PROTOCOL FOR PERIODIC ACID SCHIIF (PAS) STAINING GLOMERULAR MESANGIAL MATRIX QUANTIFICATION

Version: 1
Replaced by version: N/A
Edited by: Jeff Hodgin, MD PhD and previous Brosius lab member (Jharna Saha).

Summary: Periodic acid-Schiff (PAS) is a staining method used to detect glycogen on formalin-fixed, paraffin-embedded kidney tissue sections. PAS staining highlights basement membranes and is frequently used to diagnose glomerular mesangial matrix expansion.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Staining Jars	Fisher	22038493
Periodic acid	Sigma	P7875-25G
Schiff's reagent	Sigma-Aldrich	3952016-500ML
Gill 2 Hematoxylin	Richard-Allan Scientific	72511
Xylene	Fisher Scientific	X3P-1GAL
Ethanol (EtOH) 200 Proof	Decon-Laboratories Inc	2701
Tissue sections	N/A	N/A
Mounting Medium	Thermo Scientific	8312-4
Cover Glass	Fisher scientific	12-542-B
Universal Imaging MetaMorph® Imaging System	Molecular Devices	N/A
Scientific grade digital color CCD camera	RT SLIDER DIAGNOSTIC	N/A
Microscope and Lense	Leica DM IRB	N/A

Protocol:

WARNING HAZARDOUS CONDITION WARNED AGAINST. This comment describes a hazardous condition to which the technician may be exposed in the performance of this protocol. It also contains directions on how to avoid or minimize the danger. Warnings are always and only used for personnel safety, and precedes the first step that will expose the technician to the hazard.

Protocol 1: PERIODIC ACID SCHIFF (PAS) STAINING

1. Wash 2x 4 minutes in Xylene.
2. Wash in 100% EtOH 2x minutes
3. Wash in 95% EtOH 1x 2minutes.
4. Wash in 70% EtOH 1x 2minutes
5. Rinse in dH_2O.
6. Incubate in 0.5% Periodic Acid solution for 5min.
7. Rinse 3x in dH_2O.
8. Incubate in Schiff's reagent for 15minutes
9. Rinse under running lukewarm tap water for 5minutes.
10. Incubate in Hemotoxylin for 90 second.
11. Rinse 6x in dH_2O.
12. Wash in 70% EtOH 1x 2minutes.
13. Wash in 95% EtOH 1x 2minutes.
14. Wash in 100% EtOH 2x 2minutes.
15. Incubate in Xylene for at least 5minutes.
16. Mount slides with mounting medium 1 drop
17. Insert cover glass carefully, avoid bubble

Note: Schiff is light sensitive, mutagenic and has bad smell. *Use fume hood or flow hood for handling* Schiff's reagent.

Protocol 2: MESANGIAL MATRIX QUANTIFICATION

Pre-Operating Instructions:
Camera and Microscope should be calibrated and values loaded into MetaMorph® Program

1. Using the camera or MetaMorph® software digitizes 30 cortical glomeruli per case with a 40 X lens. Glomeruli should be chosen for a similar diameter of maximal size. Save images as uncompressed Tiff files.
2. Open the glomerulus Tiff file in MetaMorph®. Scale the image in such a way that the entire glomerulus can be seen on the screen (50-75%).
3. Using the polygon tool carefully outline the glomerular tuft. Double click to close the polygon tool.
4. From the tool bar choose **Measure**, **Calibrate Distances** and in the **Apply** window choose the calibration file for the camera from which the image was taken. Then choose **Apply**.
5. The area of the glomerular tuft can then be calculated by choosing **Measure** from the tool bar and then **Region Measurements**. Record the glomerulus area displayed.
6. To calculate the area that is PAS stained the tuft will have to be removed from the background. Select the outlined tuft and then from the tool bar choose **Edit**, **Duplicate**, **Image**. Close the full size image.
7. Size the edited image to 150 – 200 %.
8. From the tool bar select **Measure** and **Set Color Threshold**.
9. In the **Set Color Threshold** window select **Set By Example**.
10. Using **Display** on the tool bar and **Adjust Digital Contrast** the color brightness and contrast can be adjusted to best suit thresholding the area of PAS stain.
11. Once the image is adjusted use the cursor to choose the area of staining. Continue clicking the cursor over the area until the entire PAS stained area is highlighted. As each pixel is selected every pixel that color is also selected. Care must be taken to ensure that ***only*** PAS stained tissue is highlighted.

    - **NOTE:** If an area is mistakenly selected selecting **Undo Last Click** in the **Set Color Threshold** box will remove the last selection.
    - To clear all selections check the box next to **Reset color threshold range on next click** in the **Set Color Threshold** box. The next pixel chosen in the image will clear the screen. Continue selecting pixels.
    - To toggle between the selected and unselected screen, select **OFF** and **Inclusive** in the **Set Color Threshold** box

12. Once all the PAS stained area is selected choose **Measure** and **Integrated Morphometry Analysis**.
13. In the **Integrated Morphometry Analysis** box under **Set Up Parameters Fo**r: choose **Measuring** and then **Total Area** and then **Classifying** and **Total Area**.
14. Under **Display** choose **Summary**.
15. Under **Show/Log Data** choose **Current**.
16. Now choose **Measure** and the "area of PAS staining" will be the last cell in the **Summary** window under **Total**.
17. To close the **Integrated Morphometry Analysis** box choose **Reset Current** and then **Close.**
18. The percent of the glomerulus that is PAS stained is calculated as:

$$\left(\frac{\textbf{Area of PAS staining}}{\textbf{Total area of glomerulus}} \right) \textbf{X 100}$$

Reagent Preparation: N/A