AMPLIRUN® TOTAL ZIKV/DENV/CHIKV CONTROL (PLASMA)

For *in vitro* diagnostic use

MBTC023: A panel of six purified arboviruses pooled that have been inactivated to render them non-infectious and formulated in human plasma. Table 1 lists type of virus, concentration and cell-line used in the culture of each virus included in this control. This kit is intended to validate and control sample processing, amplification and detection in nucleic acid assays based on the molecular identification of arbovirus, using the product as an external run control.

INTRODUCTION:

Infections caused by arthropod-borne-viruses (arbovirus) are a major cause of hospitalization. Dengue (serotypes 1, 2, 3 and 4), Zika virus and chikungunya are arboviruses that produce similar clinical presentations. The diseases caused by these viruses are spread to people primarily through the bite of infected Aedes species mosquitoes and shares clinical features that are difficult to distinguish by symptoms observation. Recent outbreaks associated with these viruses show the importance of a rapid and precise clinical diagnosis.

CHARACTERISTICS:

The content is lyophilized. It is necessary to reconstitute it before use (refer to "Preparation of the reagents"). Total Controls are designed for single use, excess material should be discarded. Nucleic acid detection requires an extraction step that releases DNA/RNA for amplification and detection.

Product description:

Viral particles were purified from supernatants of infected cells by differential centrifugation (see Table 1 for cell-line used). Viruses were inactivated, rendering them non-infectious and diluted in human plasma.

VIRUS	STRAIN	CELL-LINE	FRAGMENT SIZE (bp)	COPIES/µl
DENGUE 1	Hawaii	LLC-MK2	71	
DENGUE 2	New Guinea C	LLC-MK2	78	
DENGUE 3	H87	LLC-MK2	167	
DENGUE 4	H241	LLC-MK2	89	
ZIKA	PRVABC59	Vero	97	
CHIKUNGUNYA	S27 Petersfield	Vero	101	

Table 1.

KIT CONTENTS:

1 VIRCELL TOTAL ZIKV/DENV/CHIKV CONTROL (PLASMA): 10 vials with a pool of lyophilized arboviruses simulating a clinical sample. Each virus is in a concentration that ranges from 4000-10000 copies/vial (See Table 1 for specific concentrations).

Lot number		
Concentration	copies/µl	See Table 1

Table 2.

Quantification validation was performed using a real-time PCR instrument from ROCHE (LightCycler®480 II).

Materials required but not supplied:
Molecular Biology grade water
Additional extraction and detection kit.

STORAGE REQUIREMENTS:

Special transport conditions not required. Store the lyophilized vial at 2-8ºC. After reconstitution, suspension should be used on the same day. Unused product should be discarded.

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations.
Use only the amount of reagent required for the test.
The product is stable until the expiry date indicated in the label, if the instructions for use are followed.

VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

1. This product is for *in vitro* diagnosis use only and for professional qualified staff.
2. Sterile tips with aerosol barrier are essential to prevent contamination.
3. Specimens should be handled as in the case of infectious samples using safety laboratory procedures. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.
4. In order to perform the test it is essential to have separate working areas.
5. Dispose of unused reagents and waste in accordance with all applicable regulations.
6. The component VIRCELL TOTAL CONTROL could include genetic material or substances of animal and/or human origin. VIRCELL TOTAL CONTROL contains inactivated microorganism, nevertheless, it should be considered potentially infectious and handled with care. Inactivation was verified by the absence of growth under same culture conditions used for each microorganism. No present method can offer complete assurance that these or other infectious agents are absent. All materials should be handled and disposed as of potentially infectious. Observe the local regulations for waste disposal.
7. Although the human plasmas have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, control and patient specimens should be handled as potentially infectious.

PREPARATION OF THE REAGENTS:

1. Add 200 µl of Molecular Biology grade water to vial 1 and mix until completely reconstituted. The concentration will be approximately 35000 copies/ml for each virus once reconstituted.
2. Shake with vortex for 30 seconds to dissolve and homogenize completely.
3. Follow diagnostic kit instructions treating TOTAL CONTROL in an identical manner to a clinical specimen using recommended amount for extraction and detection.

INTERNAL QUALITY CONTROL:

Each batch is subjected to internal quality control testing before releasing. Quality control analysis is performed using QIAamp Mini Kit (Qiagen) for sample preparation and real-time PCR run in a LightCycler®480 (Roche) for quantification. Final quality control results for each particular lot are available.

INTERPRETATION OF RESULTS AND VALIDATION PROTOCOL FOR USERS:

Refer to indications of additional extraction and detection kit.

LIMITATIONS OF METHOD:

1. This reagent is intended to be used with methods of human diagnostics. This test has not been verified with other methods.
2. The user of this kit is advised to read carefully and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
3. Use of this product should be limited only to personnel trained in molecular techniques.
4. This external run control does not substitute internal diagnostic kit controls.

5. Quantification conclusions cannot be drawn from a single point sample of known concentration. Precise clinical sample quantification could only be achieved by the standard curve method using a calibrator.

PERFORMANCES:

- **IDENTITY TEST**

Performance analysis of TOTAL CONTROL:

Performance analysis was carried out by sample preparation and PCR analysis with a specific oligo pair for each virus present in the panel. PCR fragments were visualized on a a 2% agarose gel using ethidium bromide staining. The size of each fragment is detailed in Table 1.

- **QUANTIFICATION TEST**

Quantification is based on Real-Time qPCR using the standard curve method. This method involves the use of multiple replicates of different serial dilutions of both the product and the standard of quantification.

- **INTRA-ASSAY PRECISION**

3 vials of the product were extracted under identical extraction conditions and 3 replicas of each extraction were amplified by the same operator under identical qPCR conditions. Less than 15% coefficient of variance was observed between all assays.

- **INTER-ASSAY PRECISION**

1 vial of the product was extracted and 3 replicates from this vial were amplified by 2 different operators on 3 consecutive days. Less than 15% coefficient of variance was observed between all assays.

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
	Store at x-yºC
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
RCNS Xµl	Reconstitute in x µl
SHIP	Shipment temperature
STORE	Storage temperature

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