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## Podocyte Counting and Density Analysis V.2

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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

### Summary:

This protocol describes the procedures for quantifying the podocyte count and density in the glomerulus.

### Diabetic Complication:



Nephropathy

## Materials

### Equipment:

- Universal Imaging Metamorph Imaging System (Molecular Device).
- Scientific grade digital color CCD camera (RT SLIDER DIAGNOSTIC).
- Microscope (Leica DM IRB)

## Procedure:

- 1
  - 1) Stain the Perfusion-fixed paraffin embedded sections (3.9 and 10.2 microns thick) with WT-1 antibody and immunoperoxidase and then take photograph at 40x by using Spot Advanced Software Camera.
  - 2) Photograph 50 consecutive glomerular cross-sections moving systematically from outer cortex to inner cortex and back so as to provide an equal sample from all cortical regions. Use phase contrast which gives podocyte nuclei golden color that makes them easier to count.
  - 3) From these photomicrographs (which contain 1-3 glomeruli per photograph in about 30 photographs), measure the glomerular area by using Metamorph Image Analysis Software (version 6.1) and count the podocytes in 50 sequential glomerular cross-sections at two thicknesses (3 and 9 microns).
  - 4) Measure the glomerular area by using Metamorph software (camera and microscope should be calibrated and values should be loaded into Metamorph program before outlining the glomerular tuft area). Click on the desktop icon (metamorph software icon) to open the metamorph program, and then open the images. From the menu bar, select **Measure**, then select **Calibrate Distance**. A calibration window will appear. Highlight the **40x** calibration and then click on **Apply**. Use **Polygon tool** from menu bar for outlining the glomerular tuft area. From the menu bar, select **Measure** and then **Region Measurement** to the measurement of the glomerular tuft area.
  - 5) Use a systematic method (large size cut-off) which helps to count WT-1 positive podocyte nuclei but eliminates false counting of granules. From the menu bar, choose **Measure**, then **Manually Count Objects**. Select the number **6**. Use this size restriction method to check if the number 6 from the Metamorph Image Analysis System fits within the nuclear profile. If so, count it. If the number 6 was larger than the WT-1 positive area, do not count it.

## Data Analysis:

- 2
  - 1) Count the podocyte numbers (P) and measure the glomerular tuft area (GA) from 100 consecutive cross sections per animal (50 from thick and 50 from thin section) then calculate the average for each set of 50.
  - 2) Divide the average podocyte number (P) by the average glomerular area (GA) to get podocyte number per glomerular area (P/GA) for both 3 and 9 micron sections.

3) The difference between the P/GA of the thick and the P/GA of the thin sections yielded the P/GA  $\Delta$  which is directly related to the actual difference in section thickness (6.3 $\mu$ m).

4) Calculate the average glomerular volume per podocyte (GV/P) by dividing the actual section thickness of 6.3 by the P/GA $\Delta$ .

5) Determine the glomerular volume by using the Weibel formula First, calculate the glomerular radius of both thick and thin sections by assuming circular cross sections using formula

$$\text{radius } R = (GA/\pi)^{1/2}$$

and then calculate the average radius ( $R_{av}$ ) that yielded the average maximum radius

$$R_{\max} = 4R_{av}/\pi.$$

Then calculate average glomerular volume as

$$GV = 4/3\pi(R_{\max})^3$$

Then divide the average glomerular volume by the average volume per podocyte (GV/P) to get the podocyte number per glomerulus:

$$P = GV/(GV/P)$$