RealStar® PCR Kits: Zika Virus, Chikungunya and Dengue

Here we present data on the verification and validation of three RealStar® RT-PCR Kits for parallel detection of Zika, chikungunya and dengue virus. These three viruses cause similar symptoms and are endemic in mostly the same geographic regions, therefore reliable diagnostic differentiation is urgently needed.

The flavivirus Zika virus is transmitted by the same vector *(Aedes aegypti)* as the flavivirus dengue virus and the alphavirus chikungunya virus. After transmission to humans, the above-mentioned viruses cause signs and symptoms like fever, general malaise, rash, muscle and joint pain making it difficult to identify the etiological agent.
While dengue virus has been a worldwide problem since 1950s, cases of chikungunya have only been detected in Africa and Asia until it reached the Americas in 2013.
Prior to 2015, Zika virus outbreaks occurred in some parts of Africa, Southeast Asia, and the Pacific Islands. In May 2015, the first Zika virus infection in Brazil was confirmed. In healthy adults, Zika virus infections are usually mild and self-limiting. However, they have been linked to cases of Guillain Barré Syndrome and infections in pregnant women can lead to severe forms of microcephaly of the fetus and newborn child.
Because Zika virus, dengue virus and chikungunya virus are endemic to the same geographical regions, there is need for reliable differentiation of Zika, dengue and chikungunya virus. There are no vaccines or specific treatments available for Zika, dengue or chikungunya virus infections.

altona Diagnostics GmbH developed three RT-PCR Kits (RealStar® Zika Virus RT-PCR Kit 1.0, RealStar® Dengue RT-PCR Kit 2.0 and RealStar® Chikungunya RT-PCR Kit 2.0) that facilitate simultaneous testing for the presence of Zika virus, dengue virus and chikungunya virus in the same sample. All assays contain an internal control for reliable interpretation of results.

Here we present data on the analytical specificity and sensitivity of all three RealStar® RT-PCR Kits. 16 patient samples previously tested positive for Zika virus RNA were re-analyzed with the RealStar® Zika Virus RT-PCR Kit 1.0 and the RT-PCR test published by Lanciotti et al. (2008) [1].

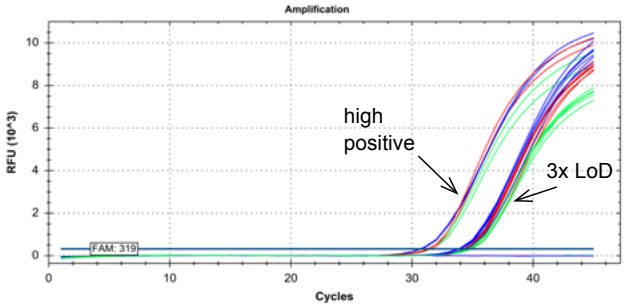

Figure 1:
Amplification plot showing the simultaneous analysis of different concentrations of virus specific *in-vitro* transcribed RNA for RealStar® Zika Virus RT-PCR Kit 1.0 (red), RealStar® Dengue RT-PCR Kit 2.0 (green) and RealStar® Chikungunya RT-PCR Kit 2.0 (blue).

