Real-time RT-PCR (TaqMan™) protocol – Mayaro virus (MAYV)

1. Master mix

Component	Volume per reaction	Volume for 10 reactions	Volume for 50 reactions
RNase/DNase-free water	6.4 µl *	64 µl *	320 µl *
reaction buffer (2x)	12.5 µl *	125 µl *	625 µl *
primer 1 (100 µM)	0.25 µl	2.5 µl	12.5 µl
primer 2 (100 µM)	0.25 µl	2.5 µl	12.5 µl
probe (25 µM)	0.15 µl	1.5 µl	7.5 µl
enzyme	0.5 µl *	5 µl *	25 µl *
Total per reaction	**20 µl**		

2. RNA

Add **5 µl** of RNA to 20 µl of master mix.
Include positive and negative **controls** to evaluate the validity of the run.

3. Cycling conditions

1 cycle:
50°C for 30 min * (reverse transcription)
95°C for 2 min * (DNA polymerase activation, "hot start")
45 PCR cycles:
95°C for 15 seconds
60°C for 1 min

4. Interpretation

positivity: Ct value ≤ 38
assay **validity**: positive and negative controls should show the expected results

5. Primers and probes

The use of the following primers and TaqMan™ probe is recommended:

Long *et al.*, *Am J Trop Med Hyg* **85**, 750-7 (2011)	
forward primer	5′-GTGGTCGCACAGTGAATCTTTC
probe	5′-FAM-ATGGTGGTAGGCTATCCGACAGGTC-TAMRA
reverse primer	5′-CAAATGTCCACCAGGCGAAG

* The volumes and times indicated are for the use of the SuperScript™ III Platinum™ One-Step qRT-PCR Kit (Invitrogen, catalog number: 11732-020 or 11732-088) and should be adjusted when other enzymes are used.