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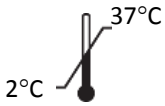
savyonDIAGNOSTICS
member of the Gamida Diagnostics Division

savvy•gen Zika virus

REF **614-01L / 614-01H**

Test kit for 48 determinations



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Intended Use

The Savvygen™ Zika Virus test allows the qualitative detection of *Zika virus* by Real-Time PCR in clinical samples. The product is intended for use in the diagnosis of *Zika virus* infections alongside clinical data of the patient and other laboratory test outcomes.

For *in-vitro* professional diagnostic use.

Background

Zika virus (ZIKV) is an emerging mosquito-borne pathogen (family Flaviviridae, genus Flavivirus) that was isolated in 1947 from a rhesus monkey in the Zika forest in Uganda. ZIKV is believed to be transmitted to humans by infected Aedes spp. mosquitoes. Several studies have demonstrated that ZIKV is endemic to Africa and Southeast Asia. In fact, in other regions, *Zika virus* infections have been reported in few cases until 2007 when an epidemic of ZIKV infection in humans occurred in Yap Island and Federated States of Micronesia, in the Pacific region.

In humans, ZIKV infection is characterized by mild fever (37.8°C–38.5°C); arthralgia (notably of small joints of hands and feet); myalgia, headache; retroorbital pain; conjunctivitis; and cutaneous maculopapular rash. Many cases of ZIKV infection are asymptomatic or have a mild medical history, no specific symptoms, and/or similar to that caused by the influenza virus. This is the reason the ZIKV infection can be misdiagnosed during the acute (viremic) phase.

Confirmation of ZIKV infections, despite its low viremia, is based mostly on detection of virus RNA in serum by using reverse transcription PCR (RT-PCR.) The serological detection of IgM antibodies against ZIKV that can be detected by ELISA, however few laboratories have this ability and Zika antibodies can present cross reactivity against other flavivirus (including Dengue). This can make difficult the serologic diagnosis, since a confirmation by neutralization assays is necessary. Consequently, the laboratory diagnosis is a challenge to be *fa*ced with the best possible approximation.

