Zika virus (ZIKV) detection & quantitation by qRT-PCR (Taqman)

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This protocol describes the real-time polymerase chain reaction with reverse transcription (qRT-PCR) approach developed for the detection and quantitation of viral titres in biological specimens using TaqMan probe based on previously published oligos for Zika virus screening of mosquitoes[1]. Zika virus is group IV ((+)ssRNA) member of the Flaviviridae family, Flavivirus genus related to the dengue, yellow fever, Japanese encephalitis, and West Nile viruses and a member of the Spondweni virus clade. There are two Zika lineages: the African lineage and the Asian lineage. Phylogenetic studies indicate that the virus spreading in the Americas is 89% identical to African genotypes but is most closely related to the Asian strain that circulated in French Polynesia during the 2013‑2014 outbreak. Like other flaviviruses, it is enveloped and icosahedral and has a nonsegmented, single-stranded, 10 Kb genome which encodes seven nonstructural proteins and three structural proteins.

Oligonucleotide primers

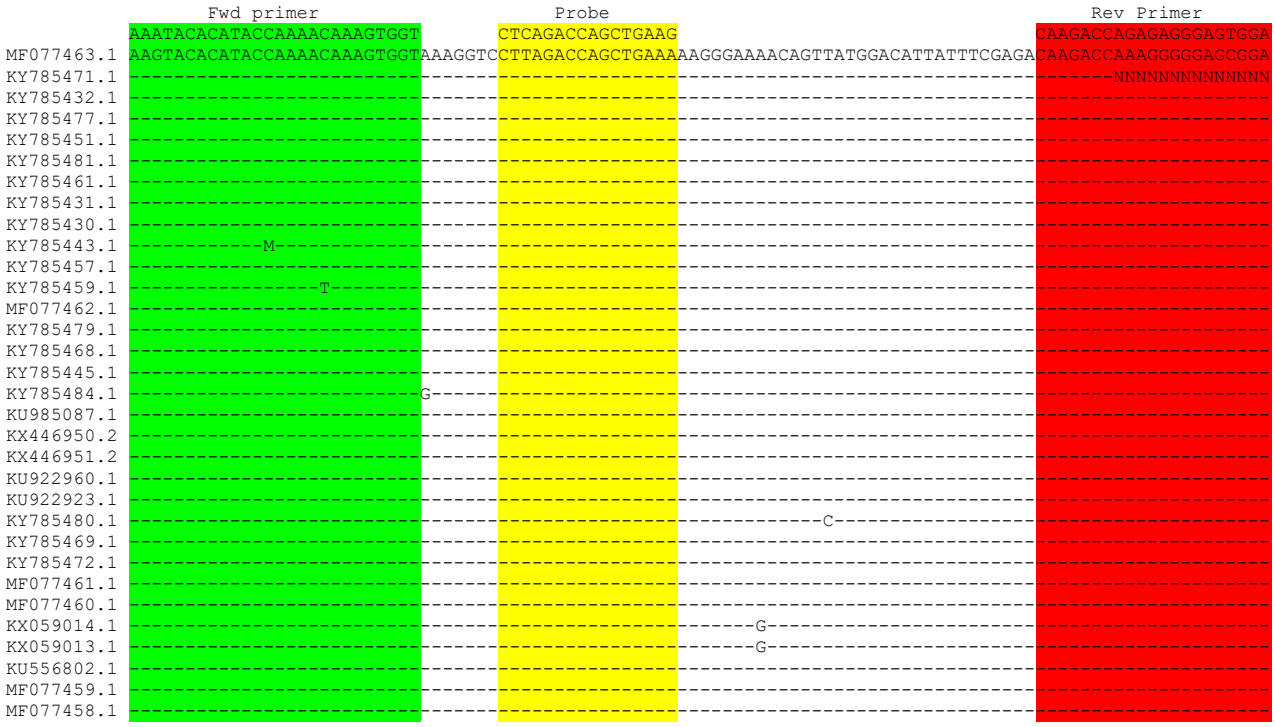
Name	Sequence[1]	bp	%GC	Tm	Hair	HomD	HetD	Size
ZIKA-F	5′-AAR-TAC-ACA-TAC-CAR-AAC-AAA-gTg-gT-3′	26	34.6	55	-1.04	-6.37	-9.9	102
ZIKA-R	5′-TCC-RCT-CCC-YCT-YTg-gTC-TTg-3′	21	62.5	58.9	0.44	- 5.85	-9.9	
Probe	FAM-5’-CTY-AgA-CCA-gCT-gAA-R-3’-BBQ	16	50	46.9	0.82	-10.24		

Note 1: Reverse oligonucleotide primer sequences given in this table are the reverse-complement of sequence present in alignments and as they should be ordered for synthesis; Hairpin (Hair), homodimer (homD) and heterodimer (HetD) ΔQ’s given in kcal/mol.

