RESEARCH LETTER

Frozen Versus Paraffin-Embedded Tissue for Glomerular Basement Membrane Thickness Measurement

To the Editor:

In kidney pathology, quantification of glomerular basement membrane (GBM) thickness is a useful marker of several kidney diseases. The standard technique to measure GBM thickness is transmission electron microscopy (TEM). In our laboratory, renal cortex is routinely submitted for TEM evaluation in a neutral-buffered formalin solution. Frozen tissue or paraffin-embedded tissue is reclaimed when wet tissue is not available for TEM or when glomeruli cannot be found in the submitted formalin-fixed tissue. It has previously been reported that the diagnostic reliability of deparaffinized formalin-fixed tissue is questionable in cases where GBM thickness is an important factor.[1] Our aim is to quantify the differences in GBM thickness measurements within a case comparing routine TEM formalin-fixed tissue to reclaimed frozen and paraffin-embedded tissue. We will determine if a preferable option of reclaimed tissue exists for collecting measurement data when formalin-fixed tissue is not available.

This study has been approved by the institutional review board at the Ohio State University (IRB 2021H0256). All samples (3 needle biopsies in total) were obtained from kidney tissues submitted for clinical evaluations. The criterion used for selecting these specimens was that all 3 processing types yielded glomeruli with open capillary loops. Although the pathological diagnosis was not considered when selecting cases for this study, the corresponding diagnoses are as follows: sample 1 was moderate chronic kidney injury with patchy interstitial fibrosis and tubular atrophy; sample 2 was mild chronic kidney injury with small subcapsular renal cortical scar; and sample 3 was mild to moderate diabetic nephropathy with diffuse and nodular diabetic glomerulosclerosis. All renal needle biopsies were evaluated utilizing standard processing protocols established within our Renal Pathology lab, including routine TEM, frozen tissue (fresh frozen or tissue transport media) and paraffin reclaimed tissue protocols. The following methods were used:

Routine TEM: Tissue was fixed in 10% formalin overnight, fixed in 3% glutaraldehyde for 45 minutes, washed with .02 M sodium cacodylate buffer, postfixed in 1% osmium tetroxide in sym-collidine buffer, washed with sym-collidine, tertiary fixed in 1% uranyl acetate (aqueous), dehydrated in a graded ethanol series, rinsed in acetone, infiltrated with 2:1 Spurr's resin: acetone overnight and embedded in 100% Spurr's Resin.

Frozen (Fresh Frozen): Tissue was flash frozen in optimal cutting temperature compound. Tissue was removed from the frozen block, placed into 10% formalin for 8-16 hours, and then processed as routine TEM tissue.

Frozen (Tissue Transport Media): Tissue was received in tissue transport media, washed in tissue transport wash solution, and then flash frozen in optimal cutting temperature compound. Tissue was removed from the frozen block, placed into 10% formalin for 8-16 hours and then processed as routine TEM tissue.

Reclaimed Paraffin: Tissue was removed from the paraffin block, placed into 1% osmium tetroxide diluted in toluene overnight, washed with acetone, infiltrated with 2:1 Spurr's Resin: acetone for 8 hours to overnight then embedded in 100% Spurr's Resin.

Semi-thin sections (750 nm) were obtained for each specimen and evaluated for adequate glomeruli. Obvious ice crystal damage was avoided on the reclaimed frozen tissue. Up to 3 glomeruli were examined for each specimen. Ultrathin sections (80-90 nm) were cut for examination on a JEOL JEM-1400 transmission electron microscope. Micrographs were captured using a Veleta digital camera (EMSIS GmbH), and GBMs were

Table 1. Comparison of Routine, TTM-frozen, and Paraffin Kidney Tissue Samples

	Patient 1 – Moderate Chronic Renal Injury			Patient 2 – Mild Chronic Renal Injury			Patient 3 – Diabetic Nephropathy		
	Routine	TTM-frozen	Paraffin	Routine	Fresh frozen	Paraffin	Routine	TTM-frozen	Paraffin
Measurements	282	288	193	483	256	311	356	378	414
Median, nm	384.73	384.33	308.16	289.405	283.11	234.99	660	667.81	529.28
Minimum, nm	175.24	210.65	158.69	136.87	136.87	113.91	235.92	223.39	194.55
Maximum, nm	960.82	873.08	709.34	1124.95	552.63	508.19	1792.86	2557.42	2351.37
Average, nm	404.60	400.90	329.83	301.48	295.57	242.70	690.00	691.51	577.61
Standard deviation, nm	117.20	114.67	100.56	87.08	77.63	64.03	206.84	240.16	240.46
Harmonic mean, nm	375.57	372.92	302.88	280.64	277.06	226.96	634.91	621.58	499.61
Corrected mean, nm	318.35	316.10	256.73	237.88	234.84	192.38	538.17	526.87	423.48
P value (compared to routine)		0.70	<0.0001		0.36	<0.0001		0.93	<0.001

Abbreviation: TTM, tissue transport medium.

measured for all samples by selecting 1 thin section with at least 1 glomerulus at a magnification of 8,000×. The entire visible areas of the glomeruli were imaged. Each image was overlayed with an equidistant 4 × 4 grid pattern. GBM width was estimated by using the orthogonal intercept method.[2] GBM measurements were obtained at each point where a grid line intersected the epithelial side of the GBM, and each measurement was made perpendicular to the GBM ending at the endothelial side of the GBM. The number of measurements taken per glomeruli ranged from 193-483. Statistical analysis was performed by using GraphPad Prism 5.0 software. Differences between the groups were analyzed by 2-tailed *t* test.

The purpose of this study was to quantify measurement differences between processing types, not to shed insight on morphological preservation. The ultrastructural preservation was suboptimal in the frozen and paraffin reclaimed tissue. However, in all specimens, the GBM outer and inner surfaces were easily identified. We assessed routine TEM and reclaimed frozen and paraffin processing types, comparing GBM measurements within each case. All 3 cases showed consistent and striking results. Our results (Table 1) indicate no statistical difference in GBM measurements between frozen-fixed and routinely processed tissue. However, the deparaffinized tissue showed a significant decrease in the mean GBM thickness as compared to routine processed tissue. The P values reported are for reclaimed frozen and reclaimed paraffin as compared to the routine processing measurements. We conclude that when GBM measurements are desired, it is preferable to use frozen-fixed tissue over deparaffinized tissue when routine formalin-fixed tissue is not available. When information other than GBM thickness is needed, the use of either form of reclaimed tissue should be utilized based on the laboratory's experience.

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