Isolation of West Nile virus on Vero cell lines

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JAN 31, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.14egn2e7pg5d/v1

Protocol Citation: Anna Nagy 2023. Isolation of West Nile virus on Vero cell lines. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egn2e7pg5d/v1

Protocol status: Working
We use this protocol and it's working

Created: Jan 31, 2023

Last Modified: Jan 31, 2023

PROTOCOL integer ID:
76151

ABSTRACT

West Nile virus (WNV), a member of the family *Flaviviridae*, genus *Flavivirus* is a mosquito-borne emerging pathogen, which is endemic in most part of Europe, especially in the central and southern regions of the continent. The vast majority of human infections remains asymptomatic, though approximately 20% of infected individuals develops milder, influenza-like disease and less than 1% has severe neurological manifestation. Besides the application of serological and molecular diagnostic techniques, virus isolation and molecular typing of virus isolates are the most important reference laboratory activities. Here we summarize a brief description of our protocol for WNV isolation on cell cultures.

WNV isolation from PCR positive clinical samples:

1 **Cell lines: Vero or Vero E6**, that are suitable for West Nile virus. Cell culture should be grown in T25 or T75 cell culture flasks.

- **Cell culture medium: DMEM** (Dulbecco's Modified Eagle Medium) high (4,5 g/L) glucose with L-Glutamine 1000L (Lonza; Catalog #: BE15-604K;).
- **5% Foetal Bovine Serum (FBS)** (Merck; Catalog F7524)
- **Antibiotic: Cell Culture Guard**, in a dilution 1:100 (ITW Reagents; Catalog A8906,0050)
- Incubator: 37°C, 5% CO_2 concentration.

Note: Prior to the virus isolation, cells should be seeded. After 24-48 hours later, when the cell monolayers are at 80-90% confluence, cell cultures can be used for virus inoculation.

2 **Samples:** PCR positive clinical specimens: serum, plasma or urine.
Note: Urine samples must be filtered on 0.22 µm pore size membrane filters and treated with 0.01 M TRIS buffer to adjust the pH to 7.2 to 7.8 prior inoculation.

3 Add 1 - 2 ml sample to a T75 cell culture flask or 0.5 ml sample to a T25 flask. Incubate at 37°C in 5% CO_2 concentration for **90 minutes.**

Note: if the sample volume is insuffiecient, complete the volume with cell culture medium.

4 After the incubation, **do not remove the virus inoculum**. Add cell culture medium (DMEM) with 5% FBS and cell culture guard in a dilution 1:100.

5 **Incubate at 37°C in 5% CO_2 concentration for up to 7 days**. Daily monitoring of the presence of any cytophatic effects is recommended.

6 **At day 7** cells should be harvested. A blind passage is recommended, using both the cell culture supernatant and harvested cells.

7 Increased viral load should be checked by real-time RT-PCR method. Ct values of the original clinical sample and cell culture supernatant should be compared.