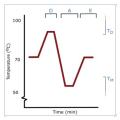

Nipah virus real-time RT-PCR (NiV-TM2018)

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ABSTRACT

The protocol aims to specifically amplify Nipah viruses (NiV) and not other viruses.
This is a modified version of a published assay.
Modifications were to account for mismatches underneath oligo target sites.
The assay targets the nucleoprotein (N) gene region and is designed as a qualitative test for investigating NiV infection of humans.

EXTERNAL LINK

https://doi.org/10.3201/eid1802.111492

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

STEPS MATERIALS

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

BEFORE STARTING

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'
NiV-N-TM2018_For	CTGGTCTCTGCAGTTATCACCATCGA
NiV-N-TM2018_Rev	ACGTA**Y**TTAGCCCATCTTCTAGTTTCA
NiV-N-TM2018_Prb	FAM – CAGCTCC**M**GACACTGCCGAGGA– BHQ1

Assay modified from Lo et al., Characterization of Nipah Virus from Outbreaks in Bangladesh, 2008–2010 , Emerg Infect Dis. 2012 Feb; 18(2): 248–255., by IanM, June 2018

MODIFICATIONS TO THE PUBLISHED ASSAY:

1. Forward primer is unmodified from that described in the original paper

2. Reverse primer has a degeneracy added to the published version
3. Probe has a degenercay added and the 3' terminal T removed, compared to the published version
4. Conditions, concentrations and reagents used all differ from those originally published
5. Addition of *in vitro* transcribed synthetic template oligonucleotide controls (PRIMER and PROBE controls)

THE DESIGN PHILOSOPHY:

- to ensure our assay detects old and new variants of NiV

Reagents

2

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

Reaction set-up

3 The assay has been used on a Rotor-Gene 6000 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	4.39	N/A
NiV-N-TM2018_For 200pmol/µl	0.05	500nM
NiV-N-TM2018_Rev 200pmol/µl	0.09	900nM
NiV-N-TM2018_Prb 100pmol/µl	0.03	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	

- 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

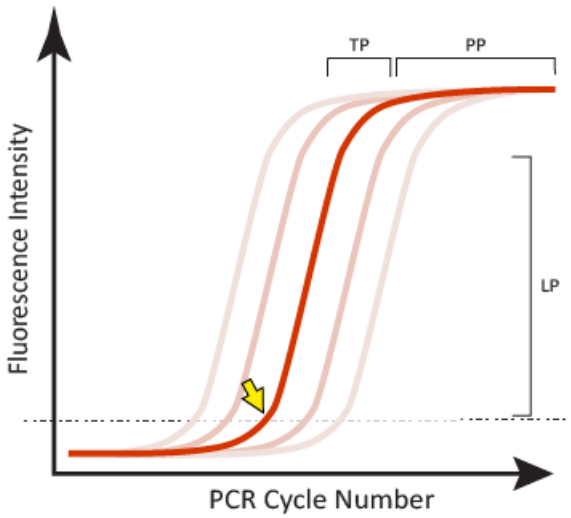

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary)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