Indirect Fluorescent antibody test (IFAT)

1. Place the antigen slides on the rack and let them dry.

2. Fix them with 4% Paraformaldehyde for 5 minutes and dry.

4% Paraformaldehyde

15 ml PBS – heat to about 55^{o}C.

0.75 g paraformaldehyde to PBS

0.1 N NaOH (Break Paraformaldehyde to formaldehyde)

Cool and adjust pH

Make up to 25 ml

3. Block them with PBS + 1% BSA for 10 minutes in a humidified chamber.

4. Wash the with TBST (TBS+0.02% Tween 20)

TBS (10X -1L)

Sodium chloride - 90 g; Tris base - 12.12 g

Make up to one liter

5. Using PBS + 1% BSA as a diluent, dilute the sera in 1.5 ml micro centrifuge tube (1:50, 1:100, and 1:200). Need to standardize while doing first time. Dilutions depend upon the concentrations of stock.

5. Place the diluted serum on antigen slides and leave in a damp chamber (humidified Chamber) for 40-60 minutes at 37°C.

6. Wash the slides three times with TBST (or TBS + 0.02% Tween 20) on a shaker for 5 minutes.

8. Air-dry the slides (no need to dry them completely)

9. Using PBS + 1% BSA as a diluents, dilute the secondary antibody with FITC conjugate in 1.5 ml micro centrifuge tube The dilutions (1:100, 1:200, and 1:400). Need to standardize while doing first time. Dilutions depend upon the concentrations of stock.

9. Incubate the slides with the conjugate. This step should be done in dark place, cover the slides with box with aluminum foil.
10. Wash 3 times as before.
11. Without drying the slides, add one or 2 drops of Fluoromount-G and put cover glass, careful not to get any air bubble in between.
12. Observe the slides under a fluorescence microscope (X200-400).

Counter stain

Evans Blue

Evans Blue can be added to the conjugate for counter stain.

FITC conjugate 50 µl,

1% Evans Blue 30 µl

PBS 920 µl

Incubate for 30-60 minutes at 37°C in dark.

Methyl Green Solution (counter stain for Immunohistochemistry)

Methyl Green Solution (0.5%)

Methyl green - 0.5 g

0.1M Sodium acetate buffer, pH4.2 - 100 ml

Mix to dissolve.

0.1M Sodium Acetate Buffer, pH4.2:

Sodium acetate, trihydrate (MW 136.1) - - 1.36 g

Distilled water - 100 ml

Mix to dissolve and adjust pH to 4.2 using concentrated glacial acetic acid

Staining Procedure

1. Wash the sections with distilled water
2. Stain in methyl green solution for 10 minutes at room temperature
3. Rinse in distilled water (sections will look blue).
4. Dehydrate quickly through 95% alcohol (10 dips, sections turn green), 2 changes of 100% alcohol (10 dips each) (alcohol used for dehydration removes some of the stain).
5. Clear in xylene
6. Mount with DPX mounting medium.