



Title: WNV Envelope-Peptide ELISA

No: RTLP-GL-EL-14

Location:
Old CCRC Tripp Lab

Approval Date:
10 September 2004

Supersedes Date:

Materials:

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- | | | | |
|-----------------------|---------------------------|--------------------|----------------------|
| •Lab coat | •Phosphate Buffered | •Acetone | •Pipettes |
| •Gloves | Saline (PBS) | •dH ₂ O | •Pipetteman |
| •Antisera (see below) | •DMEM | •Methanol | •Pipette Aid |
| •Sigmafast pNpp | •37°C, 5% CO ₂ | •Tween-20 | •Pipetteman tips |
| Alkaline-Phosphatase | incubator | •2M NaOH | •Powerblock solution |
| Substrate | •goat α-rabbit IgG | •Vero Cells | •Spectrophotometer |
| | alkaline-phosphatase | •96-well tissue | |
| | •Centrifuge | culture plate | |

Reagents and Solutions:

Antisera:

aMPV/N affinity purified Rabbit antibodies

Wash Buffer:

PBS + 0.5% Tween-20

Procedure:

1. Split Vero cells into a 96-well tissue culture plate,
NOTE: Adjust cell numbers to bring plate to 90% confluency within 24 hours.
Make sure you design plate for triplicate testing
2. Once plate is 90% confluent, gently wash cells with PBS.
3. Perform ten-fold serial dilutions of stock hMPV to be tested in serum-free DMEM.
4. Add diluted virus 10⁻¹ to 10⁻¹⁰ in triplicate to washed Vero cells. Do not add virus to rows A and H, which will serve as negative controls.
5. Incubate at 37°C, 5% CO₂ for 72 hours.
6. 72 hours post-infection (hpi), remove tissue culture supernatant and fix cells with Acetone:Methanol (60:40).

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7. Wash wells 3 times with wash buffer.
8. Block wells with 1x Powerblock for 5 minutes at 37°C.
9. Wash wells 3 times with wash buffer.
10. Dilute 1° Antibody (aMPV/N peptide Ab) 1:500 in wash buffer and add 100 µl/well.
11. Incubate at 37°C for 1 hour.
12. Wash wells 3 times with wash buffer.
13. Make a 1:1000 dilution of 2° Antibody (Goat Anti-Rabbit IgG Alkaline-Phosphatase Conjugate) in wash buffer and add 100 µl/well.
14. Incubate at 37° C for 1 hour.
15. Wash wells 3 times with wash buffer.
16. Develop Reaction 200 µl/well Sigmafast pNpp Alkaline-Phosphatase Substrate.
17. Incubate at room temperature for 10-15 minutes.
18. Stop reaction by adding 50 µl/well 2M NaOH.
19. Measure Absorbance at 405 nm by spectrophotometry.
20. Plot Absorbance vs Virus dilution. Compare to control graph. Determine OD value and correlation to Titer value.