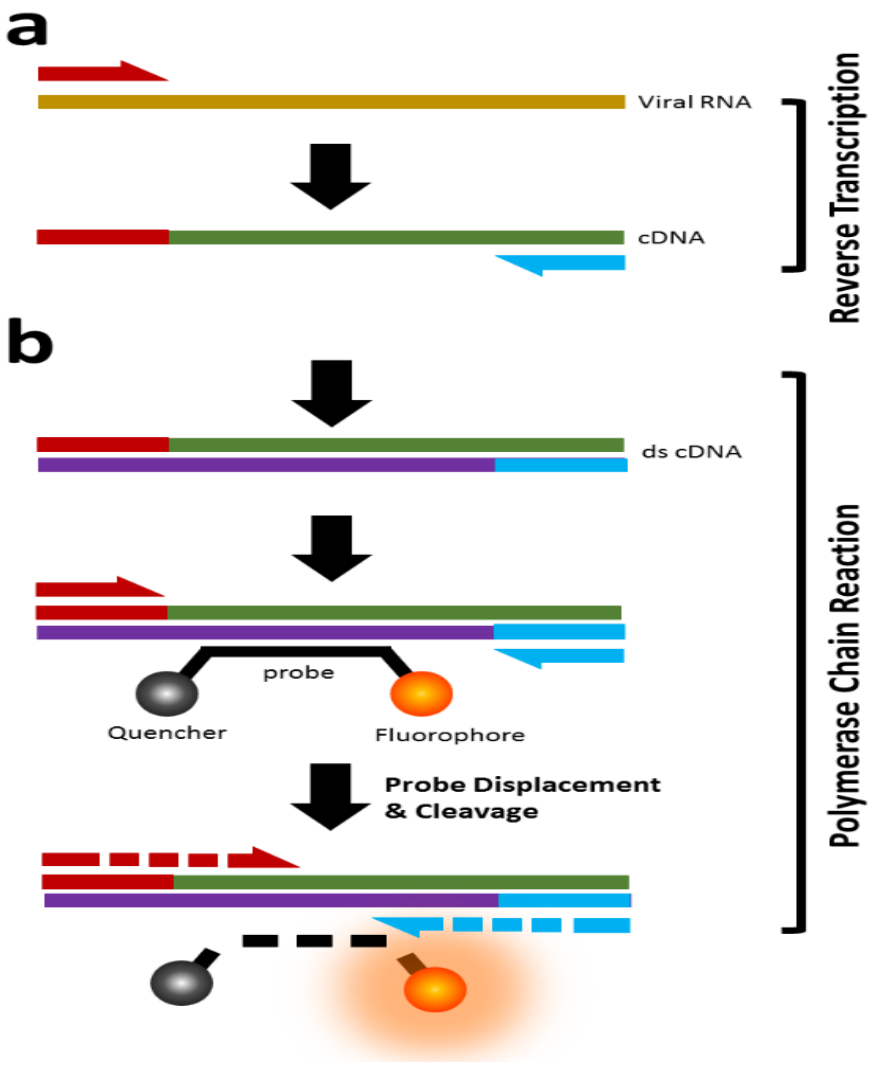
Principles of the Procedure

The Savvygen™ Zika Virus test is a ready-to use test which contains in each well all the necessary reagents for Real-Time PCR assay in a stabilized format. The test is designed for the diagnosis of *Zika virus* in clinical specimens to aid in the assessment of infections caused by this virus.

Savvygen™ Zika Virus test detection is done in one step Real-Time format where the reverse transcription and the subsequent amplification of specific target sequence occur in the same reaction well. The isolated RNA target is transcribed generating complementary DNA by reverse transcriptase, which is followed by the amplification of a conserved *envelope gene* encoded by the *Zika virus* genome using specific primers and a fluorescent–labelled probe.

Following extraction of viral RNA, the conserved fragments are reversely transcribed into cDNA in a primer-specific manner (Figure 1a). Reverse transcription is followed in a “one-pot reaction” by Taq Polymerase Chain Reaction (PCR). The assay is based on the 5’→3’ exonuclease activity of Taq DNA Polymerase (Figure 1b). A fluorophore/quencher dual-labeled probe anneals to an internal specific sequence. Upon primer elongation, Taq DNA Polymerase displaces and hydrolyzes the probe, thus releasing and activating the fluorophore. The presence of *Zika virus* is

detected by an increase in observed fluorescence during the reaction. The resulting increase in fluorescence signal is proportional to the amount of amplified product in the sample and detected by the real-time PCR instrument.



The Savvygen™ Zika Virus test is a ready to use test, which contains in each well all the necessary reagents for Real-Time PCR assay in a stabilized format. In addition, an internal control allows the detection of a possible reaction inhibition. The optical channels used for multiplexed detection of the amplified fragments are outlined in Table 1 below:

Table 1. Pathogen target and optical channel detection

Target	Optical channel
Envelope gene	FAM
Internal Control	*HEX, VIC or JOE

Materials/ Reagents Provided

Product Description	Contents
Savvygen™ Zika Virus *48 reactions. Cat.# 614-01H (High profile)* *48 reactions. Cat.# 614-01L (Low profile)*	6 x *Zika virus* 8-well strip
	1x *Zika virus* Positive Control (lyophilized)
	1x Water RNAse/DNAse free 1mL
	1x Rehydration Buffer 1.8 mL
	1x Negative Control 1 mL
	Optical caps

Additional Equipment and Material Required

- RNA extraction kit.
- Centrifuge for 1.5 mL tube.
- Vortex.
- Micropipettes (0.5-20 µL, 20-200 µL).
- Powder-free disposal gloves
- Real-Time PCR instrument (see table 2A+2B for compatible Real-Time PCRs).

