

Instruction for Use

*alpha*Cube Tropical Fever 1

For the qualitative *in-vitro* detection of RNA from Zika Virus, Chikungunya Virus, Dengue Viruses 1-4 and Yellow Fever Virus in clinical specimens.

REF

830564

830563



32

96



IVD



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1 Intended Use

The *alphaCube* Tropical Fever 1 real time RT-PCR is an assay for the detection of the RNA from Zika Virus, Chikungunya Virus, Dengue Viruses 1-4 and Yellow Fever Virus extracted from clinical specimens (e.g. blood samples, serum samples, urine samples) using real time PCR microplate systems.

2 Pathogen Information

Zika Virus disease is caused by a virus transmitted primarily by *Aedes* mosquitoes in tropical regions. But it can also be transmitted through sexual intercourse. The incubation period of Zika Virus disease is not clear, but is likely to be a few days. The symptoms are similar to other arbovirus infections such as Dengue Viruses, West Nile Virus and others and include fever, skin rashes, conjunctivitis, muscle and joint pain, malaise, and headache. These symptoms are usually mild, require no specific treatment and last for 2 to 7 days. Currently no vaccine against the Zika Virus is available. There is scientific consensus that Zika Virus is a cause of microcephaly in the unborn child and Guillain-Barré syndrome. Links to other neurological complications are also being investigated.

Chikungunya is a viral disease transmitted to humans by infected mosquitoes. The disease mostly occurs in Africa, Asia and the Indian subcontinent. However, a major outbreak in 2015 affected several countries of the Region of the Americas. After the bite of an infected mosquito, onset of illness occurs usually between 4 and 8 days but can range from 2 to 12 days. The disease shares some clinical signs with Dengue and Zika, and can be misdiagnosed in areas where they are common. It causes fever and severe joint pain. Other symptoms include muscle pain, headache, nausea, fatigue and rash. Most patients recover fully, but in some cases joint pain may persist for several months, or even years. Occasional cases of eye, neurological and heart complications have been reported, as well as gastrointestinal complaints. Serious complications are not common, but in older people, the disease can contribute to the cause of death. Treatment is focused on relieving the symptoms.

Dengue is a mosquito-borne viral infection that can be caused by four distinct, but closely related, serotypes of the virus. Dengue is found in tropical and sub-tropical climates worldwide, mostly in urban and semi-urban areas. The global incidence of Dengue has grown dramatically in recent decades. The infection causes flu-like illness, and occasionally develops into a potentially lethal complication called Severe Dengue (also known as Dengue Haemorrhagic Fever). Dengue should be suspected when a high fever is accompanied by 2 of the following symptoms: severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands or rash. Symptoms usually last for 2 to 7 days, after an incubation period of 4 to 10 days. Severe Dengue is a potentially deadly complication due to plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment. There is no specific treatment for Dengue/Severe Dengue, but a vaccine is registered and available in several countries.

Yellow Fever is an acute viral haemorrhagic disease transmitted by infected mosquitoes. The virus is endemic in tropical areas of Africa and Central and South America. Once contracted, the yellow fever virus incubates in the body for 3 to 6 days.

Many people do not experience symptoms, but when these do occur, the most common are fever, muscle pain with prominent backache, headache, loss of appetite, and nausea or vomiting. In most cases, symptoms disappear after 3 to 4 days. A small percentage of patients, however, enter a second, more toxic phase within 24 hours of recovering from initial symptoms. High fever returns and several body systems are affected, usually the liver and the kidneys. In this phase people are likely to develop jaundice, dark urine and abdominal pain with vomiting. Bleeding can occur from the mouth, nose, eyes or stomach. Half of the patients who enter the toxic phase die within 7 to 10 days. Yellow fever can be prevented by vaccination.

