AccuDiag™ Dengue IgM ELISA Kit

REF 8117-P1

IVD See External Label 2-8°C 96 Tests

Dengue IgM ELISA	
Principle	Sandwich ELISA
Detection	Qualitative
Sample	4 µL serum/plasma
Incubation Time	196 minutes
Sensitivity	91%
Specificity	99%
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The Dengue IgM ELISA is for the qualitative detection of IgM antibodies to Dengue Virus recombinant antigens (DENRA) in serum for the presumptive clinical laboratory diagnosis of Dengue virus infection. The assay is intended for use only in patients with clinical symptoms consistent with either dengue fever or dengue hemorrhagic fever. Positive results must be confirmed by Plaque Reduction Neutralization Test (PRNT), or by using the current CDC guidelines for diagnosis of this disease.

Assay performance characteristics have not been established for testing of cord blood, for testing neonates, for prenatal screening, or for general population screening.

Warning: Cross-reactivity with IgM from West Nile virus and other flaviviruses has been observed to occur with the DENV IgM ELISA. Reactive results must be reported with a caution statement regarding possible IgM cross-reactivity with other flaviviruses.

SIGNIFICANCE AND SUMMARY

Dengue is an acute viral disease that is most commonly transmitted by Aedes aegypti mosquitoes and far less so other mosquito strains. Dengue is characterized clinically by biphasic fever, rash, hematopoietic depression, and by constitutional symptoms such as malaise, arthralgia, myalgia and headache (1, 2). Infrequently, more severe disease is seen, manifested by hemorrhagic fever (DHF) which may progress to lethal shock (2-4). It is endemic in the tropics and subtropics, worldwide, where an estimated 50-100 million cases occur annually (5). During 2002, more than 30 Latin American countries reported over 10,000,000 dengue fever (DF) cases with a large number of DHF cases. In the US territories of Puerto Rico and the US Virgin Islands dengue cases reached historically high levels in 2010. In Puerto Rico, 21,000 cases were reported in 2010. In 2010, Florida reported 65 locally acquired dengue cases and a serosurvey in Key West, Florida suggest an infection rate of 5%.(6) Dengue outbreaks have also been reported in Hawaii (7), and in Laredo, Texas.

Anti-dengue virus IgM antibody is produced transiently during primary and secondary infection. In patients with primary dengue virus infection, IgM antibodies develop rapidly and are detectable by days 3 to 5 of illness in half of hospitalized patients. Anti-dengue virus IgM levels peak at about 2 weeks post infection and then decline to undetectable levels over 2 to 3 months (8,9).
In patients with secondary dengue virus infections, while the kinetics of IgM production are similar to those observed in patients with primary infections, IgM levels are significantly lower (8,9). Anti-dengue virus IgM antibodies also peak at about 2 weeks post infection, begin to wane thereafter, and are still detectable in about 30% of patients 2 months after the onset of symptoms. In contrast to primary infection, secondary infection with dengue virus results in the earlier appearance of high titers of cross-reactive IgG antibodies before or simultaneously with the IgM responses (9).

The Dengue IgM ELISA tests for IgM antibodies in human serum to Dengue derived recombinant antigens (DENRA).

ASSAY PRINCIPLE

The Dengue IgM ELISAconsists of one enzymatically amplified sandwich-type immunoassay. In this assay, Dengue IgM Negative Control, Dengue IgM Positive Control and unknown serum samples are diluted with DENV Sample Dilution Buffer, then incubated in microtiter wells which have been coated with anti-human IgM antibodies, followed by incubation with Dengue-derived recombinant antigens (DENRA) and normal cell antigen (NCA) separately. After incubation and washing, the wells are treated with a DENV-specific monoclonal antibody labeled with the enzyme horseradish peroxidase (HRP). After a second incubation and washing step, the wells are incubated with the tetramethylbenzidine (TMB) substrate. An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometers. Above a certain threshold, the ratio of the absorbencies of the DENRA and the control antigen (NCA) wells determines whether antibodies to Dengue are present.

SPECIMEN COLLECTION AND PREPARATION

- Only serum should be used for this assay, and the usual precautions for venipuncture should be observed. Blood obtained by venipuncture should be allowed to clot at room temperature (20-25°C) for 30 to 60 minutes and then centrifuged according to the Clinical and Laboratory Standards Institute (CLSI Approved Guideline – Procedures for the Handling and Processing of Blood Specimens; GP-44).
- Testing should be performed as soon as possible after collection. Do not leave sera at room temperature for prolonged periods. Separated serum should remain at 20-25°C for no longer than 8 hours. If assays are not completed within 8 hours, serum should be refrigerated at 2-8°C. If assays are not completed within 48 hours, or the separated serum is to be stored beyond 48 hours, serum should be frozen at or below -20°C.
- Avoid repeated freezing and thawing of samples since this can cause analyte deterioration. Frost-free freezers are not suitable for sample storage.
- Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use. Always quick spin before use.
- If sera are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.
- Do not use sera if any indication of microbial growth is observed.

MATERIALS AND COMPONENTS

Materials provided with the kit

Warning: Do not use any reagents where damage to the packaging has occurred.

The kit contains the following reagents:

1. **COATED MICROTITER STRIPS FOR HUMAN IGM:** Strip holder in a resealable foil pouch, containing 96 polystyrene microtiter wells coated with goat anti-human IgM antibody in each well. Stable at 2-8°C until the expiration date.
2. **DENV SAMPLE DILUTION BUFFER:** One bottle, 25 mL, ready to use. Tris-HCl buffered solution (pH 7.2-7.6) with Tween 20 (0.05%), preservative (0.05% proclin-300) and additives. Use for the dilution of test samples, positive and negative controls. Stable at 2-8°C until the expiration date.
3. **DENGUE IGM NEGATIVE CONTROL**: One vial, 50 µL. Negative serum. The Negative Control will aid in monitoring the integrity of the kit. Stable at 2-8°C until the expiration date.
4. **DENGUE IGM POSITIVE CONTROL**: One vial, 50 µL. Positive control solution containing 0.09% sodium azide. The Positive Control will aid in monitoring the integrity of the kit. Stable at 2-8°C until the expiration date.
5. **READY TO USE DENGUE RECOMBINANT ANTIGEN FOR IGM:** One bottle, 5 mL, ready to use. Tris-HCl buffered solution (pH 7.2-7.6) with Tween 20 (<0.05%), preservative (<0.05% proclin-300), antibiotics (0.0025-0.004% G418 sulphate), Dengue recombinant antigens and additives. Stable at 2-8°C until the expiration date.
6. **READY TO USE NORMAL CELL ANTIGEN (NCA) FOR DENGUE IGM:** One bottle, 5 mL, ready to use. Tris-HCl buffered solution (pH 7.2-7.6) with Tween 20 (<0.05%), preservative (<0.05% proclin-300), culture supernatant of COS-1 cell. Stable at 2-8°C until the expiration date.
7. **READY TO USE ENZYME CONJUGATE-HRP FOR DENGUE IGM:** One bottle, 9 mL, ready to use, contains flavivirus reactive monoclonal antibody (mAb) conjugated with horseradish peroxidase in Tris-Citrate buffered solution (pH 7.2-7.6) with Tween 20 (<0.05%), preservative (<0.01% thimerosal) and additives. Stable at 2-8°C until the expiration date.
   Note: The conjugate should always be stored in the light-protected bottle provided.
8. **10X WASH BUFFER:** One bottle, 120 mL. 10X concentrate of phosphate buffered saline with Tween 20 (pH 6.8-7.0). Stable at 2-8°C until the expiration date.
9. **ENWASH:** One bottle, 20 mL, ready to use. Phosphate buffered saline with Tween 20 (pH 7.2-7.6), preservative (<0.05% proclin-300). Stable at 2-8°C until the expiration date.
10. **LIQUID TMB SUBSTRATE**: One bottle, 12 mL, ready to use. Contains 3, 3', 5, 5'-tetramethylbenzidine (TMB) and hydrogen peroxide in a citric-acid citrate buffer (pH 3.3-3.8). Stable at 2-8°C until the expiration date.
    Note: The substrate should always be stored in the light-protected bottle provided.
11. **STOP SOLUTION**: One bottle, 9 mL, ready to use 1N Sulfuric Acid. Used to stop the reaction. Stable at 2-8°C until the expiration date.
    Warning: strong acid, wear protective gloves, mask and safety glasses. Dispose all materials according to applicable safety rules and regulations.