Table 2A. Compatible *Real-Time PCR instrument (Low-Profile)*

Bio-Rad	***Applied Biosystems***
CFX96 Touch™ Real-Time PCR Detection System	7500 Fast Real-Time PCR System
Roche	7500 Fast Dx Real-Time PCR System
LightCycler ®480 Real-Time PCR System	QuantStudio™ 12K Flex 96-well Fast
LightCycler ®96 Real-Time PCR System	QuantStudio™ 6 Flex 96-well Fast
Agilent Technologies	QuantStudio™ 7 Flex 96-well Fast
AriaMx Real-Time PCR System	QuantStudio™ 5 Real-Time PCR System
DNA-Technology	ViiA™ 7 Fast Real-Time PCR System
DTlite Real-Time PCR System	StepOne Plus™ Real-Time PCR System
DT prime Real-Time Detection Thermal Cycler	StepOne™ Real-Time PCR System
Qiagen	Cepheid
Rotor-Gen® Q*	SmartCycler®*

Table 2B. Compatible Real-Time PCR instrument (High-Profile)

Bio-Rad	**Applied Biosystems**
CFX96 Touch Deep Well Real-Time PCR Detection System	7300 Real-Time PCR System
iCycler iQ Real-Time PCR Detection System	7500 Real-Time PCR System
iCycler iQ 5 Real-Time PCR Detection System	7900 HT Real-Time PCR System
DNA-Technology	ABI PRISM 7700
DTlite Real-Time PCR System	QuantStudio™ 12K Flex 96-well
DT prime Real-Time Detection Thermal Cycler	QuantStudio™ 6 Flex 96-well
Stratagene /Agilent Technologies	QuantStudio™ 7 Flex 96-well
Mx3000P™ Real-Time PCR System	QuantStudio™ 3 Real-Time PCR System
Mx3005P™ Real-Time PCR System	QuantStudio™ 5 Real-Time PCR System
Abbot	ViiA™ 7 Real-Time PCR System
Abbot m2000 RealTime System	**Qiagen**
Analytik Jena Biometra	Rotor-Gen® Q*
qTOWER 2.0	**Cepheid**
TOptical	SmartCycler®*

* Once the product has been reconstituted following the appropriate procedure, transfer it into the specific Rotor-Gene® Q or SmartCycler® tubes.

Note: Savvygen™ Zika Vrus *has been validated on the following equipment: Applied Biosystems 7500 Fast Real-Time PCR System, Applied Biosystems StepOne™ Real-Time PCR System, Bio-Rad CFX96 Touch™ Real-Time PCR Detection System, Agilent Technologies AriaMx Real-Time PCR System, DNA-Technology DTprime Real-time Detection Thermal Cycler, Rotor-Gene® Q (Qiagen) and SmartCycler® (Cepheid). When using the Applied Biosystems 7500 Fast with strips it is recommend to place a plate holder to reduce the risk of crushed tube.*

Precautions

Amplification technologies can amplify target nucleic acid sequences over a billion-fold and provide a means of detecting very low concentrations of target. Care must be taken to avoid contamination of samples with target molecules from other samples, or amplicons from previous amplifications. Follow these recommendations to help control contamination.

1. Separate pre-amplification steps from post-amplification steps. Use separate locations for pre- and post-amplification. Use suitable lab equipment for each stage. Prepare samples in a laminar flow hood using suitable equipment to minimize contamination. Set up the post-amplification area in a low-traffic area with dedicated equipment.
2. The laboratory process must be one-directional, it should begin in the Extraction Area and then moved to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area where the previous step was performed.
3. Use disposable containers, disposable barrier pipette tips, disposable bench pads, and disposable gloves. Avoid washable lab wear.

4. Use a diluted bleach solution (0.2% sodium hypochlorite) to treat waste from the post-amplification and detection areas, as the waste contains amplicon. Use the bleach solution to wipe down equipment and bench areas, as well as to treat drains used to dispose of liquid waste.
5. Use negative controls to monitor for possible contamination during reaction setup. If reagent contamination is detected, dispose of the suspect reagents.
6. Do not use after expiration date.
7. Specimens must be treated as potentially infectious as well as all reagents and materials that have been exposed to the samples and handled in the same manner as an infectious agent. Take necessary precautions during the collection, storage, treatment and disposal of samples.