3 Principle of the Test

The *alphaCube* Tropical Fever 1 real time RT-PCR contains specific primers and hydrolysis probes for the detection of the nucleic acids of Zika Virus, Chikungunya Virus, Dengue Viruses 1-4 and Yellow Fever Virus extracted from clinical specimens (e.g. blood samples, serum samples, urine samples) using real time PCR microplate systems. The reverse transcription (RT) of viral RNA to cDNA and the subsequent amplification of virus specific fragments are performed in a one-step RT-PCR. The amplification can be detected when specific probes are hydrolysed by the Polymerase. The emitted fluorescence is measured in the FAM (Yellow Fever Virus and Chikungunya Virus), ROX (Dengue Viruses 1-4) and Cy5 (Zika Virus) channel.

Furthermore, the *alphaCube* Tropical Fever 1 real time RT-PCR contains a Control RNA, which is detected in a second amplification system. Added during RNA extraction, the Control RNA allows not only for the detection of RT-PCR inhibition but also detects possible mistakes during RNA extraction. This greatly reduces the risk of false-negative results. The Control RNA can also be used solely as Internal Control by adding it directly to the Mastermix. The fluorescence of the Control RNA is measured in the VIC®/HEX/JOE™/TET channel.

4 Package Contents

The reagents supplied are sufficient for 32 or 96 reactions respectively.

Table 1: Components of *alphaCube* Tropical Fever 1 real time RT-PCR kit.

Label	Lid Colour	Content	
		32	96
Reaction Mix 1 (Zika Virus, Yellow Fever Virus)	yellow	1 x 474 µl	2 x 711 µl
Reaction Mix 2 (Chikungunya Virus, Dengue Viruses 1-4)	orange	1 x 474 µl	2 x 711 µl
Enzyme	blue	1 x 12.8 µl	2 x 19.2 µl
Positive Control 1 (Zika Virus, Yellow Fever Virus)	red	1 x 75 µl	1 x 220 µl
Positive Control 2 (Chikungunya Virus, Dengue Viruses 1-4)	violet	1 x 75 µl	1 x 220 µl
Negative Control	green	1 x 150 µl	1 x 440 µl
Control RNA	colourless	1 x 320 µl	2 x 480 µl

5 Equipment and Reagents to be Supplied by User

- RNA isolation kit (e.g. *alphaClean* Pure RNA/DNA or *alphaClean* Mag RNA/DNA)
- PCR-grade Water
- Sterile microtubes
- Pipets (adjustable volume)
- Sterile pipet tips with filter
- Table centrifuge
- Vortexer
- real time PCR instrument
- Optical PCR reaction tubes with lid
- Optional: *alphaCube* LC480 Colour Compensation
- Optional: Liquid handling system for automation
- Optional: VLP-RNA (please look at page 7 for details)

6 Transport, Storage and Stability

alphaCube Tropical Fever 1 real time RT-PCR is shipped on dry ice or cool packs. All components must be stored at maximum -18°C in the dark immediately after receipt. Do not use reagents after the date of expiry printed on the package.

Up to 20 freeze and thaw cycles are possible.

For convenience, opened reagents can be stored at +2-8°C for up to 6 months.

Protect kit components from direct sunlight during the complete test run.

7 Important Notes

- *alphaCube* Tropical Fever 1 real time RT-PCR must be performed by qualified personnel only.
- Good Laboratory Practice (GLP) has to be applied.
- Clinical samples must always be regarded as potentially infectious material and all equipment used has to be treated as potentially contaminated.

8 General Precautions

- Stick to the protocol described in the Instruction for use.
- Set up different laboratory areas for the preparation of samples and for the set up of the real time RT-PCR in order to avoid contaminations.
- Pipettes, tubes and other materials must not circulate between those different laboratory areas.
- Always use filter tips.
- Regularly decontaminate equipment and benches with ethanol-free decontaminant.
- Do not combine *alphaCube* Tropical Fever 1 real time RT-PCR components of different lot numbers.

