TITLE: Direct immunofluorescence staining on formalin-fixed paraffin sections (Manual)

PURPOSE: Direct immunofluorescence (IF) on frozen tissue is the method of choice for the study of medical renal diseases. When no glomeruli are available, IF can be performed on the formalin-fixed paraffin-embedded tissue allocated for light microscopy after antigen retrieval with proteases.

SPECIMEN: Formalin-fixed paraffin-embedded renal biopsy specimen

MATERIAL: Pronase (for enzyme digestion) (Streptomyces griseus)
FITC–conjugated polyclonal rabbit antihuman antibodies to IgG, IgM, IgA, C3, C1q, kappa and lambda light chains

EQUIPMENT: Staining chamber

METHOD:
1. Cut 3 micron serial sections on organosilane-coated slides.
2. Oven dry for at 37º C overnight (or at 60 ºC for 15 mins).
3. Deparaffinize: Xylene x 10 mins (x 2), Ethanol 100% x 5 mins (x 2), 95% x 5 mins.
4. Fast wash in distilled water (20 dips)
5. Rinse in Tris buffer pH 7.6 at 37º C x 30 mins.
6. Incubate with pronase solution (Streptomyces griseus 75 mg/100 ml of Tris buffer) at 37º C x 60 mins
7. Stop enzymatic digestion with Tris buffer at 4º C x 40 mins.
8. Rinse in PBS x 10 mins
9. Incubate in a wet chamber at 4º C x 30 mins with FITC–conjugated polyclonal rabbit antibodies directed against:
   - IgG 1:20 F0202
   - IgM 1:10 F0203
   - IgA 1:10 F0204
   - C3 1:10 F0201
   - C1q 1:10 F0254
   - Kappa light chain 1:8 F0198
   - Lambda light chain 1:10 F0199
   (Dako North America)
10. Rinse with PBS 4º C x 10 mins (x 2)
12. Examine slides under a dark field ultraviolet (immunofluorescence) microscope.

MOUNTING MEDIA: Special (non-fading) medium for immunofluorescence

MICROSCOPE: Dark-field U-V light fluorescence microscope

NOTES:

Courtesy of Vivette D’Agati, Columbia University, New York, NY