Oligonucleotide primer map in artificial gene[2] (underlined primer sites) and Mexican ZIKA virus NSP5 sequences.

AAGCTCCGCGTCCTTTACCAAGGAAATAACATCACTGTAACTGCCTATGCAAACGGCGACCATGCCGTCACAGTTAAGGACGCCA
AATTCATTGTGGGGCCAATGTCTTCAGCCTGGACACCTTTCGACAACAAAATTGTGGTGTACAAAGGTGACGTCTATAATATGGA
CTACCCGCCCTTTGGCGCAGGAAGACCAGGACAATTTGG|CAGACCACGCTACGGCGTGCTACTCTGCGGAGAGTGCAGTCTGCG
ATAGTGCCCCAGGAGGACTGGGTTAACAAAGGCAAACCAACGCCCCACGCGGCCCTAG|AAGGACTAGAGGTTAGAGGAGACCAT
GTCCATGTCACCCACGGTCATCATCCTGGCATGTCTTGGGTTCTATCCTGCTGTCTCTACAGCATCATTCCAGGCACAGAACGCC
|AAGGACTAGAGGTTAGAGGAGACCCCCCCAACACAAAAACAGCATATTGACGCTGGGAAAGACCAGAGATCCTGCTGTCTCTGC
AACATCAATCCAGGCACAGAGCGCC|AAATACACATACCAAAACAAAGTGGTGAAGGTTCTCAGACCAGCTGAAGGAGGGAAAAC
AGTCATGGACATCATCTCAAGACAAGACCAGAGAGGGAGTGGA|ATGTATGTGAGTGCTGATGCCACGAAATGGTCACCAGGAGA
TAATTCGGCAAAGTTTAAGAGATTCACACAGGCATTATATGATGGCTTGTCAGATGAGAAGTTAAAATGTTGCGTTGTTGATGC|
ATGTAACACCTCTACAATGGATGCCGACAAGATTGTATTCAAAGTCAATAATCAGGTGGTCTCTTTGAAGCCTGAGATTATCGTG
GATCAATATGAGTACAAGTACCCTGC|GCATAGCTGTAGGAAGGACTCTCAAGGCATTTTTCTCCTGGTCGCTTACTGACCCTTT
AGGGAACGAAGCCCCTGGGGGATACTGTCTTGAAAAGTGGATGCTT|GCAACGCGCGATTCAGTTCCTCTTCACATAATCGCCCC
GAGCTCGCTTATCGTTTAAGCAGCTCTGCGCTACTATGGGTCCCGTGTAGAGGC|TGATGATGCCGTCGTGTGCTACAACAGTAA
CTATGCGGCTCAAGGTTTAGTAGCTAGCATTAAGAACTTTAAGGCAGTTCTGTATTATCAAGATAATGTGTTCATGTCTGAGGCA
AAATGTTGGACTGAGACTGACCTTACTAAAGGACCTCACGAATTTTGCTCACA

The original oligonucleotide and probe sequences were produced by Faye O, *et al*[1] based on sequenced NS5 protein coding regions of African ZIKV isolates. The NS5 protein encoding region alignment and primer map below shows conservation and proper hybridization of oligonucleotides and probes in 32 American ZIKV isolates (USA 6, Jamaica 2, Honduras 3, Colombia 2, Martinique 1, Puerto Rico 1, Brazil 8, Dominican Republic 1, Haiti 2 and Mexico 6).



Alignments prepared using Clustal Omega (1.2.4) EMBL-EBI and reformatted to unanimity using SURe v1.0 Sequence Unanimity Reformatting tool by UASLP's Viral & Human Genomics Laboratory (http://midasmap.uaslp.mx/suretool/). ZIKV artificial gene sequence derived from strain ArD165531 isolated in Senegal during 2002 (GenBank: KF383089).

Taqman qRT-PCR components and conditions

The Taqman qRT-PCR procedure uses One Step Ex Taq qRT-PCR Kit (Perfect Real Time) (TaKaRa Cat #RR068A)

Reagent		1x
dH_2O	cf	2 µL
2x One Step RT-PCR Buffer III	1x	5 µL
TaKaRa Ex Taq HS (5 IU/µl)	2 U	0.2 µL
RTase Enzyme Mix II	1x	0.2 µL
Oligo Fwd 10 uM	500 nM	0.6 µL
Oligo Rev10 uM	500 nM	0.6 µL
10 uM ZIKV Probe	200 nM	0.2 µL
ROX Reference Dye II	1x	0.2 µL
RNA	< 1 µg	1 µL
		vf: 10 µl

↓

Run Taqman program in Applied 7500 →

Total time: 1:00 hrs		
42 ºC	5 min	
95 ºC	10 sec	
95 ºC	5 sec	40 cycles
60 ºC[3]	34 sec	

Note 3: Data acquisition.