9 Sample Material

Starting material for the *alphaCube* Tropical Fever 1 real time RT-PCR is the nucleic acid isolated from clinical specimens (e.g. blood, serum, and urine samples).

10 Sample Preparation

The *alphaCube* Tropical Fever 1 real time RT-PCR is suitable for the detection of the RNA from Zika Virus, Chikungunya Virus, Dengue Viruses 1-4 and Yellow Fever Virus in clinical specimens (e.g. blood, serum, and urine samples) isolated with suitable isolation methods.

Commercial kits for RNA isolation such as the following are recommended:

- *alphaClean* Pure RNA/DNA
- *alphaClean* Mag RNA/DNA

Important:

In addition to the samples always run a 'water control' in your extraction as contamination control. Treat this water control analogous to a sample.

Comparing the amplification of the Control RNA in the samples to the amplification of the internal control in the water control will give insights on possible inhibitions of the real time RT-PCR. Furthermore, possible contaminations during RNA extraction will be detectable.

Please note the chapter 11 'Control RNA'.

If the real time RT-PCR is not performed immediately, store extracted RNA according to the instructions given by the manufacturer.

11 Control RNA

A Control RNA is supplied and can be used as extraction control or only as inhibition control. This allows the user to control the RNA isolation procedure and to check for possible real time RT-PCR inhibition.

The Virus-Like Particles (VLP-RNA) are not supplied, but must be added to the clinical samples directly. VLP-RNA can be used as patient-side extraction control and in automated extraction systems, when pipetting of the Control RNA to the first buffer (e.g. binding buffer) of the respective extraction kit is not possible due to extraction instrument specifications.

RNA isolation from clinical specimens (e.g. blood, serum, and urine samples).

a) Control RNA or VLP-RNA used as Extraction Control:

alphaCube Tropical Fever 1 real time RT-PCR Control RNA or VLP-RNA is added to the RNA extraction.

Add 5 µl Control RNA or VLP-RNA per extraction (5 µl x (N+1)). Mix well. Perform the RNA isolation according to the manufacturer's instructions. Please follow protocol A.

The Control RNA must be added to the Lysis Buffer of the extraction kit.

b) Control RNA used as Internal Control of the real time PCR:

If only inhibition will be checked please follow protocol B.

12 real time RT-PCR

12.1 Important Points Before Starting:

- Please pay attention to the chapter 7 'Important Notes'.
- Before setting up the real time RT-PCR familiarise yourself with the real time PCR instrument and read the user manual supplied with the instrument.
- The programming of the thermal profile should take place before the RT-PCR set up.
- In every real time RT-PCR run one Positive Control and one Negative Control should be included.
- Before each use, all reagents - except the Enzyme - should be thawed completely at room temperature, thoroughly mixed and centrifuged very briefly.

12.2 Procedure

If the Control RNA or VLP-RNA is used to control both, the real time RT-PCR and the RNA isolation procedure, please follow protocol A. If the Control RNA is solely used to detect possible inhibition of the real time RT-PCR, please follow protocol B.

Protocol A

The Control RNA or VLP-RNA was added during RNA extraction (see chapter 11 'Control RNA'). In this case, prepare the Master Mixes 1 and 2 according to Table 2. The Master Mixes contain all of the components needed for RT-PCR except the sample. Prepare a volume of Master Mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Table 2: Preparation of the Master Mix 1 or 2 (Control RNA or VLP-RNA was added during RNA extraction)

Volume per Reaction	Volume Master Mix 1 or 2
14.8 µl Reaction Mix 1 or 2	14.8 µl x (N+1)
0.2 µl Enzyme	0.2 µl x (N+1)

Protocol B

The Control RNA is used for the control of the real time RT-PCR only (see chapter 11, Control RNA'). In this case, prepare the Master Mixes 1 and 2 according to Table 3.

The Master Mixes contain all of the components needed for real RT-PCR except the sample. Prepare a volume of Master Mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Important: Dilute the Control RNA **1:10** in PCR grade Water (e.g. 1 µl Control RNA + 9 µl PCR grade Water) before adding it to the Master Mixes.

Table 3: Preparation of the Master Mix 1 or 2 (Control RNA is added directly to the Master Mix)

Volume per Reaction	Volume Master Mix 1 or 2
14.8 µl Reaction Mix 1 or 2	14.8 µl x (N+1)
0.2 µl Enzyme	0.2 µl x (N+1)
0.2 µl Control RNA* (diluted 1:10)	0.2 µl x (N+1)*

*The increase in volume caused by adding the Control RNA is not taken into account when preparing the PCR assay.

Protocol A and B: real time RT-PCR set up

- Place the number of optical PCR reaction tubes needed into the respective tray of the real time PCR instrument.
- Pipet **15 µl** of each Master Mix (1 and 2) into two separate optical PCR reaction tubes.
- Add **10 µl** of the eluates from the RNA isolation (including the eluate of the water control), the respective Positive Control and the Negative Control to the corresponding optical PCR reaction tubes (Table 4).
- Close the optical PCR reaction tubes immediately after filling in order to reduce the risk of contamination.

Table 4: Preparation of the real time RT-PCR

Component	Volume
Master Mix 1 or 2	15.0 µl
Sample	10.0 µl
Total Volume	25.0 µl

12.3 Instrument Settings

For the real time RT-PCR use the thermal profile shown in table 5.

Table 5: real time RT-PCR thermal profile

Description	Time	Temperature	Number of Cycles
<i>Reverse Transcription</i>	8 min	50°C	1
<i>Initial Denaturation</i>	3 min	95°C	1
<i>Amplification of cDNA</i>			
Denaturation	10 sec	95°C	45
Annealing and Extension	45 sec	60°C	
Aquisition at the end of this step			

Dependent on the real time instrument used, further instrument settings have to be adjusted according to table 6.

Table 6: Overview of the instrument settings required for *alphaCube* Tropical Fever 1 real time RT-PCR.

real time PCR Instrument	Parameter	Detection Channel	Notes		
LightCycler 480II	Chikungunya Virus	465-510	alphaCube LC480 Colour Compensation required		
	Yellow Fever Virus		Melt Factor	Quant Factor	Max Integration Time (sec)
	Dengue Viruses 1-4		1	10	1
	Control RNA		1	10	2
	Zika Virus		1	10	3
StratageneMx3000P / Mx3005P	Chikungunya Virus	FAM	Gain 8	Reference Dye: None	
	Yellow Fever Virus	FAM	Gain 8		
	Dengue Viruses 1-4	ROX	Gain 1		
	Control RNA	HEX	Gain 1		
	Zika Virus	Cy5	Gain 4		
ABI 7500	Chikungunya Virus	FAM	Option Reference Dye ROX: NO		
	Yellow Fever Virus				
	Dengue Viruses 1-4				
	Control RNA				
	Zika Virus	Cy5			
Rotor-Gene Q, Rotor-Gene 3000 Rotor-Gene 6000	Chikungunya Virus	Green	Gain 5		
	Yellow Fever Virus	Green	Gain 5		
	Dengue Viruses 1-4	Orange	Gain 5		
	Control RNA	Yellow	Gain 5		
	Zika Virus	Red	Gain 5		

13 Data Analysis

The virus specific amplifications are measured in the FAM, ROX, Cy5 channels. The amplification of the Control RNA is measured in the VIC[®]/HEX/JOE[™]/TET channel. The Positive Control contains *in vitro* transcripts of the respective nucleic acid sequences of Zika Virus, Chikungunya Virus, Dengue Viruses 1-4 and Yellow Fever Virus. For the Positive Control, signals in the FAM, ROX, Cy5 channels must be detected. The interpretation of the test results is described in table 7 and table 8.

Table 7: Interpretation Reaction Mix 1

Signal/Ct Values			Interpretation
FAM Channel Yellow Fever Virus	Cy5 Channel Zika Virus	HEX Channel Control RNA	
positive	positive	positive or negative*	Positive result, the sample contains Yellow Fever Virus-RNA and Zika Virus-RNA.
positive	negative	positive or negative*	Positive result, the sample contains Yellow Fever Virus-RNA.
negative	positive	positive or negative*	Positive result, the sample contains Zika Virus-RNA.
negative	negative	≤ 34**	Negative result, the sample contains no Yellow Fever Virus-RNA and Zika Virus-RNA.
negative	negative	negative or > 34**	No diagnostic statement can be made. The real time PCR is either inhibited or errors occurred while RNA extraction.

Table 8: Interpretation Reaction Mix 2

FAM Channel Chikungunya Virus	Ct Values		Interpretation
	ROX Channel Dengue Viruses 1-4	HEX Channel Control RNA	
positive	positive	positive or negative*	Positive result, the sample contains Chikungunya Virus-RNA and Dengue Viruses 1-4-RNA.
positive	negative	positive or negative*	Positive result, the sample contains Chikungunya Virus-RNA.
negative	positive	positive or negative*	Positive result, the sample contains Dengue Viruses 1-4-RNA.
negative	negative	≤ 34**	Negative result, the sample contains no Chikungunya Virus-RNA and Dengue Viruses 1-4-RNA.
negative	negative	negative or > 34**	No diagnostic statement can be made. The real time PCR is either inhibited or errors occurred while RNA extraction.

* A strong positive signal in the FAM, Cy5 and/or ROX can inhibit the IC. In such cases the result for the Control RNA can be neglected.

**Depending on the PCR instrument and/or the chosen extraction method, the Ct values might be shifted. The water control can be used as reference. If the HEX Ct value of a sample differs a lot from the water control, partial inhibition has occurred, leading to false negative results in case of weak positive samples.

Figure 1 and **Figure 2** show examples for positive and negative real time RT-PCR results.

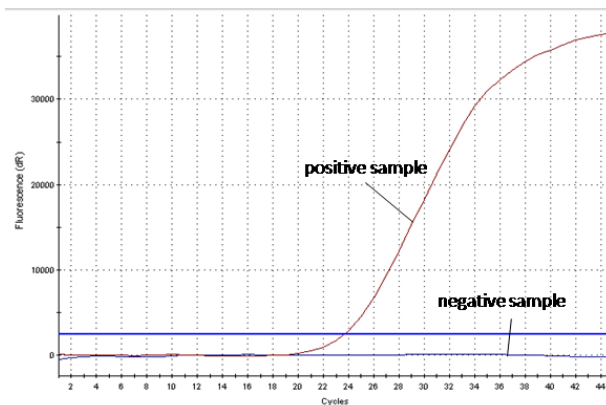


Figure 1: The positive sample shows virus-specific amplification, whereas no fluorescence signal is detected in the negative sample.

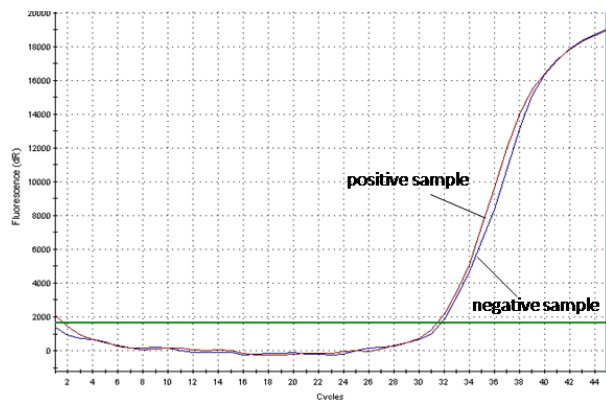


Figure 2: The positive sample as well as the negative sample show a signal in the Control RNA specific VIC®/HEX/JOE™/TET channel. The amplification signal of the Control RNA in the negative sample shows, that the missing signal in the virusspecific channel is not due to RT-PCR inhibition or failure of RNA isolation, but that the sample is a true negative.

