WEST NILE DETECTION KIT

REF VSNTD004 CE IVD

Instructions for Use

For in vitro diagnostic procedures

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I. Table of Contents

WEST NILE DETECTION KIT

REF **VSNTD004** CE IVD

For Laboratory Professional Use Only.

1. Compatible Devices

Roche LightCycler 480 Instrument II, Corbett Rotorgene, Applied Biosystems 7500 Fast, Biorad CFX 96 Realtime PCR Detection System, Biorad CFX384 Touch Realtime PCR Detection Sytem, BioMolecular Systems Bio 4 - Channel+HRM, Drawell Gentier 96E, Applied Biosystems QuantStudio 5/7/12K.

2. Intended Use

This Kit, includes a special ready-to-use system for detection in real-time PCR Device for pathogens such as; West nile virus. The West Nile Detection Kit is a in vitro nucleic acid amplification test for the detection of spesific viral nucleic acids in blood or serum samples.

3. Summary

West Nile virus (WNV) is a mosquito-borne pathogen primarily transmitted to humans and animals through the bite of an infected mosquito. It is commonly found in parts of Africa, Europe, the Middle East, and North America. Most people infected with WNV do not exhibit symptoms, but in some cases, the virus can cause mild flu-like symptoms or more severe neurological diseases, including encephalitis or meningitis.
The virus primarily affects birds, which are the main hosts, while mosquitoes spread the infection to other animals and humans. Preventing mosquito bites through the use of repellents and controlling mosquito populations is key to reducing the risk of transmission.

4. Princible of Real-Time PCR Device

The principle of real-time detection is based on the fluorogenic 5'nuclease test. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes the target DNA. This splitting results in the fluorescent signal produced by the split reporter dye, which is monitored in real time by the PCR system. The PCR cycle (Ct) in which an increase in the fluorescent signal is detected initially is proportional to the amount of the specific PCR product. Real-time monitoring of fluorescent intensities allows detection of the accumulated product without the need to reopen the reaction tube after amplification.

5. Storage

- All reagents should be stored between -10~-30°C. It is not recommended to store at +4°C. Reagents for daily use can be stored at +4°C to prevent multiple freezes and thaws.
- All reagents can be used until the expiration date indicated on the kit label. Repeated defrosting and freezing operations (> 3x) should be avoided as it will reduce the sensitivity of the test.
- Keep all reagents cold during the working steps.
- The reaction mixture should be stored in the dark.

6. Warning And Precautions

Read this instruction carefully before starting the procedure.

For in vitro diagnostic use only.

- It should be used by laboratory personnel trained in in vitro diagnostic procedures.
- Follow standard precautions. All patient samples and Positive Controls should be considered potentially infectious samples and processed accordingly.
- This test should be performed in accordance with Good Laboratory Practices.
- Do not use the kit after the expiration date.
- Do not eat, drink, smoke, apply cosmetics or touch contact lenses in areas where reagents and human samples are used.
- Treat all samples as if they are infectious using safe laboratory procedures.

7. Sample Collection, Storage and Transportation

Sample Type	Blood, serum
Sample Transfer	VISION Blood Tube
Sample Storage	2- 8°C (up to 72 hours), -20°C (If the extraction process is postponed, store the samples)
LoD (Copy/mL)	200

Table 1: Sample Information

8. Kit Content

Oligo Mix	Parameters	Channels	50 RXN	100 RXN
OLIGO MIX	West Nile Virus	FAM	500 µl	1000 µl
	None	HEX		
	None	ROX		
	RNase P (IPC)	CY5		

CONTENT		50 RXN	100 RXN
MASTER MIX	MASTER MIX FOR QPCR ASSAY	250 µL	500 µL
PC	WHOLE GENOME	150 µL	300 µL
NTC	NO TEMPLATE CONTROL	100 µL	200 µL

Table 2: Kit Content

9. Materials and Devices Required

- Biosafety cabinet
- Vortex mixer
- Microcentrifuge
- Micropipette (2 or 10 µL, 10 and 100 µL)
- Multiple micropipettes
- Rack for 1.5mL microcentrifuge tubes (5-50 µl)
- -20°C cold block for 2 x 96-well
- PCR Device
- Extraction systems or test kits
- 10% bleach solution
- Disposable dust-free gloves and surgical gown
- Pipette tip with aerosol barrier
- 1.5mL microcentrifuge tube (DNase/RNase free)
- 96 well plates or 0.1/0.2ul strips
- Plate Seal

10. Protocol

a. DNA/RNA Extraction

We recommend using the Vision Automated Extraction Kit for isolation steps.

NOTE: If the isolation method you use is suitable for working directly from the sample, vortex the sample for 10 seconds. (in case of using NAT)

b. RT-PCR Protocol

Clean the room and cabinet where the reagents will be stored, place Oligo Mix, Master Mix and controls on ice or cold block. Keep area cool drespiratoryg preparation and use.

Thaw mixtures before starting.

Before using Oligo Mix and Master Mix, the vial can be centrifuged for 5 seconds to prevent some of the contents from remaining at the tip of the vial, and then place the tube on a cold rack.

Label the 1.5 mL eppendorf tube for each run.

Determine the number of reactions (N) to set up per test. Overreaction mixing is necessary for Negative Controls, Positive Controls reactions, and pipetting error. Use the following guide to determine N.

