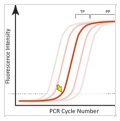

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Working

Mayaro virus real-time RT-PCR - 2016 method

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ABSTRACT

Mayaro virus (MAYV) is an Alphavirus, transmitted by mosquitos in south and central America.
This assay is designed to detect a portion of the nsP1 region of MAYV .

GUIDELINES

- If using a different brand or model of real-time thermocycler, check the concentration of ROX is adequate.
- Method assumes the user is familiar with the thermocycler and software used to run the protocol.

MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
SuperScript™ III Platinum™ One-Step qRT-PCR Kit	11732088	Life Technologies		

Oligonucleotide sequences

1

Name	5'-3'
MAYV2016TM-F	GCATCAGGHGAAGTCGTTG
MAYV2016TM-R	CTGATCTGTGTGAAGGCAAA
MAYV2016TM-P	6FAM-AGACGACCTGCAGTCAGTGATGGC-BHQ1

MAYV oligonucleotides

Reaction set-up

2

- Assay has been used on both a Rotor-Gene 6000 / Rotor-Gene Q 5-plex using 100-place rotor discs.
- Total reaction volume is 20µL.
- Prepare sufficient for number of reaction plus a 'dead volume' usually 2 extra. Adjust as necessary if using a robotic dispenser.

Reagent	Vol (µL) x1	Final reaction concentration
Nuclease free water	4.37	
MAYV2016TM-F (200pmol/ul)	0.09	900nM
MAYV2016TM-R (200pmol/ul)	0.07	700nM
MAYV2016TM-P (100pmol/ul)	0.03	150nM
2 X Reaction mix*	10	1X
Superscipt III/Platinum Taq enzyme mix*	0.4	
ROX reference dye (25uM)*	0.04	50nM
TOTAL VOLUME	**15**	

*Superscript®III Platinum® One-Step qRT-PCR kit

Dispense 15µL to each reaction well.
Add 5µL of template, extracted DNA, controls or NTC (nuclease-free water).
Total reaction volume is 20µL

Amplification

Amplification

3 The assay has been optimised and validated for the Rotor-Gene 6000 and Rotor-Gene Q thermocyclers.

PCR

1 cycle	40 cycles
50°C 5min	95°C 3 seconds
95°C 2min	60°C 30 seconds*

*Florescence acquisition step

Result Analysis

4 The definition used for a satisfactory positive result from a real-time fluorogenic PCR should include each of the following:

1. A **sigmoidal curve** – the trace travels horizontally, curves upward, continues in an exponential rise and followed by a curve towards a horizontal plateau phase
2. A **suitable level of fluorescence** intensity as measured in comparison to a positive control (y-axis)
3. A defined threshold (C_T) value which the fluorescent curve has clearly exceeded (Fig.1 arrow) and which sits early in the log-linear phase and is <40 cycles
4. A flat or non-sigmoidal curve or a curve that crosses the threshold with a C_T value >40 cycles is considered a negative result
5. NTCs should not produce a curve

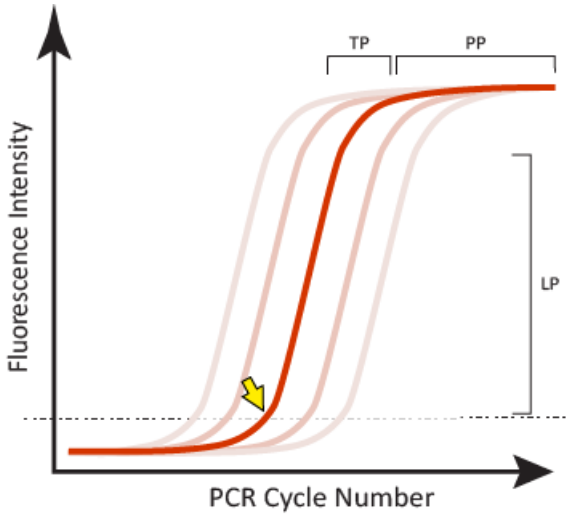

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary) threshold level as shown in this normalized linear scale depiction. LP-log-linear phase of signal generated during the exponential part of the PCR amplification; TP-a slowing of the amplification and accompanying fluorescence signal marks the transition phase; PP-the plateau phase is reached when there is little or no increase in fluorescent signal despite continued cycling.