Gene-RADAR®
Zika Virus Test
Instructions for Use

For Use Under Emergency Use Authorization (EUA) Only

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For use under Emergency Use Authorization (EUA) Only:
For *in vitro* Diagnostic Use
For Prescription Use Only

Proprietary Name
Gene-RADAR® Zika Virus Test

Common or Usual Name
Gene-RADAR® Zika Virus Test

1. Intended Use

The **Gene-RADAR® Zika Virus Test** is a real-time RT-PCR based assay intended for the qualitative detection of RNA from the Zika virus in serum samples from individuals meeting Centers for Disease Control and Prevention (CDC) Zika Virus clinical criteria (i.e., clinical signs and symptoms associated with Zika virus infection) and/or CDC Zika virus epidemiological criteria (i.e., history of residence in or travel to a geographic region with active Zika transmission at the time of travel, or other epidemiological criteria for which Zika virus testing may be indicated). Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories.

Results are for the identification of Zika virus RNA. Zika virus RNA is generally detectable in serum during the acute phase of infection (approximately 14 days following onset of symptoms, if present). Positive results are indicative of current infection. Laboratories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude Zika virus infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The **Gene-RADAR® Zika Virus Test** is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The **Gene-RADAR® Zika Virus Test** is only for use under the Food and Drug Administration's Emergency Use Authorization.

2. Test Principle

The **Gene-RADAR® Zika Virus Test** is a real-time PCR based test.

The **Gene-RADAR® Zika Virus Test** is designed to detect RNA from the Zika virus, extracted from patient serum samples utilizing a QIAamp® Viral RNA Mini Kit (Qiagen), and amplified and detected on the **Gene-RADAR® Platform.**

The **Gene-RADAR® Zika Virus Test** utilizes various controls, including: a **Gene-RADAR® Zika Virus Positive Control**, which is a synthetic RNA target sequence that can be amplified and detected by the same set of primers and probes as the Zika specific target; an **Gene-RADAR® Zika Virus Internal Process Control** which is an inactivated and stabilized MS2 Bacteriophage that contains an RNA genome, that is amplified and detected with a second pair of primers and probe included in the kit; and a **Negative Control**, which is nuclease-free water.

3. Reagents and Instruments

3.1. Materials Provided

Gene-RADAR® Zika Virus Test Kit (for up to 10 patient samples and associated positive and negative controls), includes

Gene-RADAR® Zika Virus Test Kit (Catalog No.: NBS-ZKV-100)
1 vial of Gene-RADAR® Zika Virus Kit Buffer 1, containing primers, probes and reaction buffer, (500 µL)
1 vial of Gene-RADAR® Zika Virus Internal Process Control, (50 µL)
1 vial of Gene-RADAR® Zika Virus Positive Control, (100 µL)
12 single-use Gene-RADAR® Nanochips for use with samples and controls

3.2. Materials Required But Not Supplied

Components required, but not included with the **Gene-RADAR® Zika Virus Test Kit:**

General Equipment
Clean disposable pipettes/tips reserved for RNA work only
Powder-free disposable gloves
RNAse, DNase free-disposable plasticware
Centrifuge with a rotor for 2 mL reaction tubes (Eppendorf 5415C or equivalent)
Vortex mixer (VWR 58810-163 or equivalent)
Nuclease-free water

Nucleic acid extraction kit
QIAamp® Viral RNA Mini Kit (QIAGEN 52906 or 52904)

Instrumentation
Gene-RADAR® Platform

3.3. Additional components available for purchase as needed:

Additional Components Available
Gene-RADAR® Zika Virus Internal Process Control, (50 µL) (Catalog No.: NBS-ZKV-110)
Gene-RADAR® Zika Virus Positive Control, (100 µL) (Catalog No. NBS-ZKV-120)

4. Storage Instructions

- The **Gene-RADAR® Zika Virus Test Kit** is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if the kit containers have been compromised during shipment, the product should not be used. Please contact the Technical Support team at Nanobiosym Diagnostics for assistance.
- Store **Gene-RADAR® Zika Virus Test Kit** reagents and controls at -20º C upon receipt.
- Always check the expiration date and do not use reagents beyond their expiration date.
- Reagents should not be thawed and refrozen, as this may affect the performance of the assay.
- Do not store in a frost-free freezer. Store in a non-defrosting freezer.

5. Warnings and Precautions

- This assay is for *in vitro* diagnostic use under the FDA Emergency Use Authorization only.
- Local, state, and national public health agencies (for example, county and state health departments or the US Centers for Disease Control and Prevention (CDC)) should be notified of any patient suspected to have Zika virus disease. Laboratories should consult with local, state or national public health officials on

any positive **Gene-RADAR® Zika Virus Test** result on the need for additional testing.

- Do not use reagents from other manufacturers with this assay.
- Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use segregated work areas for (a) specimen preparation, (b) reaction set-up, and (c) amplification/detection activities. Workflow in the laboratory should proceed in a unidirectional manner. Always wear disposable gloves in each area, and change them before entering different areas.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- **Gene-RADAR® Nanochips** are designed for single-use and are disposable. Do not reuse.
- Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
- Do not use components of the kit that have passed their expiration date.
- Discard samples, **Gene-RADAR® Nanochips**, and assay waste according to your local safety regulations.
- Performance of the **Gene-RADAR® Zika Virus Test** has only been evaluated for serum samples in conjunction with the QIAamp® Viral RNA Mini Kit.

6. Safety Data Sheets (SDS)

According to U.S. OSHA 29 CFR 1910.1200, and the EU Directives 67/548/EC and 1999/45/EC any products which do not contain more than 1% of a component classified as hazardous or classified as carcinogenic do not require a Safety Data Sheet (SDS).

Product is not hazardous, not toxic, and not IATA-restricted. Product is not from human, animal or plant origin. Product contains synthetic oligonucleotide primers and probes.

7. Sample Collection, Transport and Storage

The **Gene-RADAR® Zika Virus Test** is intended for detection of RNA from the Zika virus in human serum samples.

Serum samples should be collected according to the manufacturer's instructions for the sample collection device used.

Serum can be stored for up to 5 days at 2-8ºC prior to extraction per CLSI MM13-A Guidance. Purified RNA can be stored at 2-8ºC for up to 6 hours.

If nucleic acid analysis cannot be performed the same day as sample preparation, RNA should be stored at -70ºC or lower.

Laboratories should review and follow CDC recommended infection control precautions for Zika virus or other flaviviruses in handing all samples (www.cdc.gov/zika/laboratories/lab-safety.html).

8. Testing Procedure

8.1. RNA Extraction

Extract patient serum samples and controls using the QIAamp® Viral RNA Mini Kit. Follow the manufacturer's instructions for use (see QIAamp® Viral RNA Mini Kit Handbook, April 2010), including all volumes. The extraction has to be performed with a sample input volume of 50 µL. Specific details are described below.

Patient serum samples should be extracted in batches which should not exceed 10 patient serum samples.

Each batch of patient samples should be run with the following controls:

- One **Gene-RADAR® Zika Negative Control** (Nuclease-free water).
- One **Gene-RADAR® Zika Positive Control.**
- One **Gene-RADAR® Zika Internal Process Control.**

8.1.1. Preparation Steps

- Follow the manufacturer's instructions for use (see QIAamp® Viral RNA Mini Kit Handbook, April 2010), including all volumes.
- Prepare **Gene-RADAR® Zika Positive Control** and **Gene-RADAR® Zika Virus Internal Process Control** for use by gently warming in a 37ºC dry bath for 5 minutes, or until liquid. Upon melting, carefully mix by repeated inversion.

