VERSION 2

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MANUSCRIPT CITATION:

1. Molluscum contagiosum virus detection by real-time PCR
J Northill, R Simmons
5th Australian Virology Group Meeting, Mantra Erskine House, Lorne, 13-17 December, 2009.

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Protocol status: Working
We use this protocol in our group and it is working.

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Molluscum contagiosum virus real-time PCR V.2

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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_AbstractAVG2009.pdf](#)

MATERIALS

MATERIALS

SensiFAST™ Probe Lo-ROX Kit **Bioline Catalog #BIO-84002**

STEP MATERIALS

SensiFAST™ Probe Lo-ROX Kit **Bioline Catalog #BIO-84002**

SensiFAST™ Probe Lo-ROX Kit **Bioline Catalog #BIO-84002**

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:
8595

Keywords: real-time, MCV,
molluscum, PCR, TaqMan,
Rotorgene

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 SensiFast Probe Lo-ROX mix (2X)	10	1X
TOTAL VOLUME	15	

1

Bioline product
[BIO-84005](#)

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

SensiFAST™ Probe Lo-ROX Kit **Bioline Catalog #BIO-84002**

Amplification

- 3 The assay has been optimised and validated for 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 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) and 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 value >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

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.