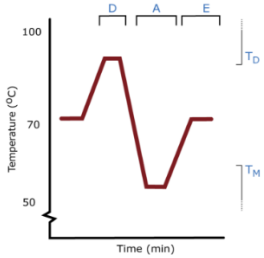
Oct 17, 2019 Version 3

Measles virus TaqMan RT-PCR (F gene; no longer in regular use; see Guidelines) V.3



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Manuscript citation:
Smith G. (2010) Measles Virus. In: Schuller M., Sloots T., James G., Halliday C., Carter I. (eds) PCR for Clinical Microbiology. Springer, Dordrecht

Protocol status: Working
We use this protocol and it's working

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Abstract

- This real-time TaqMan-MGB RT-PCR protocol aimed to amplify measles virus (MeV) strains and not other viruses.
- Michael Lyon and Mitchell Finger designed the assay in 2009 using Primer Express software.
- The method was later published by Greg Smith in 2010 (see below).
- The assay targets the fusion (F) gene region and is designed as a qualitative test for investigating MeV infection of humans.
- This was a past assay that we no longer in use. For our favoured Measles virus TaqMan test, please refer to the *MeV N TaqMan* protocol.

Materials

STEP MATERIALS

SuperScript™ III Platinum™ One-Step qRT-PCR Kit **Life Technologies Catalog #**11732088

Protocol materials

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Before start

- 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 and with PCR in general.

Oligonucleotide sequences

1

	Name	Sequence 5'-3'
	Primer Measles MGB FP	GCTCAAATTGCTCAGATACTATACAGAAA
	Primer Measles MGB RP	GCAGATATGGGGTCCCGTAA
	Probe Measles MGB Probe	FAM - CCTGTCATTATTTGGCC - MGBNFQ

FP-forward primer; MGB-minor groove binder; NFQ-non-fluorescent quencher; RP-reverse primer

Reagents

2

SuperScript™ III Platinum™ One-Step qRT-PCR Kit **Life Technologies Catalog #**11732088

Reaction set-up

3 The assay has been used on both a Rotor-Gene 6000 and a Rotor-Gene Q real-time thermocycler

Prepare sufficient mix for the number of reactions.
Include a suitable 'dead volume' as necessary if using a robotic dispenser.

	Reagent	Volume (µl) x1	Final reaction concentration
	Nuclease-free water	4.45	N/A
	Measles MGB FP 150pmol/µl	0.04	300nM
	Measles MGB RP 150pmol/µl	0.04	300nM
	Measles MGB Probe 100pmol/µl	0.03	155nM
	2X Reaction Mix[1]	10	1X
	SuperScript® III/Platinum® *Taq* Mix[1]	0.4	1X
	ROX Reference Dye (25µM)	0.04	0.05µM
	Template	5	N/A
	TOTAL	20	

1-Superscript™ III Platinum™ One-step qRT-PCR kit; MGB-minor groove binder

- Dispense 15µL to each reaction well.

- Add 5µL of template (extracted RNA, controls or NTC [nuclease-free water]).
- Total reaction volume is 20µL

Amplification

4

	50°C	5min	1X
	95°C	2min	1X
	95°C	3sec	40X
	60°C	30sec[1]	

1-Fluorescence acquisition step

Result Analysis

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

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

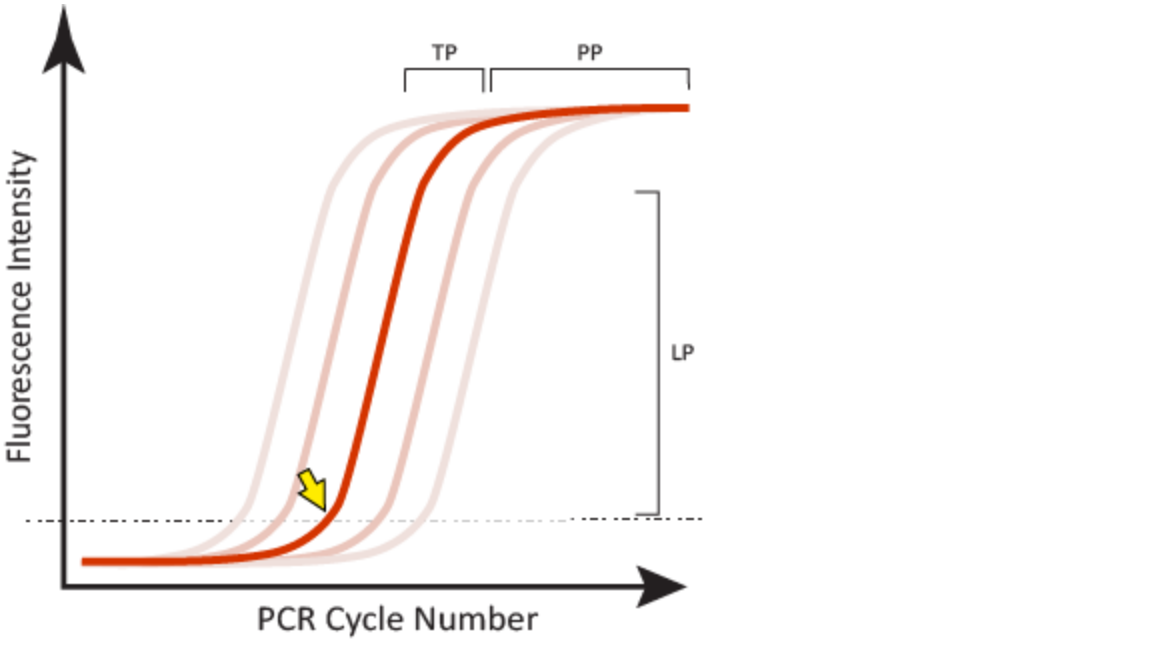

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary) 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.