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1. Molluscum contagiosum virus detection by real-time PCR
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Molluscum contagiosum real-time PCR V.1

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ABSTRACT

A real-time PCR method for the detection of Molluscum contagiosum virus from human samples. This assay targets the MC021L gene and detects both subtype 1 and 2 of the virus.

ATTACHMENTS

[AVG2009_Poster_JNorthill.pdf](#)

MATERIALS

STEP MATERIALS

⊗ TaqMan™ Fast Universal PCR Master Mix (2X) **Applied Biosystems (ThermoFisher Scientific) Catalog #4352042**

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BEFORE START INSTRUCTIONS

- 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.

PROTOCOL integer ID:
8562

Keywords: real-time, MCV,
molluscum, PCR

Oligonucleotide sequences

1

Name	5'-3'
MCVp43kF (forward primer)	GCTCACGTACGACTGCTTYGAC
MCVp43kR (reverse primer)	CGTGGAGCGCAGATTGC
MCVp43kP (probe)	6FAM-CGCTCATCTCGCAGAC-MGB

Reaction set-up

2

Assay has been used on both a Rotor-Gene 6000 / Rotor-Gene Q 5-plex using 100-place rotor discs and an ABI 7500 using 96-well plates.

Total reaction volume is 20µL and is suitable for both formats.

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	4.91	
MCVp43kF 200pmol/µL	0.03	300nM
MCVp43kR 200pmol/µL	0.03	300nM
MCVp43kP 100pmol/µL	0.03	150nM
¹ TaqMan™ Fast Universal PCR Master Mix (2X)	10	1X
Template	5	

1

Thermofisher product

[4352042](#)

Dispense 15µL to each reaction well.

Add 5µL of template, extracted DNA, controls or NTC (nuclease-free water).

Total reaction volume is 20µL

Amplification

- 3 The assay has been optimised and validated for the ABI 7500. It also is used on the Rotor-Gene 6000 and Rotor-Gene Q thermocyclers.

PCR

5
0
°
C
5
m
in
9
5
°
C
2
m
in
9
5
°
C
3
s
4
0
X
6
0
°
C
3
0
s
*

*Florescence acquisition step

Result analysis

- 4 The threshold should be placed in the exponential range above any background noise within the assay.

A positive result is one where the C_T is <40 and produces a sigmoidal curve.
NTC should not produce a curve and should be greater than $40C_T$.