Materials required but not provided

1. ELISA Spectrophotometer capable of absorbance measurement at 450 nm
2. Biological or High-Grade Water
3. Vacuum Pump
4. Plate Washer
5. 37°C Incubator without CO_2 supply or humidification
6. 1-10 µL Single-Channel Pipettors, 50-200 µL Single-and Multi-Channel Pipettors
7. Polypropylene tubes
8. Parafilm or similar plate cover
9. Timer
10. Vortex

ASSAY PROCEDURE

CAUTION: *The test procedure must be strictly followed. Any deviations from the procedure may produce erroneous results.*
Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. NOTE: For long-term storage, serum samples should not be repeatedly thawed and frozen more than three times. Sera should be further aliquoted in a smaller volume and stored at -20°C.

This kit has not been optimized by Diagnostic Automation Inc. for use with any particular automated ELISA processing system. Use with an automated ELISA processing system will require proper validation to ensure results are equivalent to the expectations described in this package insert. Modifications to the protocol of these systems and/or different volumes of reagents may be required.

Preparation of reagents

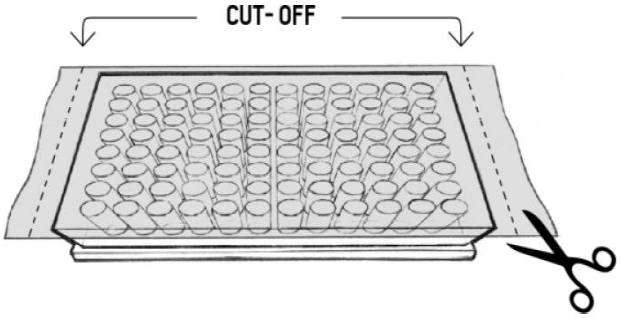
- Preparation of 1X Wash Buffer:
  Dilute the 10X Wash Buffer to 1X using Biological or High-Grade Water. To prepare a 1X Wash Buffer solution, mix 120 mL 10X wash buffer with 1080 Ml distilled (or deionized) water and rinse out any crystals. Swirl until well mixed and all crystals are dissolved. After diluting to 1X, store at room temperature for up to 6 months. Check for contamination prior to use. Discard if contamination is suspected.
- Microtiter Strip Wells:

Select the number of coated wells required for the assay. The remaining unused wells should be placed back into the pouch quickly, sealed, and stored at 2-8°C until ready to use or expiration.

Procedure

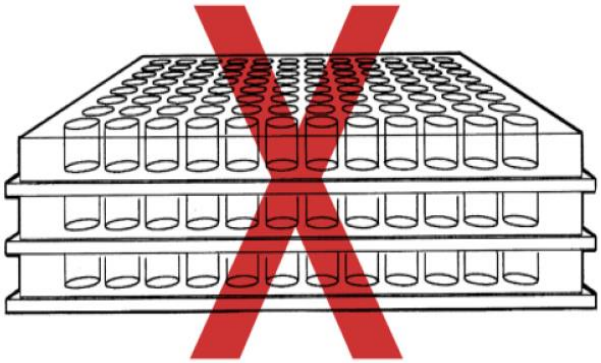
1. Positive and negative controls should be assayed in duplicate for both DENRA and NCA portions of assay. Unknown serum samples to be tested can be assayed singly or in duplicate but must be assayed for both DENRA and NCA portions of assay. Refer to flow chart at the end of this section for an example of this procedure. Up to forty-four test specimens can be tested on one 96 well plate.
2. Mark the microtiter strips to be used.
3. Using a new pipette tip each time, dilute test sera and controls to 1/100 using the provided sample diluent. Use small polypropylene tubes for these dilutions and at least 4 µL of sera and positive and negative controls. For example: mix 4 µL of serum sample plus 396 µL of DENV Sample Dilution Buffer to make a 1/100 dilution.
4. Apply 50 µL per well of 1/100 diluted test sera, Dengue IgM Negative Control, and Dengue IgM Positive Control to the plate by single or multi-channel pipetter as appropriate. An exemplary arrangement for forty-four test serum samples is shown in "Example for Serum Sample Application" chart at the end of insert. Note: All serum samples are to be tested with DENRA and NCA.
5. Cover the plate with parafilm or similar plate cover just on the well opening surface, so the bottom of the plates is not covered.

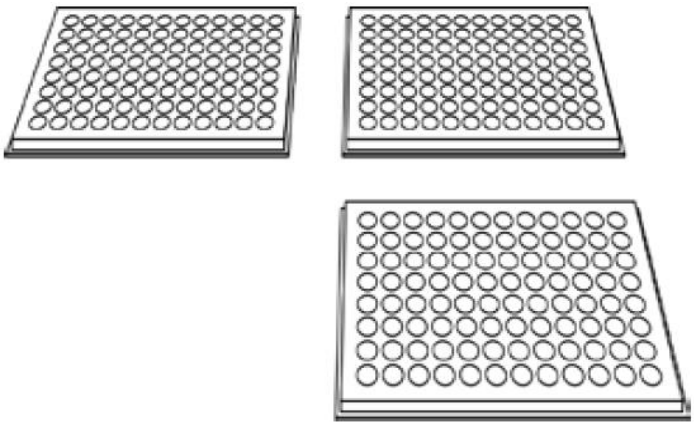
Note: This is to ensure even temperature distribution in all wells from bottom and sides; any extra parafilm can be cut off once the top is sealed to block evaporation.



