Atlas Medical

Dengue IgM ELISA

For *In-Vitro* and professional use only Store at 2° to 8° C

INTRODUCTION

Dengue virus is a single-stranded RNA virus of about 50 nm in diameter belonging to the genus Flavivirus. Dengue and dengue hemorrhagic fever are caused by one of four closely related, but antigenically distinct, virus serotypes (DEN-1, DEN-2, DEN-3, and DEN-4). Infection with one of these serotypes does not provide crossprotective immunity, so persons living in a dengue-endemic area can have four dengue infections during their lifetimes. The viruses are transmitted by Aedes aegypti, a domestic, day-biting mosquito that prefers to feed on humans. Infection with dengue viruses produces a spectrum of clinical illness ranging from a nonspecific viral syndrome to severe and fatal hemorrhagic disease. It is primarily a disease of the tropics; its global distributions is comparable to that of malaria, and an estimated 2.5 billion people live in areas at risk for epidemic transmission.- Globally, there are an estimated 50 to 100 million cases of dengue fever and several hundred thousand cases of dengue hemorrhagic fever.

- The case-fatality rate of DHF in most countries is about 5%; most fatal cases are among children and young adults.
- Important risk factors for DHF include the strain and serotype of the infecting virus, as well as the age; immune status, and genetic predisposition of the patient.
- Risk groups: residents of or visitors to tropical urban areas.

Species	Disease	Symptoms	Mechanism of Infection
Dengue virus	Dengue Dengue hemorrhagic fever (DHF) or Breakbone fever	Sudden onet of fever, servere headache, myalgias and arthralgia leucopenia, thrombocytopenia and hemorrhagic manifestations	Transmission by mosquitos (Aedes aegypti)

The presence of virus resp. infection may be identified by

- Serology: Detection of antibodies by ELISA

Infection produces lifelong immunity, but the antigenically distinct serotypes do not provide cross-protective immunity, so a person can theoretically experience four dengue infections; a dengue vaccine is not available.

INTENDED USE

ATLAS Dengue virus IgM-ELISA is intended for the qualitative determination of IgM class antibodies against Dengue virus in human serum or plasma (citrate). This kit can detect all serotypes of Dengue virus (DEN-1, DEN-2, DEN-3, and DEN-4), but at different reaction strengths .

PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of IgM-class antibodies against Dengue Virus is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microtiter strip wells are precoated with Dengue Virus antigens type 2 to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample materials horseradish peroxidase (HRP) labeled anti-human IgM conjugate is added. This conjugate binds to the captured Dengue Virus-specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of Dengue Virus-specific IgM antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450nm is read using an ELISA microwell plate reader.

MATERIALS

Reagents supplied

- **Dengue Virus Coated Wells (IgM):** 12 breakapart 8-well snap-off strips coated with Dengue Virus Type 2 Antigen; vacuum sealed, in resalable aluminum foil.
- **IgM Sample Diluent***:** 1 bottle containing 100ml of buffer for sample dilution; pH 7.2±0.2; colored green; ready to use; white cap.
- **Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Solution (20x conc.)*:** 1 bottle containing 50ml of a 20-fold concentrated buffer (pH 7.2±0.2) for washing the wells; white cap.
- **Dengue Virus anti-IgM Conjugate**:** 1 bottle containing 20ml of peroxidase labeled rabbit antibody to human IgM; coloured red, ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; yellow cap.

- **Dengue Virus IgM Positive Control***:** 1 bottle containing 2 ml; coloured yellow; ready to use; red cap.
- **Dengue Virus IgM Cut-off Control***:** 1 bottle containing 3 ml; coloured yellow; ready to use; green cap.
- **Dengue Virus IgM Negative Control***:** 1 bottle containing 2ml; coloured yellow; ready to use; blue cap.

* Contains 0.01 % kathon after dilution
** Contains 0.2 % Bronidox L
*** Contains 0.1 % kathon

Materials supplied

* 1 strip holder
* 2 Cover foils
* 1 Package insert

Materials and Equipment needed

* Elisa microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
* Incubator 37oC
* Manual or automatic equipment for rinsing wells.
* Pipetes to deliver volumes between 10 and 1000 µl
* Vortex tube mixer
* Deionised or (freshly) distilled water
* Disposable tubes
* Timer

STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at 2…8°C.

REAGENT PREPARATION

It is very important to bring all reagent, samples and controls to room temperature (20…25°C) before starting the test run!