Transport and Kit Storage

The Savvygen kits can be shipped and stored at 2-37ºC, until the expiration date stated in the label.

After resuspension of the Positive Control, store at -20ºC. Avoid repeated freeze/thaw cycles.

It is recommended to make aliquots of the positive control and store at -20ºC once resuspended, in order to avoid freeze & thaw cycles.

Test Procedure

Positive Control Preparation

Note: *The Positive Control vial contains a high copy number template of the assay targets with a contamination risk. Therefore, it is recommended to resuspend the vial in a separate laboratory area or a special cabinet.*

Open the Positive control pouch to resuspend the lyophilized *Zika virus* Positive Control (tube of red cap) with 100 µL of Water RNAse/DNAse free (transparent cap vial) that is supplied. To ensure a complete resuspension, vortex the tube thoroughly. After the first use, dispense into aliquots, in order to avoid multiple freeze-thaw cycles, and store them at -20ºC.

Specimen Collection, Processing and RNA Extraction

In order to obtain an adequate sample, the procedure for sample collection must be followed closely and according to the manufacturer's instructions. The specimens should be transported as fast as possible and be stored at the indicated temperatures conditions.

Nucleic Acid (NA) Extraction: for pretreatment and NA isolation, it is recommended to use an appropriate RNA extraction kit, according to the manufacturer´s protocol. NA Extraction may be carried out manually or automatically using commercially available extraction kits.

The assay has been validated with the following extraction kits:

- Maxwell® 16 Viral Total Nucleic Acid Purification Kit, using the Maxwell® 16 instrument (Promega).
- QIAamp Viral RNA Mini Kit, using the QIAcube instrument (QIAGEN).
- EZ1 Virus Mini Kit, using the EZ1 instrument (Qiagen).
- High Pure Viral Nucleic Acid Kit (Roche).

PCR protocol program

Set your thermocycler following the conditions below:

Table 3. Real-Time PCR profile

Step	Temperature	Time	Cycles
Reverse transcription	45ºC	15 min	1
Initial denaturation	95ºC	2 min	1
Denaturation	95ºC	10 sec.	45
Annealing/Extension*	60ºC	50 sec.	

Note: *Set the fluorescence data collection during the extension step (*) through the FAM and HEX, JOE or VIC channels.*

Depending on the equipment used select the proper detection channel (table 4). For the Applied Biosystems 7500 Fast Real-Time PCR system or the Stratagene Mx3005P™ Real-Time PCR System check that passive reference option ROX is not marked.

Preparing reaction wells

A. Reconstitute the required reaction wells.

Calculate the number of required reactions including samples and controls. It is highly recommended to run at least one positive and one negative control per run.

1. Peel off protective aluminum seal from the strips/plate.
2. Pipette 15 µL of Rehydration Buffer (Blue cap vial) into each well.

B. Add samples and controls according to real-time PCR experimental plate set up.

1. Pipette 5 µL of RNA sample into each sample well
2. Pipette 5 µL of resuspended *Zika virus* Positive Control (tube with red cap) into the positive control well.
3. Pipette 5 µL of Negative Control (tube with orange cap) into each negative control well.
4. Cover the wells with the caps provided. Spin down briefly if needed.

C. Performing PCR.

1. Place the strips in the Real-Time PCR instrument.
2. Start the run.

The fluorescence detection channels of common Real-Time PCR Thermocyclers are specified in Table 4.