6. Incubate the plate at 37°C for 1 hour in an incubator.

Note: *Do not stack plates on top of each other. They should be spread out as a single layer. This is very important for even temperature distribution. Do not use CO2 or other gas incubators. Do not place plates in contact with any wet substances such as wet paper towels, etc.*



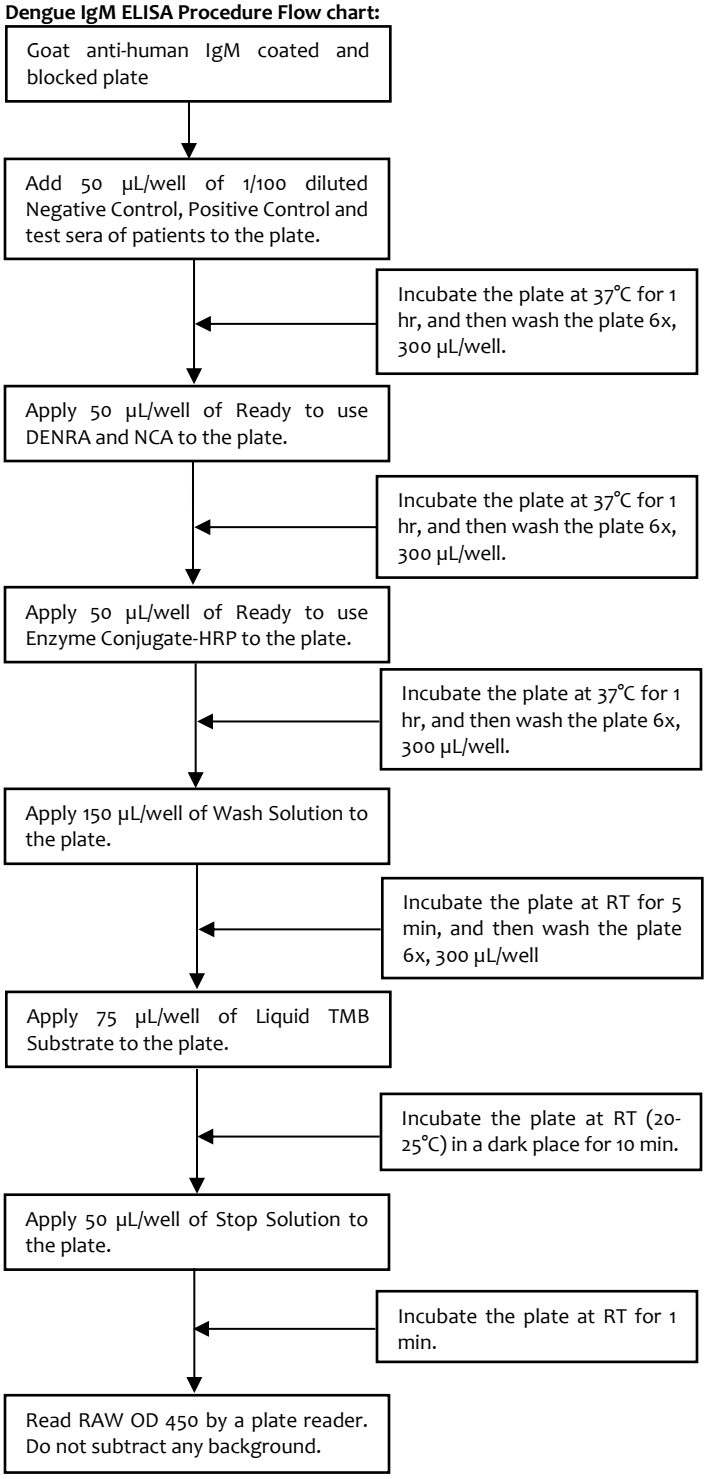


CORRECT METHOD

7. After the incubation, wash the plate 6 times with an automatic plate washer using 1X Wash Buffer. Use 300 µL per well in each wash cycle.
8. Add 50µL per well of DENRA into rows A-D and 50µL per well of NCA into rows E-H by multi-channel pipetter. An exemplary application for DENRA and NCA is shown at the end of the insert.
9. Cover the plate with parafilm or similar plate cover just on the well opening surface. The bottom of the plate should not be covered (see step 5).
10. Incubate the plate at 37°C for 1 hour in an incubator (see step 6).
11. After the incubation, wash the plate 6 times with an automatic plate washer using 1X Wash Buffer. Use 300 µL per well in each wash cycle.
12. Add 50 µL per well of ready to use Enzyme-HRP conjugate into all wells by multi-channel pipetter.
13. Cover the plate with parafilm or similar plate cover just on the well opening surface. The bottom of the plate should not be covered (see step 5).
14. Incubate the plate at 37°C for 1 hour in an incubator (see step 6).
15. After the incubation, wash the plate 6 times with an automatic plate washer using 1X Wash Buffer. Use 300 µL per well in each wash cycle.
16. Add 150µL per well of Wash into all wells by multichannel pipetter.
17. Incubate the uncovered plate at room temperature for 5 minutes.
18. After the incubation, wash the plate 6 times with an automatic plate washer using 1X Wash Buffer. Use 300 µL per well in each wash cycle.
19. Add 75 µL/well of Liquid TMB substrate into all wells using a multi-channel pipetter.
20. Incubate the plate at room temperature (20-25°C) in a dark place (or container) for 10 minutes without any cover on the plate.
21. After the incubation, add 50µL/well of Stop solution into all wells by multi-channel pipetter and incubate at room temperature for 1 minute without cover on the plate.
22. After the incubation, read the **RAW** OD 450 nm (optical density at 450 nm) value with a Microplate reader. **Do not subtract or normalize for any blank values or wells.** This may result in low NCA values and incorrect ISR values.

Please make sure the microplate reader does NOT subtract or normalize for any blank values or wells.

Dengue IgM ELISA Procedure Flow chart:



QUALITY CONTROL

Each kit contains a positive control and a negative control. Positive and negative controls must be run on each plate tested. Acceptable Immune Status Ratio (ISR) values for these controls are found in table 1 below. The negative and positive controls are intended to monitor for substantial reagent failure. The positive control will not ensure precision at the assay cutoff. The test is invalid and must be repeated if either of the controls do not meet the specifications. If the test is invalid, patient results cannot be reported. Quality Control (QC) requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and the user's own laboratory's standard QC procedures. It is recommended that the user refer to CLSI C24 and 42 CFR 493.1256 for guidance on appropriate QC practices. The results below are given strictly for guidance purposes only. Analysis is applicable when using RAW spectrophotometric readings only and where automatic subtraction of water or reagent blanks is not employed.

