Instructions for Use

For the quantitative measurement of IgM class antibodies to Chikungunya virus in Human serum and plasma.

This product is for research use only and is not intended for *in vitro* diagnostic use.

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1. Introduction

ab108717, the Chikungunya IgM ELISA Kit is intended for the qualitative determination of IgM class antibodies to Chikungunya virus in Human serum and plasma (citrate).

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-orbital 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: Chikungunya virus (Alphavirus)

Diseases: Chikungunya fever

Symptoms:-Fever, Exanthema, Joint pain, Persistent arthralgia and polyarthritis, sometimes involving joint destruction. Even rarer encephalitis and meningoencephalitis.

Mechanism of infection:- Transmission by bloodsucking mosquitoes Aedes albopictus (Africa) and Aedes aegypti (Africa, Asia).

The presence of virus resp. infection may be identified by serology: Detection of antibodies by IF and ELISA.
2. Assay Summary

Microtiter strip wells are precoated with anti Human IgM to bind corresponding antibodies of the specimen. 50\(\mu\)l of samples or control are added to the relevant wells and incubated at 37°C for 1 hour. The wells are washed to remove all unbound sample and control material.

Chikungunya antigen solution 1 is added and incubated for 30 min at room temperature. The wells are washed again.

Chikungunya antigen solution 2 is added and incubated for 30 min at room temperature. The wells are washed again.

Streptavidin conjugate is added and incubated for 30 min at room temperature. The wells are washed again.

After washing the captured Chikungunya-specific immunocomplex is visualized by adding Tetramethylbenzidine (TMB) substrate and incubated at room temperature for 15 min. 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.
3. Kit Contents

- **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 containing 15 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.
- **Chikungunya Solution 1**, lyophilised****: 6 bottles containing lyophilized Chikungunya antigen solution; red cap
- **Chikungunya Solution 2****: 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
• Strip holder
• Cover foils

4. Storage and Handling

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

5. Additional Materials Required

• Distilled or deionized water
• Precision pipettes: 5 μl, 10 μl, 50 μl, 100 μl and 1.0 ml
• Disposable pipette tips
• Microtiter well reader capable of reading absorbance at 450/620 nm.
• Vortex mixer, or equivalent
• Incubator 37°C
• Manual or automatic equipment for rinsing wells
• Disposable tubes
• Timer

6. Preparation of Reagents

It is very important to bring all reagents, samples and controls to room temperature (20-25°C) before starting the test run! After first opening components are stable until expiry date, when stored at 2-8°C.

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 ºC; stability until expiry date.*

2. **Chikungunya Solution 1**:- 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 2 days.

3. **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.

7. Preparation and Collection of Specimen

1. Use Human serum or plasma (citrate) samples with this assay.
2. 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).
3. If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
4. Heat inactivation of samples is not recommended.
5. 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 Method

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.
Assay Procedure:

1. Select the required number of microtiter strips or wells and insert them into the holder.
2. Dispense 50 µl controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
3. Cover wells with the foil supplied in the kit.
4. Incubate for 1 hour ± 5 min at 37±1 ºC.
5. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl 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*
6. Dispense 50 µl Chikungunya Solution 1 into all wells except for the blank well (e.g. A1). Cover with foil.
7. Incubate for 30 min at room temperature.
8. Repeat step 5.
9. Dispense 50 µl Chikungunya Solution 2 into all wells except for the blank well (e.g. A1). Cover with foil.
10. Incubate for 30 min at room temperature.
11. Repeat step 5.
12. Dispense 50 µl Streptavidin peroxidase conjugate into all wells except for the blank (e.g. A1). Cover with foil.
13. Incubate for 30 min at room temperature.
14. Repeat step 5.
15. Dispense 100 µl TMB solution into all wells.
16. Incubate for exact 15 min. in the dark.
17. Dispense 100 µl 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.

18. 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.

9. Data Analysis

A. 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.

B. 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*
C. 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.

D. Results in Abcam Units

Patient (mean) absorbance value x 10 = [Abcam-Units = NTU]

Cut-off

Example: \(1.216 \times 10 = 32\) NTU (Abcam Units)

\(0.38\)

Cut-off: 10 NTU

Grey zone: 9-11 NTU

Negative: <9 NTU

Positive: >11 NTU

E. Precision

<table>
<thead>
<tr>
<th>Intra-assay</th>
<th>n</th>
<th>Mean value (OD)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low positive serum</td>
<td>23</td>
<td>0.62</td>
<td>4.8</td>
</tr>
<tr>
<td>Positive serum</td>
<td>24</td>
<td>1.36</td>
<td>3.4</td>
</tr>
</tbody>
</table>
### F. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. Negative samples have been tested. The diagnostic specificity is > 90%.

### G. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. Positive samples have been tested. The diagnostic sensitivity is > 90%.

### H. Interferences

No interferences were observed when adding triglycerides, bilirubin and haemoglobin in an excess to the sample.

### 10. Limitations

- Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into account...
consideration clinical history, symptomatology as well as serological data.

- In immunosuppressed patients and newborns serological data only have restricted value.

11. Specificity

No cross reactivity were observed by using Rf-samples and samples containing antibodies against Bordetella pertussis, Chlamydia trachomatis, Chlamydia pneumoniae, Dengue Virus, TBE, Helicobacter pylori, HSV 2, Leishmania, Mycoplasma and Schistosoma.

Cross reactivity with antibodies against Borrelia, CMV and Toxoplasma cannot be excluded.

Interference with polyclonal stimulation of EBV infections is likely. In the presence of infectious Mononucleosis (Pfeiffer’s Disease, EBV infection) polyclonal stimulation of B lymphocytes can occur. This may result in non-specific reactions in the detection of antibodies of the IgM class. Therefore it is recommended to exclude an EBV infection by differential diagnosis.
12. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn't fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; concentrate samples</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td>Issue Description</td>
<td>Resolution Provided</td>
<td></td>
</tr>
<tr>
<td>--------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
<td></td>
</tr>
<tr>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
<td></td>
</tr>
<tr>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
<td></td>
</tr>
<tr>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation &amp; optimal sample storage conditions (eg. minimize freeze/thaws cycles)</td>
<td></td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed</td>
<td>Wash wells as per protocol recommendations</td>
</tr>
<tr>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
<td></td>
</tr>
<tr>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</td>
<td></td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
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For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the phone number for your region).