14 Assay Validation

Set a threshold as follows:

Negative Controls

All negative controls should be below the threshold. If there is a potential contamination (appearance of a curve in the negative control or a cluster of curves in specimens at high C_T – for example above 36), results obtained are not interpretable and the whole run (including extraction) has to be repeated.

Positive Controls

All the positive controls must show a positive (i.e. exponential) amplification curve. The positive controls must fall below a C_T of 30.

Internal Controls

All internal controls must show a positive (i.e. exponential) amplification curve. The internal control must fall below a C_T of 33. If the internal control is above C_T 34, this is an indication for problems with the extraction or a strong positive sample that can inhibit the IC. In the latter case, the assay is valid. If a water control run is performed, the IC must fall below a C_T of 33.

15 Limitations of the Method

The results must always be considered in relation to the clinical symptoms. Therapeutical consequences should be made in consideration of clinical data. A negative test result does not exclude an infection with the respective viruses.

16 Troubleshooting

The following troubleshooting guide is included to help you with possible problems that may arise when performing a real time RT-PCR.

No fluorescence signal in the FAM, ROX, Cy 5 channel of the Positive Controls

The selected channel for analysis does not comply with the protocol

Select the FAM channel for analysis of the Yellow Fever Virus or Chikungunya Virus specific amplification, the ROX channel for analysis of the Dengue Viruses 1-4 specific amplification and the Cy5 channel for analysis of the Zika Virus specific amplification.
Select the VIC[®]/HEX/JOE[™]/TET channel for the amplification of the Control RNA. Due to amplification in the specific channels, amplification of the Internal Control can be inhibited in the Positive Control.

Incorrect configuration of the real time RT-PCR

Check your work steps and compare with 'Procedure' on page 7.

The programming of the thermal profile is incorrect

Compare the thermal profile with the protocol (Table 5, page 9).

Incorrect storage conditions for one or more kit components or kit expired	Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in 'Transport, Storage and Stability', page 6.
Weak or no signal of the Control RNA and simultaneous absence of a signal in the virus specific FAM channel, ROX channel or Cy5 channel.	
real time RT-PCR conditions do not comply with the protocol	Check the real time RT-PCR conditions (page 9).
real time RT-PCR inhibited	Make sure that you use an appropriate isolation method (see 'Sample Preparation', page 6) and follow the manufacturer's instructions. Make sure that the ethanol-containing washing buffer of the isolation kit has been completely removed. An additional centrifugation step at high speed is recommended before elution of the RNA.
RNA loss during isolation process	In case the Control RNA was added before extraction, the lack of an amplification signal can indicate that the RNA isolation was not successful. Make sure that you use an appropriate isolation method (commercial kits are recommended) and stick to the manufacturer's protocol.
Incorrect storage conditions for one or more components or kit expired	Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in 'Transport, Storage and Stability', page 6.
Detection of a fluorescence signal in the FAM channel, ROX channel or Cy5 channel of the Negative Control.	
Contamination during preparation of the RT-PCR	Repeat the real time RT-PCR in replicates. If the result is negative in the repetition, the contamination occurred when the samples were pipetted into the optical PCR reaction tubes. Make sure to pipet the Positive Control last and close the optical PCR reaction tube immediately after adding the sample. If the same result occurs, one or more of the kit components might be contaminated. Make sure that work space and instruments are decontaminated regularly. Use a new kit and repeat the real time RT-PCR.
Detection of a weak fluorescence signal in the FAM channel of a sample with a strong fluorescence signal in the Cy5 channel.	
Cross-talk	Depending on the real time PCR instrument used, a strong fluorescence signal in one detection channel can lead to a weak signal (around C _T 40) in another channel due to so-called cross-talk between channels.

