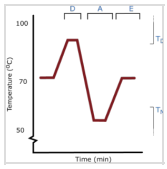

Working

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Rubella virus real-time RT-PCR

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[1]Public Health Virology, Forensic and Scientific Services

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ABSTRACT

A real-time assay for the detection of Rubella virus RNA from clinical samples. This assay was modified from a published method with oligonucleotides updated, a different kit used, and cycling times adjusted accordingly.

EXTERNAL LINK

https://doi.org/10.1016/j.jviromet.2008.01.032

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Hubschen et al. J Virol Methods, 2008. 149:246-250

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

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 TEXT

Oligonucleotide sequences

1

Name	5'-3'
RubV TAQMAN FWD 2015	TGATACCCAGACCTGTDTTCAC
RubV TAQMAN REV 2015	GGTCGATGAGGACGTGTAGG
RubV TAQMAN PRB 2015	6FAM - GATCACCCAGCACTCCACGCAA – BHQ-1

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	4.35	
RubV TAQMAN FWD 2015 200pmol/µl	0.09	900nM
RubV TAQMAN REV 2015 200pmol/µl	0.09	900nM
RubV TAQMAN PRB 2015 100pmol/µl	0.03	150nM

2X Reaction Mix	10	1X
SuperScript® III/Platinum® Taq Mix	0.4	1X
ROX Reference Dye (25µM)	0.04	50nM
TOTAL VOLUME	**15**	

[1]SuperScript® III Platinum® One-Step qRT-PCR® ™ Kit, Cat No. 11732088

NOTE

REAGENT
SuperScript ™ III Platinum ™ One-Step qRT-PCR Kit
by Life Technologies
Catalog #: 11732088

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

Amplification

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

PCR

50°C	5min	1X
95°C	2min	1X
95°C	3s	40X
60°C	30sec*	

*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

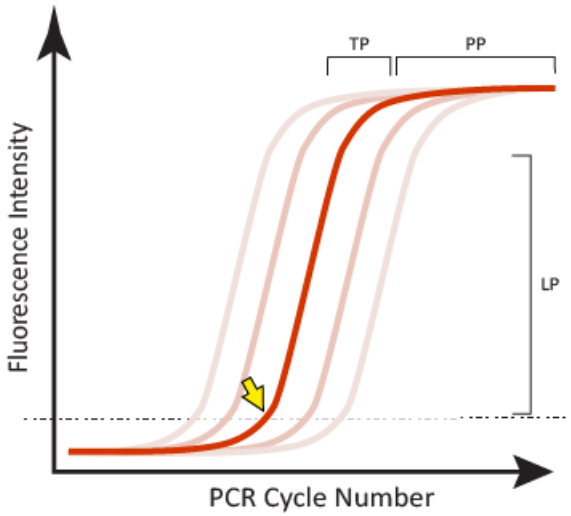

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary)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