8.1.2. Extraction Process

- For each serum sample, add 86 µL of nuclease-free water and 4 µL of **Gene-RADAR® Zika Virus Internal Process Control** into the 2 mL labelled centrifuge tube, and then add 50 µL of serum to be extracted into the 2 mL labelled centrifuge tube and mix by pulse-vortexing for 15 seconds.
- For a **Positive Control**, add 86 µL of nuclease-free water and 4 µL of **Gene-RADAR® Zika Virus Internal Process Control** into the 2 mL labelled centrifuge tube, and then add 50 µL of **Gene-RADAR® Zika Virus Positive Control** into the 2 mL labelled centrifuge tube and mix by pulse-vortexing for 15 seconds.
- For a **Negative Control,** add 136 µL of nuclease-free water and 4 µL of **Gene-RADAR® Zika Virus Internal Process Control** into the 2 mL labelled centrifuge tube.
- To ensure efficient lysis, it is essential that each serum sample or control is mixed thoroughly with Extraction Buffer AVL to yield a homogeneous solution.

Extracted serum samples and controls should be tested with the **Gene-RADAR® Zika Virus Test** within 6 hours of completing the extraction process. Residual unextracted specimens should be stored at 2-8ºC while testing is in progress. If testing cannot be completed within 6 hours, extracted samples should be stored at -70ºC or lower.

8.2. Amplification

8.2.1. Materials and equipment
Refer to Section 3 for all materials and equipment required for amplification, but not provided with this kit.

8.2.2. Samples and Controls

- Use the eluates from the Nucleic Acid Extraction procedure above.
  - *Note: Input volume on the Qiagen column is 50 µL of sample; the elution volume from the Qiagen column is 60 µL; and the volume of extracted RNA added to each amplification reaction is 15 µL. Consequently, one Qiagen column produces excess extracted RNA material, enough to run up to 4 identical amplification reactions.*
- For each extracted batch of patient serum samples and each day of testing, always include at least one **Gene-RADAR® Zika Virus Positive Control,** which has undergone the Nucleic Acid Extraction procedure above concurrent with the patient serum samples. Additional Controls need to be run if more than one kit lot is used for testing an extraction batch.

- For each extracted batch of patient serum samples and each day of testing, always include at least one **Negative Control**, which has undergone the Nucleic Acid Extraction procedure above concurrent with the patient serum samples. Best practice is to also run a Negative Control after each patient sample that tests positive.
- Additional testing of Controls may also be required at periodic intervals as dictated by local, state and country laws and by the user facility.

8.2.3. Instrument

Amplification and detection can be accomplished by using the **Gene-RADAR® Platform.**

Refer to the following documents for details:

- Gene-RADAR® Platform Operator's Manual

8.2.4. Preparation of Assay Reagents

Prepare **Gene-RADAR® Zika Virus Kit Buffer 1** for use by gently warming in a 37ºC dry bath for 5 minutes, or until liquid. Upon melting, carefully mix by repeated inversion. Each reaction requires 35 µL of the **Gene-RADAR® Zika Virus Kit Buffer 1.**

8.2.5. Set-up and Operation of the Gene-RADAR® Platform

- *Instrument Power-Up and Self-Testing*. Turn on the **Gene-RADAR® Platform** (the power switch is located at the back of the Platform). When powered up, the instrument automatically enters Self-Test mode and conducts subsystem tests to ensure proper operation. Failure of any of the Self-Tests results in the display of a warning to the user on the touchscreen. The **Gene-RADAR® Platform** will not allow a diagnostic assay to be selected or run in the event of a Self-Test failure. In the event of a self-test failure, contact an authorized Nanobiosym service representative. There are no user-serviceable parts in the **Gene-RADAR® Platform.**
- *Assay Selection*. After a successful Self-Test run, the instrument displays **Gene-RADAR® Zika Virus Test** on the touchscreen. The user should select the assay by touching the icon representing the **Gene-RADAR® Zika Virus Test**. A pop-up confirmation box will be displayed. Touching the "OK" button in the confirmation box selects the chosen assay. Touching the "Cancel" button returns the user to the assay selection menu.
- *Inserting Nanochip into Platform*. Once an assay has been selected and confirmed, the Platform will display specific instructions on the touchscreen for running the assay. The first step in running the assay is placement of a **Gene-RADAR® Nanochip** into the Platform. Carefully open a **Gene-RADAR® Nanochip** pouch by cutting with scissors along the indicated cut line, and remove the Nanochip from

the pouch. The **Gene-RADAR® Nanochip** is designed to fit into the **Gene-RADAR® Platform** in only one orientation. Align the Nanochip so it fits in the slot. Insert the Nanochip into the holder on the upper right hand side of the Platform and close the cover. When the cover closes, it will push the Nanochip into the proper position. Leave the cover closed until a sample eluate is ready for loading.

- *Prepare Eluate for Loading.* A patient serum sample eluate, or control sample eluate, prepared in the above Nucleic Acid Extraction procedure, should be prepared for loading into the Gene-RADAR® Nanochip.
  - Carefully pipette 35 µL of the **Gene-RADAR® Zika Virus Kit Buffer 1** into a fresh 0.2 mL reaction tube, and
  - Mix it with 15 µL of patient serum sample eluate, or control sample eluate. Thoroughly mix the sample or control by up and down pipetting. Close the tube and centrifuge for 30 seconds at approximately 1,000 x g (If not analyzing sample immediately, store reaction tube in iceless cooler rack at approximately 2-8ºC pending analysis), and
  - Carefully pipette the 50 µL combined mixture for loading into the **Gene-RADAR® Nanochip**.
- *Loading Sample into Nanochip.* Open the Nanochip on the **Gene-RADAR® Platform.** The sample port on the Nanochip is covered with a pre-cut, slotted protective film to minimize contamination. Penetrate the pre-cut, slotted film on the top of the Nanochip using the pipette, and carefully load the 50 µL sample into sample well of the **Gene-RADAR® Nanochip**.

  Close the **Gene-RADAR® Platform** cover. The instrument is now ready to run the **Gene-RADAR® Zika Virus Test**. Push the "Run Assay" button on the touchscreen to start the assay run. A pop-up confirmation box will be displayed. Touching the "OK" button on the confirmation box will start the assay run. Touching the "Cancel" button will cancel the assay run. A complete assay run takes approximately an hour to complete. During the assay run the instrument conducts various system checks to ensure proper operation. If a system check fails, the instrument will generate an *Error* message. In the event of an *Error* message, replace the Nanochip with a new Nanochip and sample, and re-test. Re-test may be performed with another aliquot of the same sample eluate.
- *Viewing Results*. After completion of the assay, view the Results Report by touching or clicking the "Report" icon on the touchscreen.

8.3. Interpretation of Results

The results from the **Gene-RADAR® Zika Virus Positive Control** and the **Gene-RADAR® Zika Virus Negative Control** associated with a particular group of patient serum samples, should be examined prior to interpretation of any patient serum sample results. If these controls are not valid, the patient serum sample results should not be interpreted.

