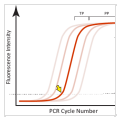

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Aichivirus real-time RT-PCR 2007 method

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1 Works for me dx.doi.org/10.17504/protocols.io.z3tf8nn

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ABSTRACT

A real-time assay to detect *Aichivirus A* (AiV-A) in human samples. It is based in the 3'UTR region and has been employed in our laboratory since 2007.

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.

STEPS MATERIALS

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

1 Oligonucleotide sequences

Name	5'-3'
AiV-F-8046	TGCTTCGGCACGCTTAGTT
AiV-R-8151	TGCARTACAACCAYGGCTTAGG
AiV-8082-FAM	6FAM-CACTCCTCCATGGTGATATAAAGACCAC-TAMRA

Aichivirus oligonucleotides

2 Reagents

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

3 Reaction Set-up

- Assay has been used on both a Rotor-Gene 6000 / Rotor-Gene Q 5-plex using 100-place rotor discs and a ABI 7500 Fast real-time machine.
- 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	4.41	
Primer AiV-F-8046 (100pmol/ul)	0.06	300nM
Primer AiV-R-8151 (100pmol/ul)	0.06	300nM
Probe AiV-8082-FAM (100pmol/ul)	0.03	150nM
2 X Reaction mix*	10.0	1X
ROX reference dye (25µM)*	0.04	50nM
Superscript III/Platinum Taq enzyme mix*	0.4	
TOTAL	**15**	

*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**

4 Amplification

1 cycle	40 cycles
50°C 5 minutes	95°C 3 seconds
95°C 2 minutes	60°C 30 seconds*

*fluorescence aquistion step

5 Result analysis

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

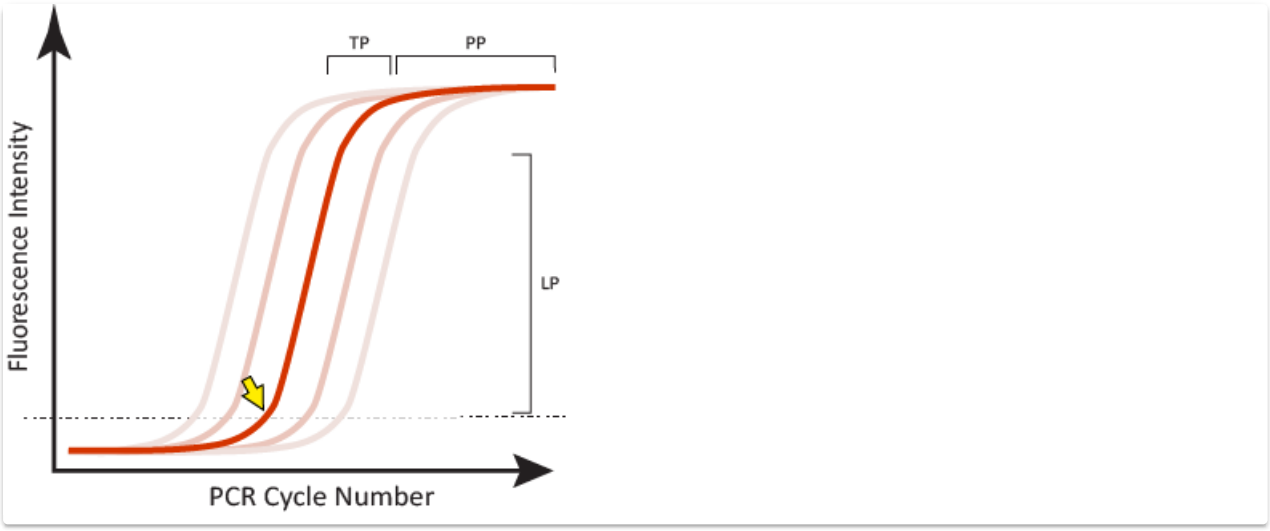

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary)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