17 Kit Performance

17.1 Analytical Sensitivity

The limit of detection (LoD) of *alphaCube* Tropical Fever 1 real time RT-PCR was determined using serial dilutions of *in vitro* transcripts in nucleic acid stabilization buffer analysed on a Stratagene Mx3000 real time PCR instrument.

The LoD of *alphaCube* Tropical Fever 1 real time RT-PCR for Chikungunya Virus, Yellow Fever Virus, and Dengue Viruses 1-4 is ≥ 10 copies per reaction each. The LoD of *alphaCube* Tropical Fever 1 real time RT-PCR for Zika Virus is ≥ 5 copies per reaction.

17.2 Analytical Specificity

The specificity of *alphaCube* Tropical Fever 1 real time RT-PCR was evaluated by in silico analysis and by amplification of RNA and DNA of other relevant viruses and bacteria found in clinical samples.

The *alphaCube* Tropical Fever 1 real time RT-PCR showed positive results for the samples containing Zika Virus, Chikungunya Virus, Dengue Viruses 1-4 and Yellow Fever Virus, whereas samples containing other pathogens were reliably tested negative. The results are shown in table 9.

Table 9: Bacterial and viral pathogens tested for the determination of the analytical specificity of *alphaCube* Tropical Fever 1 real time RT-PCR.

Pathogen	Expected Result	Result
<i>West Nile Virus</i>	negative	negative
<i>TBE Virus</i>	negative	negative
<i>Rift Valley Fever Virus</i>	negative	negative
<i>Usutu Virus</i>	negative	negative
<i>Hanta Viruses</i>	negative	negative
<i>Japanese B Encephalitis Virus</i>	negative	negative
<i>Crimean Congo Virus</i>	negative	negative
<i>Sindbis Virus</i>	negative	negative
<i>Mayaro Virus</i>	negative	negative
<i>Cytomegalovirus</i>	negative	negative
<i>HIV</i>	negative	negative
<i>HBV</i>	negative	negative
<i>HCV</i>	negative	negative

Pathogen	Expected Result	Result
<i>Influenzavirus A</i>	negative	negative
<i>Enterovirus</i>	negative	negative
<i>Respiratory Syncytial Virus</i>	negative	negative
<i>Plasmodium</i>	negative	negative
<i>Staphylococcus ssp.</i>	negative	negative
<i>Streptococcus ssp.</i>	negative	negative
<i>Klebsiella ssp.</i>	negative	negative
<i>Borrelia burgdorferi</i>	negative	negative
<i>Anaplasma phagocytophilum</i>	negative	negative
<i>Ehrlichia spec.</i>	negative	negative
<i>Babesia microti</i>	negative	negative
<i>Babesia divergens</i>	negative	negative
<i>Babesia sp. EU1 (Babesia venatorum)</i>	negative	negative
<i>Coxiella burnetii</i>	negative	negative
<i>Zika Virus</i>	positive	positive
<i>Chikungunya Virus</i>	positive	positive
<i>Yellow Fever Virus</i>	positive	positive
<i>Dengue Virus 1</i>	positive	positive
<i>Dengue Virus 2</i>	positive	positive
<i>Dengue Virus 3</i>	positive	positive
<i>Dengue Virus 4</i>	positive	positive

17.3 Reference Material and Proficiency Testing

Reference samples (extracted RNA) for Zika Virus were obtained from the Robert-Koch-Institute (RKI), Berlin. Reference samples (extracted RNA) for Zika Virus, Dengue Viruses 1-4, Chikungunya Virus, and Yellow Fever Virus were additionally obtained from a University Lab in Germany. Samples of INSTAND e.V. were tested additionally.

Table 10: Reference Material Testing using *alphaCube* Tropical Fever 1 real time RT-PCR.