The **Gene-RADAR® Platform** reports summary assay results as *Positive*, *Negative,* or *Invalid.* The **Gene-RADAR® Platform** also allows the user to display raw data with amplification plots.

8.3.1. Examination of Control Samples

Gene-RADAR® Zika Virus Test requires the user to use the summary assay results in conjunction with the amplification plots. When interpreting results, the user must view the amplification plots, and determine a Ct value for comparison against the Signal Criteria specified in this Section.

- Positive and Negative Signals
    - **Gene-RADAR® Platform**
        - A *Positive* signal is defined as a C_t value of less than or equal to **37** cycles.
        - A *Negative* signal is defined as no C_t value, or a C_t value of greater than **37** cycles.
- The **Positive Control** should be considered *Valid* if the Zika virus reaction is reported as *Positive*. In all other cases, the **Positive Control** should be considered *Invalid.*
- The **Positive Control** should not be evaluated for the Internal Process Control reaction.
- The **Negative Control** should be considered *Valid* if the Zika virus reaction is reported as *Negative*. If the Zika virus reaction is reported *Positive*, the **Negative Control** should be considered *Invalid.*
- The **Negative Control** should be considered *Valid* if the Internal Process Control reaction is reported as *Positive* and the Zika virus reaction is *Negative*. In all other cases, the **Negative Control** should be considered *Invalid.*
- In order for a patient serum sample result to be valid, the **Positive Control** and the **Negative Control** associated with that particular batch of patient serum samples must be *Valid.* See section 8.2.2 above for details.
    - If the *Positive Control* fails, the entire batch of serum samples is *Invalid*, and potential sources of amplification failure should be identified and corrected. Re-test the *Positive Control* eluate. If the Positive Control re-test is *Valid*, it may be used with its associated batch of serum samples. However, if the *Positive*

Control re-test fails, repeat the batch with fresh patient serum samples and fresh controls.

- If the *Negative Control* fails, the entire batch of serum samples is *Invalid*, and potential sources of contamination should be identified and corrected. Re-test the *Negative Control* eluate. If the Negative Control re-test is *Valid*, it may be used with its associated batch of serum samples. However, if the *Negative Control* re-test fails, repeat the batch with fresh patient serum samples and fresh controls.

8.3.2. Examination of Patient Serum Samples

Upon completion of a particular batch of patient serum samples, perform data analysis using the instrument software as described in the Gene-RADAR® Operator's Manual.

Gene-RADAR® Zika Virus Test requires the user to use the summary assay results in conjunction with the amplification plots. When interpreting results, the user must view the amplification plots, and determine a Ct value for comparison against the Signal Criteria specified in this Section.

- Positive and Negative Signals
  - **Gene-RADAR® Platform**
    - A *Positive* signal is defined as a C_t value of less than or equal to **37** cycles.
    - A *Negative* signal is defined as no C_t value, or a C_t value of greater than **37** cycles.
- A patient serum sample is considered **Positive** for Zika virus, if the Zika virus reaction is reported as *Positive,* regardless of the result for the Internal Process Control reaction.
- A patient serum sample is considered **Negative** for Zika virus, if
  - The Zika virus reaction is reported as *Negative,* and
  - The Internal Process Control reaction is reported as *Positive*
- A patient serum sample is considered **Invalid** for Zika virus, if the results do not fit either of the *Positive* or *Negative* scenarios as described above. An *Invalid* result may include following situations:
  - The Zika virus reaction is reported as *Negative*, but the Internal Process Control reaction is reported as *Negative*.

The following table summarizes the steps for the interpretation of test results:

Table 1 Summary of Result Interpretation

CONTROLS AND PATIENT SERUM SAMPLE	ZIKA VIRUS RESULT	INTERNAL PROCESS CONTROL RESULT	INTERPRETATION
Negative Control	*Negative*	*Positive*	Continue to evaluate Positive Control
	Positive	*Positive or Negative*	Re-test Negative Control eluate. If re-test Invalid, then invalidate all associated sample(s).* If re-test Valid, then continue to evaluate patient sample(s).**
	Negative	*Negative*	
Positive Control	*Positive*	*n/a*	Continue to evaluate patient sample(s)
	Negative		Re-test Positive Control eluate. If re-test Invalid, then invalidate all associated sample(s).* If re-test Valid, then continue to evaluate patient sample(s).**
Patient Sample(s)	*Positive*	*n/a*	Report as *Positive* for Zika virus
	Negative	*Positive*	Report as *Negative* for Zika virus***
	Negative	*Negative*	Report as *Invalid.* Re-test sample.

* Note: In the event a Negative Control or Positive Control is Invalid, then all associated patient samples are invalid, and the batch must be re-extracted with fresh patient samples and control samples.

** Note: In the event a Negative Control requires re-testing, all positive patient samples analyzed subsequent to the last successful Negative Control should also be re-tested. In the event a Positive Control requires re-testing, all positive patient samples analyzed subsequent to the last successful Positive Control should also be re-tested.

***A patient-matched serum specimen is currently required for serological follow up testing of negative RT-PCR results per the CDC testing algorithm (found at http://www.cdc.gov/zika/index.html).

8.3.3. Re-testing *Invalid* Results

A patient serum sample or control sample with an *Invalid* result requires re-testing the patient serum sample eluate or control sample eluate. If the *Invalid* result is repeated upon re-testing, all associated patient serum samples or control samples must be repeated with original patient serum samples and control samples.

8.4. Assay Limitations

- All results should be interpreted by a trained professional or healthcare worker in conjunction with the patient's history and clinical signs, symptoms and risk factors.
- Performance of this assay has been established only on the **Gene RADAR® Platform**. The assay is limited to use on the **Gene RADAR® Platform**.
- This test has not been validated as a quantitative test.
- Negative results do not preclude infection with Zika virus, and should not be the sole basis of a patient management decision.
- A patient matched serum specimen is required for serological follow up testing of all negative RT-PCR results, per the CDC testing algorithm. (Found at http://www.cdc.gov/zika/index.html).
- False positive results may occur from cross-contamination by target organisms, their nucleic acids or amplified product.
- Optimal performance of this test requires appropriate specimen collection, storage, and transport to the test site (refer to the storage section of this package insert). Improper sample collection, transport or storage may lead to false negative results.
- Failure to follow the assay procedures may lead to false negative results. Specimen collection conducted prior to symptom onset may lead to false negative results.
- Specimen collection after nucleic acid can no longer be found in the patient (approximately 7 days post-onset of symptoms for sera) may lead to false negative results.
- Failure to use the authorized extraction methods and/or instruments may lead to false negative results.
- Inhibitors present in a sample may lead to false negative results.

- Potential mutations within the target regions of the virus genome covered by the primer and/or probes of the test may result in failure to detect the presence of the pathogen.
- Performance of this assay has only been established for serum. Performance with other specimen types has not been evaluated.
- The impact of the administration of Zika virus vaccines and/or therapeutics on the ability to detect Zika virus RNA in patient serum samples has not been evaluated.
- Test cannot rule out diseases caused by other bacterial or viral pathogens.

9. Analytical Performance Evaluation

9.1. Analytical Sensitivity

The analytical sensitivity of the **Gene-RADAR® Zika Virus Test** was assessed by determining its Limit of Detection (LoD) through an estimation study and a confirmatory study using Zika virus (Puerto Rico isolate PRVABC59) spiked into human serum.