Table 4: Detection fluorescence channels of different Real-Time PCR systems

Real-Time PCR THERMOCYCLER	*System Detection channels*	*Savvygen probes channels*	*Remarks*
Roche LightCycler® 96 or LightCycler®480II	465/510	FAM	Color Compensation is required only for LC480 system
	533/580	HEX	
	533/610	ROX	
	618/660	Cy5	
Applied Biosystems ABI 7500 fast	FAM	FAM	Passive reference option ROX is not mark
	VIC	HEX	
	ROX	ROX	
	Cy5	Cy5	
Bio-Rad CFX96 ™	FAM	FAM	
	HEX	HEX	
	ROX	ROX	
	Cy5	Cy5	
Agilent AriaMx	FAM	FAM	
	HEX	HEX	
	ROX	ROX	
	Cy5	Cy5	
DNA-Technology DTlite / DTprime	FAM	FAM	
	HEX	HEX	
	ROX	ROX	
	Cy5	Cy5	
Smartcycler® Cepheid	Channel 1	FAM	
	Channel 2	HEX	
	Channel 3	ROX	
	Channel 4	Cy5	
Abbott m2000rt	FAM	FAM	
	HEX	HEX	
	ROX	ROX	
	Cy5	Cy5	
Rotor-Gene®Q Qiagen	Green	FAM	
	Yellow	HEX	
	Orange	ROX	
	Red	Cy5	

Interpretation of results

Interpretation of results can be automatically performed if programed by the user using the RT-qPCR instrument software following the manufacturer´s instructions. It is required to run assay controls (positive and negative controls) in each run to validate the reaction. See table 5.

Note: *The positive controls used in each run must show an amplification curve for Zika virus target, which validates the reaction, while the negative control well should demonstrate an absence of signal (except internal control target).*

Table 5. Results interpretation

Interpretation	Zika target gene (FAM)	Internal control (**HEX/VIC/JOE**)	Negative Control	Positive Control
Zika virus Positive	POS	POS / NEG	NEG	POS
Zika virus Negative	NEG	POS	NEG	POS
Invalid Run	POS	POS	POS	POS
Invalid Run	NEG	NEG	NEG	NEG

POS: a positive amplification signal;
NEG: a negative amplification signal (no signal)

- **Positive Sample**: A sample is considered positive for *Zika virus* if it shows a signal in the **FAM** channel and a Ct less than 40.
- **Negative Sample:** A sample is considered Negative, if the sample shows no amplification signal in the detection channel but the internal control has a positive signal in the **HEX/VIC/JOE** channel.
- **Invalid run-** The assay should be considered as invalid and a new run should be performed, if there is signal of amplification for the pathogen in the negative control well or absence of signal in the positive control well.

Note: *If an amplification curve for the internal control is not shown, the sample should be retested by dilution of the original sample 1:10. Alternatively it is recommended to repeat the nucleic acid extraction due to possible problems caused by PCR inhibitors.*

Limitations of the test

- This test provides a presumptive diagnosis of *Zika virus* infection. All results must be interpreted together with other clinical information and laboratory findings available to the physician.
- This assay should be used only with blood, serum, CSF, amniotic fluid and urine samples. The use of other samples has not been validated.
- Error results may occur from improper sample collection, handling, storage, technical error, sample mix-up, or because the number of organisms in the sample is below the analytical sensitivity of the test.
- The presence of PCR inhibitors may cause invalid results.
- A false positive result with other targets is possible due to contamination with PCR products from previous testing.
- As with all PCR-based *in-vitro* diagnostic tests, extremely low levels of target below the analytical sensitivity of the assay may be detected, but results may not be reproducible.
- If a certain sample result is Invalid then the sample should be repeated from RNA extraction.

Quality Control

In order to confirm the appropriate performance of the molecular diagnostic technique, an Internal Control (IC) is included in each reaction. Besides, a positive and a negative control must be included in each assay to interpret the results correctly.