Calculation of the Negative Control: Calculate the mean Dengue IgM Negative Control values with DENRA and with the control antigen (NCA):

Example 1: Dengue IgM Negative Control

	DENRA	NCA
Replicate 1	0.108	0.066
Replicate 2	0.082	0.061
Sum	0.190	0.127

Average DENRA = 0.190 ÷2 = 0.095
Average NCA = 0.127 ÷ 2 = 0.064
Calculate the DENRA / NCA ratio (ISR):
0.095 ÷ 0.064 = 1.48

Any Dengue Negative Control DENRA/NCA ratio greater than 1.65 indicates that the test procedure must be repeated.

Calculation of the Positive Control:
Calculate Dengue IgM Positive Control values with DENRA and with the NCA.

Example 1: Dengue IgM Positive Control

	DENRA	NCA
Replicate 1	0.635	0.105
Replicate 2	0.655	0.115
Sum	1.290	0.220

Average DENRA = 1.290 ÷2 = 0.645
Average NCA = 0.220 ÷ 2 = 0.110
Calculate the DENRA / NCA ratio (ISR):
0.645 ÷ 0.110 = 5.86

Any Dengue IgM Positive Control DENRA/NCA ratio less than 5.0 indicates that

the test procedure must be repeated.

The values in the Table 1 below must be obtained in order to report results of the assay. Non-fulfillment of these criteria is an indication of deterioration of reagents or an error in the test procedure and the assay must be repeated.

Table 1

Factor (For Assay Verification)	Tolerance
Mean Dengue Negative Control OD in DENRA	<0.300
Mean Dengue IgM Positive Control OD in DENRA	>0.350
Dengue IgM Positive Control Immune Status Ratio (ISR)	>5.000
Dengue Negative Control Immune Status Ratio (ISR)	<1.650

CALCULATIONS FOR UNKNOWN SAMPLE ANALYSIS

Calculation of the Immune Status Ratio (ISR): If unknowns were assayed in duplicate, compute the average of the two replicates with the DENRA, and the two replicates with the NCA, then calculate the DENRA/NCA ratio (ISR) by dividing the average DENRA OD by the average NCA OD values. If unknown samples were assayed singly, divide the DENRA OD value by the NCA OD value.

Selection of the Cut-off and Equivocal Range Determination: One hundred nine True Positive and 97 True Negative serum samples were used for the determination of the optimal ISR cut-off and for establishing an equivocal range. All testing was performed according to this package insert. Two-graph ROC analysis was used to determine the optimal cut-off for the ISR values, giving equal weighting to Sensitivity and Specificity. An optimal cut-off of ISR = 1.87 was found. An equivocal range was established and two thresholds were determined. ISR values ≥ 2.84 determined test positivity, corresponding to a test specificity of 99% with a sensitivity of 91%. ISR values ≤ 1.65 determined test negativity, corresponding to a test sensitivity of 96% and a test specificity of 94%. Values between 1.65 and 2.84 are considered equivocal.

RESULTS

ISR	Result	Interpretation
≤ 1.65	Negative	No detectable IgM antibody, individual does not appear to be infected with Dengue virus. The result does not rule out Dengue virus infection. An additional sample should be tested within 7-14 days if early infection is suspected. Other Dengue virus assays such as Dengue NS1 assays, PCR or culture should be performed to rule out early acute infection.
1.65<ISR<2.84	Equivocal	Equivocal samples should be repeated in duplicate and the average ISR value should be evaluated. Samples that remain equivocal after repeat testing should be reported that Dengue virus IgM antibody cannot be determined, and should be repeated by an alternative method or another sample should be collected.
≥2.84	Positive	Presence of detectable IgM antibody, presumptive infection with Dengue virus. The result should be confirmed by plaque reduction neutralization test (PRNT) or by using the latest CDC guideline for diagnosis of this disease. A positive IgM result may not indicate a recent infection **because IgM may persist for several months after infection.**

LIMITATIONS OF THE ASSAY

- All reactive samples must be confirmed by Plaque Reduction Neutralization Test (PRNT) or by using the latest CDC guideline for diagnosis of this disease. Review the latest information on diagnosis at the CDC website: http://www.cdc.gov/dengue/clinicalLab/laboratory.html.
- Since this is a presumptive assay, the presence of false positive and false negative results must be considered.
- Serological cross-reactivity across the flavivirus group is very common. Certain sera from patients infected with Japanese Encephalitis, West Nile, and/or Saint Louis viruses may give false positive results. Therefore, any Dengue positive sera must be confirmed with other tests.
- Cross-reactivity with Malaria IgM has not been evaluated with the DENV IgM Capture ELISA.
- Assay performance characteristics have not been established for visual result determination.
- Assay performance characteristics have not been established for matrices other than serum.
- Results from immunosuppressed patients must be interpreted with caution.
- Assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient.
- High cholesterol levels (> 300 mg/dL) appear to give variable results and may affect the DENRA OD values.
- High triglyceride levels (> 3000 mg/dL) appeared to exhibit a slight effect of raising the ISRs of low positive sera.
- Hemoglobin (> 1600 mg/dL) appears to affect some serum samples by lowering the ISRs.

EXPECTED VALUES

Endemic Population

The DENV IgM Capture ELISA was used at a clinic in an endemic country in South America in 2009 to prospectively test serum samples from symptomatic individuals displaying symptoms similar to Dengue infection. Sixty-six individuals were screened at the visit at the onset of fever. Samples consisted of both primary and secondary infections. The reactivities of the samples with the DENV IgM Capture ELISA are summarized in tables 3 and 4 below.

Table 3: Expected Results from Endemic Region with Patients Displaying Symptoms

			DENV IgM Results[a]			
Age (years)	# Male	# Female	Non-reactive	Equivocal	Reactive	Prevalence
9-20	3	10	12	0	1	7.7%
21-30	5	7	8	1	3	25%
31-40	6	14	13	2	5	25%
41-50	7	6	9	2	2	15.4%
51-60	2	3	3	0	2	40%
61-70	2	0	2	0	0	0%
71-80	1	0	1	0	0	0%
Total	26	40	48	5	13	19.7%

A: all reactive samples were confirmed by PRNT.

Non-Endemic Population

Serum samples (n = 200) from non-symptomatic individuals were collected from Florida, Texas and Pennsylvania during March 2004. The samples represent a variety of ages, both male and female. The reactivities of the screened archived serum samples are summarized in table 4 below.

Table 4: Expected Results from Non-endemic Region with individuals not Displaying Symptoms

			Dengue IgM Results			
Age (years)	# Male	# Female	Non-reactive	Equivocal	Reactive	Prevalence
10-20	5	13	18	0	0	0.0%
21-30	38	30	68	0	0	0.0%
31-40	27	38	64	1	0	0.0%
41-50	23	20	42	0	1[a]	2.3%
51-60	5	1	6	0	0	0.0%
Total	98	102	198	1	1	0.5%

A: This sample was Dengue-positive by PRNT.

