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Version 3

Influenza A H3 virus TaqMan assay V.3

DOI

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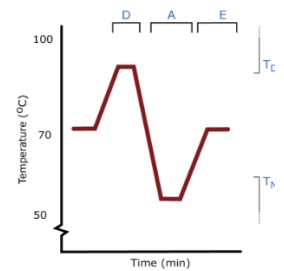
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Protocol status: Working

We use this protocol and it's working

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Keywords: World Health Organization, h3 virus taqman, h3 taqman, influenza virus, influenza, hemagglutinin, molecular diagnosis, who information for molecular diagnosis, world health organization, test, modification to the world health organization, gene,

Abstract

This test is a modification to the World Health Organization's influenza A H3 TaqMan documented in 'WHO information for molecular diagnosis of influenza virus - update 1' (see file below). New primers were added and the WHO primers discarded.

The test targets the hemagglutinin (HA) gene.

Attachments



[Influenza_WHO_update..](#)

1.1MB

Materials

STEP MATERIALS

 SuperScript™ III Platinum™ One-Step qRT-PCR Kit [Life Technologies Catalog #11732088](#)

Protocol materials

 SuperScript™ III Platinum™ One-Step qRT-PCR Kit [Life Technologies Catalog #11732088](#)

Before start

- If using a different brand or model of real-time thermocycler, check that the concentration of ROX is adequate.
- Method assumes the user is familiar with the thermocycler and software used to run the protocol.

Oligonucleotides

1

Name	5'-3' SEQUENCE
H3hFor1	GGTACGGYTTTCAGGCAT
H3hRev1	TCAATCTGATGGAATTTCTCGTTG
H3h-1144dProbe	6FAM-CTGCTGCTTGTCTCTTCCCT-BHQ1

- The oligoprobe is from the World Health Organization protocol linked below.
- New primers were designed to improve assay performance.
http://www.who.int/entity/influenza/gisrs_laboratory/molecular_diagnosis_influenza_virus_humans_update_201403rev201505.pdf?ua=1

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	Vol (μL) X1	Final reaction concentration
Nuclease-free water	4.43	
H3hFor1 (200pmol/μL)	0.05	500nM
H3hRev1 (200pmol/μL)	0.05	500nM
H3h-1144dProbe (100pmol/μL)	0.03	150nM
2X Reaction Mix ¹	10.0	1X
ROX reference Dye (25μM) ^{1,2}	0.04	50nM
SuperScript™ III/Platinum™ Taq Mix ¹	0.4	
TOTAL	15	

1-Superscript™III Platinum™ One-step qRT-PCR kit; 2-See Guidelines



- 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 RT-PCR

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

*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) 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

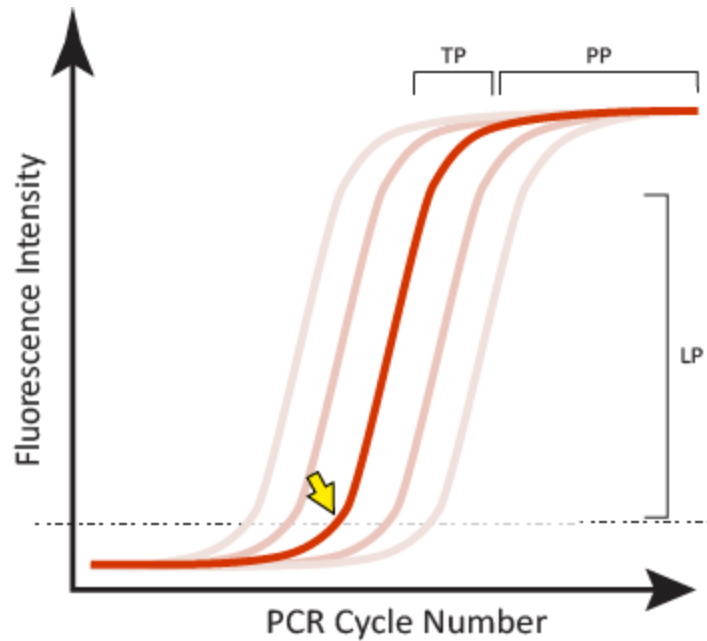


Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary)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