The estimation of sensitivity study was performed using the **Gene-RADAR® Zika Virus Test** following the Instructions for Use for the Qiagen QIAamp Viral RNA Mini Kit for Nucleic Acid Extraction, and the **Gene-RADAR® Platform** for amplification and detection. Study results were evaluated in accordance with Section 8.3.2.

An estimated LoD was first determined using Zika virus-negative pooled serum spiked with nine (9) concentration levels of live Zika virus, (Puerto Rico isolate PRVABC59), and analyzed in triplicate. The results of the Estimated Limit of Detection study are shown below in **Table 2**. The lowest concentration at which all 3 replicates were positive **(200 PFU/mL)** was designated as the Estimated LoD.

Table 2 Gene-RADAR® Platform, Estimated Limit of Detection

Zika Virus Strain	Stock Titer	PFU/reaction	PFU/mL	Average C_t	Call Rate	Estimated Limit of Detection (LoD)
Zika Virus, (Puerto Rico isolate PRVABC59)	1.58×10^8 $TCID_{50}$/mL	2.5×10^5	20,000,000	17.7	3/3	**200 PFU/mL (or 2.5 PFU/reaction)**
		2.5×10^4	2,000,000	21.3	3/3	
		2.5×10^3	200,000	24	3/3	
		2.5×10^2	20,000	27	3/3	
		2.5×10^1	2,000	30.7	3/3	
		2.5×10^0	**200**	**33**	**3/3**	
		2.5×10^{-1}	20	34	2/3	

Zika Virus Strain	Stock Titer	PFU/reaction	PFU/mL	Average C_t	Call Rate	Estimated Limit of Detection (LoD)
		2.5×10^{-2}	2	37	1/3	
		2.5×10^{-3}	0.2	36	1/3	

In the confirmation of sensitivity study, 20 serum sample replicates were spiked with Zika virus, (Puerto Rico isolate PRVABC59), at the Estimated Limit of Detection concentration. The results of the confirmatory LoD testing are shown in **Table 3** below. Confirmation of the Limit of Detection required positive detection for at least 19 out of 20 replicates (i.e. $\geq$ 95%).

Table 3 Gene-RADAR® Platform, Confirmed Limit of Detection

Concentration (PFU/mL)	Zika Virus Positive Result	Hit Rate	IPC Assay Positive Result
200	20/20	100%	20/20

The claimed Limit of Detection for the Gene-RADAR® Zika Virus Test is 200 PFU/mL.

Using a standard curve constructed with *in vitro* transcribed RNA, 1 PFU of Zika Virus was experimentally determined to be equivalent to approximately 90 RNA copies.

FDA Sensitivity Study: The analytical sensitivity of the **Gene-RADAR® Zika Virus Test** in serum was also evaluated using reference materials (S1 and S2) and a standard protocol provided by the FDA, which included a LoD range finding study and a confirmatory LoD study. Results of the FDA Sensitivity Study on the **Gene-RADAR® Platform** are presented below in **Table 4**.

Table 4 Summary of LoD Confirmation Results Using FDA Reference Materials

Reference Materials	Specimen Type	Confirmed LoD* in RNA NAAT Detectable Units/mL
S1	Serum	3,333
S2	Serum	5,000

*Study performed according to an FDA issued protocol

9.2. Analytical Reactivity

Our primer sequences are designed to amplify a highly conserved sequence within the Zika virus genome. The primers were selected using NCBI Primer-

BLAST with a target region of the Zika Puerto Rico isolate PRVABC59 as the input sequence.

The analytical reactivity of the Gene-RADAR® Zika Virus Test was evaluated by wet testing five different Zika virus strains spiked in pooled serum, in triplicate, on the Gene-RADAR® Platform. Results of the reactivity analyses are summarized below in **Table 5.**

Table 5 Analytical Reactivity (Wet tested on Gene-RADAR® Platform)

Zika Virus Isolate	Country	GenBank Accession No.	Source/Sample Type	Concentration	Average Ct (3 Replicates)
Zika Virus, Human/2015/Colombia	Columbia	KU820897	BEI Resources, Cat. No. NR-50183	400 PFU/mL	27
Zika Virus, H/PAN/2015/CDC-259359	Panama	KX156775	BEI Resources, Cat. No. NR-50219	400 PFU/mL	25
Zika Virus, H/PAN/2015/CDC-259349	Panama	KX156774	BEI Resources, Cat. No. NR-50220	400 PFU/mL	28
Zika Virus, H/PAN/2015/CDC-259364	Panama	KX156776	BEI Resources, Cat. No. NR-50221	400 PFU/mL	25
Zika Virus, H/Puerto Rico/2015, Strain PRVABC59	Puerto Rico	KU501215.1	ATCC, Cat. No. VR-1843	400 PFU/mL	31

The analytical reactivity of the Gene-RADAR® Zika Virus Test was also evaluated by performing *in silico* analyses of different isolates of the Zika virus obtained from the National Center for Biotechnology Information (NCBI). Results of the *in silico* reactivity analyses are summarized below in **Table 6A** and **Table 6B. (Strains that were wet tested have been highlighted.)**

Table 6A Analytical Reactivity (Tested *in silico*)

New World Zika Virus Isolates				Query function	F-primer	R-primer	Probe
				Query length	17	24	31
				Strand match	plus plus	plus minus	plus plus
GenBank	**Region**	**Country**	**Date**	**Strain/Isolate**	**percent identity with primer**		
KU686218	N. America	Mexico	2015	MEX/InDRE/14	100%	100%	97%
KU922923	N. America	Mexico	2016	MEX/InDRE/Lm	100%	100%	100%
KU922960	N. America	Mexico	2016	MEX/InDRE/Sm	100%	100%	100%
KU985088	N. America	Mexico	2015	MEX/InDRE/Zika-2	100%	100%	100%

New World Zika Virus Isolates				Query function	F-primer	R-primer	Probe
				Query length	17	24	31
				Strand match	plus plus	plus minus	plus plus
GenBank	**Region**	**Country**	**Date**	**Strain/Isolate**	**percent identity with primer**		
KU853012	Caribbean	Domin. Rep.	2016	PD1	100%	100%	100%
KU853013	Caribbean	Domin. Rep	2016	PD2	100%	100%	100%
KU509998	Caribbean	Haiti	2014	Haiti/1225/2014	100%	100%	100%
KX051563	Caribbean	Haiti	2016	Haiti/1/2016	100%	100%	100%
KU647676	Caribbean	Martinique	2015	MRS_OPY_Martinque_PaRi	100%	100%	100%
KU501215	Caribbean	Puerto Rico	2015	PRVABC59	100%	100%	100%
KX087101	Caribbean	Puerto Rico	2015	PRVABC59	100%	100%	100%
KU501216	C. America	Guatemala	2015	103344	100%	100%	100%
KU501217	C. America	Guatemala	2015	8375	100%	100%	100%
KU870645	C. America	Guatemala	2016	FB-GWUH-2016	100%	100%	100%
KX156774	C. America	Panama	2015	CDC-259349_V1-Vx	100%	100%	100%
KX156775	C. America	Panama	2015	CDC-259359_V1-Vx	100%	100%	100%
KX156776	C. America	Panama	2015	CDC-259364_V1-Vx	100%	100%	100%
KU321639	S. America	Brazil	2015	ZikaSPH2015	100%	100%	100%
KU365777	S. America	Brazil	2015	BeH818995	100%	100%	100%
KU365778	S. America	Brazil	2015	BeH819015	100%	100%	100%
KU365779	S. America	Brazil	2015	BeH819966	100%	100%	100%
KU365780	S. America	Brazil	2015	BeH815744	100%	100%	100%
KU497555	S. America	Brazil	2015	Brazil-ZKV2015	100%	100%	100%
KU527068	S. America	Brazil	2015	Natal RGN	100%	100%	100%
KU646827	S. America	Brazil	2015	Si323	100%	100%	100%
KU707826	S. America	Brazil	2015	SSABR1	100%	100%	100%
KU729217	S. America	Brazil	2015	BeH823339	100%	100%	100%
KU729218	S. America	Brazil	2015	BeH828305	100%	100%	100%
KU926309	S. America	Brazil	2016	Rio-U1	100%	100%	100%
KU926310	S. America	Brazil	2016	Rio-S1	100%	96%	100%
KU940224	S. America	Brazil	2015	Bahia09	100%	100%	100%