PERFORMANCE CHARACTERISTICS

Who Panel Study

A well characterized reference panel of samples was created by a joint effort of the United Nations International Children's Emergency Fund / United Nations Development Programme / World Bank / World Health Organization Special Program for Research and Training in Tropical Diseases and the Pediatric Dengue Vaccine Initiative. The reference panel was created by establishing a network of seven laboratories which contributed serum specimens and evaluated the samples (10). A subset of the reference panel was available for screening with the Diagnostic Automation Inc. DENV IgM ELISA. **The panel was tested by the CDC in Puerto Rico and the results are shown below in Table 5.**

Category	Number tested	Dengue IgM ELISA result	Count	Category	Number tested	Dengue IgM ELISA result	Count
Dengue IgM Negative	27	Negative	27	ST. Louis encephalitis (SLE)	2	Negative	1
		Equivocal	0			Equivocal	1
		Positive	0			Positive	0
Dengue IgM Positive	109	Negative	4	Lyme IgG	9	Negative	9
		Equivocal	5			Equivocal	0
		Positive	100			Positive	0
West Nile Virus IgG	11	Negative	10	Rheumatoid Factor (RF)	10	Negative	10
		Equivocal	1			Equivocal	0
		Positive	0			Positive	0
West Nile Virus IgM	25	Negative	21	Systemic lupus erythematosus	2	Negative	2
		Equivocal	2			Equivocal	0
		Positive	2			Positive	0
Yellow Fever (YF) IgM	4	Negative	3	New World hantavirus (HTN) IgM	7	Negative	7
		Equivocal	1			Equivocal	0
		Positive	0			Positive	0

The positive percent agreement (PPA) and negative percent agreement (NPA) are tabulated throughout by considering the "worst-case scenario." That is, equivocal samples are considered false negative for the PPA and equivocal samples are considered false positive for the NPA.

Positive Percent Agreement: (100/109) 91.7% (95% CI: 84.9-95.8%).
Negative Percent Agreement: (90/97) 92.8% (95% CI: 85.6-96.7%).

Clinical Studies

Study Site 1:

This retrospective study utilized serially collected archived samples from individuals displaying signs and symptoms of Dengue infection. Samples were collected from a select date onwards until a predetermined number of reactive samples were reached. The study was conducted using 197 subjects' sera obtained from a reference laboratory in Southeast Asia. Two sample draws (394 total samples collected 1-2 weeks apart) were available and confirmation of DENV was assessed by different methods in the reference laboratory. The final diagnosis for each subject was determined by the reference laboratory using a diagnostic algorithm (validated in-house IgM test result, and/or PCR result, and/or a rising IgG titer, and/or a four-fold rise of HAI titer between acute and convalescent blood draw). Any one test was used to confirm a positive diagnosis.

All the above samples were sequentially collected and tested by the Diagnostic Automation Inc. Dengue IgM ELISA kit. Positive and negative percent agreements with the reference laboratory final diagnosis are tabulated below as a function of the number of days post-onset of fever.

Table 6: Dengue IgM ELISA Performance from Study Site 1

Days post onset fever	Positive Percent Agreement	Negative Percent Agreement	# Equivocal samples with final diagnosis of Positive	# Equivocal samples with final diagnosis of Negative
2-3 days	28.6% (2/7)	100.0% (4/4)	1	0
4-5 days	40.3% (27/67)	78.8% (26/33)	19	7
6-7 days	75.9% (63/83)	88.6% (31/35)	14	3
8-10 days	88.8% (71/80)	97.1% (33/34)	7	1
11-15 days	91.7% (22/24)	100.0% (21/21)	2	0
16-19 days	100.0% (5/5)	100.0% (1/1)	0	0

The positive percent agreement (PPA) and negative percent agreement (NPA) are tabulated throughout by considering the "worst-case scenario." That is, equivocal samples are considered false negative for the PPA and equivocal samples are considered false positive for the NPA. Note: The above summary compares the Diagnostic Automation Inc. assay test results to the final diagnosis determined by the reference lab using PCR, HAI, rise in IgG titer and the in-house IgM ELISA.

Study Site 2:

A retrospective study of 212 serially collected archived samples from individuals displaying symptoms of Dengue infection were evaluated in a reference lab in the Western United States. Samples from 2008-2009 were collected from a select date onwards until a predetermined number of reactive samples was reached. The majority of the samples originated from the Caribbean and the southern and southeastern regions of the United States (Texas and Florida); however a minority of the samples may also have originated from Africa and Asia. Of the 212 samples tested, 116 were negative, 67 were positive. Twenty nine specimens fell in the equivocal range and were repeated according to the package insert specifications. Upon retest, 13 equivocal samples were subsequently categorized as negative, 11 were again equivocal, and 5 were categorized as positive.

Due to lack of sample volume or access, confirmatory PRNT was conducted on only 5 of the 11 equivocal samples (with 3/5 or 60% being confirmed as DENV positive) and 70 of the 72 positive samples (with 62/70 or 88.6% being confirmed as DENV positive). A total of 130 samples were not screened by PRNT. All 130 samples that were not screened by PRNT were screened by the CDC Dengue MAC-ELISA at the CDC and categorized as negative, equivocal, indeterminate or positive as determined by the CDC MAC-ELISA protocol. Thirteen of the samples screened by the CDC MAC-ELISA were categorized as indeterminate (n=9) or equivocal (n=4) and were subsequently tested by PRNT to clarify the sample status. Only 3 of the 9 (33.3%) indeterminate samples screened PRNT positive and 2 of the 4 (50%) of the equivocal samples screened PRNT positive.

Table 7a: Reactivity of Study Site 2 Samples – Confirmed by PRNT

		Final Diagnosis by PRNT		
		Dengue Positive	Dengue Negative	Total
Dengue IgM ELISA Result	Positive	62	8	70
	Equivocal	3	2	5
	Negative	4	3	7
	Total	69	13	82

Table 7b: Reactivity of Study Site 2 Samples – Confirmed by MAC-ELISA

		Final Diagnosis by PRNT		
		Dengue Positive	Dengue Negative	Total
Dengue IgM ELISA Result	Positive	1	1	2
	Equivocal	1	5	6
	Negative	4	118	122
	Total	6	124	130

a: 13 samples were CDC MAC-ELISA Indeterminate or Equivocal and were tested by PRNT to ultimately classify sample status. Positive and negative percent agreements are calculated by tabulating the results from both PRNT (Table 7a) and CDC MAC-ELISA (Table 7b).
Positive Percent Agreement: (63/75) 84.0% (95% CI: 73.9-90.8%).
Negative Percent Agreement: (121/137) 88.3% (95% CI: 81.8-92.8%).