1. Coated snap-off Strips

The ready to use breakapart snap-off strips are coated with Dengue Virus Type 2 Antigen. Store at 2…8°C. The strips are vacuum sealed. Immediately after removal of strips, the remaining strips should be resealed in the aluminum foil along with the desiccant supplied and stored at 2…8°C. After first opening stability until expiry date when stored at 2…8°C.

2. Dengue Virus anti IgG Conjugate

The bottles contain 20 ml of a solution with anti human IgM horseradish peroxidase, buffer, stabilizer preservatives and inert red dye. The solution is ready to use. Store at 2…8°C. After first opening stability until expiry date when stored at 2…8°C.

3. Controls

The bottles labeled with Positive, Cut-off and Negative Control contain a ready to use control solution. It contains 0.1% kathon and has to be stored at 2…8°C. After first opening stability until expiry date when stored at 2…8°C.

4. IgM Sample Diluent

The bottle contains 100ml phosphate buffer, stabilizers, preservatives and inert green dye. It is used for the dilution of the patient specimen. The solution contains anti human IgM class antibodies to eliminate competitive inhibition from specific IgM class antibody to remove rheumatoid factor. This ready to use solution has to stored at 2…8°C.. After first opening until expiry date when stored at 2…8°C.

5. Washing Solution (20xconc.)

The bottle contains 50ml of a concentrated buffer, detergents and preservatives. Dilute washing solution 1+19; e.g. 10 ml washing solution +190 ml fresh and germ free redistilled water. The diluted buffer is stable for 5 days at room temperature. Crystals in the solution disappear by warning up to 37°C in a water bath. After first opening the concentrate is stable until the expiry date.

6. TMB Substrate Solution

The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to stored at 2…8°C , a way from the light. The solution should be colourless or could have a slight blue ting. If the substrate turns into blue. It may have become contaminated and should be thrown a way. After first opening stability until expiry date when stored at 2…8°C.

7. Stop Solution

The bottle contains 15ml 0.2M sulphuric acid solution This ready to use solution has to be stored at 2…8°C. After first opening stability until expiry date.

SPECIMEN COLLECTION AND PREPERATION

Use human serum or plasma (citrate) samples with this assay. If they assay is performed within 24 hours after sample collection, the specimen should be kept at 2…8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing. *Avoid repeated freezing and thawing*.

Sample Dilution.

Before assaying, all samples should be diluted 1+100 with IgM Sample Diluent. Dispense 10µl sample and 1 ml IgM Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex. *Positive and negative controls are ready to use and must not be diluted.*

ASSAY PRCEDURE

Test Preparation

Please read the Package insert carefully **before** performing the assay. Result reliability depends on strict adherence to the Package insert as described. if performing the test on ELISA automatic systems we recommend to increase the washing steps form three to five and the volume of washing solution from 300µl to 350µl to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplies in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:

1 well	(e.g. A1)	For the substrate blank
1 well	(e.g. B1)	For the negative control,
2 wells	(e.g. C1+D1)	For the cut-off control and
1 well	(e.g. EL)	For the positive control.

It is recommended to determine controls and patient samples in duplicate, if necessary.

Perform all assay steps in the order given and without any appreciable delays between the steps.
A clean, disposable tip should be used for dispensing each control and samples.
Adjust the incubator to 37° ± 1°C.

1- Dispense 100µl controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
2- Cover wells with the foil supplied in the kit.
3- Incubate for 1 hour ± 5 min at 37±1°C.
4- When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl of Washing Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be>5 sec. at the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
Note: - Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
5- Dispense 100µl Dengue Viruses anti-IgM Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6- **Incubate for 30 min at room temperature**. Do not expose to direct sunlight.
7- Repeat step 4.
8- Dispense 100µl TMB Substrate Solution into all wells.
9- Incubate for exactly 15 min at room temperature in the dark.
10- Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.

Any blue color developed during the incubation turns to yellow

Note: Highly positive patient samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Predilution of the sample with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the sample 1+100 with dilution buffer and multiply the results in ATLAS Unit by 2.

11- Measure the absorbance of the specimen at 450/620 nm within 30 min after addition of the stop solution.

Measurement

Adjust the ELISA Microwell Plate Reader to zero using the **substrate blank in well A1.**
If – due to technical reasons – the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!
Measure the absorbance of all wells at **450 nm** and record the absorbance values for each control and patient sample in the distribution and identification plan.
Dual wavelength reading using 620 nm as reference wavelength is recommended.
Where applicable calculate the **mean absorbance values** of all duplicates.