Pathogen	Expected Result	Result
<i>Zika Virus (RKI Standard)</i>	positive	positive
<i>Zika Virus</i>	positive	positive
<i>Chikungunya Virus</i>	positive	positive
<i>Yellow Fever Virus</i>	positive	positive
<i>Ampli Run Yellow Fever Virus (Viracell)</i>	positive	positive
<i>Dengue Virus 1</i>	positive	positive
<i>Dengue Virus 2</i>	positive	positive
<i>Dengue Virus 3</i>	positive	positive
<i>Dengue Virus 4</i>	positive	positive

Table 11: Proficiency Testing using *alphaCube* Tropical Fever 1 real time RT-PCR.

Sample	Expected Result - Dilution	Result
403001	Zika Virus positive – African Lineage 1:250	positive
403002	Zika Virus positive – African Lineage 1:25	positive
403003	Zika Virus negative	negative
403004	Zika Virus positive – African Lineage 1:2500	positive
403013	Zika Virus positive – Asian Lineage 1:500	positive
403014	Zika Virus positive – Asian Lineage 1:50	positive
403015	Zika Virus negative	negative
403016	Zika Virus positive – African Lineage 1:400	positive
369021	Dengue Virus positive – DENV-2 1:400	positive
369022	Dengue Virus positive – DENV-4 1:7,5	positive
369023	Dengue Virus positive – DENV-1 1:70	positive
369024	Dengue Virus positive – DENV-2 1:4	positive
369029	Dengue Virus negative	negative
369030	Dengue Virus positive – DENV-2 1:40	positive
369031	Dengue Virus positive – DENV-1 1:140	positive
369032	Dengue Virus positive – DENV-2 1:400	positive
392021	Chikungunya Virus positive – 1:4500	positive
392022	Chikungunya Virus positive – 1:13500	positive
392023	Chikungunya Virus positive – 1:1500	positive
392024	Chikungunya Virus negative	negative
392005	Chikungunya Virus positive – 1:10000	positive
392006	Chikungunya Virus positive – 1:1000	positive
392007	Chikungunya Virus positive – 1:10000	positive
392008	Chikungunya Virus positive – 1:10000	positive
12 copies per µl	AmpliRun Yellow Fever Virus	positive
1.2 copies per µl	AmpliRun Yellow Fever Virus	positive
0.12 copies per µl	AmpliRun Yellow Fever Virus	positive

18 Abbreviations and Symbols

cDNA	complementary Deoxyribonucleid Acid
RNA	Ribonucleid Acid
PCR	Polymerase Chain Reaction
RT	Reverse Transcription

REACTION MIX 1	Reaction Mix 1
REACTION MIX 2	Reaction Mix 2
ENZYME	Enzyme
CONTROL 1 +	Positive Control 1
CONTROL 2 +	Positive Control 2
CONTROL —	Negative Control
CONTROL RNA IC	Control RNA



Catalog number



Contains sufficient for <n> tests



Upper limit of temperature



Manufacturer



Use by YYYY-MM-DD



Batch code



Content



Consult instructions for use

*In vitro* diagnostic medical device**gerbion GmbH & Co. KG**

Remsstr. 1
70806 Kornwestheim
Germany
phone: +49 7154 806 20 0
fax: +49 7154 806 20 29
e-mail: info@gerbion.com
www.gerbion.com

Distributor**Mikrogen GmbH**

Floriensbogen 2-4
D-82061 Neuried
Germany
phone: +49 (0) 89-54801-0
fax: +49 (0) 89-54801-100
e-mail: mikrogen@mikrogen.de
www.mikrogen.de

19 Literature

- [1] Zika Virus. WHO updated fact sheet 06 September 2016.
- [2] Chikungunya. WHO fact sheet, updated April 2017.
- [3] Dengue and Severe Dengue. WHO fact sheet, updated April 2017.
- [4] Yellow Fever, WHO fact sheet, updated May 2016