In the above calculations for Table 7b, the percent agreements incorporate both the data from the PRNT and CDC MAC-ELISA tables. CDC MAC-ELISA equivocal and indeterminate samples (n = 13) were classified by PRNT.

Study Site 3:
The specificity of the Dengue IgM ELISA was evaluated at a public health lab located in the upper Midwestern United States. This retrospective study used 289 archived samples (collected 2005-2008) from symptomatic subjects with and without other diseases (including 136 individuals with West Nile Virus, Hepatitis A, B or C, HIV, Legionnaire's Disease, RMSF, and Lyme disease). All testing and diagnosis was performed at the public health laboratory. All samples were collected from individuals from the same upper Midwest state, which has never had an outbreak of Dengue virus. Therefore these samples were assumed to be Dengue virus negative based on the history of dengue incidences in this general area (http://doh.sd.gov/ID/AnnualReport/1997-2007.pdf).

After initial testing and rescreening of equivocal samples, 215 samples tested negative, 22 samples repeatedly tested equivocal and 52 samples tested positive. Samples that were either test positive or equivocal in the initial screening were included for PRNT testing (74 samples). It was observed that virtually all of the cross-reactivity was due to West Nile Virus.

Table 8: Reactivity of Retrospective Samples from Dengue Non–Endemic Area (Site 3) in the US

		Final Diagnosis		
		Dengue Negative, no other disease present	DENV Negative, other diseases present	Dengue PRNT Positive
Dengue IgM ELISA Result	Positive	1	40[b]	11
	Equivocal	0	16[b]	6
	Negative	100	115	0
	Total	101	171	17

a: Virtually all observed DENV PRNT positive samples had low PRNT titers and were identified as West Nile positive serum samples, indicative of WNV cross-reactivity with DENV PRNT (11).
b: Note: All of the observed false positives and the high number of equivocal samples are due solely to the cross-reactivity observed with West Nile positive samples (see Table 9).

The samples may be further subdivided by the disease status of the individual. For instance, individuals who are West Nile Virus (WNV) positive may cross-react with the DENV IgM ELISA. The results for Study Site 3 are shown in the following tables.

Table 9: Cross-reactivity of the Dengue IgM ELISA Using Samples from Study Site 3

		Sample Status	
		WNV Negative, other disease present	WNV Positive
Dengue IgM ELISA Result	Positive	0	40
	Equivocal	0	16
	Negative	35	80
	Total	35	136

Negative Percent Agreement for samples with no disease detected: (100/101) 99.0% (95% CI: 94.1-100%). [See Table 8, above].
Negative Percent Agreement for samples with diseases other than West Nile Virus: (35/35) 100% (95% CI: 88.2-100%).
Negative Percent Agreement for samples with West Nile Virus: (80/136) 58.8% ((95% CI: 50.4-66.7%).

None of the 35 samples from subjects without DENV but with RMSF (n = 5), Legionnaires' Disease (n=2), Lyme Disease (n=2), HIV (n=8), Hep A(n=2), Hep B (n=5) or Hep C (n=11) were equivocal or positive by the Diagnostic Automation Inc. DENV IgM ELISA. In the 136 subjects without DENV but who had West Nile Virus, 80 were Dengue IgM ELISA test negative, 16 were in the equivocal range and 40 were test positive.

Study Site 4:
The specificity of the DENV IgM Capture ELISA was evaluated at a State Dept. of Health located in Southern US using 199 archived samples (collected from 2004-2008) from symptomatic subjects presumed to be DENV negative. Most patients displayed symptoms of headache and fever while others also displayed neurological symptoms. In initial testing, 183 samples were test negative, 10 fell in the equivocal range and were repeated according to the package insert specifications, and 6 were test positive. Upon retest, all 10 samples in the equivocal range were subsequently categorized as test negative.

All 199 samples were further screened at the CDC using the CDC Dengue IgM (MAC) ELISA to classify the specimens as Dengue negative, equivocal, positive or uninterpretable (non-specific background too high). Please note these classifications are the CDC classifications for their kit. 16 samples were considered uninterpretable by the CDC Dengue IgM (MAC) ELISA but were confirmed negative using PRNT testing. These samples are considered negative in the tables below. One sample tested equivocal and one sample tested positive with the CDC Dengue IgM ELISA.

Table 10: reactivity of Retrospective Samples from Dengue Non-Endemic Area (Study Site 4)

		CDC Dengue IgM (MAC) ELISA		
		Dengue Positive	Dengue Equivocal	Dengue Negative
Dengue IgM ELISA Result	Positive	0	0	6
	Equivocal	0	0	0
	Negative	1	1	191
	Total	1	1	197

Negative Percent Agreement: (191/197) 97.0% (95% CI: 93.4-98.7%)

Study Site 5:

In a prospective study of 55 symptomatic subjects (mean age, 35.1 years – samples collected in 2009) from a Dengue endemic region in South America), each subject had samples collected at presentation as well as at a second visit 4-14 (mean 9) days later and both samples were tested with the DENV IgM ELISA. Equivocal samples at both visits were re-tested using the DENV IgM ELISA. Confirmatory PRNT testing was performed on samples at visit 1 and at visit 2. PRNT changes of 4-fold or greater between visits 1 and 2, indicative of current Dengue infection (12), were present in 39 subjects. Samples are considered to have a 4-fold increase in PRNT levels if the PRNT value increases from, for instance, PRNT = 10 on the first visit to PRNT = 40 by the second visit for a given Dengue subtype. Samples that demonstrated a PRNT value of <10 that only increased to a PRNT = 10 (n = 4 samples) were not considered to have a 4-fold increase.

Table 11: Reactivity of Prospective Samples from Dengue Endemic Area (Site 5) at First Visit[a]

		Final Diagnosis	
		Recent Dengue infection[b]	No signs of recent Dengue infection[c]
Dengue IgM ELISA Result	Positive	13	0
	Equivocal	4	1
	Negative	22	15
	Total	39	16

a: Positive Percent Agreement: (13/39) 33.3% (95% CI: 20.6-49.1%).
Negative Percent Agreement: (15/16) 93.8% (95% CI: 69.7-100%).
b: PRNT positive and ≥ 4-fold increase in PRNT between first and second visits
c: PRNT positive and <4-fold PRNT increase between first and second visits

The results for serum samples from the second visit by the patients (4-14 days later) are shown in the following tables.

As can be readily noted below, the sensitivity of the assay increases by this second visit time point.

Table 12: Reactivity of Prospective Samples from Dengue Endemic Area (Site 5) at Second Visit

		Final Diagnosis	
		Recent Dengue infection[b]	No signs of recent Dengue infection[c]
Dengue IgM ELISA Result	Positive	31	1
	Equivocal	2	0
	Negative	6	15
	Total	39	16

a: Positive Percent Agreement: (31/39) 79.5% (95% CI: 64.2-89.5%).
Negative Percent Agreement: (15/16) 93.8% (95% CI: 69.7-100%).
b: PRNT positive and ≥ 4-fold increase in PRNT between first and second visits
c: PRNT positive and <4-fold PRNT increase between first and second visits

It should be recalled, as noted in the "Interpretation of Results" section, that equivocal samples should be repeated and sent for confirmatory testing if they remain equivocal.