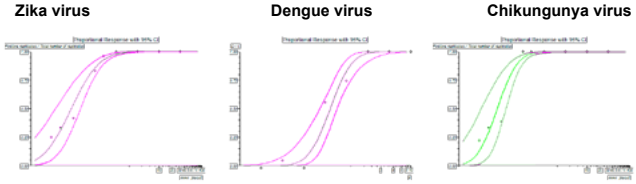

Figure 2:
Probit analysis for the RealStar® Zika Virus RT-PCR Kit 1.0, RealStar® Dengue RT-PCR Kit 2.0 and RealStar® Chikungunya RT- PCR Kit 2.0.
The analytical sensitivity (Limit of Detection: 95% LoD) was determined for each kit by testing replicates of half-logarithmic dilutions of virus specific in-vitro transcribed RNA. The X-axis shows the concentration of RNA and the Y-axis the proportion of positive results.
Sensitivity of RealStar® Zika virus RT-PCR Kit 1.0 is 0.61 copies/µl (95% confidence interval 0.39 to 1.27 copies/µl). The LoD for RealStar® Dengue RT-PCR Kit 2.0 (DENV type 4) is 0.58 copies/µl (95% confidence interval 0.34 to 1.77 copies/µl). Analytical sensitivity of RealStar® Chikungunya RT-PCR Kit 2.0 is 0.321 copies/µl (95% confidence interval 0.21 to 0.67 copies/µl).

The cross-reactivity was tested in between the assays with Zika, dengue and chikungunya virus RNA and with RNA of Sudan ebolavirus, Zaire ebolavirus, Marburg virus, Japanese encephalitis virus, St. Louis encephalitis virus, West Nile virus, yellow fever virus and Murray Valley encephalitis virus. No unspecific cross-reactivity was observed.

To evaluate the clinical performance of the RealStar® Zika Virus RT-PCR Kit 1.0 a total of 208 clinical specimens from 153 patients with signs and symptoms of Zika virus infection (106 female (F) and 47 male (M)) were analyzed retrospectively in a blinded fashion. From the 208 samples tested 103 were serum and 105 were urine specimens. The Samples of all specimens were extracted using the QIAamp Viral RNA kit. The RNA samples were tested with RealStar® Zika Virus RT-PCR Kit 1.0 and with a published RT-PCR method of Lanciotti et al. [1] as a reference test.

Table 1: Result summary for the detection of Zika virus RNA in serum samples.
Total of serum samples was 103

Results from real-time RT-PCR assay described by Lanciotti et al.	**Results of the RealStar® Zika Virus RT-PCR Kit 1.0**		
	Positive		Negative
62 Positive	60		2
41 Negative	2		39
Total (103 samples)	62		41
			95% CI
Positive Percent Agreement	60/62	96.8%	89.0% - 99.1%
Negative Percent Agreement	39/41	95.1%	83.9% - 98.7%

Table 2: Result summary for the detection of Zika virus RNA in urine samples.
Total of urine samples was 105

Results from real-time RT-PCR assay described by Lanciotti et al.	**Results of the RealStar® Zika Virus RT-PCR Kit 1.0**		
	Positive		Negative
49 Positive	46		3
56 Negative	6		50
Total (105 samples)	52		53
			95% CI
Positive Percent Agreement	46/49	93.9%	83.5% - 98.0%
Negative Percent Agreement	50/56	89.3%	78.5% - 95.0%

The RealStar® assays for the detection of Zika virus, dengue virus and chikungunya virus are highly sensitive and allow the detection of low-level of virus RNA in patient samples. The Internal Control included in all assays enables reliable diagnostics.
The RealStar® Zika Virus RT-PCR Kit 1.0, RealStar® Dengue RT-PCR Kit 2.0 and Chikungunya RT-PCR Kit 2.0 represent valuable tools for simultaneous detection of all three viruses in parallel in one PCR run. Furthermore, the RealStar® Zika Virus RT-PCR Kit 1.0 was assessed under the Emergency Use Assessment and Listing Procedure (EUAL) by the WHO [2] and has been accepted August 2106 as the first Zika virus diagnostic test eligible for procurement agencies and member states.

[1] Lanciotti, R. S., Kosoy, O. L., Laven, J. J., Velez, J. O., Lambert, A. J., Johnson, A. J., ... & Duffy, M. R. (2008). Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis, 14(8), 1232-9.
[2] (2016): „Emergency Use Assessment and Listing (EUAL) Procedure for Zika Virus Disease (IVDs)“. World Health Organization. Retrieved 2016.10.11. from http://www.who.int/diagnostics_laboratory/eual-zika-virus/zika/en/.

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