DENGUE Ag ELISA TEST
(SERUM / PLASMA)

REF E0312

2°C 8°C

IVD

INTENDED USE

The *Aria* Dengue Ag ELISA Test is a solid-phase enzyme-linked immunosorbent assay for the qualitative detection of dengue NS1 antigen (DEN1, 2, 3, 4) in human serum or plasma. It is intended for professional use only as an aid in the diagnosis of an acute infection with dengue viruses.

INTRODUCTION

Dengue virus is an enveloped, single-stranded, positive-sense RNA virus that comprises four related but distinct serotypes (DEN1, 2, 3, and 4). The virus is transmitted by mosquitoes of the daytime-biting *Stegomyia* family, principally *Aedes aegypti* and *Aedes albopictus*. Currently, more than 2.5 billion people living in the areas of tropical Asia, Africa, Australia and the Americas are at risk for dengue infection. An estimated 67-136 million cases of dengue fever and 20,000 deaths occur worldwide annually[1-3].

Dengue NS1 antigen is released into the blood during viral replication in an infected patient, and is detectable from the first day after the onset of fever up to Day 9[4,5]. NS1 antigen can be identified before the formation of antibodies, thus making it a beneficial biomarker for early detection of dengue infection, allowing for prompt management of dengue fever[5, 6]. Immune responses to a dengue infection vary depending on the immune status of the patient. During a primary infection, IgM anti-dengue virus starts to appear approximately 4-6 days after the onset of fever, peaks after approximately two weeks, and remains in circulation for about 2-3 months[5]. IgG anti-dengue virus levels begin to increase slowly, peak around 14-21 days, and then decrease to low levels, persisting for the duration of life[6]. During secondary infection, NS1 antigen can be detected in patients for up to 9 days after the onset of illness. However it was reported that NS1 detection could be compromised by pre-existing anti-dengue IgG antibodies[7].

The *Aria* Dengue Ag ELISA Test utilizes pairs of specific polyclonal and monoclonal anti-dengue antibodies for the detection of all four serotypes of dengue NS1 antigen (DEN1, 2, 3, 4) in human serum or plasma.

TEST PRINCIPLE

The *Aria* Dengue Ag ELISA Test is a solid-phase enzyme-linked immunosorbent assay based on the principle of the antibody sandwich technique for the detection of dengue NS1 antigen in human serum or plasma.

The *Aria* Dengue Ag ELISA Test is composed of two key components:
1) Solid microwells pre-coated with rabbit anti-pan dengue NS1 antibody,
2) Liquid conjugates composed of monoclonal antibodies recognize NS1 antigen from DEN1, 2, 3 and 4 conjugated with horseradish peroxidase (HRP-anti-dengue NS1 Conjugates).

During the assay, the test specimen is first incubated in the coated microwell. The dengue NS1 antigen, if present in the specimen, binds to the antibody coated on the microwell surface, and any unbound specimen is then removed by a wash step. During a second incubation with the HRP-anti-dengue NS1 conjugates, the dengue NS1 antigen absorbed on the surface of microwell binds to the conjugates, forming a conjugate complex. Unbound conjugates are then removed by washing. After addition of the TMB substrate, the presence of the conjugate complex is shown by development of a blue color resulting from a reaction between the enzyme and substrate. This reaction is then quenched by addition of the Stop Solution, and the absorbance value for each microwell is determined using a spectrophotometer at 450/620-690 nm.

MATERIALS AND REAGENTS

Materials and reagents provided with the kit

Item	Description	Quantity	Catalog
1	Anti-dengue NS1 Ab Coated Microwells	8 wells x 12 strips	E0312W
2	HRP–anti-dengue NS1 Conjugates	11 mL	E0312H
3	Dengue NS1 Antigen Positive Control	1.5 mL	E0312P
4	Dengue NS1 Antigen Negative Control	1 mL	E0312N
5	Wash Buffer (30X Concentrate)	5 mL	E0312WB
6	Sample Diluent	2	E0312SD
7	TMB Substrate A	6 mL	TME2000A
8	TMB Substrate B	6 mL	TME2000B
9	Stop Solution	1 mL	E1002
10	ELISA Working Sheet	2	E0001ES
11	Product Insert	1	PI-E0312

