WHO Emergency Use Assessment and Listing for Zika IVDs
PUBLIC REPORT

Product: careGENE™ Zika Virus RT-PCR kit
WHO EUAL reference number: EAZ 0009-007-00

Emergency Use Assessment and Listing of In Vitro Diagnostics Procedure

WHO has developed an Emergency Use Assessment and Listing (EUAL) procedure to expedite the availability of in vitro diagnostics (IVDs) needed in public health emergency situations. This EUAL procedure will generate WHO recommendations in order to provide advice to procurement agencies and Member States on the acceptability of a specific IVD in the context of a public health emergency, based on a minimum set of available quality, safety, and performance data and an agreed plan for further evaluation.

The EUAL procedure is comprised of three components that aim to assess the safety, quality and performance of the IVD:

- a review of the manufacturer's quality management system documentation;
- a review of the documentary evidence of safety and performance; and
- an independent performance evaluation

careGENE™ Zika Virus RT-PCR kit with product code **MZS-N09682** manufactured by **WELLS BIO, Inc.**, 16, Magokjungang 8-ro 1-gil, Gangseo-gu, Seoul, 07795, Republic of Korea, **CE marked regulatory version**, was listed as eligible for WHO procurement on 26 June 2018

Intended Use

The careGENE™ Zika Virus RT-PCR kit is an in vitro diagnostic medical device, based on real-time RT-PCR technology utilizing reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA). It is intended for the qualitative detection of Zika virus RNA. Serum specimens are validated for use. The function of the assay as an aid for diagnosis of Zika virus infection in patients with symptoms of Zika infection. Reactive results should be interpreted together with other clinical information available to the physician. The assay is manually operated and to be used with QIAamp Viral RNA Mini Kit (Qiagen, Cat. No. 52906) and real-time PCR instrumentation ABI 7500. The assay is for use by a laboratory professional trained to use real-time PCR in a laboratory setting.

Test kit contents:

Reagents	Quantity sufficient for 96 tests
4X 1 Step RT-PCR Mix	480 µL/vial
Zika primer/probe Mix	480 µL/vial
Nuclease free water	600 µL/vial
Instructions for use	1

Items required but not provided:

Materials	
RNA extraction kits	• QIAamp Viral RNA Mini Kit (Qiagen, Cat. No. 52906)
Reagents	• Positive control (Cat. No. MZC-E09682)
Real-Time PCR instrumentation	• Applied Biosystems® 7500 Real-Time PCR
Other equipment requirements	• Centrifuge • Vortex mixer
Consumables	• PCR tube • Micropipette • Disposable powder-free gloves

Storage: The test kit should be stored at minus 20°C or lower.

Shelf-life upon manufacture: 12 months.

Warnings/limitations: Positive control must be ordered separately

Summary of the WHO EUAL assessment for careGENE™ Zika Virus RT-PCR kit

WELLS BIO, Inc. submitted an expression of interest for WHO emergency quality assessment of **careGENE™ Zika Virus RT-PCR kit** on 04 April 2016.

Review of quality management documentation
To establish the eligibility for WHO procurement, WELLS BIO, Inc. was asked to provide up-to-date information about the status of their quality management system.

Based on the review of the submitted quality management system documentation, it was established that sufficient information was provided by WELLS BIO, Inc. to fulfil the requirements described in the "Invitation to manufacturers of in vitro diagnostics for Zika virus to submit an application for emergency use assessment and listing by WHO".

Quality management documentation for Emergency Use Assessment and Listing conclusion: **Acceptable**

Product dossier assessment
WELLS BIO, Inc. submitted documentation in support of safety and performance for **careGENE™ Zika Virus RT-PCR kit** as per the "Invitation to manufacturers of in vitro diagnostics for Zika virus to submit an application for emergency use assessment and listing

by WHO"[1]. The information submitted in the product application was reviewed by WHO staff and external experts (reviewers) appointed by WHO. The findings of the reviews were reported in accordance with "Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting Zika Virus Nucleic Acid or Antigen" (WHO document PQDx_0240).

