Sensitive and Reliable Detection of Zika Virus with the Clonit Quanty Zika RT-PCR Test Using VERSANT kPCR Sample Prep

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Abstract

Background: Zika virus (ZIKV) is a flavivirus first isolated in 1947 in Uganda that is transmitted through the bite of an Aedes mosquito. Infection with ZIKV during pregnancy is associated with fetal microcephaly and other serious birth defects. The quanty Zika assay* (Clonit, Milan, Italy) is a real-time RT-PCR–based in vitro diagnostic test intended for quantitative detection of Zika virus RNA in clinical specimens (serum, urine, and saliva). The quanty RT-PCR test contains reagents for reverse transcription and specific amplification of the NS5 region of Zika virus RNA. In addition, the assay has a built-in standard curve for quantitation and extraction control to confirm the validity of the extraction process. In this study, we compare the performance of the quanty Zika PCR assay with an assay from Lanciotti et al. (2008).[1]

Method: Zika RNA was extracted from serum and urine samples with the VERSANT® kPCR Sample Prep and VERSANT Sample Preparation 1.0 Reagents and then amplified on the Thermo Fisher Scientific QUANTSTUDIO 5 Real-Time PCR System. A comparison with an assay using primers/probe from Lanciotti et al. was conducted on 42 paired serum and urine specimens previously confirmed PCR-positive or suspected positive by a physician.

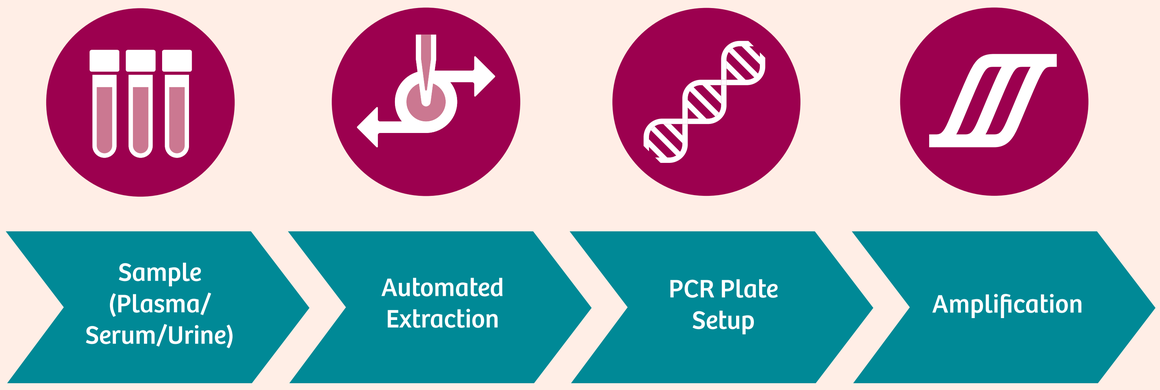

Figure 1. VERSANT kPCR Sample Prep with the VERSANT MiPLX Software Solution for automated nucleic acid extraction and PCR plate setup.

Results: The Clonit quanty Zika assay detected 17 positive specimens (80%) and the Lanciotti assay detected 18 positive specimens (85%) among the 21 serum specimens. The Clonit quanty Zika assay detected 16 positive specimens (76%) and the Lanciotti assay detected 13 positive specimens (62%) among the 21 urine specimens.

Conclusion: The data demonstrates that the Clonit quanty Zika assay results are comparable in serum and more sensitive in urine when compared to CDC. Clinical specificity testing is in progress.

Introduction

ZIKV is an emerging mosquito-borne public-health threat, with reports of transmission in the United States and three U.S. territories. An estimated 80% of persons infected with ZIKV show few to no symptoms.[2] Associated symptoms are mild fever, myalgia, headache, conjunctivitis, and cutaneous maculopapular rash.[2] Amid ZIKV outbreak in Brazil, ZIKV RNA was identified in tissues from a significant number of infants with microcephaly and from fetal losses in women who were infected during pregnancy.[3,4,5] Other outcomes such as Guillain-Barré syndrome are being studied in association with the ZIKV infection. The ability to accurately detect and diagnose ZIKV infection is very critical.