New World Zika Virus Isolates				Query function	F-primer	R-primer	Probe
				Query length	17	24	31
				Strand match	plus plus	plus minus	plus plus
GenBank	**Region**	**Country**	**Date**	**Strain/Isolate**	**percent identity with primer**		
KU940228	S. America	Brazil	2015	Bahia07	100%	100%	100%
KU991811	S. America	Brazil	2016	INMI1	100%	100%	100%
KX173842	S. America	Brazil	2016	16Z08	100%	100%	100%
KX101066	S. America	Brazil	2015	Bahia01	100%	100%	100%
KX101061	S. America	Brazil	2015	Bahia03	100%	100%	100%
KX197192	S. America	Brazil	2015	PE243	100%	100%	100%
KX173844	S. America	Brazil	2016	16Z62	100%	100%	100%
KX173843	S. America	Brazil	2016	16Z62	100%	100%	100%
KX173841	S. America	Brazil	2016	16Z11	100%	100%	100%
KX173840	S. America	Brazil	2016	16Z10	100%	100%	100%
KX101060	S. America	Brazil	2015	Bahia02	100%	100%	100%
KU646828	S. America	Columbia	2015	Si322	100%	100%	100%
KU820897	S. America	Columbia	2015	FLR	100%	100%	100%
KX087102	S. America	Columbia	2015	FLR	100%	100%	100%
KX198135	S. America	Panama	2016	BEI-259634_V4	100%	100%	100%
KU312312	S. America	Suriname	2015	Z1106033	100%	100%	100%
KU312313	S. America	Suriname	2015	Z1106032	100%	100%	100%
KU312314	S. America	Suriname	2015	Z1106031	100%	100%	100%
KU312315	S. America	Suriname	2015	Z1106027	100%	100%	100%
KU937936	S. America	Suriname	2016	ZIKVNL00013	100%	100%	100%
KU744693	S. America	Venezuela	2016	VE_Ganxian	100%	100%	97%

Table 6B Analytical Reactivity (*in silico*)

Old World Zika Virus Isolates	Query function	F-primer	R-primer	Probe
	Query length	17	24	31

				Strand match	plus plus	plus minus	plus plus
GenBank	**Region**	**Country**	**Date**	**Strain/Isolate**	**percent identity with primer**		
KJ634273	S.Pacific	Cook Islands	2014	CK-ISL 2014	100%	100%	100%
AB908162	S.Pacific	French Poly.	2014	ZIKV Hu/Tahiti/01u/2014NIID	100%	100%	100%
KJ776791	S.Pacific	French Poly.	2013	H/PF/2013	100%	100%	100%
KM212966	S.Pacific	French Poly.	2013	NC13(FP)-26112013-22072	100%	-[1]	100%
KM212963	S.Pacific	N.Caledonia	2014	NC14-23012014-250	100%	-	100%
KM212964	S.Pacific	N.Caledonia	2014	NC14-17042014-4554	100%	-	100%
KM212965	S.Pacific	N.Caledonia	2013	NC13(FP)-20112013-22015	100%	-	100%
JN860885	S.E. Asia	Cambodia	2010	FSS13025	100%	100%	100%
KU955593	S.E. Asia	Cambodia	2010	FSS13025	100%	100%	100%
KU740184	S.E. Asia	China	2016	GD01	100%	100%	100%
KU761564	S.E. Asia	China	2016	GDZ16001	100%	100%	100%
KU820898	S.E. Asia	China	2016	GZ01	100%	100%	100%
KU820899	S.E. Asia	China	2016	ZJ03	100%	100%	100%
KU866423	S.E. Asia	China	2016	SZ01	100%	100%	100%
KU955589	S.E. Asia	China	2016	Z16006	100%	100%	100%
KU955590	S.E. Asia	China	2016	Z16019	100%	100%	100%
KU963796	S.E. Asia	China	2016	SZ-WIV01	100%	100%	100%
KX056898	S.E. Asia	China	2016	GZ02	100%	100%	100%
KX185891	S.E. Asia	China	2016	SZ02	100%	100%	100%
KX117076	S.E. Asia	China	2016	Zhejiang04	100%	100%	100%
HQ234499	S.E. Asia	Maylasia	1966	P6-740	100%	96%	97%
EU545988	S.E. Asia	Micronesia	2007	ZIKV 2007	100%	100%	97%
KU681082	S.E. Asia	Philippines	2012	CBC-0740	100%	100%	94%
KF993678	S.E. Asia	Thailand	2013	PLCal_ZV	100%	100%	100%
KU681081	S.E. Asia	Thailand	2014	SV0127-14	100%	100%	100%
KF268948	Africa	C. African Rep.	2013	ARB13565	94%	88%	94%
KF268949	Africa	C. African Rep.	2013	ARB15076	94%	88%	100%
KF268950	Africa	C. African Rep.	2013	ARB7701	94%	88%	94%
HQ234500	Africa	Nigeria	1968	IbH_30656	94%	88%	97%

[1] "-" indicates that no significant similarities were found beween our reverse primer and this organism.

Old World Zika Virus Isolates				Query function	F-primer	R-primer	Probe
				Query length	17	24	31
				Strand match	plus plus	plus minus	plus plus
GenBank	**Region**	**Country**	**Date**	**Strain/Isolate**	**percent identity with primer**		
KJ634273	S.Pacific	Cook Islands	2014	CK-ISL 2014	100%	100%	100%
KF383115	Africa	Nigeria	1968	ArB1362	94%	88%	100%
KF383116	Africa	Nigeria	1968	ArD7117	94%	88%	97%
KF383117	Africa	Nigeria	1997	ArD128000	94%	96%	100%
KF383121	Africa	Nigeria	2001	ArD158095	88%	96%	100%
KU963574	Africa	Nigeria	1968	IbH-30656_SM21V1-V3	94%	88%	97%
HQ234501	Africa	Senegal	1984	ArD_41519	88%	88%	97%
KU955591	Africa	Senegal	1984	41525-DAK	88%	88%	97%
KU955592	Africa	Senegal	1984	41662-DAK	88%	88%	97%
KU955595	Africa	Senegal	1984	41671-DAK	88%	88%	97%
KX198134	Africa	Senegal	1984	DAK-AR-41524_A1C1-V2	88%	88%	97%
AY632535	Africa	Uganda	1947	MR 766	88%	96%	100%
DQ859059	Africa	Uganda	1947	MR 766	94%	88%	100%
HQ234498	Africa	Uganda	1947	MR_766	88%	96%	100%
KF383119	Africa	Uganda	2001	ArD158084	88%	96%	100%
KU720415	Africa	Uganda	1947	MR 766	88%	96%	100%
KU955594	Africa	Uganda	1947	MR-766	88%	96%	100%
LC002520	Africa	Uganda	1947	MR766-NIID	88%	96%	100%
KU963573	Africa	Uganda	1947	MR-766_SM150-V8	88%	96%	100%