Others 3 x Microplate Sealers and 1 x Zip-loc Bag

Materials and reagents required but not provided in the kit
1. Pipette capable of delivering 50 µL and 100 µL with a precision better than 98.5%
2. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450 nm wavelength is acceptable
3. Absorbent paper for blotting the microwells
4. Timer
5. Distilled or de-ionized water

STORAGE AND STABILITY

All reagents except the concentrated wash buffer are ready to use as supplied. Store all components at 2-8°C. Do not freeze. Avoid strong light. Reseal the microwells after removing the desired number of wells. Ensure that the reagents are brought to room temperature before opening. All the reagents are stable through the expiration date printed on the label if not opened. Place unused wells in the zip-lock bag provided and return to 2-8°C. Open vial stability from this point is 8 weeks at 2-8°C, or until the labeled expiration date, whichever is earlier.

SPECIMEN COLLECTION AND PREPARATION

- Serum or plasma should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
- This kit is designed for use with serum or plasma specimen without additives only.
- If a specimen is not tested immediately, refrigerate at 2-8°C. If a storage period greater than three days is anticipated, the specimen should be frozen (-20°C). Avoid repeated freezing-thawing of specimens. If a specimen is to be shipped, pack in compliance with federal regulation covering the transportation of etiologic agents.
- Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assay.
- Do not use specimens demonstrating gross lipemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

PREPARATION OF THE REAGENTS PRIOR TO ASSAYING

1. Bring all reagents, controls to room temperature (20-25°C).
2. **Preparation of working Wash Buffer**

   Warm up the concentrated Wash Buffer to 37°C to dissolve the precipitant if it appears. Dilute concentrated Wash Buffer 30 fold with water as follows:

Plate	DI water	Wash buffer (30X)	Final volume
1 strip	58 mL	2.0 mL	60 mL
2 strips	116 mL	4.0 mL	120 mL
3 strips	174 mL	6.0 mL	180 mL
4 strips	232 mL	8.0 mL	240 mL

The diluted wash buffer can be stored at 2-8°C for up to 3 days.

3. Mix each reagent before adding to the test wells.
4. Determine the number of strips needed and mark on the ELISA Working Sheet with the appropriate information. Positive and Negative Controls should be run in duplicate to ensure accuracy.

ASSAY PROCEDURE

1. Remove the desired number of strips and secure them in the microwell frame. Place unused strips into the zip-lock bag and seal for later use.
2. Add 50 µL Sample Diluent into each Control Well and Test Well, respectively according to the designation on the ELISA Working Sheet.
3. Add specimens to the plate according to the designation on the ELISA Working Sheet:

3. 1 Blank Wells: Do not add any reagents in the blank wells.

3. 2 Control Wells: Add 50 µL Dengue NS1 Positive Control and 50 µL Dengue NS1 Negative Control into the designated control wells respectively.

3. 3 Test Wells: Add 50 µL test specimens to each test well.

3. 4 Gently rock the wells for 20 seconds, and then cover the plate with a sealer.

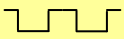
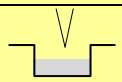
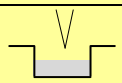
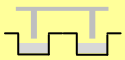
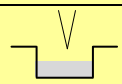
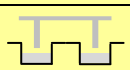
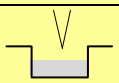
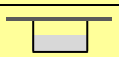
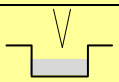
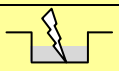
4. Incubate the wells at 37°C for 60 minutes.
5. Wash Step (Can be performed manually or with automated washing):

   Manual washing: Carefully remove the incubation mixture by disposing the solution into a waste container. Fill each well with 350 µL diluted wash buffer and rock gently for 20-30 seconds. Discard the wash solution completely. Repeat 4 more times. After completing the last wash step, tap plate on absorbent paper to remove residual liquid.

   Automatic washing: Automated plate washer must be calibrated to ensure efficient washing. Aspirate incubation mixture from all wells completely. Fill each well with 350 µL diluted wash buffer and soak for 20-30 seconds. Aspirate all wells completely. Repeat 4 more times.

