Protocol: Isolation and Cultivation of Andesvirus (ANDV) from Clinical Material Using Vero-E6 Cells

Authors

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Biosafety

All procedures involving ANDV propagation must be conducted in a certified Biosafety Level 3 (BSL-3) laboratory, following institutional and national biosafety guidelines.

Materials and Reagents

- Vero-E6 cells (ATCC® CRL-1586™)
- Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% fetal bovine serum (FBS), 1% Penicillin-Streptomycin (Pen-Strep)
- Clinical samples **PCR positive and serologically negative** for ANDV
- Phosphate-buffered saline (PBS), pH 7.4
- Trypsin-EDTA (0.05%)
- 6-well cell culture plates
- T25 cell culture flasks
- Lysis buffer containing Equine Arteritis Virus (EAV) for internal PCR control
- Sterile pipettes and filter tips
- Personal protective equipment (PPE) appropriate for BSL-3

Day –1: Cell Seeding

1. Harvest and count Vero-E6 cells to obtain a single-cell suspension.
2. Seed cells into a sterile 6-well plate at a density of 1.5×10^5 cells/mL, adding 3 mL of the cell suspension per well.
3. Incubate the plate overnight at 37°C with 5% CO_2 to allow cells to reach approximately 80% confluency.

Day 0: Inoculation with Clinical Material

1. Confirm that the Vero-E6 monolayers are ~80% confluent.
2. Aspirate and discard the culture medium from all 6 wells.
3. Designate wells as follows:
    - Two ANDV PCR positive clinical samples, each inoculated in duplicate wells (total: 4 wells).
    - One uninfected cell control, also in duplicate (total: 2 wells).
4. Add 150 µL of ANDV-positive clinical sample to each of the 4 designated wells.
5. Add 600 µL of DMEM with 5% FBS and 1% Pen-Strep to each inoculated well, ensuring the cell layer is fully covered.
6. Add 750 µL of DMEM with 5% FBS and 1% Pen-Strep to each control well.
7. Incubate the plate for 1 hour at 37°C with 5% CO_2 to allow viral adsorption.
8. After incubation, add 2.25 mL of DMEM with 5% FBS and 1% Pen-Strep to each well, resulting in a final volume of 3 mL per well.
9. Immediately following medium addition, collect a 200 µL aliquot from each well and store at –80°C or as required. This aliquot is used to determine the initial viral load (Ct-value) at the start of culture via PCR.

Routine Maintenance and Sampling

Medium Change and Sampling (Weekly, e.g., DPI 7, 21, etc.)

1. Collect aliquots from each well as follows:
    - 1 mL for long-term storage at –80°C.
    - A separate aliquot of 200 uL for PCR analysis: transfer directly to 270 uL lysis buffer containing EAV internal control.
2. Remove the remaining supernatant from the wells and discard according to BSL-3 waste protocols.
3. Add 3 mL of fresh DMEM with 5% FBS, 1% Pen-Strep to each well and return the plate to the incubator.

Cell Passage (Every Two Weeks, e.g., DPI 14, 28, etc.)

1. Gently mix and collect supernatant as described above (1 mL for storage, 200 uL aliquot for PCR).
2. Remove any remaining supernatant from each well.
3. Wash each well once with PBS (add sufficient PBS to cover the cell layer, gently rock the plate).
4. Remove the PBS completely.
5. Add 0.5 mL trypsin-EDTA (0.05%) to each well.
6. Incubate at room temperature until the cells visibly detach from the surface (monitor microscopically).
7. Add 2,5 mL of DMEM with 5% FBS and 1% Pen-Strep to each well to neutralize the trypsin and resuspend the cells by gentle pipetting.
8. For further culturing:
    - If passaging to a new 6-well plate: transfer 1 mL of the cell suspension into a new well and add 2 mL DMEM with 5% FBS and 1% Pen-Strep (final volume: 3 mL per well).
    - If passaging to a T25 flask: transfer 1 mL of the cell suspension into a T25 flask and add 4 mL DMEM with 5% FBS and 1% Pen-Strep (final volume: 5 mL per flask).
9. Incubate the new cultures at 37°C with 5% CO_2.

Table 1. Example timeline for the isolation and cultivation of Andes Hantavirus (ANDV) on Vero-E6 cells.

Time Point	Date	DPI	Procedure
Day -1	28-05-2026	—	Seed Vero-E6 cells (3 mL per well in 6-well plate)
Day 0	29-05-2026	0	Inoculate with clinical sample, incubate for 1 hour, adjust to 3 mL, collect baseline aliquot (Ct-value)
Day 7	05-06-2026	7	Sampling (supernatant for storage and PCR), medium change
Day 14	12-06-2026	14	Sampling (supernatant for storage and PCR), **Cell passage** (split 1:3 to new plate/flask), medium change
Day 21	19-06-2026	21	Sampling (supernatant for storage and PCR), medium change
Day 28	26-06-2026	28	Sampling (supernatant for storage and PCR), **Cell passage** (split 1:3 to new plate/flask), medium change
Day 35	03-07-2026	35	Sampling (supernatant for storage and PCR), medium change
...	...	...	Weekly: sampling and medium change; every two weeks: cell passage

Abbreviations:

- DPI = Days Post Infection;
- Sampling = 1 mL supernatant for storage, separate aliquot for PCR;
- Medium change = replace with fresh DMEM + 5% FBS;
- Cell passage = split cells 1:3 as described in the protocol.

Protocol adapted from: Coelho R, Kehl S, Periolo N, Biondo E, Alonso D, Perez C, et al. (2025) Virological ocharacterization of a new isolated strain of Andes virus involved in the recent person to-person transmission outbreak reported in Argentina. PLoS Negl Trop Dis 19(6): e0013205. https://doi.org/10.1371/journal.pntd.0013205.