Here we assess the Clonit quanty Zika RT-PCR test using the VERSANT Molecular Sample Prep. The quanty RT-PCR test contains reagents for reverse transcription and specific amplification of the NS5 region of Zika virus RNA. In addition, the assay has a built-in standard curve for quantitation and extraction control to confirm the validity of extraction process.

Methods

Sample: Serum and urine samples were used for nucleic acid extraction of ZIKV. 21 clinically matched Zika-positive samples were used for both serum and urine. In addition, 20 clinical Zika-negative serum and urine samples were tested.

Nucleic Acid Extraction: A Siemens Healthineers automated system—the VERSANT kPCR Sample Prep with the VERSANT MiPLX Software Solution and VERSANT Sample Preparation 1.0 Reagents—was used for sample (serum or urine) extraction (250 µL input; 50 µL elution).

RT-PCR Amplification: Purified RNA was added to a PCR plate containing Zika Enzyme Mix and Zika Primer/Probe Mix, and the wells were sealed. The Zika Enzyme Mix contains dNTPs, reference dye, and enzymes for nucleic acid amplification. The Thermo Fisher Scientific QUANTSTUDIO 5 Real-Time PCR System was used for amplification. The following thermal profile was used:

Cycles	denaturation	annealing	extension
1	50° C 20 min		
1	95° C 2 min		
45	95° C 15 sec	58° C 45 sec	72° C 15 sec

A comparison with an assay using primers/probe from Lanciotti et al. was conducted on 42 paired serum and urine specimens previously confirmed PCR-positive or suspected positive by a physician.

Results

The Clonit quanty Zika assay detected 17 positive specimens (80%) and the Lanciotti assay detected 18 positive specimens (85%) among the 21 serum specimens (Table 1). The Clonit quanty Zika assay detected 16 positive specimens (76%) and the Lanciotti assay detected 13 positive specimens (62%) among the 21 urine specimens (Table 2).

Table 1. Comparison between Clonit quanty Zika assay and the Lanciotti assay for clinical serum and urine samples.

Patient	Clonit Assay		Lanciotti Assay	
ID	Serum	Urine	Serum	Urine
102461	-	36.5	-	37.4
102471	-	34.3	37.4	34.5
102521	32.9	35.2	30.5	35.3
102531	39.0	31.4	37.1	31.8
102551	34.1	39.9	32.6	-
102561	36.1	-	34.3	37.3
102591	31.8	38.0	31.2	35.6
102621	41.3	32.0	-	32.7
102651	35.1	36.7	34.8	-
102661	-	36.3	38	34.5
102711	-	34.9	36.9	31.6
102721	35.6	39.0	34.4	-
102741	36.9	-	-	26.5
102761	37.1	-	36.2	-
102781	31.5	40.2	31.5	37.9
102811	36.9	39.4	34.8	37.0
102831	33.4	-	32.6	-
102841	30.8	40.0	30.5	-
102852	32.7	41.2	32.8	34.5
102871	34.1	-	32.34	-
102921	33.3	38.4	32.28	-
# Detected	17	16	18	13

Table 2. Comparison between Clonit quanty Zika assay and the Lanciotti assay for clinical serum samples.

	Serum	Clonit quanty Assay		
		Positive	Negative	Total
Lanciotti Assay	Positive	17	1	18
	Negative	2	1	3
	Total	19	2	21

Table 3. Comparison between Clonit quanty Zika assay and the Lanciotti assay for clinical urine samples.

	Urine	Clonit quanty Assay		
		Positive	Negative	Total
Lanciotti Assay	Positive	13	2	15
	Negative	3	3	6
	Total	16	5	21

Clinical specificity

Negative clinical samples were tested with both the Clonit quanty Zika assay as well as the Lanciotti assay. All samples were determined to be negative when the Clonit quanty Zika assay was compared to the Lanciotti assay.

Conclusions

- The Clonit quanty Zika assay is comparable to the Lanciotti assay for serum sample testing. The Clonit quanty assay detected 80% of the clinical serum samples, compared to 85% detection with the Lanciotti assay.
- The Clonit quanty Zika assay is more sensitive than the Lanciotti assay for urine sample testing. The Clonit quanty assay detected 76% of the clinical urine samples, compared to 62% detection with the Lanciotti assay.
- Both the Clonit quanty Zika assay and the Lanciotti assay were clinically specific.

References

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*Clonit quanty Zika assay is CE-marked for IVD use.

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