Safety and performance documentation for Emergency Use Assessment and Listing conclusion: **Acceptable**

Laboratory evaluation
Analytical testing

Testing was conducted with the international standard (IS, code 11468/16) developed by the Paul-Ehrlich-Institut (PEI), Langen, Germany on behalf of WHO. The standard has been assigned a potency of 7.70 log10 units/mL based on the results of an international collaborative study and was approved by the Expert Committee on Biological Standardization (ECBS) in October 2016.

The study was performed under the BSL-2 conditions. Reference material was reconstituted in 0.5 ml of nuclease-free water.

Three independent dilution series of 11468/16 were prepared in Zika virus-negative pooled human plasma for the evaluation. In the initial testing, 11468/16 was diluted in 10-fold dilution steps down to 10^8 IU. Sufficient volume was prepared so that duplicate extractions could be performed. Dilutions between log 10^{-4} and log 10^{-8} ($5x10^7$ IU to $5x10^1$ IU/ml) were tested in order to determine the end-point. The duplicate RNA extracts were each tested in duplicate PCR reactions.

Subsequently, 11468/16 was tested in half $\log_{10}$ dilution steps between log 10^{-4} and log $10^{-6.5}$ ($1.58x10^3$ to $1.58E10^1$ IU/ml). Five replicates of each dilution were extracted and PCR was performed singly on each sample, per dilution.

A third round of testing was performed using 11468/16 tested in half $\log_{10}$ dilution steps – between 10^{-4} and log $10^{-6.5}$. Five replicates of each dilution were extracted and PCR was performed singly on each sample, per dilution.

One hundred and forty µl sample volume was used for the extraction. The final elution volume was 2 x 40 µl of which 5 µl were used for the amplification/detection reaction (corresponding to a sample volume equivalent of 8.75 µl). Testing was performed on the ABI 7500 instrument (Thermo Fisher, Darmstadt, Germany) in accordance with manufacturer's recommendations and as per the instrument's user manual.

[1] Invitation to manufacturers of in vitro diagnostics for Zika virus to submit an application for emergency use assessment and listing by WHO.

The analytical sensitivity (95% limit of detection [LoD]) of **careGENE™ Zika Virus RT-PCR kit** was determined by Probit analysis. The LOD (at 95% hit rate) was 285.2 IU/ml (combined value from all three runs), with the 95% confidence interval (CI) ranging from (95% CI: 139.2-584.3) IU/ml.

Table 1 Dose for centile 95 (95% LoD) and confidence interval (CI)

	Serum
95% LoD (CI) IU/ml	285.2 IU/ml (95% CI: 139.2-584.3)

Laboratory evaluation for Emergency Use Assessment and Listing conclusion: **Acceptable**

WHO Emergency Use Assessment and Listing Decision

Based on the review of the manufacturer's submitted data, as well as data generated from the limited laboratory evaluation **careGENE™ Zika Virus RT-PCR kit** is eligible for WHO procurement.

Post market surveillance to monitor the performance of **careGENE™ Zika Virus RT-PCR kit** is highly recommended.

Scope and duration of procurement eligibility

careGENE™ Zika Virus RT-PCR kit with product code MZS-N09682, manufactured by WELLS BIO, Inc. is considered to be eligible for WHO procurement. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO prequalified.

As part of the on-going requirements for listing as eligible for WHO procurement WELLS BIO, Inc. must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. WELLS BIO, Inc. is required to notify WHO of any complaints, including adverse events related to the use of the product within 7 days of receipt. Furthermore, WHO will continue to monitor the performance of the assay in the field.

WHO reserves the right to rescind eligibility for WHO procurement, if additional information on the safety, quality and performance comes to WHO's attention during post-market surveillance activities.

