RIDA®GENE Zika Virus: A new commercial real-time RT-PCR assay for sensitive and reliable detection of zika virus in urine and plasma samples

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Introduction

Besides endemic areas such as Africa and South East Asia, high numbers of zika virus infections were recently reported in South America, particularly in Brasil. In February 2016, the World Health Organisation (WHO) issued a Public Health Emergency of International Concern since microcephaly and other neurological disorders were increasingly reported in newborns of pregnant women with zika virus infections.[1] Zika virus belongs to the genus of *flavivirus* and similarly to other members of the *flavivirus* genus, transmission of zika virus occurs via mosquitos, in particular mosquitos of the genus *Aedes*.[2] Cross reactions with other *flaviviruses*, such as dengue virus or chickungunya virus, are often observed upon antibody-specific diagnostic testing so that confirmatory testing is required, in particular in areas where there have been possible co-infections.
Real-time RT-PCR is a suitable method to specifically detect zika virus RNA within the first week after onset of symptoms in urine and plasma samples.
This study aimed to evaluate a new real-time RT-PCR assay for the detection of zika virus in urine and plasma samples.

Picture 1: RIDA®GENE Zika Virus, Art. No. PG6205RUO

Methods

The RIDA®GENE Zika Virus real-time RT-PCR assay detects zika virus-specific RNA by targeting the NS2A gene. An internal control RNA detects PCR inhibition, monitors reagent integrity and confirms successful nucleic acid extraction.

The analytical reactivity of the RIDA®GENE Zika Virus assay was tested using proficiency testing samples (INSTAND e.V.). Analytical specificity was determined using known quality control standards (ZeptoMetrix). Spike experiments were carried out to determine the analytical sensitivity in comparison to three other commercially available assays. A clinical evaluation of known positive and negative urine and plasma samples which were pre-characterised by two other test systems was performed on the LightCycler® 480II (Roche).

Results

Due to the high emergence for diagnostic assays, reliable proficiency samples for NAT testing are now available as well. These were used for evaluation of the analytical reactivity of the RIDA®GENE Zika Virus real-time RT-PCR assay (Table 1). Commercially available *flavivirus* strains were used for evaluation of the analytical specificity. No cross-reactivity to other *flaviviruses* including dengue virus, chickungunya virus and west nile virus was detected using the assay (Table 2).

Clinical evaluation of the RIDA®GENE Zika Virus real-time RT-PCR assay with known positive and known negative urine and plasma samples showed concurrent results when compared to pre-characterised clinical material. All positive and negative samples were identified correctly by the RIDA®GENE Zika Virus real-time RT-PCR assay. Overall, the positive and negative agreement for zika virus was 100 % (Figure 1).

Analytical sensitivity was tested in comparison to three other commercially available assays using spiked zika virus samples and performing a dilution series. The RIDA®GENE Zika Virus real-time RT-PCR assay showed excellent correlation to the tested competitor assays (Table 3).

An example run of the RIDA®GENE Zika Virus real-time RT-PCR assay with the LightCycler® 480II is illustrated in Figure 2.

An analytical sensitivity of 50 copies/reaction was achieved with the LightCycler® 480II/LC2.0, Mx3005P, Rotor-Gene Q, ABI7500, CFX96 and SmartCycler II real-time PCR instruments.

Table 1: Analytical reactivity of the RIDA®GENE Zika Virus real-time RT-PCR assay

Samples	Zika virus
1 (403001)	positive
2 (403002)	positive
3 (403003)	negative
4 (403004)	positive

Table 2: Analytical specificity of the RIDA®GENE Zika Virus real-time RT-PCR assay

Strain	Reactivity
Zika virus	positive
Chikungunya virus	negative
Dengue virus	negative
West-Nile virus	negative

		Reference method +	Reference method -	Total
RIDA®GENE Zika Virus	+	6	0	6
	-	0	10	10
	Total	6	10	16

Positive agreement: 100 %
Negative agreement: 100 %

Figure 1: Clinical performance of the RIDA®GENE Zika Virus real-time RT-PCR assay using urine and plasma samples

Table 3: Analytical sensitivity of the RIDA®GENE Zika Virus real-time RT-PCR assay

Dilution series	RIDA®GENE Zika Virus Ct value	Competitor 1 Ct value	Competitor 2 Ct value	Competitor 3 Ct value
1	18.71	16.84	19.76	15.73
2	21.87	20.80	23.07	19.44
3	25.28	26.41	27.96	22.5
4	28.98	28.43	30.97	26.42
5	31.86	31.99	33.72	29.55
6	34.00	34.35	36.60	31.90
7	38.62	38.93	n.d.	36.36

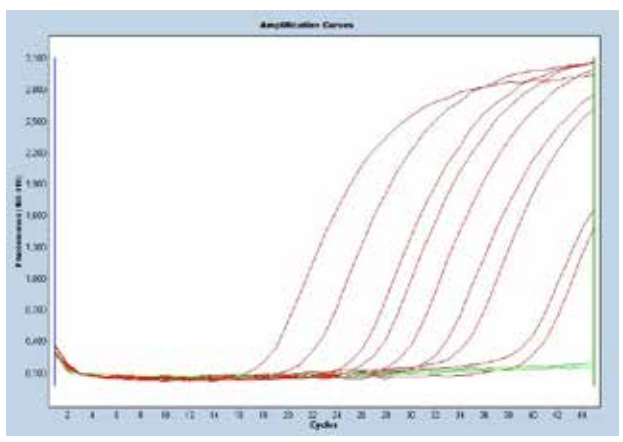

Figure 2: RIDA®GENE Zika Virus real-time RT-PCR assay example run on the LightCycler® 480II

Conclusion

The RIDA®GENE Zika Virus real-time RT-PCR assay is a sensitive and reliable assay for the detection of zika virus. The assay is highly specific for zika virus without known cross-reactivity to other *flaviviruses* such as dengue virus or chickungunya virus. The validation of different common real-time PCR instruments provides broad flexibility and allows for rapid pathogen detection in the routine diagnostics laboratory.

References
1. *World Health Organization* http://www.who.int/mediacentre/news/statements/2016/emergency-committee-zika-microcephaly/en/
2. www.cdc.gov/zika/transmission

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