6. Pipette 100 µL HRP-anti-dengue NS1 Conjugates into each well, except the blank well. Gently rock the microwell to ensure thorough mixing.
7. Incubate at 37°C for 60 minutes.
8. Wash the plate as described in step 5.
9. Add 50 µL TMB Substrate A and 50 µL TMB Substrate B into each well including the blank well. Gently rock the microwells for 20 seconds to ensure thorough mixing.
10. Incubate at room temperature (20-25°C) in dark for 15 minutes.
11. Stop the reaction by adding 100 µL Stop Solution into each well. Gently mix for 30 seconds. It is important to make sure that all the blue color completely changes to a color yellow.
12. Set the microplate reader wavelength at 450 nm. Measure the absorbance (OD) of each well against the Blank Well within 30 minutes after adding Stop Solution. A filter of 620-690 nm can be used as a reference wavelength to optimize the assay result.

Flow chart of assay procedure

1.	Secure strips in microwell frame		Number of strips
2.	Add sample diluent, except Blank Wells		50 µL
3.	Add controls or specimens Gently rock		50 µL 20 seconds
4.	Incubate		37°C, 60 minutes
5.	Wash: manual or automatic		5 times
6.	Add HRP-anti-dengue NS1 Conjugates, except Blank Wells		100 µL
7.	Incubate		37°C, 60 minutes
8.	Wash: manual or automatic		5 times
9.	Add TMB Substrate A and B. Gently rock		50 µL + 50 µL 20 seconds
10.	Incubate in dark		RT (20-25°C) 15 minutes
11.	Add Stop Solution. Gently rock		100 µL, 30 seconds
12.	Read result		450/620-690 nm within 30 minutes

INTERPRETATION OF RESULTS

A. Set up the cut-off value

The cut-off value = 0.20 + N
N: Mean OD of the negative control. Use N=0.10 for calculation of the cut-off value if the mean OD is less than 0.10.

B. Calculation of specimen OD ratio

Calculate an OD ratio for each specimen by dividing its OD value by the cut-off value as follows:

$$\text{Specimen OD ratio} = \frac{\text{Specimen OD}}{\text{Cut-off Value}}$$

C. Assay validation

The mean OD value of the positive controls should be ≥ 0.8
The mean OD value of the negative controls should be ≤ 0.2
Check the assay procedure including incubation time and temperature and repeat assay if above criteria is not met.

D. Interpretation of the results

	Specimen OD ratio
Negative	< 1.0
Positive	≥ 1.0

1. A negative result indicates that there is no detectable dengue NS1 in the specimen.
2. Results just below the cut-off value (lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to re-test in duplicate the corresponding specimens when it is applicable).
3. Specimens with OD ratio ≥ 1.0 are initially considered to be positive by the *Aria* Dengue Ag ELISA Test. They should be retested in duplicate before a final interpretation is made.
   If after re-testing of a specimen, the absorbance value of the 2 duplicates are less than the cut-off value, the initial result is non-repeatable and the specimen is considered to be negative with the *Aria* Dengue Ag ELISA Test.
   Non-repeatable reactions are often caused by:
   - Inadequate microwell washing,
   - Contamination of negative specimens by serum or plasma with a high concentration of NS1 antigen,
   - Contamination of the TMB Substrate by oxidizing agents (bleach, metal ions, etc.),
   - Contamination of the Stop Solution.

   If after retesting the absorbance of one of the duplicates is equal to or greater than the cut-off value, the initial result is repeatable and the specimen is considered to be positive with the *Aria* Dengue Ag ELISA Test, subject to the limitations of the procedure, described below.

PERFORMANCE CHARACTERISTICS

1. Analytical Sensitivity

The *Aria* Dengue Ag ELISA Test can detect recombinant dengue type 2 NS1 antigen at a level as low as 0.3 ng/mL, when spiked into negative specimens.

2. Clinical performance

A total of 301 specimens were collected from susceptible subjects and tested by *Aria* Dengue Ag ELISA Test and by a commercial leading brand EIA. Comparison for all subjects is shown in the following table:

	***Aria* Dengue Ag ELISA Test**		
Reference EIA	**Positive**	**Negative**	**Total**
Positive	25	0	25
Negative	6	270	276
Total	31	270	301

Relative Sensitivity: 100%, Relative Specificity: 97.8%, Overall agreement: 98.0%

3. Precision

a. Intra-assay Precision was determined by assaying 15 replicates of three different samples.

Panel	N	OD	SD	CV
Negative	15	0.193	0.017	8.82%
Low positive	15	0.803	0.063	7.81%
High positive	15	1.509	0.109	7.10%

b. Inter-assay Precision was determined by assaying three patient samples in 10 separate runs. Data was analyzed by ANOVA (analysis of variance).