9.3. Analytical Specificity

Cross Reactivity: The analytical specificity of the **Gene-RADAR® Zika Virus Test** was evaluated by wet testing selected pathogens known to cause Zika-like clinical symptoms. In this study, the **Gene-RADAR® Zika Virus Test** was evaluated with samples containing RNA purified from nine (9) other pathogens, spiked in pooled serum, in triplicate, at clinically relevant levels, on the **Gene-RADAR® Platform**. All tested pathogens showed negative reactivity with the **Gene-RADAR® Zika Virus Test**. Results are summarized in **Table 7** below.

Table 7 Analytical Specificity (Wet Tested on Gene-RADAR® Platform)

Organism	Source/Sample Type	Catalog No.	Spiked Concentration	Result
Dengue Virus 1	BEI Resources, Genomic RNA	NR-2768	8.0×10^5 copies/mL	Negative
Dengue Virus 1	BEI Resources, Genomic RNA	NR-2768	8.0×10^6 copies/mL	Negative
Dengue Virus 2	Vircell, AMPLIRUN Dengue 2 Virus RNA Control	MBC056	8.0×10^5 copies/mL	Negative
Dengue Virus 3	BEI Resources, Genomic RNA,	NR-2771	8.0×10^5 copies/mL	Negative
Dengue Virus 3	BEI Resources, Genomic RNA,	NR-2771	8.0×10^6 copies/mL	Negative
Dengue Virus 4	BEI Resources, Genomic RNA	NR-4289	8.0×10^5 copies/mL	Negative
Dengue Virus 4	BEI Resources, Genomic RNA	NR-4289	8.0×10^6 copies/mL	Negative
Yellow Fever (Vaccine strain)	Vircell, AMPLIRUN Yellow Fever Virus RNA Control	MBC100	1.21×10^6 copies/mL	Negative
West Nile Virus	Vircell, AMPLIRUN West Nile Virus RNA Control	MBC069	1.0×10^6 copies/mL	Negative
Chikungunya Virus	Vircell, AMPLIRUN Chikungunya Virus RNA Control	MBC099	1.44×10^6 copies/mL	Negative
Parvovirus (B19)	Zeptometrix, NATtrol Human Parvovirus B19	NATPARVO-0004	1.04×10^6 copies/mL	Negative
Plasmodium falciparum	ATCC, Genomic DNA	PRA-405D	8.0×10^5 copies/mL	Negative

The cross-reactivity of the **Gene-RADAR® Zika Virus Test** was also evaluated by *in silico* testing for these pathogens, and other pathogens known to cause Zika-like clinical symptoms. *In silico* results are summarized in **Table 8** below. **(Strains that were wet tested have been highlighted.)**

Table 8 Gene-RADAR® Zika Virus Test, *In Silico* Cross-Reactivity

	query function	F-primer	R-primer	Probe
	query length	17	24	31
	strand match	plus/plus	plus/minus	plus/plus
Organism	**tax ID**	**percent identity with primer**		
Dengue Virus 1	11053	94%	50%	32%

	query function	F-primer	R-primer	Probe
	query length	17	24	31
	strand match	plus/plus	plus/minus	plus/plus
Organism	**tax ID**	**percent identity with primer**		
Dengue Virus 2	11060	100%	46%	39%
Dengue Virus 3	11069	59%	54%	32%
Dengue Virus 4	11070	94%	46%	29%
Yellow Fever	40005	65%	54%	32%
Yellow Fever (Vaccine strain)	11090	65%	38%	32%
West Nile Virus	11082	76%	50%	32%
Chikungunya Virus	37124	59%	38%	35%
Mayaro virus	59301	59%	46%	35%
Parvovirus (B19)	10798	53%	42%	32%
Plasmodium falciparum	5833	71%	58%	39%
St. Louis encephalitis virus	11080	59%	54%	35%
Japanese encephalitis virus	11071	65%	42%	32%
Spondweni virus	64318	47%	33%	29%
Hepatitis C virus	11102	82%	58%	42%
Eastern Equine Encephalitis Virus (EEE)	11021	59%	54%	35%
Western Equine Encephalitis Virus (WEE)	11038	47%	42%	35%
Ross River virus	11029	53%	38%	29%
Barmah Forest virus	11020	47%	33%	42%
O'nyong-nyong virus	11027	47%	38%	29%
Sindbis virus	11034	53%	50%	42%
Tonate virus	60877	47%	29%	39%
Una virus	59304	47%	33%	29%
Measles virus	11234	65%	42%	48%
Rubella virus	11041	65%	42%	29%
Enterovirus all serotypes	12059	71%	54%	42%
Adenovirus all serotypes	10508	76%	54%	42%
Hepatitis B virus	10407	65%	63%	39%
HIV	11676	71%	58%	48%
Varicella Zoster virus	10335	59%	46%	32%
Cytomegalovirus (CMV)	10358	71%	58%	45%
Epstein Barr Virus (EBV)	10376	71%	46%	39%
Rickettsia sp.	780	65%	54%	39%
Borrelia burgdorferi	139	59%	58%	39%
Group A *Streptococcus*	1301	0%	0%	0%

	query function	F-primer	R-primer	Probe
	query length	17	24	31
	strand match	plus/plus	plus/minus	plus/plus
Organism	**tax ID**	**percent identity with primer**		
Leptospirosis	171	71%	63%	42%
Plasmodium vivax	5855	71%	58%	48%
Trypanosoma cruzi (Chagas)	5693	88%	63%	52%
Schistosomiasis	31245	88%	67%	61%
Hepatitis A virus vaccine - BIOVAC-A brand	12092	59%	54%	32%
Salmonella typhi (Typhoid-Ty21a vaccine)	90370	71%	54%	39%
Escherichia coli (E.coli)	562	76%	58%	61%

Interference Study. Due to high homology noted for some organisms in the *in silico* Cross-Reactivity Study, selected potential interfering organisms were further evaluated in a wet testing study. Potential interfering organisms were spiked in contrived Zika-positive serum samples which were prepared with live Zika virus, (Puerto Rico isolate PRVABC59), spiked at 3 x LoD. Study samples were analyzed by the Gene-RADAR® Zika Virus Test. Results are presented in **Table 9** below. None of the interfering organisms tested below interfered with the **Gene-RADAR® Zika Virus Test**'s ability to detect Zika Virus.

