Chikungunya Virus

IgM µ-capture ELISA

Enzyme immunoassay for the qualitative determination of IgM-class antibodies against Chikungunya virus in human serum serum or plasma

For laboratory use only.

Product Number: NB-06-0272 (96 Determinations)

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1. INTRODUCTION

Chikungunya virus is an arthropod borne virus of the genus Alphavirus (family Togaviridae). The Alphavirus genus contains at least 24 distinct species. These are lipid-enveloped virions with a diameter of 50 to 60 nm.
Alphavirus infections are initiated by the bite of an infected mosquito, which results in the deposition of virus in subcutaneous and possibly cutaneous tissues. After an incubation period of 1 to 12 days the Chikungunya fever develops.
Chikungunya fever (Chikungunya means "that which bends up", in reference to the crippling manifestations of the disease) is an acute viral infection characterized by a rapid transition from a state of good health to illness that includes severe arthralgia and fever.
Temperature rises abruptly to as high as 40°C and is often accompanied by shaking chills. After a few days, fever may abate and recrudesce, giving rise to a "saddleback" fever curve. Arthralgia is polyarticular, favoring the small joints and sites of previous injuries, and is most intense on arising. Patients typically avoid movement as much as possible. Joints may swell without significant fluid accumulations. These symptoms may last from 1 week to several months and are accompanied by myalgia. The rash characteristically appears on the first day of illness, but onset may be delayed. It usually arises as a flush over the face and neck, which evolves to a maculopapular or macular form that may be pruritic. The latter lesions appear on the trunk, limbs, face, plams and soles, in that order of frequency. Petechial skin lesions have also been noted. Headache, photophobia, retro-orbitral pain, sore throat with objective signs of pharyngitis, nausea and vomiting also occur in this setting. Occasionally, however persistent arthralgia and polyarthritis (lasting months or even years) do occur, sometimes involving joint destruction. Even rarer, sequelae include encephalitis and meningoencephalitis with high lethality rates.
The virus has major importance in Africa and Asia. From 20% to more than 90% of the population of tropical and subtropical show serologic evidence of infection. Because Aedes mosquitoes are increasingly prevalent in North Africa and South America, where the population would be uniformly susceptible to infection, the possibility for epidemics is evident. Chikungunya virus infections are imported to central Europe mainly by travellers to tropical and subtropical countries.

Species	Diseases	Symptoms	Mechanism of infection
Chikungunya virus (Alphavirus)	Chikungunya fever	Fever Exanthema Joint pain Persistent arthralgia and polyarthritis, sometimes involving joint destruction. Even rarer encephalitis and meningoencephalitis	Transmission by bloodsucking mosquitoes Aedes albopictus (Africa) Aedes aegypti (Africa, Asia)

The presence of virus resp. infection may be identified by

- Serology: Detection of antibodies by IF, ELISA

2. INTENDED USE

The Chikungunya IgM µ-capture ELISA is intended for the qualitative determination of IgM class antibodies to Chikungunya virus in human serum and plasma (citrate).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of IgM-class antibodies to Chikungunya is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiter strip wells are precoated with anti human IgM to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample and control material Chikungunya antigen solution is added. After a further washing step a mixture of biotinylated Chikungunya antibody and Streptavidin conjugate is added. After washing the captured Chikungunya-specific immuncomplex is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of Chikungunya-specific IgM antibodies in the patient specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Chikungunya Microplate (IgM):** 12 breakapart 8-well snap-off strips coated with anti human IgM, in resealable aluminium foil.
- **Sample Diluent** ***: 1 bottle containing 100 ml of ready to use buffer for sample dilution; pH 7.2 ± 0.2; coloured yellow; white cap.
- **Stop Solution**: 1 bottle containing 15 ml. Ready to use sulphuric acid, 0.2 mol/l; red cap.
- **Washing Solution (20x conc.)*:** 1 bottle each containing 50 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.
- **Chikungunya antigen, lyophilised:** 6 bottles containing lyophilized Chikungunya antigen solution; red cap

