ZIKV/DENV/CHIKV REALTIME PCR KIT

REF RTPCR004 Σ 48

CE 0123 For *in vitro* diagnostic use

INTENDED PURPOSE

Real Time RT-PCR kit to detect nucleic acid from Zika, chikungunya and dengue viruses in human serum and plasma samples.
The device is intended to be used with general population with suspected infection by the microorganism.
The test is a qualitative and automated assay, intended to be used as an aid to diagnosis.

INTRODUCTION

Zika virus (ZIKV) is an enveloped and icosahedral single-stranded RNA (+) virus belonging to the genus *Flavivirus*, member of the *Flaviviridae* family and therefore evolutionarily related to other mosquito-borne arboviruses such as dengue, yellow-fever and West Nile virus. Zika is predominantly an asymptomatic or mild disease with symptoms lasting for several days to a week after being bitten by an infected mosquito. Clinical picture is characterized as a 'dengue-like' syndrome, most common symptoms of Zika are fever, rash, joint pain, and conjunctivitis (red eyes). However, Zika virus infection during pregnancy can cause microcephaly. During the French Polynesian Zika outbreak, an unexpectedly high number of Guillain-Barré syndrome cases were observed suggesting that ZIKV was the cause of this syndrome.
Chikungunya virus (CHIKV) belongs to the family *Togaviridae*, which includes enveloped, icosahedral, single-stranded RNA (+) viruses with a diameter of approximately 70 nm. Chikungunya is an acute febrile illness with sudden onset of fever and joint pains, particularly affecting the hands, wrists, ankles and feet. The virus is transmitted from human to human by the bites of infected female mosquitoes, most commonly, *Aedes aegypti* and *Aedes albopictus*.
Dengue viruses (DENV) are enveloped, polyhedral, single-stranded RNA (+) virus with a diameter of 40 to 60 nm. Dengue (DF) and dengue hemorrhagic fever (DHF) are caused by one of four closely related, but antigenically distinct, virus serotypes (DENV-1, DENV-2, DENV-3, and DENV-4), of the genus *Flavivirus*. DF and DHF are primarily diseases of tropical and sub-tropical areas, and the four different dengue serotypes are maintained in a cycle that involves humans and the *Aedes* mosquito. Infections produce a spectrum of clinical illness ranging from a nonspecific viral syndrome to severe and fatal hemorrhagic disease. Clinical manifestations include rash, sudden onset of fever, chills, severe headache, nausea, myalgias and arthralgias, leukopenia, thrombocytopenia and hemorrhagic manifestations. It occasionally produces shock and hemorrhage, leading to death. Important risk factors for DHF include the strain of the infecting virus, as well as the age, and especially the prior dengue infection history of the patient.
Zika, dengue and chikungunya share some clinical signs, and can be misdiagnosed in areas where they are prevalent. Recent outbreaks associated with these viruses show the importance of a rapid and precise clinical diagnosis.

TEST PRINCIPLE

It is based on the reverse transcription (RT) and amplification in the same reaction well of specific fragments of Zika virus (ZIKV), chikungunya virus (CHIKV) and dengue virus (DENV) by real time PCR.
One lyophilized master mix (RT-PCR MIX) is provided for screening and confirmation using one independent target for each virus.
PCR mix targets a specific fragment of the *E* gene for ZIKV, a specific fragment of *nsP2* gene for CHIKV and specific fragment of 3' untranslated region (3'UTR) for DENV.
An amplification control is included to check the absence of carry-over of amplification inhibitors and the correct amplification set-up. This control consists of a bacteriophage MS2 RNA and a specific oligo pair/probe for its amplification.
The technique is divided into 2 main steps: 1) RNA extraction and 2) reverse transcription and amplification/detection with specific oligo pairs and probes. ZIKV RNA is detected in FAM channel, CHIKV RNA is detected in Texas/ROX channel and DENV RNA is detected in HEX/VIC channel. The internal control (MS2 RNA) is detected in Cy5 channel.

KIT FEATURES

VIRCELL RT-PCR MIX and VIRCELL POSITIVE CONTROL are lyophilized. It is necessary to reconstitute them before use (see "Preparatory treatment of the device" section). The rest of the reagents are ready to use.

This kit is based on reverse transcription, amplification and detection using real time PCR.

MATERIALS PROVIDED

[1] VIRCELL ZCD RT-PCR MIX: 6 vials containing reverse transcriptase, Taq polymerase, buffer and specific primers/probe for Zika virus (*E* gene), dengue virus (3'UTR) and chikungunya virus (*nsP2* gene). Also, as internal control, primers/probe for bacteriophage MS2 RNA. 8 reactions per vial. Lyophilized.
[3] VIRCELL ZCD POSITIVE CONTROL: 1 vial containing a mixture of lyophilized non-infectious nucleic acids to be used as positive control. Red cap.
[4] VIRCELL NEGATIVE CONTROL: 200 µl of deionized water to be used as negative control. Green cap.
[5] VIRCELL PCR MIX RECONSTITUTION SOLUTION: 2 x 1 ml of aqueous solution to reconstitute the PCR mix. Yellow cap.
[6] VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION: 500 µl of aqueous solution to reconstitute the positive control. Brown cap.

Special materials required but not provided:
-Microbiological safety cabinet.
-DNA/RNA extraction kit (see recommendations in "Assay procedure").
-Real Time PCR thermocycler (FAM, HEX/VIC, Texas/ROX and Cy5 detection).
-Precision micropipettes.
-Sterile tips with aerosol barrier.
-Microcentrifuge.
-PCR cabinet (recommended).
-Vortexer.

STORAGE AND HANDLING CONDITIONS

Store at 2-8°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8°C.

IN-USE STABILITY

VIRCELL POSITIVE CONTROL reconstituted: store it between -25°C and -15°C and use until expiration date. Avoid more than 10 freeze-thaw cycles during this time period. Store it between 2ºC and 8ºC and use before 60 minutes.
VIRCELL RT-PCR MIX reconstituted: store it between -25°C and -15°C and use until expiration date. Avoid more than 5 freeze-thaw cycles during this time period. Store it between 2°C and 8°C and use before 60 minutes.
Rest of reagents: Refer to package label for expiration date (at 2-8°C).
VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use only. For professional use only.
2. The product should be limited to personnel who have been trained in the technique.
3. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
4. Use only protocols described in this insert. Conditions other than specified may give erroneous results.
5. EUH210: Safety data sheet available on request.
6. Wear personal protective equipment when handling samples and reagents. Wash hands properly after handling the samples and reagents. All procedures must be carried out in accordance with the approved safety standards.
7. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
8. Never pipette by mouth.
9. Do not use in the event of damage to the package.
10. Do not use the kit after expiration date.
11. Do not leave the reagents at temperature different to the recommended longer than absolutely necessary.
12. Keep containers for samples and reagents closed while they are not being handled.
13. Avoid using samples subjected to repeated freeze-thaw cycles.
14. Handle in aseptic conditions to avoid microbial contaminations.
15. Reagents in this kit could include nucleic acids. Observe the local regulations for waste disposal.
16. Dispose of unused reagents and waste in accordance with all applicable regulations.
17. Use kit components only. Do not mix components from different kits or manufacturers. Only VIRCELL NEGATIVE CONTROL, VIRCELL PCR MIX

RECONSTITUTION SOLUTION and VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION are compatible with the equivalents in other RTPCR VIRCELL references and lots.
18. The glass elements contained in kits could cause physical damage in the event of break. Handle with care.
19. 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.
20. Testing of all the samples at the earliest interval following collection will help ensure the most accurate test results. Variation in storage times during specimen shipment has not been assessed.
21. It is recommended to have two different areas to perform the test: Pre-Amplification and Amplification areas.
22. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of kit reagents or amplification mixtures. All reagents should be closely monitored to purity.
23. It is recommended to use conventional RNA purification kits.
24. Any serious incident that occurs in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

CONDITIONS FOR COLLECTION, HANDLING AND PREPARATION OF THE SPECIMEN

The kit has been validated for samples of serum and plasma.
Use of sterile or aseptic techniques will preserve the integrity of the specimen and avoid microbial contamination. Plasma should be obtained from blood collected in tubes with EDTA or citrate as anticoagulant.
It is recommended to avoid delay on transport and laboratory investigations. Separated serum/plasma should remain at room temperature for no longer than 8 hours. If immediate delivery to the laboratory is not possible, store specimens in a refrigerator (2 to 8ºC). Store specimens for which testing will be delayed beyond 48h after collection at -20ºC or lower; avoid freezing at higher temperatures and freeze-thaw cycles.
Purified RNA can be stored at 2-8ºC for up to one week, and for longer-term storage, it should be kept at -70ºC or lower. To minimize the risk of contamination and to avoid repeated freeze-thaw cycles, it is recommended to prepare aliquots for subsequent analysis.
Recommended guidance: Separated Serum or Plasma. p. 5.5.1.1.1. GP44-A4_Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests, 4th ed. CLSI.

PREPARATORY TREATMENT OF THE DEVICE

All reagents supplied are ready to use, except for the VIRCELL RT-PCR MIX [1] and the VIRCELL POSITIVE CONTROL [3].
[1] VIRCELL RT-PCR MIX. For reconstitution add 120 µl of VIRCELL PCR MIX RECONSTITUTION SOLUTION [5] per vial. Mix thoroughly using a vortex for 2-3 seconds.
⚠The reconstituted VIRCELL RT-PCR MIX must be used within 60 minutes of adding the reconstitution solution stored at 2-8ºC if the start of the test is delayed. In this case, a freeze rack is recommended.

The excess of reconstituted PCR mix can be frozen at temperature between -25°C and -15°C to be used in subsequent reactions.
[3] VIRCELL POSITIVE CONTROL. Follow the next steps to reconstitute it:

- Centrifuge the corresponding tube for 5 seconds at 5000 g.
- Add 100 µl of VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION [6]
- Mix with vortex for 1-2 seconds.
- Centrifuge the tube for 5 seconds at 5000 g.

After reconstitution, the VIRCELL POSITIVE CONTROL [3] can be frozen at temperature between -25°C and -15°C to be used in subsequent reactions.

ASSAY PROCEDURE

1. DNA/RNA extraction (performed in the Pre-Amplification area):
   1.1. It is recommended to use a commercial extraction kit for DNA/RNA extraction. In order to use commercial extraction kits, follow the manufacturer instructions. Consult with Customer Service.
2. Amplification using RT-PCR (performed in the Amplification area):
   2.1. Preparation of the RT-PCR tubes: Label and allocate in freeze rack the number of tubes/strips of tubes needed. One tube will be required for each sample plus one tube for the negative control and another one for the positive control.
   2.2. Reconstitution of VIRCELL RT-PCR MIX: Add 120 µl of VIRCELL PCR MIX RECONSTITUTION SOLUTION [5] per vial. Mix thoroughly using a vortex for 2-3 seconds. Maintain cold after reconstitution/thawing.
   2.3. Pipet 15 µl of Mix to a PCR tube.
   2.4. Addition of the sample: Add 5 µl of each extracted RNA sample to each tube. Add 5 µl of VIRCELL POSITIVE CONTROL [3] and VIRCELL NEGATIVE CONTROL [4] to the corresponding tubes. The negative control is water. Secure caps on the tubes.
   2.5. RT-PCR program: Insert the PCR tubes in the real time thermocycler and run the following program*:

1 cycle	51 ºC	20 minutes
1 cycle	95 ºC	2 minutes
45 cycles	95 ºC	15 seconds
	58 ºC	45 seconds*

* Fluorescence data (FAM, HEX/VIC, Texas/ROX and Cy5) should be collected.

INTERNAL QUALITY CONTROL

Each batch is subjected to internal quality control testing before releasing, complying with strict specifications.

VALIDATION PROTOCOL FOR USERS

It is recommended to include one negative control in each run performed. The negative control will monitor reagent or environmental contamination.
The positive control is recommended to be included on each run. The positive control monitors for reagent failures and for correct operation of essential procedure.
The thermocycler software is likely to automatically calculate the baseline fluorescence value (threshold) based on the amplification curve for each target (fluorescence detection). Nevertheless, it is recommended to set the thresholds for the different detection channels individually. In order to set a threshold for each target, it is recommended to use as a reference the amplification curves of the positive and negative controls. The threshold should be fixed at the beginning of the exponential reading of fluorescence and above the background signal.
The controls result interpretation is as follows:

CONTROL	ZIKV (FAM)	DENV (HEX/VIC)	CHKV (Texas/ROX)	IC (Cy5) [1]	Interpretation
VIRCELL ZCD POSITIVE CONTROL	Amplification (Ct <40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Correct
	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	Invalid
VIRCELL NEGATIVE CONTROL	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	Amplification (Ct < 40)	Correct
	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	No amplification or Ct >40	Invalid

INTERPRETATION OF RESULTS

The result interpretation is described in the tables below:

RESULT	ZIKV (FAM)	DENV (HEX/VIC)	CHKV (Texas/ROX)	IC (Cy5) [1]	Interpretation
1	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	Invalid (sample/kit/setup related)

RESULT	ZIKV (FAM)	DENV (HEX/VIC)	CHKV (Texas/ROX)	IC (Cy5) [1]	Interpretation
2	No amplification or Ct >40	No amplification or Ct >40	No amplification or Ct >40	Amplification (Ct < 40)	Negative
3	Amplification (Ct < 40)	No amplification or Ct >40	No amplification or Ct >40	Amplification (Ct < 40) or no amplification	ZIKV
4	No amplification or Ct >40	No amplification or Ct >40	Amplification (Ct < 40)	Amplification (Ct < 40) or no amplification	CHIKV
5	No amplification or Ct >40	Amplification (Ct < 40)	No amplification or Ct >40	Amplification (Ct < 40) or no amplification	DENV
6	Amplification (Ct < 40)	No amplification or Ct >40	Amplification (Ct < 40)	Amplification (Ct < 40) or no amplification	ZIKV + CHIKV
7	Amplification (Ct < 40)	Amplification (Ct < 40)	No amplification or Ct >40	Amplification (Ct < 40) or no amplification	ZIKV + DENV
8	No amplification or Ct >40	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40) or no amplification	CHIKV + DENV
9	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40) or no amplification	ZIKV + CHIKV + DENV

[1] In case of a high copy number of the target nucleic acid, the amplification of the internal control (IC) in results 3 to 9 may be affected. The late amplification or absence of IC amplification does not change the interpretation of the result.

In case of invalid or inconclusive result, it is recommended to re-extract DNA/RNA from original specimen and re-test it. In the case of failure of amplification of internal control, improper extraction of nucleic acids or inhibition of amplification could be assumed. Testing a new sample is recommended.

LIMITATIONS OF USE

1. The performance with specimens other than human serum and plasma has not been evaluated.
2. The device is intended for the detection of the presence of the infectious agent; it is not intended to detect the exposure to the infectious agent.
3. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures.
4. Detection of the pathogens nucleic acids depends on the organism load present in the specimen and may be affected by specimen collection methods, patient factors, stage of infection and/or strain. False negative results may also occur if amplification inhibitors are present in the specimen. The kit was validated with a specific nucleic acid extraction method. Alternative extraction procedures might be also appropriate but require user validation. A 260/280 purity ratio among 1.8-2.0 is acceptable.
5. The test provides qualitative results. No correlation can be drawn between the magnitude of a positive result and the number of microorganisms in the sample.
6. The test only works within the limits of the genomic regions from which the primers and probes have been chosen. The test targets highly conserved regions, however due to the high variability of RNA genomes it is possible that certain subtypes might not be detected. At design time, mutations of the target regions were not detected.
7. A negative test result does not exclude the presence of the target organism at levels below the detection limit of the assay.
8. A positive test does not rule out the possibility that other pathogens may be present.
9. This kit is designed for generic detection of DENV, it is not possible to differentiate among DENV-1, DENV-2, DENV-3 and DENV-4 serotypes. If distinction of specific DENV serotypes is needed, additional testing is required.
10. The values obtained in the sensitivity and specificity performance study correspond to the total number of samples tested and may vary depending on the type of sample.
11. The performance results showed were generated using the thermocycler CFX96 (Bio-Rad).
12. Nucleic acids tested in the performance evaluation were processed using the OptiPure Viral Kit on Maelstrom 48 series or Maelstrom 96 series (TANBead).
13. The performance results showed correspond to comparative studies with commercial predicate devices in a defined population sample. Small differences can be found with different populations or different predicate devices.
14. A summary of safety and performance is available on EUDAMED or it can be requested at email address customerservice@vircell.com.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

CHIKV

Positive human serum samples (n=54), positive human plasma samples (n=70), external quality control samples (n=31) and previously confirmed negative human serum samples (n=139) and negative human plasma samples (n=68) were analysed. Samples were tested against commercial Real-time PCR kits.

Samples were extracted using OptiPure Viral kit on Maelstrom 48 series or Maelstrom 96 series (TANBead) and run in CFX96 (Bio-Rad).

The results were as follows:

Samples No.		362
Sensitivity (%)		99
	95% CI	96-100
Specificity (%)		100
	95% CI	98-100
PPV (%)		100
NPV (%)		100
LR+/LR-		>1000 / 0.01
True Positive		154
True Negative		207
False Positive		0
False Negative		1
Borderline		0

CI: Confidence intervals
PPV: Positive predictive value
NPV: Negative predictive value
LR+: Positive likelihood ratio
LR-: Negative likelihood ratio

DENV

Positive human serum samples (n=141), positive human plasma samples (n=59), external quality control samples (n=25) and previously confirmed negative human serum samples (n=139) and negative human plasma samples (n=68) were analysed. Samples were tested against commercial Real-time PCR kits.

Samples were extracted using OptiPure Viral kit on Maelstrom 48 series or Maelstrom 96 series (TANBead) and run in CFX96 (Bio-Rad).

The results were as follows:

Samples No.		432
Sensitivity (%)		100
	95% CI	98-100
Specificity (%)		100
	95% CI	98-100

PPV (%)	100
NPV (%)	100
LR+/LR-	>1000 / 0.00
True Positive	224
True Negative	207
False Positive	0
False Negative	1
Borderline	0

CI: Confidence intervals
PPV: Positive predictive value
NPV: Negative predictive value
LR+: Positive likelihood ratio
LR-: Negative likelihood ratio

ZIKV

Positive human serum samples (n=26), positive human plasma samples (n=28), external quality control samples (n=48) and previously confirmed negative human serum samples (n=139) and negative human plasma samples (n=68) were analysed. Samples were tested against commercial Real-time PCR kits.

Samples were extracted using OptiPure Viral kit on Maelstrom 48 series or Maelstrom 96 series (TANBead) and run in CFX96 (Bio-Rad).

The results were as follows:

Samples No.		309
Sensitivity (%)		99
	95% CI	95-100
Specificity (%)		100
	95% CI	98-100
PPV (%)		100
NPV (%)		100
LR+/LR-		>1000 / 0.01
True Positive		101
True Negative		207
False Positive		0
False Negative		1
Borderline		0

CI: Confidence intervals
PPV: Positive predictive value
NPV: Negative predictive value
LR+: Positive likelihood ratio
LR-: Negative likelihood ratio

PRECISION

4 samples (2 positive and the positive and negative controls) were amplified twice in 2 runs per day in 2 different qRT-PCR thermocyclers on 20 consecutive days. Samples were run in CFX96 (Bio-Rad). Within-run precision, between-run precision, between-day precision and within-laboratory precision were determined.

The results were as follows:

CHIKV

Sample	Within-run precision %CV	Between-run precision %CV	Between-day precision %CV	Within-laboratory precision %CV
Positive control	0.7	2.1	0.9	2.4
Positive sample 1	1.2	1.4	1.9	2.6
Positive sample 2	0.8	2.3	1.6	3.0
Negative control	No amplification	No amplification	No amplification	No amplification

CV: Coefficient of variation

DENV

Sample	Within-run precision %CV	Between-run precision %CV	Between-day precision %CV	Within-laboratory precision %CV
Positive control	0.4	0.4	0.6	0.9
Positive sample 1	1.1	1.6	1.3	2.3
Positive sample 2	1.2	0.5	1.9	2.2
Negative control	No amplification	No amplification	No amplification	No amplification

CV: Coefficient of variation

ZIKV

Sample	Within-run precision %CV	Between-run precision %CV	Between-day precision %CV	Within-laboratory precision %CV
Positive control	0.4	0.8	0.5	1.0
Positive sample 1	1.0	0.6	1.6	2.0
Positive sample 2	0.7	0.8	1.3	1.7
Negative control	No amplification	No amplification	No amplification	No amplification

CV: Coefficient of variation

INTERFERENCES

A study has been performed to evaluate the effect of potentially interfering substances.

Samples were extracted using OptiPure Viral kit on Maelstrom 48 series (TANBead) and run in CFX96 (Bio-Rad).

The results were as follows:

Interfering substances	Samples No.	Maximum added concentration without interference
Azathioprine	2	10.8 µmol/L
Bilirubin	2	342 µmol/L
Cyclosporine	2	5 mg/L
EDTA	2	3.4 µmol/L
Hemoglobin	2	2 g/L
Heparin	2	3000 U/L
Human genomic DNA	2	10 ng/µL
Human serum albumin	2	60 g/L
Ibuprofen	2	2425 µmol/L
Metronidazole	2	701 µmol/L
Sodium citrate	2	129 mmol/L
Triglycerides	2	500 mg/dL

CROSS REACTIVITY

A study has been performed to evaluate the effect of potentially cross-reactive microorganisms. Samples were run in CFX96 (Bio-Rad).

The results were as follows:

Microorganism	Samples No.	Positives No.
Acinetobacter baumannii	1	0
Adenovirus	1	0
Bacteroides fragilis	1	0
Bartonella henselae	1	0
Bordetella pertussis	1	0
Borrelia burgdorferi	1	0
Brucella abortus	1	0
Candida albicans	1	0
Chlamydia trachomatis	1	0
Coxiella burnetii	1	0
Cryptococcus neoformans	1	0
Cytomegalovirus	1	0
Eastern equine encephalitis virus	1	0
Echovirus 30	1	0
Enterobacter cloacae	1	0
Enterovirus 68	1	0
Epstein-Barr virus	1	0
Escherichia coli (EAEC)	1	0
Haemophilus influenzae	1	0
HCoV-229E	1	0
Herpes simplex virus type 1	1	0
Herpes simplex virus type 2	1	0
Human herpesvirus 6 (HHV-6)	1	0
Influenza A virus	1	0
Japanese encephalitis virus	1	0
John Cunningham virus	1	0
Legionella pneumophila	1	0
Leishmania chagasi	1	0
Leptospira interrogans	1	0
Listeria monocytogenes	1	0
Mayaro virus	1	0
Mycobacterium tuberculosis	1	0
Neisseria meningitidis	1	0
Oropouche virus	1	0