Table 9 Interference Study (Gene-RADAR® Platform)

Organism	Source/Sample Type	Catalog No.	Spiked Concentration	Zika Virus Concentration	Result
Hepatitis C Virus	ATCC	VR-3233SD	12,500 copies/reaction	7.5 PFU/reaction	31
Adenovirus 5	ATCC	VR-5D	12,500 copies/reaction	7.5 PFU/reaction	31
HIV-1	ATCC	VR-3245SD	12,500 copies/reaction	7.5 PFU/reaction	30
Cytomegalovirus	ATCC	VR-538	12,500 copies/reaction	7.5 PFU/reaction	30
Epstein-Barr Virus	ZeptoMetrix	NATEBV-0006	12,500 copies/reaction	7.5 PFU/reaction	30
Leptospirosis	ATCC	BAA-1107	12,500 copies/reaction	7.5 PFU/reaction	31
Plasmodium vivax	ATCC	30138	12,500 copies/reaction	7.5 PFU/reaction	30
Trypanosoma cruzi (Chagas)	BEI Resources	NR-40347	12,500 copies/reaction	7.5 PFU/reaction	31

Organism	Source/Sample Type	Catalog No.	Spiked Concentration	Zika Virus Concentration	Result
Schistosomiasis	BEI Resources	NR-41320	12,500 copies/reaction	7.5 PFU/reaction	31
Salmonella typhi	ATCC	700931D-5	12,500 copies/reaction	7.5 PFU/reaction	31
Control (Zika PRVABC59)	ATCC	VR-1843	n/a	7.5 PFU/reaction	31

Because the nucleic acid extraction and RT-PCR methods utilized in the **Gene-RADAR® Zika Virus Test** are well-established, endogenous interference testing was not required.

10. Clinical Performance Evaluation

10.1. Clinical Study

To predict clinical performance of the **Gene-RADAR® Zika Virus Test**, serum samples were obtained from 25 unique Zika-positive donors in the Dominican Republic with signs and symptoms of Zika virus infection. The 25 samples were determined to be Zika-positive based on the results of an EUA comparator assay.

Twenty-five (25) additional contrived positive serum samples were prepared by spiking Zika virus (Puerto Rico isolate PRVABC59, ATCC Catalog No. VR-1843) into unique donor serum samples obtained from Innovative Research of Novi, MI (Normal, Healthy Human Single Donor Serum, Catalog No. IPLA-SERS). Thirteen (13) of the contrived serum samples were spiked at the Limit of Detection, and 12 contrived serum samples were spiked at higher concentrations.

Fifty (50) unique donor serum samples obtained from Innovative Research of Novi, MI (Normal, Healthy Human Single Donor Serum, Catalog No. IPLA-SERS) were used as Zika negative samples.

All samples were analyzed in a randomized blind test using the **Gene-RADAR® Zika Virus Test** following the Instructions for Use for the Qiagen QIAamp Viral RNA Mini Kit for nucleic acid extraction and the **Gene-RADAR® Platform** for amplification and detection.

The 25 Zika-positive serum samples from donors in the Dominican Republic, and the 50 Zika-negative unique donor serum samples were also analyzed by an EUA comparator assay (Altona RealStar Zika Virus RT-PCR Kit, on a Roche LightCycler instrument). From a total of 75 matched samples analyzed, 25 matched samples agreed as positive between the **Gene-RADAR® Zika Virus Test** and the EUA comparator assay (positive agreement of 100%), and 50 matched samples agreed as negative between the **Gene-RADAR® Zika Virus Test** and the EUA comparator assay (negative agreement of 100%).

Summary results of the clinical study using the **Gene-RADAR® Zika Virus Test** on the **Gene-RADAR® Table 9**.

Table 9 Gene-RADAR® Platform Natural and Contrived Samples Results Summary (vs. the comparator results or expected results)

SPECIMEN CATEGORY	NUMBER TESTED	GENE-RADAR® ZIKA VIRUS TEST	
		POSITIVE	NEGATIVE
Zika Negative Specimens, obtained from Innovative Research of Novi, MI tested at Nanobiosym by the Altona RealStar Zika Virus RT-PCR Kit U.S. EUA to be negative	50	---	50/50
Natural Zika Virus Positive Specimens obtained from Boca Biolistics, LLC tested at Nanobiosym by the Altona RealStar Zika Virus RT-PCR Kit U.S. EUA to be positive	25	25/25	---
Contrived Zika Virus Positive Specimens 1 x LOD	13	13/13	---
Contrived Zika Virus Positive Specimens 2 x LOD	4	4/4	---
Contrived Zika Virus Positive Specimens 3 x LOD	4	4/4	---
Contrived Zika Virus Positive Specimens 5 x LOD	4	4/4	---
Total (100)		50/50	50/50
Positive Percent Agreement	100% (50/50), 95% CI: 92.9 – 100%		
Negative Percent Agreement	100% (50/50), 95% CI: 92.9 – 100%		

11. Technical Assistance

For customer support, please contact our Technical Support team:

email: GeneRADAR@nanobiosym.com

Nanobiosym Diagnostics, Inc.
245 First Street
Cambridge, MA 02142
Phone: +1-781-391-7979

Nanobiosym Diagnostics, Inc.
245 First Street
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Gene-RADAR® Zika Virus Test
Document Number: IFU-ZKV
Version 1.0
Date: November 2016

Gene-RADAR®
Zika Virus Positive Control
Instructions for Use

For Use Under Emergency Use Authorization (EUA) Only

Nanobiosym Diagnostics, Inc.
245 First Street
Cambridge, MA 02142
www.nanobiosym.com

Gene-RADAR® Zika Virus Positive Control
Instructions for Use

For use under Emergency Use Authorization (EUA) Only:
For *in vitro* Diagnostic Use

Read this Package Insert and the **Gene-RADAR® Zika Virus Test** Package Insert completely prior to performing the test; failure to do so may cause inaccurate test results.

Catalog Number: NBS-ZKV-120

12. Intended Use

The **Gene-RADAR® Zika Virus Positive Control** is intended for use as a positive control material in the Gene-RADAR® Zika Virus Test for the detection of RNA from Zika virus.

The **Gene-RADAR® Zika Virus Positive Control** is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The **Gene-RADAR® Zika Virus Positive Control** is only for use under the Food and Drug Administration's Emergency Use Authorization.

13. Explanation of Control

The **Gene-RADAR® Zika Virus Test** utilizes various controls, including: a **Positive Control**, which is a synthetic RNA target sequence that can be amplified and detected by the same set of primers and probes as the Zika specific target.

14. Materials Provided

Gene-RADAR® Zika Virus Positive Control (100 µL) is sufficient for up to 10 patient samples and associated controls.

Positive Control
Gene-RADAR® Zika Virus Positive Control, (100 µL) (Catalog No. NBS-ZKV-120)

4. Storage Instructions

- The **Gene-RADAR® Zika Virus Positive Control** is shipped on dry ice. The control should arrive lrozen. If it is not frozen upon receipt, or if the container has been compromised during shipment, the product should not be used. Please contact the Technical Support team at Nanobiosym Diagnostics for assistance.
- Store **Gene-RADAR® Zika Virus Positive Control** at -20ºC upon receipt.

- Always check the expiration date and do not use reagents beyond their expiration date.
- Reagents should not be thawed and refrozen, as this may affect the performance of the assay.
- Do not store in a frost-free freezer. Store in a non-defrosting freezer.

