

Supplemental Data

Supplementary Table 1: Primers and Taqman hydrolysis probe used for ANDV detection

Oligonucleotide name	Oligonucleotide sequence	Sense*	Localization	Tm
AF004660				
ANDV-Forward	GCAGCTGTGTCTACATTGGAGAC	S	187-209	57.3°C
ANDV-Reverse	GCTCCTATAGCCTTCCAATCAGC	A	413-391	58.7°C
Taqman Probe	FAM-ACAAAACCAGTTGATCCA-MGBNFQ	S	268-285	66.5°C

* S= sense, A= antisense

Supplementary Table 2 Reaction conditions for ANDV RT-qPCR assays.

	Two-step RT-qPCR		One-step RT-PCR	
Platforms	480 LightCycler	LightCycler 2.0	480 LightCycler	LightCycler 2.0
Reaction volume	10 µL	10 µL	10 µL	10 µL
Reverse Transcriptase	MMLV enzyme ¹	MMLV enzyme ¹	Tth DNA Polymerase ²	Tth DNA Polymerase ²
PCR kit	LightCycler TaqMan Master kit ²	LightCycler TaqMan Master kit ²	LightCycler 480 RNA Master Hydrolysis Probe ²	LightCycler 480 RNA Master Hydrolysis Probe ²
Primer (final concentration)	0,3 mM	0,3 mM	0,3 mM	0,3 mM
MGB probe (final concentration)	0,3 mM	0,3 mM	0,3 mM	0,3 mM

Master Mix (final concentration)	1X	1X	1X	1X
Mn(OAc)₂	∅	∅	3,25 mM	3,25 mM
Template	1 µL cDNA	1 µL cDNA	1 µL RNA	1 µL RNA
Cycling conditions				
	Two-Step RT-qPCR		One-Step RT-qPCR	
Reverse Transcription		72°C, 10min 4°C, 5 min 42°C, 1h 70°C, 15 min 4°C, ∞		63°C, 3 min
Time Real PCR	Denaturation	95°C, 10 min, Ramp 20°C/seg, Fluorescence Acquisition: none	Denaturation	95°C, 10 min, Ramp 20°C/seg, Fluorescence Acquisition: none
40 repeats	Amplification	95°C, 10 seg, Ramp 20°C/seg, Fluorescence Acquisition: none	Amplification	95°C, 10 seg, Ramp 20°C/seg, Fluorescence Acquisition: none
	Annealing	60°C, 30 seg, Ramp 20°C/seg, Fluorescence Acquisition: none	Annealing	60°C, 30 seg, Ramp 20°C/seg, Fluorescence Acquisition: none
	Extension	72°C, 1 seg, Ramp 20°C/seg, Fluorescence Acquisition: single	Extension	72°C, 1 seg, Ramp 20°C/seg, Fluorescence Acquisition: single

¹Invitrogen, CA, USA.

² Roche Appl Biosc, IN, USA

Supplementary Table 3: Efficiency for one step and two step reactions in two real time platforms

Platform	LC 2.0			LC 480	
Assay	RNA one-step	cDNA two-step	plasmid two-step	RNA one-step	cDNA two-step
Efficiency (%)	82.7	91.8	97.3	63.6	93.5