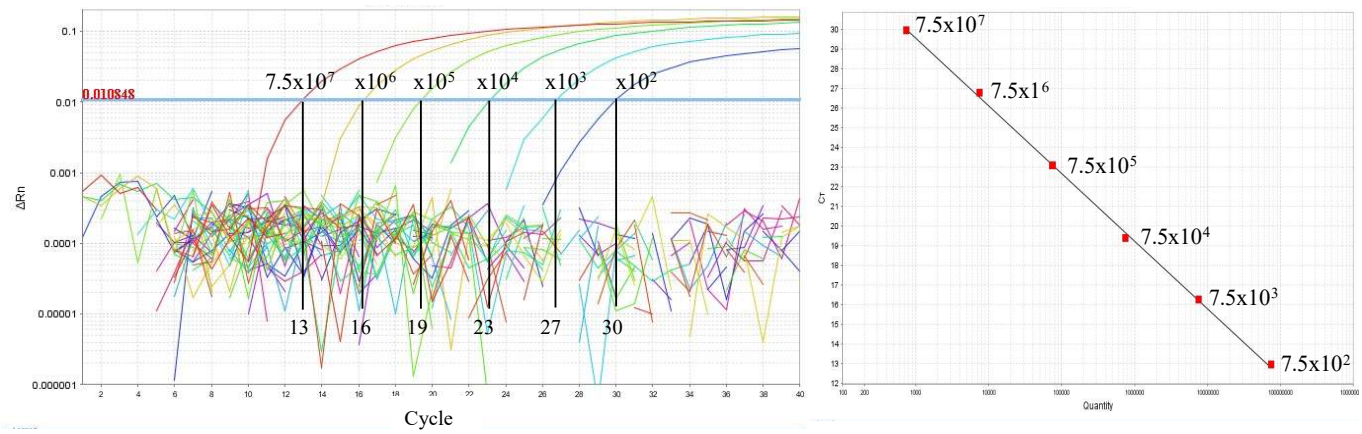
Titration curve preparation for quantitative analysis of viral titres

Using Artificial Gene (AG) sequence stock having all viral references (CHIKV, WNV, DENV1-3, DENV2-4, ZIKV, SNV, RABV, TCRV, MERS and SARS) prepare the following log titres (stock currently at $7.51x10^9$ cp/µL, working dilution of AG prepared at $7.51x10^7$ cp/µL).

		AG stock	dH_20 vol	Ct
Working	$7.5x10^7$ cp/µL	10 µL	90 µL	12.95 (±0.01)
1st	$7.5x10^6$ cp/µL	10 µL	90 µL	16.31 (±0.07)
2nd	$7.5x10^5$ cp/µL	10 µL	90 µL	19.47 (±0.12)
3rd	$7.5x10^4$ cp/µL	10 µL	90 µL	23.07 (±0.00)
4th	$7.5x10^3$ cp/µL	10 µL	90 µL	26.63 (±0.22)
5th	$7.5x10^2$ cp/µL	10 µL	90 µL	29.95 (±0.00)

Add 90 µL to each of the 6 PCR 0.2 mL tubes. Take 10 µL of initial working stock (at $7.51x10^7$ cp/µL) and dispense into 1st PCR tube, wash tip 30 times, cap, vortex for 10 seconds and spin down for 10 seconds. Retrieve 10 µL from volumetric centre of PCR tube and dispense into 2nd PCR tube repeating exactly the same procedure for further dilutions.

Performance summary



Standard curve: ***m* = -3.373, Y = 39.449, R^2 = 1.0**
Limit of detection (LODet): **$7.51x10^2$ cp/µL**
Limit of discrimination (LODis): **$7.51x10^2$ cp/µL**

References

1. Faye, O., Faye, O., Diallo, D., Diallo, M., Weidmann, M., & Sall, A. (2013). Quantitative real-time PCR detection of Zika virus and evaluation with field-caught Mosquitoes. Virology Journal, 10(1), 311.

Revision history

1.0 Original document.

2.0 Optimized protocol.

3.0 Changes to document format only.

4.0 Changes to reagent volumes for greater PCR yield on Miniamp Cyclers.