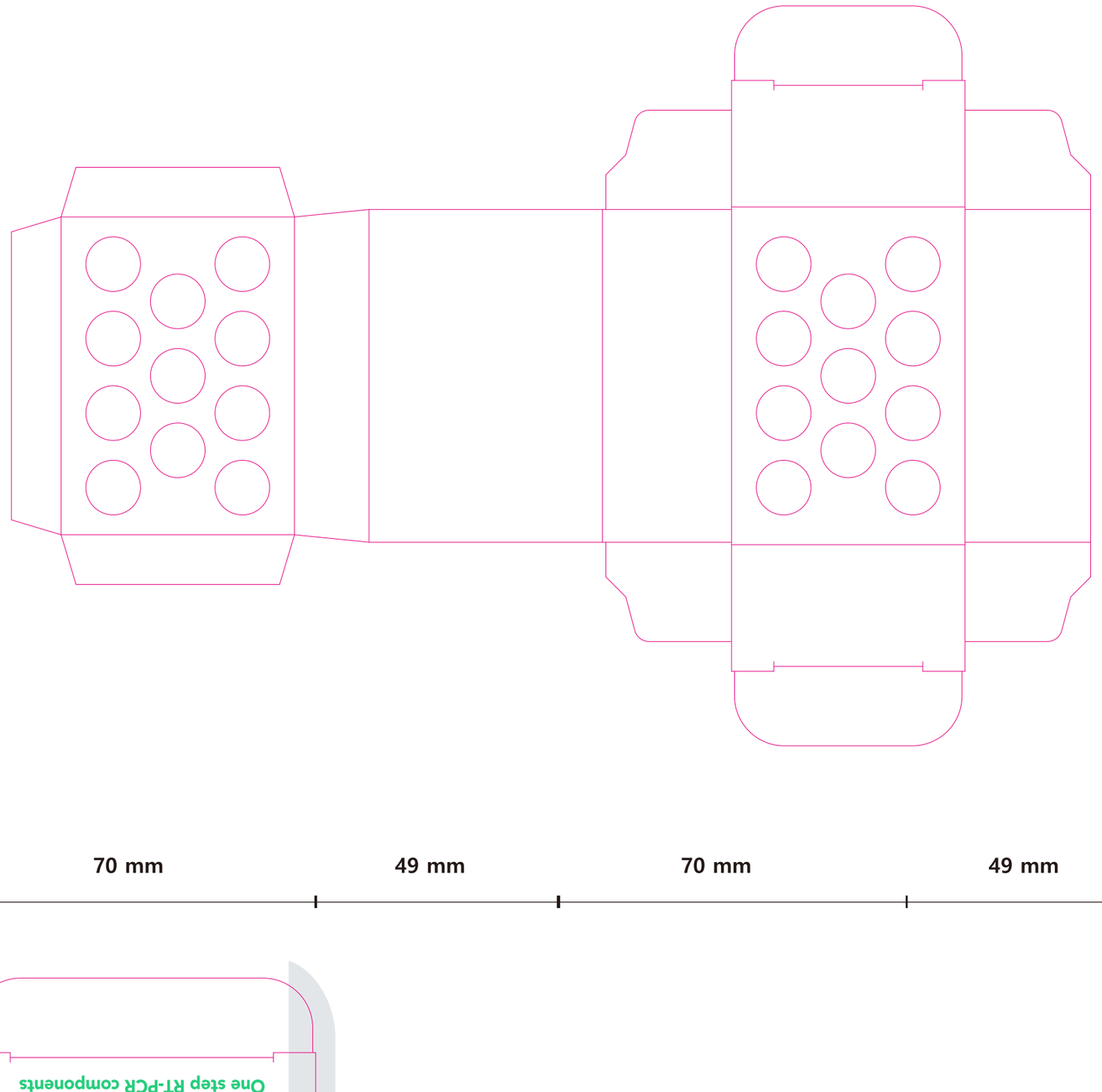
World Health Organization

Labelling

1. **Labels**
2. **Instructions for use**

World Health Organization

1. Labels



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World Health Organization

World Health Organization

2. Instructions for use

careGENE™ Zika Virus RT-PCR kit

INTENDED USE

The careGENE™ Zika Virus RT-PCR kit is an *in vitro* diagnostic medical device, based on real-time RT-PCR technology utilizing reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA). It is intended for the qualitative detection of Zika virus RNA. Serum specimens are validated for use. The function of the assay as an aid for diagnosis of Zika virus infection in patients with symptoms of Zika infection. Reactive results should be interpreted together with other clinical information available to the physician. The assay is manually operated and to be used with QIAamp Viral RNA Mini Kit (Qiagen, Cat. No. 52906) and real-time PCR instrumentation ABI 7500. The assay is for use by a laboratory professional trained to use real-time PCR in a laboratory setting.