Performance Characteristics

Clinical sensitivity and specificity

Overall, 61 clinical samples from symptomatic patients were tested by Real-Time PCR using: i) the Savvygen™ Zika Virus, ii) RealStar® Zika Virus RT-PCR Kit (Altona Diagnostics) and/or iii) in-house Real-Time PCR assay (reported by Faye et al., 2013 and/or Lanciotti et al., 2008 (recommended by the CDC)). *Zika virus* was detected in 6 samples by the Savvygen™ Zika Vrus test. The results show a high sensitivity and specificity to detect *Zika virus* using Savvygen™ Zika Virus (see table 6)

Table 6. Clinical study results

Pathogen	Positive Agreement		Specificity	
	TP/ (TP+FN)	Percent	TN/ (TN+FP)	Percent
Zika virus	6/6	100%	55/55	100%

Analytical sensitivity

In series of experiments to establish the limit of detection for *Zika virus,* a 10-fold dilution of 10^7 to 10^1 copies/reaction was conducted. According to the results, this assay has a detection limit of ≥10 viral RNA copies per reaction (Figure 1)

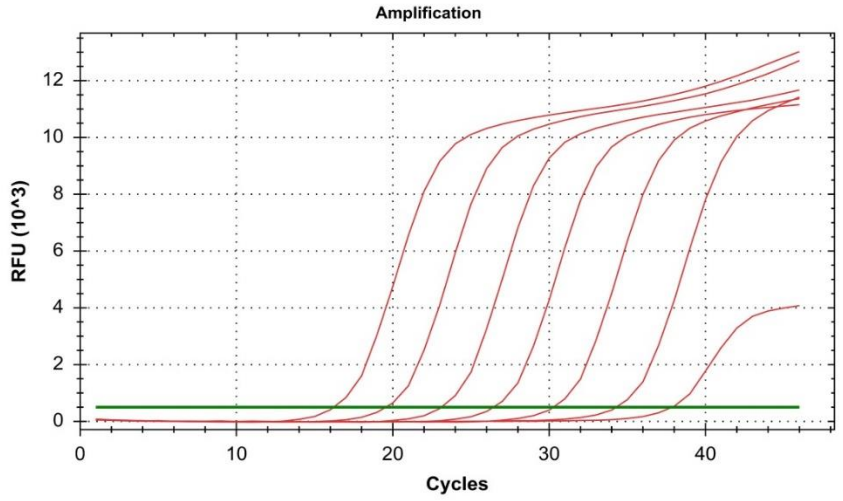


Figure 1. Amplification plot for 10-fold dilution series of *Zika virus* template ranging from 10^7 to 10^1 copies/rxn

Analytical specificity

The analytical specificity for the Savvygen™ Zika Virus assay was tested within the panel of the following microorganisms. No cross-reactivity was observed for any of the listed species (table 7).

Table 7. Cross-reactivity testing.

Pathogen	Cross-Reactivity Test
	Savvygen™ Zika Virus
Chikungunya virus strain S27 Petersfield	-
Dengue 1 virus strain Hawaii	-
Dengue 2 virus strain New Guinea C	-
Dengue 3 virus strain H87	-
Dengue 4 virus strain H241	-
St Louis Encephalitis virus strain 17D	-
West Nile virus strain H160/99	-
West Nile virus Heja	-
West Nile virus Ug37	-
Yellow Fever virus strain 17D	-

Analytical reactivity

The reactivity of the Savvygen™ Zika Virus test was confirmed by the Real-Time amplification using *Zika virus strain MR 766* as template.

Bibliography

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4. Musso D, Roche C, Nhan TX, Robin E, Teissier A, Cao-Lormeau VM. Detection of *Zika virus* in saliva. J Clin Virol. 2015; 68:53-5.
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6. Centers for Disease Control and Prevention. Zika Virus (http://www.cdc.gov/zika/index.html).
7. World Health Organization. *Zika virus* and potential complications (http://www.who.int/emergencies/zika-virus/en/).

Symbols for IVD Components and Reagents

Manufacturer	Use by	For i*n vitro* diagnostic use only
Lot number	Temperature limitation	Consult instructions for use
Catalogue number	Contains sufficient for <n> test	Buffer (sample diluent)
Keep Dry		