5. Warnings and Precautions

- This control is for use with the **Gene-RADAR® Zika Virus Test** only.
- Do not use reagents from other manufacturers with this control.
- Controls and specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling controls and specimens. Use personal protective equipment (PPE) consistent with current guidelines.
- Avoid microbial and nuclease (DNase/RNase) contamination of the control.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling controls.
- Use segregated work areas for (a) specimen preparation, (b) reaction set-up, and (c) amplification/detection activities. Workflow in the laboratory should proceed in a unidirectional manner. Always wear disposable gloves in each area, and change them before entering different areas.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Do not use controls that have passed their expiration date.
- Discard controls and assay waste according to your local safety regulations.

6. Safety Data Sheets (SDS)

According to U.S. OSHA 29 CFR 1910.1200, and the EU Directives 67/548/EC and 1999/45/EC any products which do not contain more than 1% of a component classified as hazardous or classified as carcinogenic do not require a Safety Data Sheet (SDS).

Product is not hazardous, not toxic, and not IATA-restricted. Product is not from human, animal or plant origin. Product contains synthetic oligonucleotide primers and probes.

7. Procedure

Refer to the **Gene-RADAR® Zika Virus Test** Instructions for Use.

When utilizing the **Gene-RADAR® Zika Virus Test,** patient serum samples should be extracted in batches which should not exceed 10 patient serum samples. Additionally, a batch of patient serum samples must be extracted within a time period not exceeding 24 hours in duration.

At least one **Gene-RADAR® Zika Virus Positive Control** must be performed for each batch of patient serum samples.

7.1 Preparation Steps

- Follow the extraction kit manufacturer's instructions for use (see QIAamp® Viral RNA Mini Kit Handbook, April 2010), including all volumes.
- Prepare **Gene-RADAR® Zika Positive Control** for use by gently warming in a 37ºC dry bath for 5 minutes, or until liquid. Upon melting, carefully mix by repeated inversion.

7.2 Extraction Process

- For a **Positive Control**, add 86 µL of nuclease-free water and 4 µL of **Gene-RADAR® Zika Virus Internal Process Control** into the 2 mL labelled centrifuge tube, and then add 50 µL of **Gene-RADAR® Zika Virus Positive Control** into the 2 mL labelled centrifuge tube and mix by pulse-vortexing for 15 seconds.
- To ensure efficient lysis, it is essential that each control is mixed thoroughly with Extraction Buffer AVL to yield a homogeneous solution.

7.3 Amplification

- For each extracted batch of patient serum samples and each day of testing, always include at least one **Gene-RADAR® Zika Virus Positive Control,** which has undergone the Nucleic Acid Extraction procedure above concurrent with the patient serum samples. Additional Controls need to be run if more than one kit lot is used for testing an extraction batch.

8. Technical Assistance

For customer support, please contact our Technical Support team:

email: GeneRADAR@nanobiosym.com

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Gene-RADAR® Zika Virus Positive Control
Document Number: IFU-ZKV-PC
Version 1.0
Date: February 2017

Gene-RADAR®
Zika Virus Internal Process Control Instructions for Use

For Use Under Emergency Use Authorization (EUA) Only

Nanobiosym Diagnostics, Inc.
245 First Street
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Gene-RADAR® Zika Virus Internal Process Control Instructions for Use

For use under Emergency Use Authorization (EUA) Only:
For *in vitro* Diagnostic Use

Read this Package Insert and the **Gene-RADAR® Zika Virus Test** Package Insert completely prior to performing the test; failure to do so may cause inaccurate test results.

Catalog Number: NBS-ZKV-110

15. Intended Use

The **Gene-RADAR® Zika Virus Internal Process Control** is intended for use as an internal process control material in the Gene-RADAR® Zika Virus Test for the detection of RNA from Zika virus.

The **Gene-RADAR® Zika Virus Internal Process Control** is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The **Gene-RADAR® Zika Virus Internal Process Control** is only for use under the Food and Drug Administration's Emergency Use Authorization.

16. Explanation of Control

The **Gene-RADAR® Zika Virus Test** utilizes various controls, including: an **Internal Process Control**, which is an inactivated and stabilized MS2 Bacteriophage that contains an RNA genome, that is amplified and detected with a pair of primers and probe different from the primers and probe for the Zika specific target.

17. Materials Provided

Gene-RADAR® Zika Virus Internal Process Control (50 µL) is sufficient for up to 10 patient samples and associated controls.

Internal Process Control
Gene-RADAR® Zika Virus Internal Process Control, (50 µL) (Catalog No. NBS-ZKV-110)

4. Storage Instructions

- The **Gene-RADAR® Zika Virus Internal Process Control** is shipped on dry ice. The control should arrive frozen. If it is not frozen upon receipt, or if the container has been compromised during shipment, the product should not be used. Please contact the Technical Support team at Nanobiosym Diagnostics for assistance.

- Store **Gene-RADAR® Zika Virus Internal Process Control** at -20ºC upon receipt.
- Always check the expiration date and do not use reagents beyond their expiration date.
- Reagents should not be thawed and refrozen, as this may affect the performance of the assay.
- Do not store in a frost-free freezer. Store in a non-defrosting freezer.

6. Warnings and Precautions

- This control is for use with the **Gene-RADAR® Zika Virus Test** only.
- Do not use reagents from other manufacturers with this control.
- Controls and specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling controls and specimens. Use personal protective equipment (PPE) consistent with current guidelines.
- Avoid microbial and nuclease (DNase/RNase) contamination of the control.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling controls.
- Use segregated work areas for (a) specimen preparation, (b) reaction set-up, and (c) amplification/detection activities. Workflow in the laboratory should proceed in a unidirectional manner. Always wear disposable gloves in each area, and change them before entering different areas.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Do not use controls that have passed their expiration date.
- Discard controls and assay waste according to your local safety regulations.

7. Safety Data Sheets (SDS)

According to U.S. OSHA 29 CFR 1910.1200, and the EU Directives 67/548/EC and 1999/45/EC any products which do not contain more than 1% of a component classified as hazardous or classified as carcinogenic do not require a Safety Data Sheet (SDS).

Product is not hazardous, not toxic, and not IATA-restricted. Product is not from human, animal or plant origin. Product contains synthetic oligonucleotide primers and probes.

9. Procedure

Refer to the **Gene-RADAR® Zika Virus Test** Instructions for Use.

When utilizing the **Gene-RADAR® Zika Virus Test,** patient serum samples should be extracted in batches which should not exceed 10 patient serum samples. Additionally, a batch of patient serum samples must be extracted within a time period not exceeding 24 hours in duration. Each batch of serum samples must contain at least one **Positive Control** and one **Negative Control.**

Each serum sample and control must contain a **Gene-RADAR® Zika Virus Internal Process Control**.

7.1 Preparation Steps

- Follow the extraction kit manufacturer's instructions for use (see QIAamp® Viral RNA Mini Kit Handbook, April 2010), including all volumes.
- Prepare **Gene-RADAR® Zika Virus Internal Process Control** for use by gently warming in a 37ºC dry bath for 5 minutes, or until liquid. Upon melting, carefully mix by repeated inversion.

7.2 Extraction Process

- For each serum sample and control, add 4 µL of **Gene-RADAR® Zika Virus Internal Process Control** into the 2 mL labelled centrifuge tube, and mix with serum sample or control by pulse-vortexing for 15 seconds.

10. Technical Assistance

For customer support, please contact our Technical Support team:

email: GeneRADAR@nanobiosym.com

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