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Zikavirus protocol qRT-PCR Erasmus MC, 17 March 2016

Total nucleic acid of EDTA-plasma and/or urine was extracted using MagnaPureLC (Roche Diagnostics, Almere, the Netherlands) and the total nucleic acid isolation kit, with an input volume of 200µl and output volume of 100µl. The extraction was internally controlled by the addition of a known concentration of phocine distemper virus (PDV, <http://www.european-virus-archive.com/viruses/phocine-distemper-virus>).

One step Fast virus mastermix Applied biosystems (P/N 4444432)	
One step mastermix (4x)*	5 ul
Primer/probe mix	0.4 ul
DNase/RNase free H ₂ O	6.6 ul
Total RT-PCR mix volume	12 ul
Input RNA	8 ul
Total reaction volume	20 ul

*Including UNG (1 U/ul)

One step Fast viral mastermix thermal profile on ABI7500 or LC480 (Roche), white 96-wells plate.

One step Fast viral mastermix		
2 min	50 °C	
20 sec	95 °C	
3 sec	95 °C	45 cycli
30 sec*	60 °C [#]	

*on ABI7500 is a min. of 31sec detection allowed

acquisition at each cycle.

Primer probe sequences (5'-3') and concentrations per reaction

Virus	target	Product	Sequence 5'-3'		Primer conc (pmol/µl)	Reference
ZIKV	E	77 bp	ZIKV_1086_fwd	CCGCTGCCCAACACAAG	30	Lanciotti et al, Emerging Infectious Diseases 2008
			ZIKV_1107_probe	FAM- AGCCTACCTTGACAAGCAGTC AGACACTCAA-BHQ1	5	
			ZIKV_1162c_rev	CCACTAACGTTCTTTTGCAGA CAT	30	
PDV (IC)	HA	78 bp	Probe*	FAM-atgcaagggccaatt-MGB	10	Hoek et al., SCJID2012
			Primer-fwd	cgggtgcctttacaagaac	30	
			Primer-rev	ttcttctcaacctgtcc	7.5	

This qRT-PCR was validated for Asian and African zika virus strains. Erasmus MC has positive zikavirus controls (Ct15) available in lysisbuffer on request (<http://www.european-virus-archive.com/viruses/zika-virus-strain-suriname-2016>).

Confirmatory qRT-PCR: RealStar® Zika Virus RT-PCR Kit, Altona (Germany).