INTRODUCTION

Zika virus belongs to Flaviviridae and has a novel single-stranded, positive-sense RNA. Zika virus is believed to be transmitted to humans by infected Aedes spp. mosquitoes. Studies indicated that Zika virus has been endemic in Africa and Southeast Asia. In 2007, an epidemic of Zika virus infection in humans occurred in Yap, Federated States of Micronesia, in the Pacific region. During 2007-2013, a few cases of Zika virus infected travelers returning from Africa or Southeast Asia were reported. The outbreak of Zika fever began in April 2015 in Brazil, and subsequently spread to other countries in South America, Central America and the Caribbean.

Zika virus infection is believed to be asymptomatic or mildly symptomatic in most cases. Thus, Zika virus infection could be misdiagnosed during the acute (viremic) phase because of no specific Zika virus symptoms. Because Zika, Dengue and Chikungunya virus are endemic in the same geographical regions and cause similar symptoms, definite identification of the etiological agent is only possible with laboratory testing. Zika virus detection by means of realtime RT-PCR should be done early (up to 7 days) after illness onset. Confirmation of Zika virus infections is based mostly on detection of virus RNA in serum by using reverse transcription PCR (RT-PCR).

PRINCIPLE

careGENE™ Zika Virus RT-PCR kit was developed based on real-time RT-PCR technology utilizing reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with fluorescent reporters and quencher dyes. The careGENE™ Zika Virus RT-PCR kit contains of 3 components; 4X 1 Step RT-PCR Mix, Zika primer/probe Mix, and Nuclease free water. The careGENE™ Zika Virus RT-PCR kit is compatible with most conventional qPCR machines. (ABI 7500)

Probes for Zika virus specific sequence are labeled with the fluorophore FAM and CY5/Alexa647 for dual detection. The dual detection of FAM and CY5/Alexa647 Probe for Zika virus can increase the specificity of the test kit. The probe for the internal control (IC; Housekeeping gene) specific sequence is labeled with the fluorophore VIC/HEX. The careGENE™ Zika Virus RT-PCR kit includes an endogenous internal control (enIC), which can be used as internal control for the sample preparation procedure (nucleic acid extraction) and/or as a RT-PCR inhibition control.

MATERIALS PROVIDED (96 tests / kit)

Amplification components

Components	Volume	Storage
4X 1 Step RT PCR Mix	480 µL	Below -20℃
Zika primer/probe Mix	480 µL	Below -20℃
Nuclease free water	600 µL	Below -20℃

※ Positive control is not included in the careGENE™ Zika Virus RT-PCR kit. Contact WELLS BIO, INC. or local supplier for purchasing the Positive control.(Cat. No. MZC-E09682)

MATERIALS REQUIRED BUT NOT PROVIDED

- Appropriate (optical) 96-well reaction plate or tube.
- Micropipette
- Sterilized pipette tips with filter barriers
- Centrifuge, Vortex mixer
- Disposable powder-free gloves
- Real Time PCR machine (ABI 7500)

WARNINGS & PRECAUTIONS

1. Read provided instructions for use before using the test kit.
2. The test procedure, precautions and interpretation of results must be closely followed when testing.
3. Observe storage condition indicated on the vials and outer package.
4. Do not swallow the solution of components.
5. Avoid microbial and DNase/RNase contamination of the specimen and the components of the kit.
6. Do not use the kit beyond the expiration date that is indicated on the outer package.
7. Always use DNase/RNase-free disposable pipette tips with filter barriers.
8. Use disposable protective gloves while handling potentially infectious materials and performing the assay. Wash hands thoroughly afterwards.
9. Clean up spills thoroughly using an appropriate disinfectant such as 70% ethanol.
10. Do not open the reaction tubes post amplification, to avoid contamination with amplicons.
11. Discard sample and assay waste according to your local safety regulations.

LIMITATION

1. A specimen for the test should be appropriately collected, transported, handled and processed.
2. Do not use components from other lots.
3. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.

TEST PROCEDURE

Specimen collection and handling

1. Blood is collected in a serum separator tube and incubated for 30 min at room temperature(15-25℃). After incubation, centrifuge at 1,800 x g for 10 min to separate serum and transfer the serum into a clean tube.
2. Serum should be shiped on refrigerant gel packs or dry ice.
3. Separated specimen be stored at 2 - 8℃ up to 72 hrs after collection. For longer period of storage, should be stored below -20℃ in aliquots.