RESULTS

Run Validation Criteria

In order for an assay to considered valid, the following criteria must be met:

- **Substrate blank** In A1: Absorbance **value lower than 0.100.**
- **Negative control** in B1: Absorbance Value **lower than 0.200.**
- **Cut-off control** in C1 and D1: Absorbance value **between 0.250 and 0.900.**
- **Positive control** in E1: Absorbance value equal to or greater than the cut-off value.

Calculation of Results

The cut-off is the mean absorbance value of the Cut-off control determinations.
Example: Absorbance value Cut-off control 0.49 + absorbance value Cut-off control 0.47 =0.96 / 2= 0.48
Cut-off = 0.48

Interpretation of Results

Samples are considered **POSITIVE** if the absorbance value is higher than 10% over the cut-off.
Samples with an absorbance value of 10% above or below the cut-off should not be considered as clearly positive or negative → **grey zone**

It is recommended to repeat the test again 2-4 weeks later with afresh sample. If results in the second test are again in the grey zone the sample has to be considered **NEGATIVE >**
Samples are considered **NEGATIVE** if the absorbance value is lower than 10% below the cut-off.

Results In ATLAS Units

Patient (mean) absorbance value x 10 / Cut-off = ATLAS UNIT

Example: 1.786 x 10 / 0.48 = 37 ATLAS Unit

Cut-off:	10	ATLAS Unit
Grey zone:	9-11	ATLAS Unit
Negative:	<9	ATLAS Unit
Positive:	>11	ATLAS Unit

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

Interassasy	n	Mean	Cv (%)
Pos. Serum	20	1.18	4.8

Intrassay	n	Mean	Cv (%)
Pos Serum	7	0.97	3.1

Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 97.6%.

Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte it is 82.3%.

Interferences

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml hemoglobin, 5mg/ml triglycerides and 0.2 mg/ml bilirubin.

Cross-Reactivity

Acute Infection	**ATLAS Elisa Dengue IgM**
Adenovirus	neg.
CMV	neg.
EBV	neg.
HBV	neg.
Echinococcus	neg.
Influenza	neg.
Leptospira	neg.
Mycoplasma	neg.
Picorna	neg.
RSV	neg.
Rubella	neg.
Syphilis	neg.
Toxoplasma	neg.
VZV	neg.
Q-Fever	pos.

Note: The results refer to the groups of samples investigated; these are not guaranteed specifications.

LIMITATIONS OF THE PROCEDURE

The **Flaviviridae family** includes the serotypes of Dengue virus as well as the **yellow fever**, **Japanese encephalitis** viruses and **Tiek borne encephalitis (TBE).** There is a cross reactivity among flaviviruses, due to the presence of common antigen determinants. Diagnosis of Dengue infection should be made in conjunction with other clinical signs and symptoms and laboratory findings. Known cross reactions among Dengue antigens must be considered during interpretation since epitopes are known to react with other flaviviruses. It is recommended to test a reactive samples wit a second serological method. Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance value. Diagnosis of an infectious should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history. Sympotmatology as well s serological data. In immunocompromised patients and newborns serological data only have restricted value.

PRECAUTIONS AND WARNING

- Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the test kits with analyzers and similar equipment has to be validated.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of the test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- After first opening and subsequent storage check conjugate and control

vials for microbial contamination prior to further use.

- To a void cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.

WARNING:	In the used concentration Bronidox L has hardly any toxicological risk upon contract with skin and mucous membranes!
WARNING:	Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contract with the eyes, rinse thoroughly with water and consult a doctor!

12.1 Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulation. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. LITERATURE

HAYES E.B., Bubler D.J., Dengue and dengue hemorrhagic fever, Pediatric Inf. Dis.J. 11.:311-317,1992

CDC: Imported Dengue-United States 1193-1994, J.Am.Med.Assn.274:113,1995.

Rigau-Perez J.G.Gubler D.J., Vorndam A.V. et al, Dengue surveillance-United States 1986-1992, Morbidity and Mortality Weekly Report 43:7-19, 1994,

CDC: DEngue feveramong U.S. military personnel-Haiti, September-November, 1994, J.Am.Med.Assn.,273:14-15, 1994

Sharp T.W., Wallance M.R. Hayes C.G.et al, Dengue fever in US troops during Operation Restore Hope, Somalia 1992-1993, Am.J.Trop.Med.Hyg.53:89-94

ATLAS MEDICAL
Unit 4, William James House
Cowley Rd, Cambridge, CB4 0WX
Tel: ++44 (0) 1223 858 910
Fax: ++44 (0) 1223 858 524
PPI460 A01
Revision A (18.06.2007)