Title: Phospholipase A2 receptor staining on paraffin embedded tissue with routine immunofluorescence

Purpose: For determination of primary versus secondary membranous glomerulopathy

Specimen: Formalin-fixed paraffin embedded renal biopsy tissue

Materials:
- Proteinase K (Dako Ready-to-use, S3020)
- Rabbit polyclonal anti-PLA2R1 antibody (Sigma-Aldrich, HPA012657 SIGMA)
- Alexa Fluor 488 goat anti-rabbit IgG (Life Technologies)

Method:
1. Cut 3-μm-thick section from formalin-fixed paraffin-embedded tissue.
2. Deparaffinization: Heat in oven for 12 minutes at 60 degrees. Xylene (5 min x 2), 100% ethanol (5 min), 95% ethanol (5 min), and then a fast clean in distilled water.
3. Enzyme pretreatment with protease K (Dako), 30 min at room temperature.
4. PBS rinse.
5. Rabbit polyclonal anti-PLA2R1 antibody (Sigma-Aldrich), 1:50 (diluted in PBS), 30 min at room temperature.
6. PBS rinse.
7. Alexa Fluor 488 goat anti-rabbit IgG (Life Technologies), 1:100 (diluted in PBS), 30 min at room temperature.
8. PBS rinse
9. Cover slip with aqueous mounting media (Dako)
10. Examine slides with a dark field ultraviolet light (immunofluorescence microscope)

Mounting media: Special (non-fading) aqueous mounting medium for Immunofluorescence (Dako)

Microscope: Dark field U-V light fluorescence microscope

Interpretation:
Positive membranous glomerulopathy cases will have a pattern of staining identical to the IgG. While most cases show strong staining, it is interpreted as either positive or negative with positive staining representing PLA2R-associated membranous glomerulopathy.

Notes:
Tissue staining for PLA2R1 in glomeruli has been shown to have a sensitivity of 75% (95% CI 65-84%) and a specificity of 83% (95% CI 72-90%) for primary membranous glomerulopathy.

This stain does not require confocal microscopy. It is extremely important to run a negative control (secondary antibody only) with every case. On rare cases there will see a blush of staining in the glomeruli (trace) which usually indicates staining by the secondary antibody only. When this is the case, there will also be a blush of staining in the negative control which matches what you see in the PLA2R stain. We find that there is more background staining when used on fresh frozen tissue making the test much more difficult to interpret and therefore would not recommend it.
Reference:

Courtesy of: Dr. Christopher P. Larsen, Nephropath, Arkansas