Sample preparation and Storage

The sample is the Zika virus RNA prepared by conventional viral RNA isolation kit. QIAamp Viral RNA Mini Kit (QIAGEN, Cat. No.52906) is recommended. Isolated viral RNA has to be stored below -20℃.

Real-time PCR Master Mix set up

1. Mix the components following the table below.

Number of test	1 test	16 tests	48 tests	96 tests
4X 1 Step RT-PCR Mix	5 µL	80 µL	240 µL	480 µL
Zika primer/ probe Mix	5 µL	80 µL	240 µL	480 µL
Nuclease free water	5 µL	80 µL	240 µL	480 µL
Total	15 µL	240 µL	720 µL	1,440 µL

2. Pipette 15 µL of the Master Mix into each required well of 96-well reaction plate or reaction tube.
3. Add 5 µL of the sample(eluate from the nucleic acid extraction) or 5 µL control(positive control or negative control).
   * To determine the validity of the experiment, positive control(PC) and negative control(NC) are used per run.
4. Set the PCR machine with appropriate detection channel.(Set the ROX signal as a passive reference in case of ABI 7500)

*** Fluorescent Reporter**

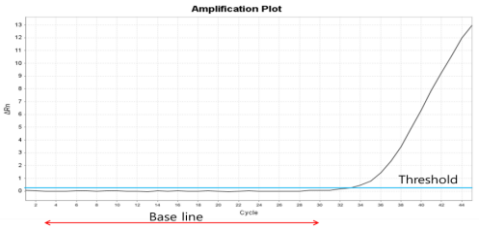
Detection target	Reporter
Zika virus RNA target 1	FAM
Zika virus RNA target 2	CY5/Alexa647
Internal Control	VIC /HEX

5. Perform PCR amplification step as follows.

cDNA synthesis	Pre-denaturation	Amplification	
50℃	95℃	95℃	58℃
15 min	20 sec	15 sec	60 sec
1 cycle	1 cycle	40 cycles	

DATA ANALYSIS

1. Analysis setting
   (1) Set the baseline of all PCR results using flat signal in an initiation phase
   (2) The threshold is 0.03 (delta Rn).
   ※ Threshold can be set up differently depending on the machine to use. The threshold set up in this analysis is optimized for ABI 7500 machine as an example.



2. Acceptance Criteria
   – Positive: Ct value of signal is less than 36.
   – Negative: Ct value of signal is over 36 or not detected.
   ※ Cut-off value set up in this analysis is optimized for ABI 7500 machine as an example. Cut-off value might be different and should be validated by the user when using other RT-qPCR machines

3. Interpretation of Results

Example	Zika virus Target 1	Zika virus Target 2	Internal Control	Results interpretation
	FAM	CY5 (Alexa647)	VIC (HEX)	
1	positive	positive	positive	Zika virus positive
2	positive	negative	positive	Zika virus positive
3	negative	positive	positive	Zika virus positive
4	negative	negative	positive	Zika virus negative
5	positive	positive	negative	Invalid
6	positive	negative	negative	Invalid
7	negative	positive	negative	Invalid
8	negative	negative	negative	Invalid
Positive control	positive	positive	positive	Valid.
Negative control	negative	negative	negative	Valid.

※ To run a valid diagnostic test, the results of positive, negative controls and internal control must be valid. If any of the control results are not valid, repeat the test.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity (Limit of Detection)

To test the sensitivity and limit of detection(LOD) of careGENE™ Zika Virus RT-PCR kit, two runs of test were made each day in duplicate for 5 days using CDC reference material (ATCC, PRVABC59). LOD is determined as 0.1 PFU for serum specimen.

	PFU	Mean Ct	Result in agreement	Percent agreement
Target 1	1000	16.20	20/20	100%
	100	21.03	20/20	100%
	10	24.42	20/20	100%
	1	28.16	20/20	100%
	0.1	32.47	20/20	100%
	0.05	N/A	0/20	0%

	PFU	Mean Ct	Result in agreement	Percent agreement
Target 2	1000	18.27	20/20	100%
	100	22.54	20/20	100%
	10	26.18	20/20	100%
	1	29.80	20/20	100%
	0.1	33.32	20/20	100%
	0.05	N/A	0/20	0%

Cut off Value

Limit of detection was decided as the cutoff value. A Ct value of 36 was set as the cut-off of careGENE™ Zika Virus RT-PCR kit

Cross Reactivity

To test cross reactivity, careGENE™ Zika Virus RT-PCR kit was tested using the pathogens as shown in the table below.

