



Description of Methods for use of Antiviral Antibodies in Indirect Immunofluorescence

REAGENTS

1. Acetone-fixed cells infected with appropriate virus (positive control).
2. Acetone-fixed uninfected cells (negative control).
3. Appropriate antibody at dilutions for titration.
4. Secondary FITC-conjugated antibody eg Fluorescein Anti-Mouse IgG (H + L), made in horse, Cat. No. FI-2000 and VECTASHIELD® Mounting Medium (H-1000).
5. Cells to test.

EQUIPMENT

Fluorescence microscope, dark humid slide incubation tray, 37 °C incubator.

PROCEDURES

1. Allow slides to reach 25 °C before starting.
2. Apply antibody at appropriate dilution (20µl/spot).
3. Incubate for 30 minutes at 37 °C in a dark, humid slide incubation tray.
4. Rinse 3 x 5 minutes in phosphate buffered saline (pH7.4).
5. Air dry.
6. Apply FI-2000 (1:300 dilution in buffer).
7. Incubate for 30 minutes at 37 °C in a dark, humid slide incubation tray.
8. Rinse 3 x 5 minutes in phosphate buffered saline (pH7.4).
9. Apply H-1000 and coverslip.
10. Read under oil using 50x oil objective on fluorescence microscope.