Influenza B virus VICTORIA lineage TaqMan 2018 / FluB-VICT-TM2018 Version 2

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Abstract

This protocol was designed and developed at this laboratory.

The protocol specifically aims to amplify strains of Influenza B VICTORIA virus lineage and not strains of the YAMAGATA virus lineage or other virus species. The assay targets the haemagglutinin (HA) region and is designed as a qualitative lineage-typing test for human cases of seasonal influenza virus type B infections.

FluB-VICT-TM2018 is ideally used alongside its companion protocol, 'Influenza B virus YAMAGATA lineage TaqMan 2018' (FluB-YAMA-TM2018), which aims to target influenza B virus YAMAGATA lineage strains exclusively. The two assays perform best as UNIPLEX protocols; a drop in sensitivity was observed when combined in a DUPLEX format.

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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.

Materials

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

Protocol

Oligonucleotide sequences

Step 1.

Name	Sequence 5'-3'
FluB-HA-lineageF	ACCAG**R**GGGAAACTATGCCC
FluB-HA-lineageR1	CC**D**GATGT**R**A**Y**AGG**YY**TG**R**C**Y**T
FluB-HA-lineageR2	CCGGATGT**D**ACAGGT**Y**TGAC**Y**T
FluB-HA-lineageR3	CCAGATGTAA**Y**AGGTCT**K**A**Y**TT
FluB-HA-lineageR4	CC**D**GATGTAACAGGTCTG**R**C**Y**T

FluB-HA-Vict-Probe	*CY5* - CAGACCAAAATGCAC**R**GGGAA**H**ATACC– *BHQ-3*

Reagents

Step 2.

READAGENTS

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

Reaction set-up

Step 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.

MIX PREPARATION

Reagent	Volume (µl) x1	Final reaction concentration
Nuclease-free water	3.46	N/A
FluB-HA-lineageF 200pmol/µl	0.07	700nM
FluB-HA-lineageR1 200pmol/µl	0.05	500nM
FluB-HA-lineageR2 200pmol/µl	0.05	500nM
FluB-HA-lineageR3 200pmol/µl	0.05	500nM
FluB-HA-lineageR4 200pmol/µl	0.05	500nM
FluB-HA-Vict-Probe 100pmol/µl	0.03	150nM
2X Reaction Mix[1]	10	1X
$MgSO_4$ 50mM	0.8	5mM
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

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

Amplification

Step 4.

CYCLING CONDITIONS

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

[1]Florescence acquisition step

Result Analysis

Step 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

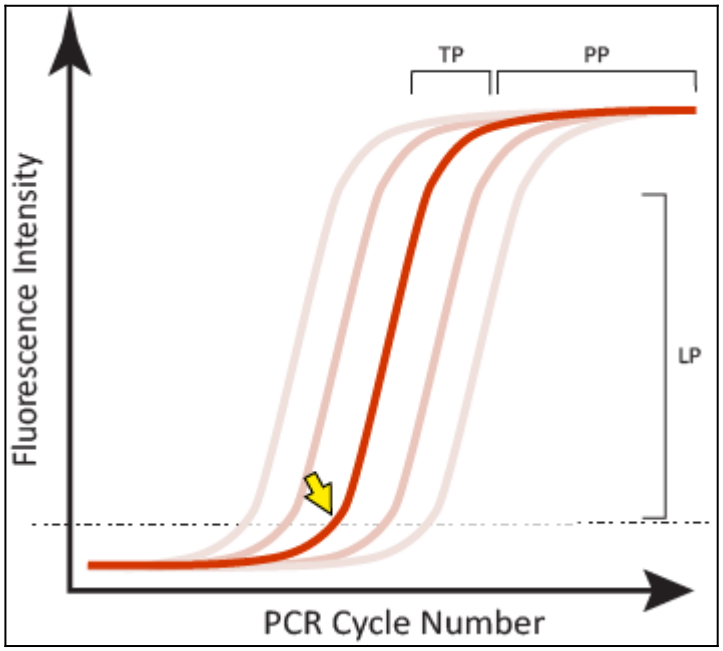

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary)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