Dia.Pro
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Extracted from

Design Dossier

Chikungunya virus IgG

Ref. CHIKVG.CE

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1. PRODUCT DESCRIPTION

The devise named CHIKV IgG, coded CHIKVG.CE, is an In Vitro Diagnostic Device or IVDD, classified according to EDMA definitions as a devise for the determination of Viral Markers, code n° 15.04.40.25. The product is not classified by the IVDD directive 98/79/EC and belongs to the category of self-certification.
Specifically, the device is an Enzyme Linked Immuno Sorbent Assay (or ELISA) intended to be used for the qualitative and semi-quantitative determination of class IgG antibodies to Chikungunya Virus in human plasma and sera. The kit is intended for the follow-up of CHIKV-infected patients.

The kit is composed of a box that contains all the components and the instructions for use necessary for 96 tests.

In order to preserve the performances of the device, the kit has to be always stored and shipped at +2…8°C. At the customer site, the kit has to be stored at 2..8°C and returned to that temperature, after use.

The kit has to be used by skilled and qualified personnel in a laboratory of diagnostic analysis, under the control and supervision of a medical doctor, responsible of the management of the laboratory. The laboratory has to be qualified by a certified body or similar entity to carry out in vitro diagnosis of human diseases.

The device contains the following components:

1. Microplate
2. Negative Control
3. Mid Positive Control
4. High Positive Control
5. Wash buffer concentrate
6. Enzyme conjugate
7. Chromogen/Substrate
8. Sulphuric Acid
9. Specimen Diluent
10. Plate sealing foils
11. Package insert

The kit has to be used in combination with the following essential tools, not supplied by Dia.Pro Diagnostic Bioprobes srl.:

- Automated ELISA Microplate washer
- ELISA Microplate reader
- ELISA Microplate incubator
- Precision micropipettes and disposable tips

Alternatively the device can be used in a fully automated ELISA workstation (example: DIA.BLOOd by DiaPro srl) provided that the Quality Control parameter reported in the IFU are duly matched and that the automated assay method strictly follows the indications reported in there.

Products of human origin are used only in the formulation of the Controls (human IgG).
The human CHIKV IgG positive and negative plasma used there is certified negative for HBsAg, HIV Ab and HCV Ab by the supplier (expired donations).

As the product is intended to be used to test human sera and plasma, the package insert reports measures for personal and environmental safety (gloves, glasses and lab coats) to be used by the laboratory personnel when carrying out the assay.

Procedures of waste handling and disposal are also given to the end user.

No variants to the standard format of the device are present.

Should a Distributor want to put the name of its Company on the external box of the device, a specific label (see Annex for Labels) will take over for the one reporting the name of Dia.Pro Diagnostic BioProbes s.r.l.., positioned on the external box, upper label.
No modification of name, code, method of analysis and packaging has been introduced

Important Note:
No Common Technical Specifications have been elaborated by the European Community for CHICK virus determination yet. Dia.Pro Diagnostic BioProbes s.r.l. has therefore defined the internal technical specifications, or ITS, for the device taking into consideration:

a. what reported by EC CTS for the markers of viral hepatitis, not used for blood screening, concerning clinical specificity and sensitivity;
b. the specifications for Immunological Testing for Infectious Diseases – Approved Guideline – second Edition – code I/LA18-A2 – defined by NCCLS, USA
c. the performances and the assay characteristics of the kits for the determination of IgG to CHIKV and those of DENG.CE and WNAB.CE , considering these kits part of the same family of Flavi&Toga Viruses.

2. PRODUCT INFORMATION

2.1 Intended use

This device CHIKVG.CE is a qualitative and semi quantitative determination of IgG antibodies to Chikungunya Virus (CHIKV) in human plasma and sera.
The kit is intended for the follow-up of CHIKV-infected patients.

The CHIKVG.CE test can be used manually or automatically.
The CHIKVG.CE test is intended exclusively for *in vitro* diagnostic use.

2.2 Intended users

The kit CHIKVG.CE has to be used by skilled and qualified personnel in a laboratory of diagnostic analysis, under the control and supervision of a medical doctor, responsible of the management of the laboratory. The laboratory has to be qualified by a notified body to carry out in vitro diagnosis of human diseases.

2.3 Photographs of kit

The standard device is intended as 96 tests format code CHIKVG.CE.
A picture of the Product is reported in the Figure below:

Figure 2-1: Illustration of the CHIKVG.CE with all components out of the kit

2.4 Principle of the assay

The method of analysis used is based on the following principle:

Microplates are coated with Chikungunya Virus immunodominant synthetic antigens derived from ENV region.
In the 1st incubation, the solid phase is treated with diluted samples and anti CHIKV IgG are captured, if present, by the antigens. After washing out all the other components of the sample, in the 2nd incubation bound anti-CHIKV IgG are detected by the addition of anti hIgG antibody, labeled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti-CHIKV IgG antibodies present in the sample.
The test can be made semi quantitative by means of three level controls determining the content of IgG in ArbU/ml.

2.5 Specimen collection

The device has been validated for use with serum and plasma that are prepared using standard techniques of preparation of samples for clinical laboratory analysis.

2.5.1 Serum and plasma collection and transport

Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results.
Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
Sera and plasma can be stored at +2°..8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8um filters to clean up the sample for testing.
Do not use heat inactivated samples as they could give origin to false reactivity.

2.6 For instruments of automated assays: a description of the appropriate assay characteristics or dedicated assays

Any ELISA automated work station can be used following some recommendations:

- When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the IFU (sections "Internal Quality Control"). The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended for blood screening when the number of samples to be tested exceed 20-30 units per run.
- It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

2.7 Product workflow

The time required to perform the test after clinical specimen collection is about 3h 20' considering that:

1- The components of the kit have to reach room temperature (about 1 hour) before their use in the assay (pre-assay operations)

2- The time required to perform the Assay procedure is about 2h 20' (washing steps excluded) according to the following Assay Scheme:

Method	**Operations**
Controls Samples diluted 1:101	100 µl 100 µl
1st incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µl
2nd incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	100 µl
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm