ANDV specific PCR (PHAS)_V1 (2026-05-15)

Extraction:

Sample Inactivation (done at BSL-3 at PHAS, each laboratory should conduct their own risk assessment):

Mix 200 µl of sample and 600 µl of TRIzol reagent and vortex thoroughly to ensure complete mixing. Incubate at room temperature for 5 minutes.

Phase Separation (BSL-2):

1. Add 160 µl Chloroform, vortex thoroughly to ensure complete mixing and incubate at room temperature for 3 minutes.
2. Centrifuge the tube at 4°C 12000 x g (RCF) for 15 minutes.
3. After Centrifugation, a phase separation will form.
4. Transfer 200 µl of the upper aqueous phase to a new tube without disturbing the interphase.
5. Continue with automated MagLEAD Extraction or use QIAamp Viral Mini kit for manual extraction according to manufacturer's instruction.

Automated MagLEAD Extraction:

According to manufacturer's instruction with elution volume of 50 µl.

One step Real-time RT-PCR for detection of Andes virus:

The ANDV RT-PCR assay is carried out in 25 µL reaction mixtures containing TaqMan Fast Virus 1-step Master Mix (Applied Biosystems, Thermo Fisher Scientific), 5 µL template RNA, DNase/RNase-free H2O (Life Technologies, Thermo Fisher Scientific), 0.9 µM of each primer, and total 0.2 µM of two TaqMan probes (0,18 µM ANDV_P and 0,02 µM ANDV_P2) (Applied Biosystems).

Amplification and detection of the amplicon is performed in a StepOne Plus real-time PCR system (Applied Biosystems).

To ensure adequate RNA extraction, the presence of Beta-actin mRNA in clinical samples is analysed using a TaqMan gene expression assay (Applied Biosystems).

Primer and probe sequences:

Target	**Sequence (5'–3')**
ANDV_F	[GCTACTRCTGCRAAAGCTGGA]
MPRLV_F	[GCTACTGCTACAAAAGCTGTG]
ANDV_R	[GCTGYTGTTCGTGTGCDGTGAT]
ANDV_P	[FAM-ATGAGCAMCCTCCAAGAA-MGB (NFQ)]
ANDV_P2	[FAM-ATGAGCAMCCTCCAGGAA-MGB (NFQ)]

Reaction mixture:

Master mix for 25 µL reaction	Final konc.	µL /reaction
TaqMan FAST Virus 1-step MM (4x)	1x	6,25
ANDV_F (10 µM)	0,9 µM	2,25
MPRLV_F (10 µM)	0,9 µM	2,25
ANDV_R (10 µM)	0,9 µM	2,25
ANDV_P (10 µM)	0,18 µM	0,45
ANDV_P2 (10 µM)	0,02 µM	0,05
H_2O	NA	6,5
Volyme		20
RNA-template		5

Positive control: Andes virus RNA (5 µl/PCR reaction)

The cycling profile:

50°C for 5 min; 95°C for 20 s; 45 cycles of 95°C for 3 s and 60°C for 30 s.

Samples were considered positive if target amplification was recorded within 40 cycles (cycle threshold (Cq) ≤ 40). The baseline and threshold were set using the auto-baseline and auto-threshold features in the StepOne software (Applied Biosystems).

Performance

Detection limit is not established. The ANDV S real-time RT-PCR has been evaluated with:

- Negative serum (n = 20)
- Negative EDTA whole blood (n = 9)
- Serum from Puumala virus-positive serum (n = 6)
- RNA extracted from Puumala virus, Hantaan virus, Seoul virus, Dobrava virus, Sin Nombre virus, Tula virus, and Prospect Hill virus.

None of the above resulted in non-specific amplification. The ANDV S assay has also been evaluated with previously confirmed ANDV-positive patient samples from Argentina.