**Indication:** Test system for the in vitro determination of antibodies against Chikungunya virus in human serum or plasma for the diagnosis of the following diseases: Chikungunya fever, Chikungunya fever associated arthritis and haemorrhagic fever.

**Clinical significance:** The Chikungunya virus is the pathogenic agent of Chikungunya fever, an infectious tropical disease characterised by fever and joint pain. It is transmitted by mosquitoes of the genus Aedes aegypti (Yellow fever mosquito) and Aedes albopictus (Asian tiger mosquito) that are active day and night.

Chikungunya fever was first reported in 1952-53 during an epidemic in the Makonde plateau, which is the border region between Tanzania and Mozambique, East Africa. In the Makonde language the term Chikungunya stands for “crookedly walking patient” due to its main symptom of severe joint and muscle pains accompanied by a high sensitivity to touch in the whole body (70% to 99% of cases). In addition to the generally rapidly rising high fever (38.5-40°C), Chikungunya virus infections are characterised by lymph node swelling, maculo-papular rash with little or moderate itching (approx. 50%), rarely occurring punctual bleeding of the skin (petechia), milder forms of mucosa bleeding, e.g. of the nose or gums (approx. 25%), headache, fatigue and ophthalmitis. Placental or congenital transmission has also been described. The incubation period is 2 to 3 days (1 to 12 days are also possible). Chikungunya fever subsides after around 10 days, generally without any lasting damage. Approx. 10% of patients experience joint pains which persist for more than 3 weeks or even months and years. In some cases, fulminant hepatitis or neurological complications such as encephalopathy or meningoencephalopathy have been observed. Chikungunya virus infections lead to life-long immunity. Asymptomatic infections have also been described.

The Chikungunya virus is a small enveloped single-stranded RNA virus belonging to the genus Alphavirus from the Togaviridae family and to the group of Arboviruses, which are transmitted by the bite of arthropods. It is sensitive to heat (over 58 °C), dehydration, soap and disinfectants.

Potential transmission cycles (human to human = urban cycle, or animal to human = sylvatic cycle) and clinical symptoms partially resemble those of dengue fever and yellow fever. The Chikungunya virus is closely related to the O’nyong-nyong virus, the causative agent of O’nyong-nyong fever. So far, reservoir hosts have been monkeys and rodents. According to its varying geographic distribution, the virus is divided into five subspecies which can be clearly genetically differentiated: west African, central African, east and south African, Asian and Indian Ocean subtypes.

**Relevance of serological diagnostics:** The detection of Chikungunya viruses or virus particles can only be performed up to the 7th day after the onset of symptoms due to a short viraemic phase and is generally negative when antibody titers are found. Specific antibodies (IgM, IgG) can be detected at the earliest 6–8 days after the onset of the first clinical symptoms. Seroconversion of IgM takes place approximately from the 50th day after onset of the disease. In laboratory diagnostics, suspected cases are confirmed by a positive anti-Chikungunya virus IgM result or a fourfold titer increase of isolated anti-Chikungunya virus IgG in 2 samples or, alternatively, by a positive PCR result. The determination of Chikungunya viral antigens or a positive anti-Chikungunya virus IgM result is of major importance in the screening of blood reserves.
Test Characteristics

Anti-Chikungunya Virus IIIFT (IgG/IgM)

Test principle: The indirect immunofluorescence test is an in vitro assay for the determination of specific antibodies against Chikungunya virus. BIOCHIPS coated with Chikungunya virus infected and non-infected cells are fixed onto the reaction fields of a microscope slide. In the case of positive reactions, specific antibodies of the class IgG and IgM will bind to the viral antigens. In a second step, the attached antibodies are stained with fluorescein-labelled anti-human antibodies and made visible with the fluorescence microscope.

Test procedure: EUROIMMUN BIOCHIP slides are incubated using the proprietary TITERPLANE Technique. Results are evaluated by fluorescence microscopy. Incubation of the substrates with the positive and negative controls provided in each kit verifies correct performance of the test and aids evaluation.

Inter-lot reproducibility: Positive and negative control sera were incubated on 3 different slide lots. For IgG and IgM the fluorescence intensities of the investigated IIFT resulted in deviations within the required specification of a maximum of +/- 1 intensity level.

Reference range: Titer 1:≥10 (IgG bzw. IgM). The following antibody prevalence was found in healthy blood donors: IgG: 0% (n = 203); IgM: 1.3% (n = 150).

Specificity and sensitivity:

<table>
<thead>
<tr>
<th>Substrate/Ig class</th>
<th>Reference (number and origin of samples)</th>
<th>Specificity</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chikungunya virus (IgG)</td>
<td>Anti-Chikungunya Virus IIIFT (n=290, France1 and Germany2,3)</td>
<td>100%</td>
<td>96%</td>
</tr>
<tr>
<td>Chikungunya virus (IgM)</td>
<td>Anti-Chikungunya Virus IIIFT (n=290, France1 and Germany2,3)</td>
<td>95%</td>
<td>96%</td>
</tr>
</tbody>
</table>

1National Reference Centre for Arboviruses, Institut Pasteur, Lyon, France (n=100 sera from patients with Chikungunya fever)
2Reference Centre for Imported Viral Diseases, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany (n=50 sera from patients with Chikungunya fever)
3University of Schleswig-Holstein, Campus Lübeck, Germany (n=100 sera from healthy blood donors)

Technical data:

Antigen substrate: Chikungunya virus infected cells (species EU 14).

Sample dilution: Serum or plasma.
Qualitative evaluation: 1:10 (IgG and IgM).
Quantitative evaluation: 1:10/100/1000 etc. (IgG and IgM).
There is no upper limit to the measurement range.

Test procedure: 30 min (sample)/30 min (conjugate), room temperature.

Microscopy: Objektive 20x
Excitation filter: 488 nm, colour separator: 510 nm, blocking filter: 520 nm, light source: EUROIMMUN LED, EUROStar Bluelight or mercury vapour lamp, 100 W.

Reagents: Ready for use, with the exception of the PBS-Tween buffer (for dilution and washing step).

Stability: All kit components are stable for at least 18 months from the date of manufacture.

Kit formats: 10 or 20 slides, each containing 5 or 10 test fields.
Kits include all necessary reagents (RF absorption additionally requires EUROSORB, order no. ZF 1270-0145).

Order no.: FI 293a-1005 G or M
(Test kit contains 10 slides with 5 test fields each.)