Panel	Runs	OD	SD	CV
Negative	10	0.159	0.011	6.81%
Low positive	10	0.846	0.040	4.72%
High positive	10	1.[illegible]	0.058	4.12%

4. Cross-reactivity

No false positive Dengue NS1 test results were observed on 3-10 positive specimens from each of the following disease states or special conditions, respectively:

HCV	HBsAg	HIV	*H. pylori*	Malaria
Syphilis	ANA	HAMA	RF (up to 8,400 IU/mL)	

5. Hook effect

No hook effect was observed at the concentration of the Dengue NS1 Ag up to 0.1 mg/mL.

6. Interference

Common substances (such as pain and fever medication and blood components) may affect the performance of the *Aria* Dengue Ag ELISA Test. Interference was studied by spiking these substances into 3 dengue antigen clinical specimens: negative, low positive and high positive. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the *Aria* Dengue Ag ELISA Test.

List of potentially inferring substances and concentrations tested:

1. Salicylic acid	4.34 mmol/L	5. Glucose	55 mmol/L
2. Sodium citrate	1.3 %	6. Heparin	3,000 U/dL
3. Creatinine	0.5 mmol/L	7. Bilirubin	10 mg/dL
4. EDTA	3.4 µmol/L		

WARNING AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not use expired kits.
3. Bring all reagents to room temperature (20-25°C) before use.
4. Do not use the components in any other type of test kit as a substitute for the components in this kit.
5. Do not use serum derived from hemolyzed blood specimens for testing.
6. Do not ingest the reagents. Avoid contact with eyes, skin and mouth. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
10. At the beginning of each incubation, and after adding Stopping Solution, gently rock the microwells to ensure thorough mixing. Avoid the formation of air bubbles which results in inaccurate absorbance values. Avoid splashing liquid while rocking or shaking the wells.
11. Do not allow the microwells to dry between the end of the washing operation and the reagent distribution.
12. The enzyme-substrate reaction is very sensitive to metal ions. Therefore do not allow any metal element to come into contact with the conjugate or TMB Substrate.
13. The enzyme-substrate is temperature dependent. Ensure that the room temperature during incubation falls between 20-25°C.
14. The TMB Substrate must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The TMB Substrate B must be stored in the dark.
15. Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and TMB Substrate.
16. **The wash procedure is critical. Wells must be aspirated completely before adding the Wash buffer or liquid reagents. Automatic washers must be validated with the test kit prior to use. Insufficient washing will result in poor precision and falsely elevated absorbance values.**
17. **Microplate reader must be calibrated per manufacturer's instruction to ensure accurate determination of absorbance. Non-calibrated readers often leads to invalid test results.**
18. Avoid exposure to strong light during color development.

LIMITATION OF THE TEST

1. The Assay Procedure and the Interpretation of Results must be followed closely when testing for the presence of dengue NS1 antigen in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *Aria* Dengue Ag ELISA Test is limited to the qualitative detection of dengue virus NS1 antigen in human serum or plasma. The intensity of the color does not have linear correlation with the dengue NS1 antigen concentration in the specimen.
3. The *Aria* Dengue Ag ELISA Test cannot be used to differentiate between primary or secondary dengue infections. No information on the dengue serotype(s) present in a specimen can be provided with this test.
4. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect results.
5. Infection may progress rapidly. If the symptoms persist, while the result from the *Aria* Dengue Ag ELISA Test Test is negative, it is recommended to test with an alternative test method.
6. Any use of interpretation of this test results must also rely on other clinical findings and the professional judgment of health care providers.

REFERENCES

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Index of Symbols

	See instructions for use	CONJ	Conjugates
IVD	For *in vitro* diagnostic use only	ANTIGEN	Antigen
REF	Catalog #	ENZ DILT	Enzyme diluent
LOT	Lot number	CONTROL +	Positive control
N	Tests per kit	CONTROL -	Negative control
	Do not reuse	SAMP DILT	Sample diluent
	Manufacturer	TMB SUBS	TMB substrate
	Date of manufacture	STOP SOLN	Stop solution
EC REP	Authorized representative	WASH BUFF	Wash buffer
2°C 8°C	Store between 2-8°C	MICROWELLS	Coated microwells
	Use by		

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For Export Only, Not For Re-sale In The USA

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