Preparing LR White Embedded Tissue with mPrep/s Specimen Capsules

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LR White (LRW) is an acrylic embedding resin with advantages for immuno-labeling, enzyme-chemistry, and cyto-chemistry TEM. Because LRW is hydrophilic, it enables aqueous reagents to diffuse into thin sections without prior etching which can denature antigens. In comparison to epoxies, LRW helps maintain the antigenicity required for immuno-labeling. However, a problem with LRW (and similar acrylics) is that because immuno-labeled tissue is usually not OsO_4 treated to preserve antigenicity, it can be difficult to orient or even find the tissue because some tissues are nearly invisible in resin. In this report, we provide a technique to embed tissue specimens in LRW for TEM using mPrep/s specimen capsules. This solves the tissue orientation and tissue location problem while also minimizing handling and providing rapid preparation.

LRW is a low viscosity resin that must be cured anaerobically. Prior known methods for mPrep/s specimen processing used epoxy resins that have a higher viscosity, and which may be polymerized in air. The low viscosity of LRW, while enabling quick infiltration, presents problems with mPrep/s capsules because resin can leak out when placing mPrep/s capsules into the mPrep silicone bench to seal capsule bottoms. Secondly, the anaerobic polymerization requirement for LRW also presents a problem since mPrep/s capsules do not have an air-tight lid. Herein, we solved both the leakage and sealing problem by modifying an existing method. As shown in Figure 1, mPrep/s capsules were themselves embedded within widely available '00' gelatin capsules, which are often used for LRW preparation. These "gel caps" provide a liquid-tight seal and exclude oxygen during heat polymerization. Moreover, this method enables specimens to be orientated, labeled and processed entirely in mPrep/s capsules.

Protocol: Rat tissues were perfusion fixed in Sorensen's phosphate buffer with 4% paraformaldehyde and 1% glutaraldehyde, while mouse tissue samples were excised and immersed in 4% paraformaldehyde and 0.25% glutaraldehyde. Specimens were then transferred to the EM lab and stored in 1/10 strength fixative at 4 C to maintain antigenicity prior to further processing. Tissue samples were then cut to size (1-2 mm) and oriented in mPrep/s capsules. The mPrep/s capsules were then attached to the ASP-1000 automated specimen processor (ASP). The ASP then automatically exchanged reagents to process specimens through buffer rinses, alcohol dehydration, and LRW infiltration. No OsO_4 post-fixation was used to preserve antigenicity. The total ASP processing time from rinse to resin infiltration was ~30 minutes. After processing, 100% LRW resin was dispensed from the mPrep/s capsules and the capsules were immediately removed from the ASP and placed into gel caps filled with 100% LRW. As the mPrep/s capsules sank into the fresh resin, the specimens were re-infiltrated (Figure 1). Since mPrep/s capsules are just slightly smaller than 00 gel caps, the mPrep/s capsules remain vertically aligned. The mating half of the gel caps were then used to cover the resin filled gel cap bottoms. The covered gel caps with entrapped mPrep/s capsules were then placed in a 60 C oven for overnight polymerization.

Specimens were sectioned directly in the gelatin capsule (holding the mPrep capsule) as shown in Figure 1d. Thin sections (70 nm) were grid stained with 4% uranyl acetate in methanol

followed by Reynolds lead citrate. Micrographs were obtained using a Philips CM120 TEM equipped with a BioSprint camera running AMT Capture Engine V700.

Results and Discussion: Tissues were fully embedded and well preserved. Figure 2 shows liver with densely clustered mitochondria with "white" cristae and membranes, because no OsO_4 post fixation was used. Figure 3 shows well preserved extraocular striated muscle, while Figure 4 shows fully embedded and well preserved dorsal root ganglion nervous tissue.