- **Chikungunya antibody Solution****:** 1 bottle containing 6 ml of biotinylated Chikungunya antibody, ready to use; coloured blue; white cap
- **Streptavidin conjugate**:** 1 bottle containing 6 ml Streptavidin conjugated with peroxidise, ready to use; coloured red, black cap
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; yellow cap.
- **Chikungunya IgM Positive Control****:** 1 bottle containing 1.5 ml; coloured yellow; ready to use; red cap.
- **Chikungunya IgM Cut-off Control****:** 1 bottle containing 2 ml; coloured yellow; ready to use; green cap.
- **Chikungunya IgM Negative Control***:** 1 bottle containing 1.5 ml; coloured yellow; ready to use; blue cap.

* contains 0.1 % Bronidox L after dilution
** contains 0.2 % Bronidox L
*** contains 0.1 % Kathon
**** contains 0.02 % Kathon and 0.02% Bronidox L after reconstitution

4.2. Materials supplied

- 1 Strip holder
- 1 Cover foils
- 1 Test protocol
- 1 distribution and identification plan

4.3. Materials and Equipment needed

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

5. STABILITY AND STORAGE

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

6. REAGENT PREPARATION

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

6.1. Microplate

The ready to use breakapart snap-off strips are coated with anti human IgM. Store at 2...8°C. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C; stability until expiry date.*

6.2. Chikungunya Antigen

The bottles contain lyophilized Chikungunya antigen solution. The content of each vial has to be resolved in 1 ml diluted washing solution by turning it slowly (no vortex) and 15 min incubation at room temperature. The reconstituted solution is at 2...8°C stable for 1 days.

6.3. Chikungunya Antibody Solution

The bottle contains 6 ml biotinylated Chikungunya antibody, stabilizers, preservatives and an inert blue dye. This ready to use solution has to be stored at 2...8°C. *After first opening stability expiry date when stored at 2…8°C.*

6.4. Controls

The bottles labelled with Positive, Cut-off and Negative Control contain a ready to use control solution. They have to be stored at 2...8°C. *After first opening stability until expiry date when stored at 2…8°C.*

6.5. Washing solution (20xconc.)

The bottle contains 50 ml 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. *After first opening the concentrate is stable until expiry date when stored at 2…8°C.*

6.6. Sample Diluent

The bottle contains 100 ml sample dilution buffer, detergents and preservatives. It is used for the dilution of the patient specimen. *After first opening the buffer solution is stable until expiry date when stored at 2…8°C.*

6.7. Streptavidin Conjugate

1 bottle contains 6 ml Streptavidin conjugated with peroxidase, detergents and preservatives, coloured red. The reagent is ready to use and has to be stored at 2….8°C. *After first opening the conjugate is stable until expiry date when stored at 2…8 °C.*

6.8. TMB Substrate Solution

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

6.9. Stop Solution

The bottle contains 15 ml 0.2 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8 °C. *After first opening stability until expiry date.*

7. SPECIMEN COLLECTION AND PREPARATION

Use human serum or plasma (citrate) samples with this assay. If the assay is performed within 5 days 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.*
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgM Sample Diluent. Dispense 10µl sample and 1ml IgM Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. If performing the test on ELISA automatic systems we recommend to increase the washing steps from 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 supplied 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. E1)	for the positive control.

It is left to the user 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 sample.

Adjust the incubator to 37° ± 1°C.