Reproducibility Study

The reproducibility of the Dengue IgM ELISA was evaluated at three sites and by two different operators at each site for five days. All samples (including controls) were run in triplicate. The study was conducted at a Public Health Lab in Florida, at Diagnostic Automation Inc, and at a reference laboratory in the central U.S. Four serum specimens using clinical specimens diluted into an analyte negative matrix, plus a positive and a negative control, were used. The four serum specimens (not including positive and negative controls) included a negative specimen, a specimen just below the equivocal range, a specimen within the equivocal range, and a positive specimen. The serum dilutions selected also ensured that the analyte concentration in the specimens represented a clinically relevant range. The results are shown in the following table

			Intra-Assay (within-run)		**Day-to-Day**		**Operator-to-Operator**		**Site-to-Site**		**Total**	
Sample ID	n	Mean ISR	S_{wr}	$\%CV_{wr}$	S_{DD}	$\%CV_{DD}$	S_{OO}	$\%CV_{OO}$	S_{SS}	$\%CV_{SS}$	S_T	$\%CV_T$
Panel A	90	1.133	0.148	13.06	0.056	4.96	0.066	5.86	0.064	5.63	0.158	13.97
Panel B	90	1.587	0.245	15.41	0.187	11.79	0.182	11.44	0.081	5.11	0.308	19.40
Panel C	90	2.433	0.621	25.52	0.668	27.46	0.522	21.44	0.207	8.52	0.912	37.49
Panel D	90	5.821	1.18	20.28	2.014	34.59	1.072	18.41	0.693	11.91	2.334	40.10
Positive Control	90	11.944	1.692	14.17	2.437	20.40	2.085	17.46	1.546	12.94	2.967	24.84
Negative Control	90	1.148	0.152	13.25	0.088	7.67	0.064	5.61	0.051	4.47	0.176	15.31

All values are calculated as DENRA/NCA ratios
Sx = Standard Deviation of "x" (wr or DD) wr: within run, DD: between day, OO: between operator, SS: between site
%CV: = % Coefficient of Variation

Cross-reactivity Study:

The Dengue IgM ELISA assay was screened against a number of serum samples containing IgM antibodies to several different diseases (see the following table). Samples were initially tested in duplicate.

Any equivocal and positive samples were retested in triplicate. Samples that tested positive were evaluated for Dengue virus exposure using the plaque reduction neutralization test. Significant cross-reactivity was only observed with West Nile Virus.

Table 14: DAI Dengue IgM ELISA Cross-reactivity

Disease or infectious agent	Number of samples	DENV IgM ELISA		Total # of Positive and Equivocal	% Positive or Equivocal
		Equivocal	Positive		
Eastern Equine Encephalitis (EEE)‡[a]	10	0	0	0/10	0%
Japanese Encephalitis (JE)[a]	2	0	0	0/2	0%
Saint Louis encephalitis (SLE)[a]	4	0	0	0/4	0%
Hepatitis B virus[a]	10	0	0	0/10	0%
Epstein Barr Virus[a]	15	0	0	0/15	0%
Rheumatoid Factor	7	0	0	0/7	0%
Hepatitis C virus[a]	10	0	0	0/10	0%
Cytomegalovirus	10	0	0	0/10	0%
Anti-nuclear Antibodies (ANA)[a]	10	0	0	0/10	0%
Varicella zoster virus[a]	10	0	0	0/10	0%
Lyme Disease[a]	5	0	0	0/5	0%
Leptospira[a,b]	11	0	0	0/11	0%
West Nile Virus[a]	24	4	8	12/24	50%
Total	112	4	8	12/128	9.4% (12/128)

a: The screened samples contained specific IgM antibodies to their respective analyte. Cross-reactivity with Malaria IgM antibodies have not been evaluated with the Diagnostic Automation Inc. Dengue IgM assay.

b: One leptospirosis sample that screened reactive with the Diagnostic Automation Inc Dengue IgM assay was tested in PRNT and was found to be positive in the PRNT assay (it is not included in this table as it is considered a true positive sample).

The total number of samples tested for cross-reactivity sera is 128 after removing three sera (one WNV positive, one JEV positive and one leptospirosis positive sample) that were also confirmed positive for Dengue Virus by PRNT. Twelve West Nile IgM positive samples out of 24 screened as positive or equivocal after retesting in the DENV IgM ELISA. The overall percent cross-reactivity with West Nile IgM was 50%.

Interference Study:
Four potentially interfering substances commonly occurring in serum were tested for their effect on the Dengue IgM ELISA test. One Dengue negative sample and four Dengue positive samples were used ranging in strength from weakly positive to strongly positive. The four potentially interfering substances were Bilirubin (1& 2 mg/dL), Triglycerides (500 & 3000 mg/dL), Hemoglobin (1600 & 16,000 mg/dL), Cholesterol (300 and 500 mg/dL). Bilirubin did not exhibit any effect on the test results. High levels of triglycerides (3000 mg/dL) appeared to exhibit a slight effect of raising the ISRs of low positive sera. Hemoglobin appears to affect some serum samples by lowering the ISRs at high hemoglobin levels and possibly at low hemoglobin levels. Cholesterol appeared to give variable results from repeated studies. As a result it can only be stated that it is possible that cholesterol affects DENRA OD values at both cholesterol levels (300 and 500 mg/dL). The table below shows the % change in the observed ISR from the interfering substance when compared to the reference sample.

Table 15: Percent Change in ISR Value in the Presence of Interfering Substances

	Control ISR Value	Bilirubin		Triglyceride		Hemoglobin		Cholesterol	
		1 mg/dL	2 mg/dL	500 mg/dL	3,000 mg/dL	1,600 mg/dL	16,000 mg/dL	300 mg/dL	500 mg/dL
Positive Control	20.84	16.4%	-1.0%	-1.7%	9.8%	-67.2%	-80.6%	21.8%	-1.9%
Negative Control	1.18	-1.0%	-4.8%	-0.4%	7.2%	-1.3%	3.7%	7.0%	29.4%
Sample D	14.40	-0.9%	-2.6%	20.8%	31.6%	-43.5%	-62.6%	-10.8%	-17.3%
Sample E	6.36	25.1%	8.4%	-19.4%	-28.6%	-59.8%	-69.6%	-49.5%	-5.1%
Sample F	0.94	15.5%	0.1%	-7.5%	-12.9%	-0.1%	12.2%	4.0%	1.1%

WARNING AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE. A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert.

