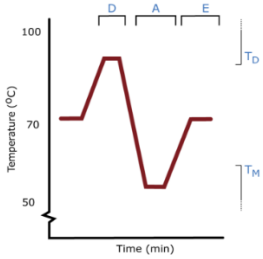
Jul 10, 2018

Dengue virus type 2 (DENV-2) capsid-Thai TaqMan assay (no longer in use; see Guidelines)



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Ian M Mackay[1], Judy A Northill[1], Alyssa Pyke[1]

[1]Public Health Virology, Forensic and Scientific Services

Public Health Virology, F...

Ian M Mackay
Public Health Virology, Forensic and Scientific Services

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Manuscript citation:
Development and Evaluation of Serotype- and Group-Specific Fluorogenic Reverse Transcriptase PCR (TaqMan) Assays for Dengue Virus
http://jcm.asm.org/content/39/11/4119.abstract

Protocol status: Other
Archived former assay.

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Protocol Integer ID: 13176

Abstract

This protocol was designed and developed at this laboratory and incorporates a previously published oligoprobe (see below).
The protocol specifically aims to amplify DENV02 viruses and not other dengue viruses. The assay targets the capsid region and is designed as a qualitative test for investigating suspected human cases of DENV-2 infections.
This assay has been superseded by the **Dengue virus type 2 (DENV-2) MGB TaqMan (DENV2-2016MGB) assay.**

Guidelines

This was a past assay that we no longer in use.
For the best DENV-2 TaqMan assay, please refer to our recommended protocol:
Dengue virus type 2 (DENV-2) MGB TaqMan (DENV2-2016MGB) assay
https://www.protocols.io/view/dengue-virus-type-2-denv-2-mgb-taqman-denv2-2016mg-n7kdhkw

Materials

MATERIALS

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

STEP MATERIALS

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

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'
	D2TaqC(b)-f[1]	TTCATGGCCCT**K**GTGGC
	D2Cor05r[1]	CCCCATCT**Y**TT**Y**A**R**TATCCCTG
	D2TaqCor-FAM[2]	*FAM* - TCCTTCGTTTCCTAACAATCC– *TAMRA*

1

Designed by Alyssa Pyke, Public Health Virology, 2005;

2

Previously published, Callahan
et al
.
http://jcm.asm.org/content/39/11/4119.abstract

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	4.44	N/A
	D2TaqC(b)-f 150pmol/µl	0.04	300nM
	D2Cor05r 150pmol/µl	0.04	300nM
	D2TaqCor-FAM 75pmol/µl	0.04	150nM
	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 TM III Platinum TM One-step qRT-PCR kit

- 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

Florescence 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:

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), 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 >40 cycles is considered a negative result.
5. NTCs should not produce a curve

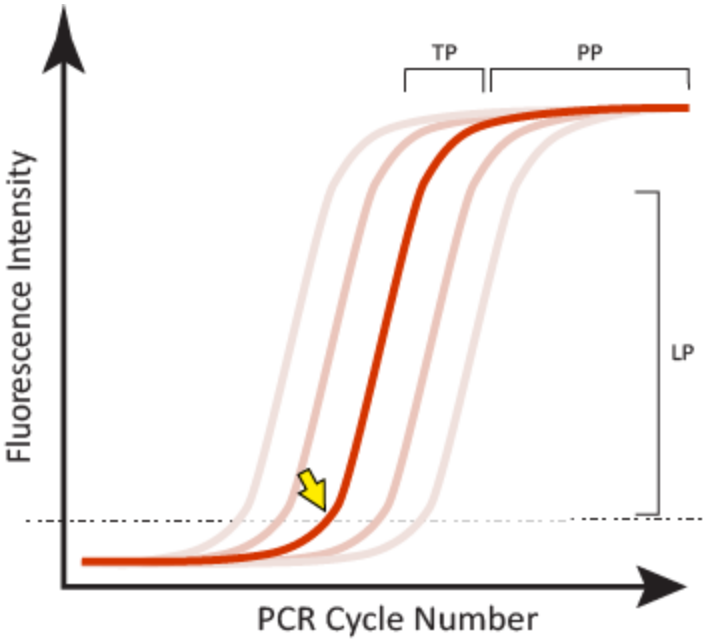

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary)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