative control, one positive control and two calibrator should be included in each subsequent runs.

b. Unused wells should be stored at 2-8°C with desiccant in an 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 desicant along with clamp & rod.

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

- **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 480ml. of distilled or deionized water. Wash buffer is stable for 2 months when stored at 2-8°C.

- **Preparation of Working Conjugate:**

Dilute conjugate concentrate 1:50 in Diluent. **Do not store working conjugate.** Prepare a fresh dilution for each assay in a clean glass vessel. Determine the quantity of working conjugate solution to be prepared from the table below. 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
Enzyme Conjugate Concentrate (µl)	20	40	60	80	100	120	140	160	180	200	220	240
Diluent (ml)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0

- **Preparation of working substrate solution:**

Mix TMB substrate and TMB Diluent in 1:1 ratio 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 Susbstrate (ml)	0.8	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8.0	8.8	9.6
TMB Diluent (ml)	0.8	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8.0	8.8	9.6

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.

14. WASH PROCEDURE:

1. Incomplete washing will adversely affect the test outcome.
2. Aspirate the well contents completely into a waste container. Then fill the wells completely with wash buffer (300 - 350 µl) avoiding overflow of buffer from one well to another and allow to soak (approx. 30 seconds). Aspirate completely and repeat the wash and soak procedure 5 additional times for a total of 6 washes.
3. Automated washer if used should be well adjusted to fill each well completely without over filling.
4. Tap upside down on absorbant sheet till no droplets appear on the sheet, taking care not to dislodge the wells.

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°C) before testing begins. Return the reagents to 2-80C after use.
2. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
3. All reagents must be mixed well before use.
4. To avoid contamination, do not touch the top or bottom of strips or edge of wells.
5. All pipetting steps should be performed with utmost care and accuracy. Cross contamination between reagents and samples will invalidate results.
6. Prevent evaporation during sample incubation by covering the strips with sealer; remove sealer before washing.
7. 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-Dengue NS1 antibody 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 Diluent in all the wells.
2. Add 50 µl Negative Control in A-1 well.
3. Add 50 µl Calibrator in B-1, C-1 & D-1 well.
4. Add 50 µl Positive Control in E-1 well.
5. Add 50 µl sample in F-1 well onwards.
6. Add 100 µl of working Conjugate Solution in each well.
7. Ensure thorough mixing of controls, samples to be tested & working conjugate to get reproducible results.
8. Apply cover seal.
9. Incubate at 37°C ± 1°C for 90 min. ± 1min.
10. While the samples and working Conjugate are incubating, prepare working Wash Solution as specified in preparation of reagents.
11. Take out the plate from the incubator after the incubation time is over and, wash the wells 6 times with working Wash Solution.
12. Add 150 µl of working substrate solution in each well.
13. Incubate at room temperature (20-30°C) for 30 ± 2 minutes in dark.
14. Add 100 µl of stop solution and leave at room temperature (20-30°C) for 1 minute.
15. Read absorbance at 450 nm within 5 minutes of adding stop solution in an ELISA READER. (Bichromatic absorbance measurement with a reference wavelength 600-650 nm is recommended when available).

16. SUMMARY OF PROCEDURE

Add controls, calibrator and samples		50 µl Diluent 50 µl controls, calibrator and samples
Prepare Working conjugate		No. of Strips 1 2 3 4 5 6 7 8 9 10 11 12 Enz. conc (µl) 20 40 60 80 100 120 140 160 180 200 220 240 Diluent (ml) 1 2 3 4 5 6 7 8 9 10 11 12
Add Conjugate		100 µl
Cover the plate & incubate		90 mins. at 37°C
Wash		6 Cycles
Prepare Chromogenic Substrate		No. of Strips 1 2 3 4 5 6 7 8 9 10 11 12 TMB Substrate (ml) 0.8 1.6 2.4 3.2 4.0 4.8 5.6 6.4 7.2 8.0 8.8 9.6 TMB Diluent (ml) 0.8 1.6 2.4 3.2 4.0 4.8 5.6 6.4 7.2 8.0 8.8 9.6
Add Substrate		150 µl
Incubate in dark		30 mins. ± 2 minutes at Room Temp.
Add Stop Solution		100 µl
Read Results		450 nm./630 nm.