With this technique, specimens were sectioned directly in gel cap blocks, which in turn held mPrep/s capsules. This protocol enabled tissue orientation to be established after *in situ* aldehyde fixation when the tissue is easily seen and more easily handled then when in resin. This is especially important because non-osmicated tissue can be difficult to find in fluid or polymerized resin. Because the tissue was held in a known centered position in the mPrep/s capsule, this made it easy to find the tissue for sectioning. This therefor lowers the risk of "losing" a nearly invisible tissue sample because it is in an unknown location in a polymerized resin block. Finally, the full reagent protocol following aldehyde fixation required only 30 minutes prior to resin curing.
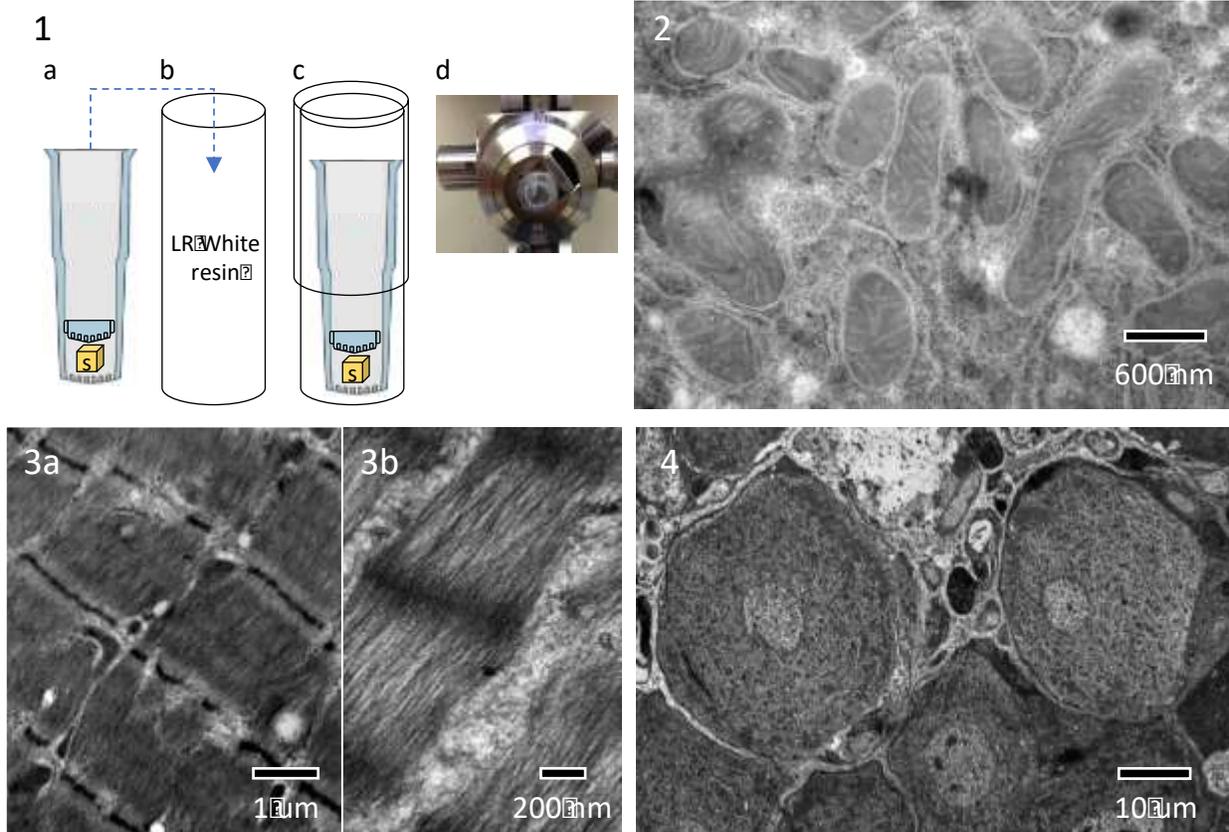

Figure 1: Specimen oriented and entrapped in mPrep/s capsule (a) is placed into 00 size gel cap filled with 100% LRW resin (b). After immersing mPrep capsule in a gel cap, the gel cap is covered with the mating half of the gel cap (c), which is then transferred to a 60 C oven for curing. Gel cap with embedded specimen in mPrep/s capsule is directly clamped in microtome for sectioning (d).
Figure 2: Liver (rat) with mitochondria. Membranes are white due to absence of OsO_4 staining.
Figure 3: Extraocular muscle (mouse) at low (a) and higher (b) resolution.
Figure 4: Dorsal root ganglion nerve tissue (mouse).