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Comparative study of two diagnostic tests for detection of RNA Zika (ZIKV) in clinical samples

Laura Alonso Acero*¹, Ana Sarria², Silvia Garcia-Bujalance¹, Beatriz Domínguez Calero¹, Dolores Montero¹, Julio García Rodríguez¹

¹*Hospital Universitario La Paz*

²*Hospital Universitario La Paz; Microbiology*

Background: Considering the last Zika epidemic reported in the Americas in 2015 and the severe pathology associated with this infection in fetuses and newborns, an early molecular diagnosis of this virus is essential.

In this study we compared two different diagnostic tests for detection of RNA Zika Virus (ZIKV) in clinical samples: urine (2); placenta (1) and maternal milk (1) from an infected pregnant woman; blood from the umbilical cord (1); total blood (2); amniotic fluid (1); semen (3) from the same patient, testing also the supernatant from the semen culture (2); and serum (1) and urine (1) from a symptomatic woman.

Material/methods: 19 samples were extracted with easyMAG® (BioMeriëux), using external lysis in some cases.

The two tests are based in a RT-PCR for detection of ZIKV. The target of Viasure® Zika Virus Real Time PCR Detection Kit (Certest Biotec) is a preserved region of the *envelope* gene while the target of RealStar® Zika Virus RT-PCR kit 1.0 (Altona Diagnostics GmbH) is not stated by the manufacturer.

Serial dilutions of a known number of copies from the first semen sample (4.2x10³ copies/mL, 1.2x10³ copies/mL, 1.2x10² copies/mL, 12 copies/mL) were performed to determine the analytical sensitivity of the techniques. Serial dilutions with the standard of ZIKV provided by RealStar® for quantification of the virus could not be quantitatively done by Viasure® due to the use of different targets.

Clinical data was obtained from the electronic medical history.

Results:

Patient	Sample	Day of illness (DOI)	RealStar® Zika Virus	Viasure® Zika Virus
Man, 28 y.o. (Santo Domingo)	Semen #1	20	Positive (Ct: 31.8)	Positive (Ct: 35.18)
	• Semen culture		Positive (Ct: 31.1)	Positive (Ct: 34.3)
	• 4.2x10 ³ copies/mL		Positive (Ct: 33)	Positive (Ct: 35.5)
	• 1.2x10 ³ copies/mL		Positive (Ct: 36.7)	Positive (Ct: 37.3)
	Semen #2	50	Positive (Ct: 33)	No data
	• Semen culture		Positive (Ct: 22.9)	Positive (Ct: 24.9)
	Semen #3	100	Negative	Not conclusive (Ct: 38)*
Woman, 57 y.o. (Nicaragua)	Urine	7	Positive (Ct: 30.2)	Positive (Ct: 31.7)
	Serum		Positive (Ct: 35.8)	Positive (Ct: 37.07)

*Not conclusive. The fluorescence curve showed an unspecific signal.

The clinical samples not shown on the table were negative in both tests. Serial dilutions with 1.2x10² copies/mL and 12 copies/mL were negative.

Conclusions: Overall, there were no discrepancies between tests and they may be both used for diagnosis of ZIKV. However, Ct results were higher with Viasure®, despite starting with an equal number of copies. Therefore, it is not recommended to interchange Ct values between techniques.

Molecular diagnosis of ZIKV is a necessary tool. In semen samples RNA ZIKV can be detected as much as two months after symptom onset.