Microorganism	Samples No.	Positives No.
Parechovirus	1	0
Plasmodium falciparum	1	0
Poliomavirus hominis type 1 (BK virus)	1	0
Rhinovirus	1	0
Rickettsia conorii	1	0
Rift Valley fever virus	1	0
Ross River virus	1	0
Rotavirus	1	0
Salmonella typhi	1	0
Sandfly fever Naples virus	1	0
Sandfly fever Sicilian virus	1	0
Seul virus	1	0
St. Louis encephalitis virus	1	0
Staphylococcus aureus	1	0
Streptococcus pneumoniae	1	0
Tick-borne encephalitis virus (TBEV)	1	0
Toscana virus	1	0
Toxoplasma gondii	1	0
Trypanosoma cruzi	1	0
Varicella-zoster virus	1	0
Venezuelan equine encephalitis virus	1	0
West Nile virus	1	0
Western equine encephalitis virus	1	0
Yellow fever virus	1	0
TOTAL	58	0

In addition, an in-silico analysis of the primers/probes sequences comparing to other microorganisms that could be found in clinical samples was performed.
The results were as follows:

	Homology >80%		
Microorganism	CHIKV	DENV	ZIKV
Chapare virus	No	No	No
Crimean-Congo hemorrhagic fever virus	No	No	No
Ebola virus	No	No	No
Junin virus	No	No	No
Hepatitis C virus	No	No	No
Human immunodeficiency virus type 1	No	No	No
Guanarito virus	No	No	No
Kyasanur virus	No	No	No
Lassa virus	No	No	No
Lymphocytic choriomeningitis virus	No	No	No
Machupo virus	No	No	No
Marburg virus	No	No	No
Omsk virus	No	No	No
Rift Valley fever virus	No	No	No
Sabiá virus	No	No	No

A homology greater than 80% was not found in any analyzed microorganism.

ANALYTICAL SENSITIVITY

A preliminary LoD (limit of detection) was determined by testing serial dilutions of quantified ZIKV, DENV1, DENV2, DENV3, DENV4 and CHIKV samples. Samples were extracted using OptiPure Viral kit on Maelstrom 48 series (TANBead) and run in CFX96 (Bio-Rad).
Once an approximated LoD is determined, the final concentration was confirmed by testing 3 serial dilutions. A minimum of 20 replicates is tested for each dilution. The LoD is determined as the lowest concentration where ≥ 95% of the replicates are positive.

	CHIKV	DENV1	DENV2	DENV3	DENV4	ZIKV
LoD (copies/µl)	0.2	1.5	1.7	2	1.9	0.6
LoD (copies/ml)	42	389	453	520	504	159
LoD (copies/reaction)	0.8	7.3	8.5	9.8	9.5	3

INCLUSIVITY

An in-silico analysis for the primered genes included in the assay was performed to determine the inclusivity for the different ZIKV, DENV-1, DENV-2, DENV-3, DENV-4 and CHIKV sequences available.
The criteria selected for including the different sequences in the analysis was geographic and the date when the sequence was deposited. Different lineages, types or subtypes were included in the analysis of each virus.
GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) was used for accessing sequences.
The results of the in-silico analysis show that the kit is predicted to detect all genome variants included in the analysis.

EXTERNAL CONTROL

Controls that are required but not provided with the kit will be the following:
- as positive extraction control, AMPLIRUN® TOTAL ZIKV/DENV/CHIKV CONTROL (PLASMA) Cat. MBTC023 (Vircell).

External controls help monitoring any cross-contamination that occurs during the extraction process, additionally serve as validation tools for extraction reagents.

SYMBOLS USED IN LABELS

In vitro diagnostic medical device


Use-by (expiry date)

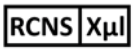
Store at x-y °C

Contains sufficient for <n> test

Batch code

Catalogue number


Consult instructions for use



Reconstitute in <X> µl


Manufacturer

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Update in section: IN-USE STABILITY, CONDITIONS FOR COLLECTION, HANDLING AND PREPARATION OF THE SPECIMEN, LIMITATIONS OF USE, PERFORMANCE CHARACTERISTICS