1. Dispense 50µl 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 >5sec. 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 50µl Chikungunya antigen into all wells except for the blank well (e.g. A1). Cover with foil.
6. **Incubate for 30 min at room temperature.**
7. Repeat step 4.
8. Dispense 50 µl Chikungunya Antibody Solution into all wells except for the blank well (e.g.A1). Cover with foil.
9. **Incubate for 30 min at room temperature.**

10. Repeat step 4.
11. Dispense 50 µl Streptavidin peroxidase conjugate into all wells except for the blank (e.g. A1). Cover with foil.
12. **Incubate for 30 min at room temperature.**
13. Repeat step 4.
14. Dispense 100 µl TMB solution into all wells
15. **Incubate for exact 15 min. in the dark**.
16. Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB substrate.
    Any blue colour developed during the incubation turns into 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 negative matrix for example 1+1, is recommended. Then dilute the sample 1+100 with dilution buffer and multiply the results in NTU by 2.
17. Measure the absorbance of the specimen at 450/620nm within 30 min after addition of the Stop Solution.

8.2. 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.

9. RESULTS

9.1. Assay Validation Criteria

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

▪ **Substrate blank**	in A1:	Absorbance value **< 0.100**
▪ **Negative control**	in B1:	Absorbance value **< cut-off**
▪ **Cut-off control**	in C1 and D1:	Absorbance value **0.150 - 1.300**
▪ **Positive control**	in E1:	Absorbance value > cut-off

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The cut-off is the mean absorbance value of the Cut-off control determinations.

Example: Absorbance value Cut-off control 0.39 + absorbance value Cut-off control 0.37 =0.76 / 2 = 0.38

Cut-off = 0.38

9.3. 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 a fresh 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.

9.3.1. Results in NeoBiotech Units

$$\frac{\text{Patient (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NeoBiotech-Units = NTU]}$$

Example: $\frac{1.216 \times 10}{0.38} = 32\ NTU$

Cut-off:	10	NTU
Grey zone:	9-11	NTU
Negative:	<9	NTU

- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- The GenWay ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

WARNING:	In the used concentration Bronidox L has hardly any toxicological risk upon contact with skin and mucous membranes!
WARNING:	Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact 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 regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Product Number: 40-521-475066 Chikungunya Virus IgM µ-capture ELISA (96 Determinations)

BIBLIOGRAPHY

Gerald L. Mandell, John E. Bennett, Raphael Dolin, Principles and practice of Infectious diseases, 2005, Chapter 147, 1913–1919

Patrick Hochedez, Staphane Jaureguiberry, Monique Debruyne, Philippe Bossi, Pierre Hausfater, Gilles Brucker, Francois Bricaire, Eric Caumes, Chikungunya infection in travellers, Emerging Infectios Diseases Vol. 12, No. 10, October 2006, 1565-1566

F.H. Kayser, K.A. Bienz, J. Eckert, R.M. Zinkernagel, Medical Microbiology, Stuttgart 2005, 440-441

SCHEME OF THE ASSAY

Chikungunya Virus IgM µ-capture -ELISA

Assay preparation

Prepare reagents and samples as described. Establish the distribution and identification plan for all specimens and controls on the form supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Assay procedure

	Substrate blank (e.g. A1)	Negative control	Positive control	Cut-off control	Sample (diluted 1+100)
Negative control	-	50µl	-	-	-
Positive control	-	-	50µl	-	-
Cut-off control	-	-	-	50µl	-
Sample (diluted 1+100)	-	-	-	-	50µl
Cover wells with foil supplied in the kit **Incubate for 1 h at 37°C** Wash each well three times with 300µl of washing solution					
Solution 1	-	50µl	50µl	50µl	50µl
Cover wells with foil supplied in the kit **Incubate for 30 min at room temperature** Wash each well three times with 300µl of washing solution					
Mixture of Solution 2 /Streptavidin conjugate (1 +1)	-	50µl	50µl	50µl	50µl
Cover wells with foil supplied in the kit **Incubate for 30 min at room temperature** Wash each well three times with 300µl of washing solution					
TMB	100µl	100µl	100µl	100µl	100µl
Cover wells with foil supplied in the kit **Incubate for exact 15 min at room temperature in the dark**					
Stop solution	100µl	100µl	100µl	100µl	100µl
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

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