SAFETY PRECAUTIONS

- All human source materials used in the preparation of controls have been either heat-inactivated or tested negative for antibodies to HIV 1&2, Hepatitis C and Hepatitis B surface antigen. However, no test method can ensure 100% efficiency. Therefore, all human controls and antigen should be handled as potentially infectious material. The Centers for Disease Control and Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at Biosafety Level 2.
- Wear protective clothing, eye protection, and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
- Do not eat, drink, smoke, or apply cosmetics where immunodiagnostic materials are being handled.
- Do not pipette by mouth.

TECHNICAL PRECAUTIONS

- This test must be performed on serum only. The use of whole blood, plasma or other specimen matrix has not been validated.
- Do not mix various lots of any kit component within an individual assay.
- **Do not heat-inactivate test sera.**
- All reagents must be equilibrated to room temperature (20-25°C) before commencing the assay. The assay will be affected by temperature changes.
- Avoid repeated freezing and thawing of the serum specimens to be evaluated.
- While diluting the controls and test sera in sample dilution buffer for use in ELISA testing, it is critical that a **new pipette tip** be used for each sample to avoid cross contamination.
- All reagents are susceptible to contamination, thus, it is advisable to dispense reagents directly from bottles using clean pipettes or by carefully pouring. Pipettes should be used **only once** to avoid contamination of the components.
- Unused microwells must be resealed immediately and stored in the presence of desiccant. Failure to do so may cause erroneous results with those unused microwells.
- Do not use any component beyond the expiration date shown on its label.
- Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.
- Do not use a humidified incubator or a water bath for 37°C incubation steps. Doing so may lead to erroneous results.
- Some reagents may form a slight precipitate, mix gently before use.
- Incomplete washing will adversely affect the outcome and assay performance.
- To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed used to add the TMB solution.
- Avoid microbial contamination of reagents, especially for the Ready to Use Enzyme Conjugate HRP for Dengue IgM. Avoid contamination of the TMB Substrate Solution with the Enzyme Conjugate-HRP.
- Cover working area with disposable absorbent paper.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL
This kit contains reagents made with human serum or plasma. The serum or plasma used has been heat-inactivated unless otherwise stated. Handle all sera and kits used as if they contain infectious agents. Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for the proper disposal of specimens.

CHEMICAL HAZARD
Safety Data Sheets (SDS) are available for all components of this kit. Review all appropriate SDS before performing this assay. Avoid all contact between hands and eyes or mucous membranes during testing. If contact does occur, consult the applicable SDS for appropriate treatment.

Example for Serum Sample Application

	1	2	3	4	5	6	7	8	9	10	11	12
A	Negative Control	Sample #1	Sample #5	Sample #9	Sample #13	Sample #17	Sample #21	Sample #25	Sample #29	Sample #33	Sample #37	Sample #41
B	Negative Control	Sample #2	Sample #6	Sample #10	Sample #14	Sample #18	Sample #22	Sample #26	Sample #30	Sample #34	Sample #38	Sample #42
C	Positive Control	Sample #3	Sample #7	Sample #11	Sample #15	Sample #19	Sample #23	Sample #27	Sample #31	Sample #35	Sample #39	Sample #43
D	Positive Control	Sample #4	Sample #8	Sample #12	Sample #16	Sample #20	Sample #24	Sample #28	Sample #32	Sample #36	Sample #40	Sample #44
E	Positive Control	Sample #4	Sample #8	Sample #12	Sample #16	Sample #20	Sample #24	Sample #28	Sample#32	Sample #36	Sample #40	Sample# 44
F	Positive Control	Sample #3	Sample #7	Sample #11	Sample #15	Sample #19	Sample #23	Sample #27	Sample#31	Sample #35	Sample #39	Sample #43
G	Negative Control	Sample #2	Sample #6	Sample #10	Sample #14	Sample #18	Sample #22	Sample #26	Sample 30	Sample #34	Sample #38	Sample #42
H	Negative Control	Sample #1	Sample #5	Sample #9	Sample #13	Sample #17	Sample #21	Sample #25	Sample 29	Sample #33	Sample #37	Sample #41

Example for DENRA and NCA Application

	1	2	3	4	5	6	7	8	9	10	11	12
A	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA
B	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA
C	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA
D	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA	DENRA
E	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA
F	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA
G	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA
H	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA

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TROUBLESHOOTING

Problem	Possible Cause	Possible Resolution
High Absorbances for DENRA/NCA	Incorrect component used	Do not combine controls or reagents between different lots of the ELISA kits.
	Samples incorrectly diluted	Sera should be diluted 1:100 in kit's sample dilution buffer.
	Cross contamination of wells	A new tip must be used for every test or control sera.
	Incomplete washing of wells	Wells must be completely filled and emptied 6 times during each wash cycle.
	Incubation times too long	Incubation times vary, please refer to the "Assay Procedure" section for correct times.
	Incubations in humidified chamber or water bath	37°C incubators without humidification and without CO_2 supply should be used for sample, antigen, and conjugate incubation steps.
	Conjugate contamination with TMB	It is recommended to use a new pipette/pipette tip each time to dispense conjugate and TMB.
	Incorrect wavelength filter	The optical density readings must be read with only a 450nm filter. There must not be any background subtraction.
Low Absorbances for DENRA/NCA	Samples incorrectly diluted	Sera should be diluted 1:100 in kit's sample dilution buffer.
	Kit expiration date and storage	Verify that incubators are calibrated and that the temperatures are monitored.
	Incorrect component used	Do not combine controls or reagents between different lots of the ELISA kits.
	Component temperatures	All kit components must be equilibrated at room temperature for optimal performance.
	Incubation times too short	Incubation times vary, please refer to the "Assay Procedure" section for correct times.
	Incubation temperature too low	Verify that incubators are calibrated and that the temperatures are monitored.
	DENRA/NCA contamination	The antigens are very susceptible to contamination. It is recommended to use a new pipet/pipette tip each time to dispense conjugate. Keep the lid on the conjugate unless in use. When possible, use a hood for dispensing of conjugate.
	Conjugate contamination	The conjugate is very susceptible to contamination. It is recommended to use a new pipet/pipette tip each time to dispense conjugate. Keep the lid on the conjugate unless in use. When possible, dispense conjugate in a clean laminar flow hood or biological safety cabinet.
	TMB contamination with Stop solution	It it recommended to use a new pipet/pipette tip each

Problem	Possible Cause	Possible Resolution
		time to dispense TMB and stop solution.
	Use of reagents in the wrong sequence, or omission of step(s)	Check the "Assay Procedure" section and component labels prior to use.
	Incorrect wavelength filter	The optical density readings must be read with only a 450nm filter. There must not be any background subtraction.

MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

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Date Adopted	2024-04
Brand Name	AccuDiag™
REF 8117-P1	AccuDiag™ - Dengue IgM ELISA
Revision Date: 10-01-2019	