Cross reactivity pathogens	
West nile virus `	*Dengue virus serotype 3*
Yellow fever virus	*Dengue virus serotype 4*
La Crosse virus	*Varicella zoster virus*
Measles virus	*Chikungunya virus,*
Dengue virus serotype 1	*Parvo virus*
Dengue virus serotype 2	*Plasmodium falciparum*

careGENE™ Zika Virus RT-PCR kit did not show cross reactivity with pathogens above.

Interference

To test effect of interference material (Bilirubin, Albumin or EDTA), careGENE™ Zika Virus RT-PCR kit was tested using two concentrations of sample and three types of interference material. careGENE™ Zika Virus RT-PCR kit is not affected by potential interfering substances, which are potentially present in specimen or RNA extraction kit as Bilirubin, Albumin or EDTA.

Reproducibility

Two runs of test were made each day. Each test was repeated twice with three different lots of careGENE™ Zika Virus RT-PCR kit in three different places by two different experimenters for 5 days. Inter-assay variability was measured using first run of five separate qPCR assays performed on five different days. All Cv values are below 5%.

	Strong positive		Weak positive	
	Ct range	Mean Cv	Ct range	Mean Cv
Target 1 intra-assay	29.96~31.37	1.61%	31.10~35.32	1.54%
Target 2 intra-assay	30.04~31.72	1.69%	32.36~33.64	1.42%.
GAPDH intra-assay	32.52~33.48	1.03%	32.08~33.67	1.63%

Repeatability

Two runs of test were made each day in duplicate for 20 days. Intra-assay variability was measured by average coefficient of variation of single qPCR. All Cv values are below 5%.

	Strong positive		Weak positive	
	Ct range	Mean Cv	Ct range	Mean Cv
Target 1 intra-assay	29.98~31.37	0.94%	31.12~33.32	4.83%
Target 2 intra-assay	30.28~31.72	3.28%	32.36~33.64	2.74%
GAPDH intra-assay	23.52~33.48	2.06%	32.10~33.65	3.34%

Clinical performance

The careGENE™ Zika Virus RT-PCR kit was tested to detect Zika infection using 200 human serum samples (50 positives and 150 negatives) by PECET in Columbia and Korea National University Guro Hospital in Korea.

	No. of specimens tested	No. of valid tests	Test Result		Result (%)
			Positive	Negative	
Sensitivity	50	50	50/50	0/50	100
Specificity	150	150	0/150	150/150	100

The performance of careGENE™ Zika Virus RT-PCR kit was both sensitivity and specificity came out to be 100%.

STORAGE CONDITION

careGENE™ Zika Virus RT-PCR kit components: Store below -20℃ (sealed). It is stable and can be used for 12 months from date of manufacture.

REFERENCES

1. Oumar Faye, Ousmane Faye (2008). One-step RT-PCR for detection of Zika virus. Journal of Clinical Virology 43 96-101.
2. Pranav Patel, Olfert Landt (2013). Development of one-step quantitative reverse transcription PCR for the rapid detection of flaviviruses. Virology Journal 10:58

Description of Symbol Used

Symbol	Description	Symbol	Description
REF	Catalogue number	IVD	In vitro diagnostic medical device
LOT	Batch code		Caution
	Use-by date		Manufacturer
	Upper limit of temperature		Consult instructions for use
EC REP	Authorized representative in the EC	CE	CE mark

WELLS BIO, INC.
16, Magokjungang 8-ro 1-gil,
Gangseo-gu, Seoul, 07795,
Republic of Korea
Manufacturing Site/ Factory:
155, Jeongwangcheon-ro,
Siheung-si, Gyeonggi-do,
15107, Republic of Korea

☏ +82-2-3660-6900
🖷 +82-2-3660-6990
✉ info@wellsbio.net
www.wellsbio.net

EC REP **EU Representative:**
MDSS GmbH
Schiffgraben 41, 30175
Hannover, Germany

CE IVD