If the number of samples, together with the controls (n) is between 1 and 14; N = n+1 If the number of samples is greater than (n) 15 with controls; N = n+2

For each run, calculate the amount of reagents to be added to the mixture (N = number of reactions)

Transfer the reagents to the labeled 1.5 mL microcentrifuge tubes sequentially. After adding the reagents, mix the reaction mixture by pipetting.

NOTE: DO NOT USE THE VORTEX.

Reaction mixes are obtained by mixing 10 µL of oligo mix and 5 µL of master mix per reaction. Dispense 15 µL of Reaction Mixes into appropriate wells.

NOTE: Each Sample must be added separately to each reaction mix.

c. Sample Addition

Vortex the sample tubes for approximately 10 seconds.

Samples should be added to the specific assay being tested in columns

Carefully pipette 5 µL of the first sample into the well labeled for that sample. Keep other sample wells closed drespiratoryg addition. Change pipette tips after each addition.

To prevent cross contamination and allow sample tracking, tightly close the well into which the sample was added.

Frequently change gloves to avoid contamination.

Cover the entire reaction plate and move the reaction plate to the positive control processing area.

Pipette 5 µL of positive control solution into column.

Securely close the wells after addition of control DNA.

NOTE: If using 8-tube strips, label the TAB of each strip to indicate sample position.

Briefly centrifuge the reaction tube strips for 10-15 seconds. Place on cold rack after centrifugation.

d. Real-Time PCR Thermal Profile Set-Up

VISION WEST NILE DETECTION KIT	TEMPERATURE	TIME	CYCLE
	Celcius	Sec.	
REVERSE TRANSCRIPTION	55	900	1
INITIAL ACTIVATION	95	30	1
DENATURATION	95	15	40
EXTENSION	60	45	

Table 3: Thermal Profile

11. Threshold Settings

Just above the maximum negative control level.
Quality Control: Negative control and positive control must be loaded correctly, otherwise the sample results are invalid.

12. Controls

Controls that will be provided with the test kit are provided.

Control	Descriptions	Results
PC	Viral/Bacterial genome	+
NTC	No template Control	-
IPC	Rnase P Gene	+
EXC	None	-

Table 4: Control Design

13. Result Interpretation

Negative: The sample tested is negative for the tested agent.

Positive: The sample tested is positive for the tested agent.

Contamination: Repeat the analysis paying attention to the “Warnings and precautions” section.

Invalid: Sampling isn’t successfully done or there is a problem drespiratoryg the sample transportation. A new sample from the same source should be collected and tested again.

Reagent Problem: Test the PC(s) provided with the kit setting up the PC reactions. If the test result is positive, the run is valid.

Cq Value	Results
Cq<18	High Positive
18<Cq<27	Positive
27<Cq<35	Low Positive
35<Cq	Negative or repeat test

Table 5: Result interpretation

14. Performance Characteristics

a. Analytical Sensitivity (Limit of Detection)

Standard materials of each of the respiratory pathogens were diluted into 4 concentrations (500, 200, 100, 10 copy/mL). The test was performed 3 times on each concentration for each pathogens. The limit of detection is calculated as 95%.

Parameters	LoD (Copy/mL)
West Nile Virus	**200**

Table 6: Sensitivity

b. Analytical Spesifity

Cross Reactivity: No potential for cross-reactivity among themselves or with other pathogens.

Interfering Substances: Interfering substances were tested at certain concentrations. No effects were observed.

Spesifity: Specificity was found to be 98.1% in false positive studies with negative samples.

15. Quality Control

NTC consists of using nuclease-free water instead of RNA/DNA in RT-PCR reactions. NTC reactions for all mixtures of oligos should not exhibit fluorescent growth curves that cross the threshold line. If any of the NTC reactions exhibit a growth curve that exceeds the cycle threshold, reaction mixture contamination may have occurred. If this happens, repeat the operation strictly adhering to the manual. Positive Control (PC) consists of in vitro replicated DNA. Contains specific gene regions for target regions. RNase P (Internal Control),All clinical samples should exhibit fluorescent growth curves that cross the threshold in the RNase P reaction within 40.00 cycles <35.00 Ct), this indicating the presence of the human RNase P gene. Failure to detect RNase P in any clinical specimen may indicate:

- Incorrect extraction of nucleic acid from clinical materials resulting in RNA loss and/or RNA degradation
- Lack of adequate human cellular material due to insufficient collection or loss of sample integrity.
- Improper assay setup and execution.
- Reagent or equipment failure.

16. Symbols

Symbol	Title of Symbol	Symbol	Title of Symbol	Symbol	Title of Symbol
	In vitro diagnostic medical device.		Batch code		Contains sufficient for <*n*> tests
	Manufacturer		Catalogue number		Keep away from sunlight
	Use-By Date		Non-Sterile		Protect from heat and radioactive sources
	Control		Consult *instructions for use* or consult electronic *instructions for use*		Do not use if package is damaged and consult *instructions for use*
	Ngative Control		CE Marking		Keep dry
	Positive Control		Temperature limit		Keep it upright

For serious adverse events related to the product, report to Vision Biotechnology and the competent authority where the user and/or patient is located.

17. Contact


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