17. CALCULATION OF RESULTS

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 ≥ 0.35. If it is not so, the run is invalid and must be repeated.
iv) Cut off value must be ≥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.

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 NS1 Ag units : Calculate by multiplying the sample O.D. ratio by 10.

Dengue NS1 Ag 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 NS1 Ag units = 1.761 x 10 = 17.61

18. INTERPRETATION OF RESULTS

a. If the Dengue NS1 Ag Units is < 9 then interpret the sample as Negative for Dengue NS1 Antigen.
b. If the Dengue NS1 Ag Units is between 9 - 11 then interpret the sample as Equivocal for Dengue NS1 Antigen.
c. If the Dengue NS1 Ag Units is > 11 then interpret the sample as Positive for Dengue NS1 Antigen.

Result	Interpretation
Negative	Dengue NS1 antigen absent. This finding does not definitively rule out a dengue infection; a convalescent sample should undergo serological testing in 7–14 days.
Equivocal	Retest all equivocal samples. If they remain borderline, switch to a different testing methodology or request a new patient sample.
Positive	The presence of the dengue NS1 antigen confirms the infection. Run serology panels on future follow-up samples for comprehensive confirmation.

We **recommend to include the following disclaimer** in the final patient test report: 'These results were determined using the Dengue NS1 Ag Microlisa by J. Mitra. Values derived from different assays are non-interchangeable. Additionally, a result's magnitude beyond the cut-off threshold does not quantify total antigen volume. "Please **ensure all results are documented solely as positive, negative, or equivocal**, avoiding numeric values."

19. PERFORMANCE CHARACTERISTICS

19.1 Clinical Performance:

Diagnostic Sensitivity and Specificity

a) In House Evaluation:

The kit has been evaluated in-house with the known panel of fresh as well as frozen Dengue NS1 antigen positive of all 4 dengue serotypes and Negative samples. The samples also included cross-reacting samples; Epstein-Barr virus, Malaria, Rheumatoid factor, Leptospirosis, Japanese encephalitis, yellow fever and West Nile viruses. Following are the result of evaluation:

No. of Samples	Status	Dengue NS1 Ag Microlisa		Sensitivity	Specificity	Sensitivity at 95% CI*	Specificity at 95% CI*
		Positive	Negative				
50	Dengue NS1 Ag Positive	50	0	100%	-	92.87% - 100%	-
250	Dengue NS1 Ag Negative	1	249	-	99.60%	-	97.77% - 99.43%

External Evaluation:

i) Site:1

Sensitivity with RT-PCR confirmed positive samples The sensitivity of Dengue NS1 Ag Microlisa kit has been evaluated at International Centre for Genetic Engineering and Biotechnology (ICGEB) on 58 retrospective serum samples from patients with dengue infection and 50 negative samples confirmed by Dengue RT-PCR. The samples were from the four different dengue serotypes (DEN 1, DEN 2, DEN 3 & DEN 4). The sensitivity of the Dengue NS1 Ag Microlisa kit when compared with Dengue RT- PCR is as follows:

Diagnostic Sensitivity				Diagnostic Specificity		
Serotype	Dengue RT PCR Positive Samples	Result of Dengue NS1 Ag Microlisa	Sensitivity of Dengue NS1 Ag Microlisa	No. of Negative samples	Dengue RT PCR Negative Samples	Result of Dengue NS1 Ag Microlisa
Den 1	25	25	100%	50	50	50
Den 2	8	7	87.5%			
Den 3	22	22	100%			
Den 4	3	3	100%			
Sensitivity	98.28%			Specificity	100%	
Sensitivity at 95% CI*	90.86% to 99.70%			Specificity at 95% CI*	92.86% to 100.00%	

I) Site:2

The Dengue NS1 Ag MICROLISA kit has been extensively evaluated using both serum and plasma samples across multiple study sites, including NABH/NABL-accredited laboratories and WHO collaborating centres such as **National Institute of Virology (NIV)**. The evaluation included RT-PCR confirmed samples, retrospective specimens, and comparative testing against CE-marked reference assays. These studies consistently demonstrated high diagnostic sensitivity and specificity of the assay. The overall diagnostic sensitivity and specificity observed of the Dengue NS1 Ag MICROLISA kit is as follows:

No. of Samples	Dengue Status	Dengue NS1 Ag Microlisa		Sensitivity	Specificity	Sensitivity at 95% CI*	Specificity at 95% CI*
		Positive	Negative				
234	Dengue NS1 Ag Positive	234	0	100%	-	98.38% to 100.00%	-
314	Dengue Negative	1	313	-	99.68%	-	98.22% to 99.94%

* Confidence Interval

19.2 Analytical Performance:

a) Analytical Specificity:

A total of 158 specimens from patients with confirmed disease other than dengue was tested to establish the analytical specificity of the Dengue NS1 Ag Microlisa. These specimens were from patients with disease that have potential for cross-reactivity. Each of the specimens included in the study were characterized with respect to diseases diagnosis prior to the analysis with the Dengue NS1 Ag Microlisa. No cross-reactivity was observed across 158 specimens. Refer to below table:

Disease type	Total Specimens	Positive results	Interference (when spiked with Dengue NS1 Ag positive sample)
HIV	20	0/20	No interference observed
HCV	20	0/20	No interference observed
HBV	20	0/20	No interference observed
Syphilis	20	0/20	No interference observed
Chikungunya IgM	8	0/8	No interference observed
TB	13	0/13	No interference observed
RF	3	0/3	No interference observed
Antenatal	5	0/5	No interference observed
Influenza	7	0/7	No interference observed
Malaria	5	0/5	No interference observed
ANA	6	0/6	No interference observed
lipemic	5	0/5	No interference observed
HAMA	5	0/5	No interference observed
elevated IgG	2	0/2	No interference observed
elevated IgM	5	0/5	No interference observed
high total protein	5	0/5	No interference observed
Scrub Typhus	2	0/2	No interference observed
Leptospira IgM	2	0/2	No interference observed
Haemoglobin	5	0/5	No interference observed

Precision:

i) **Reproducibility** (Inter Assay Variation) by testing 10 replicates at different time interval for sensitivity- 3 Dengue NS1 Ag positive samples (1 weak positive, 1 medium positive & 1 strong positive), and for specificity 2 negative samples. The C.V. (%), weak positive, medium positive & strong positive values were within 10%. The C.V. (%), and of negative samples were observed within 20%.

ii) **Repeatability** (Intra Assay Variation) by testing 10 replicates in a single run for sensitivity- 3 Dengue NS1 Ag positive samples (1 weak positive, 1 medium positive & 1 strong positive), and for specificity 2 negative samples. The C.V. (%), weak positive, medium positive & strong positive values were within 10%. The C.V. (%), and of negative samples were observed within 20%.

IMPORTANT

In case user have any problems or come across any serious incident that has occurred in relation to the device shall be reported to the manufacturer. Please call our Technical Customer Service Cell at New Delhi Phone: +91-11-47130300.

20. LIMITATIONS OF THE TEST

1. The test is intended for the detection of Dengue NS1 antigen in serum or plasma only and should not be used with other body fluids.
2. **This is a screening test and indicates only the presence or absence of Dengue NS1 antigen.** All reactive samples must be confirmed using an appropriate confirmatory test. For definitive diagnosis, results should be interpreted along with the patient's clinical history, symptoms, and serological findings.
3. Clinical diagnosis must not rely solely on this test. Results should always be interpreted in conjunction with clinical signs and symptoms and other relevant laboratory findings.
4. The test is not intended for screening of the general population. Testing should be limited to patients with clinical suspicion of dengue infection or known exposure risk. The positive predictive value depends on disease prevalence.
5. Serological cross-reactivity is common within the flavivirus group, including dengue serotypes 1, 2, 3, and 4, as well as other related viruses like Murray Valley and encephalitis, Japanese encephalitis, yellow fever and West Nile viruses. This occurs in less than 1% of the sample tested. These infections should be excluded before confirming a diagnosis.
6. All samples testing positive by Dengue NS1 Ag Microlisa should be referred to a reference laboratory for confirmation and for epidemiological recording.

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 for use, 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

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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 2-8°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 provided with the kit.
	d) Inactivated substrate, improper storage or preparation	Use freshly prepared substrate solution. Recheck procedure, repeat assay
	e) Omission of any step in test procedure	Follow the procedure meticulously & repeat assay.