



Title: *Detection of Human Metopneumovirus (HMPV) by ELISA*

No: RTLP-GLP-HMPV-2

Location:
Old CCRC Tripp Lab

Approval Date:
10 September 2004

Supersedes Date:

Materials:

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|----------------------|--|---------------------------|------------------|
| •Lab coat | •Phosphate Buffered | •4°C cold storage | •Pipettes |
| •Gloves | Saline (PBS) | •Sigmafast pNpp | •Pipetteman |
| •HMPV Antigen | •Tween-20 | substrate | •Pipette Aid |
| • NaHCO ₃ | •2° Ab: goat α-mouse | •37°C, 5% CO ₂ | •Pipetteman tips |
| •Dry non-fat milk | | incubator | |
| •1° Antibodies | hybridomas)-alkaline
phosphatase-conjugated | | |

Procedure:

1. Mix your antigen (HMPV infected LLCMK2 or HMPV infected Veros 1:1 with DPBS. Plate 6-10 ug/well of antigen. Protein determination of antigen can be determined by BCA assay from Pierce.
2. Add 100 µl/well and incubate at 37°C for 1 hour or overnight at 4°C.
3. Wash wells 3 X with PBS/0.5% Tween 20.
4. Block with 5% dry milk/PBS for 1 hour at 37°C or overnight at 4°C.
5. Add primary antibody (This can be a positive control, test sera, or hybridoma supernatant) to wells at appropriate dilution. For HMPV positive control use rabbit Pub anti-HMPV G peptide antibody. Negative control can be either normal rabbit sera, no primary or an isotype control. Use 1:500 dilution for positive and negative control; start with undiluted (“Neat”) with hybridoma supernatants.
6. Incubate at 37°C for 1 hour or overnight at 4°C.
7. Wash wells 3 X with PBS/0.5% Tween 20.

8. Add secondary antibody. For positive control, PaB anti-HMPV G peptide antibody, use Goat -anti Rabbit IgG whole molecule alkaline phosphatase conjugated. Use at 1:1000 diluted in 0.5% milk 100 µl/well. (Use Goat Anti-mouse G whole molecule alkaline phosphatase conjugated for hybridomas or test sera.
9. Incubate at 37°C for 1 hour or overnight at 4°C.
10. Wash 3 X with PBS/0.5% Tween 20
11. Make up the pNpp (Sigmafast) substrate accordingly. Need 20 ml/plate.
12. Add 200 µl of substrate/well and incubate for 10-15 minutes based on reaction and background.
13. Measure absorbance at OD 405/495.