

Protocol for the detection of Andes virus by real-time RT-PCR

1. Extraction

External lysis (BSL-3): 400 µl of sample and 1 ml of lysis buffer (NUCLISENS® easyMAG® Lysis Buffer, Biomérieux).

Internal control: 20 µl of CDV (canine distemper virus).

EMag extraction according to manufacturer's instruction with elution volume of 50 µl.

2. Real-time RT-PCR

| Target | Primers/probe | Sequences (5'-3') | Final conc. (nM). |
|--------|----------------------|---|-------------------|
| Andes | ANDV F (forward) | GCAGCTGTGTCTACATTGGAGAC | 900 |
| | ANDV R (reverse) | GTCCTATAGCCTTCCAATCAGC | 900 |
| | ANDV TMGB1 (probe 1) | FAM-ACAAAACCAGTTGATCCA-NFQ MGB | 200 |
| | ANDV TMGB2 (probe 2) | FAM-ATCCAACAGGGCTTGAG-NFQ MGB | 200 |
| CDV | CDV F (forward) | GCTACCCAAGAAACCGTCATTG | 900 |
| | CDV R (reverse) | GCATGGCAGGGACGAGTT | 900 |
| | CDV P (probe) | VIC-CGTTCCAGGGAGTCCAGGACTACGTCAAC-TAMRA | 200 |

Positive control: Plasmide Andes Thermo Fisher Scientific (pYES2)

Andes:

12.5 µl 2X RT-PCR Buffer (Applied Biosystems, ref: AM1005M)

1 µl 25X RT-PCR Enzyme Mix (Applied Biosystems, ref: AM1005M)

1.5 µl primers and probes Andes

10 µl sample or control

CDV:

12.5 µl 2X RT-PCR Buffer (Applied Biosystems, ref: AM1005M)

1 µl 25X RT-PCR Enzyme Mix (Applied Biosystems, ref: AM1005M)

1.5 µl primers and probes CDV

10 µl sample or control

Cycles:

50°C 15 min

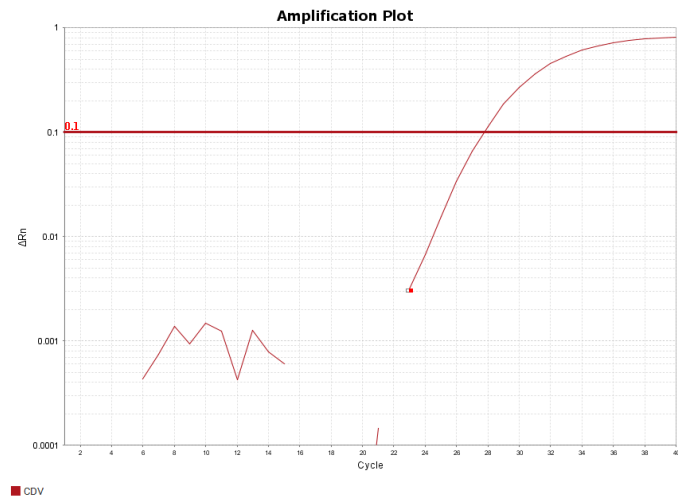
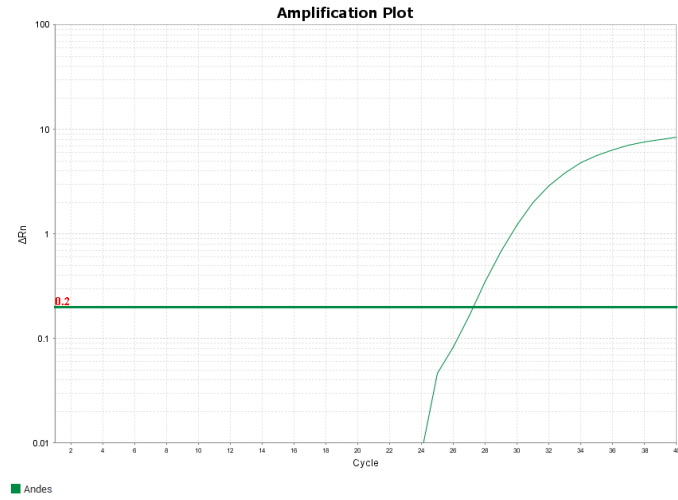
95°C 10 min

40 cycles:

95°C 8 sec

60°C 34 sec

3. Results (QuantStudio5 with thresholds: 0.2 for Andes and 0.1 for CDV)



4. Reference for Andes PCR: Kramski, Marit, et al. "Detection and typing of human pathogenic hantaviruses by real-time reverse transcription-PCR and pyrosequencing." *Clinical chemistry* 53.11 (2007): 1899-1905.