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Automated Rapid Preparation of Tissue Specimens for TEM Pathology

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The typical time to prepare clinical tissue specimens for TEM varies from 1-2 days to several hours, with microwave processing heretofore providing the most rapid preparation [1, 2]. Microwave processing is accomplished by the manual exchange of each processing reagent alternated by placing the reagent-immersed specimens into a microwave oven. Following the final resin infiltration step, each tissue samples is then manually transferred to an embedding mold for polymerization. This manual process requires the full attention of the microscopist for the entire protocol followed by an hour to transfer specimens to embedding molds [2]. In the present study, mammalian tissues were automatically prepared for TEM in 2 hours or less (prior to resin curing) with an ASP-1000 Automatic Specimen Processor. Rather than microwaves, the ASP used rapid mixing to accelerate processing by enhancing reagent diffusion. Specimens remained in the same labeled mPrep capsule from initial fixation through sectioning, thus eliminating tedious manual transfers to embedding molds and ensuring continuous specimen labeling.

Mouse tissues were excised, cut into 1-2 mm pieces, inserted into mPrep/s capsules and fixed overnight at 5C in phosphate buffered 4% paraformaldehyde and 1% glutaraldehyde. Rats were perfusion-fixed, tissues were excised and then subsequently handled as per mouse tissues. Specimens in mPrep/s capsules were attached to the ASP and processed with a conventional chemical protocol of 1% phosphate buffered OsO_4, water washes, graded ethanols, acetone, and Embed 812. Rapid processing was achieved with reagent exchanges as fast as to 2 per second, and 600 exchanges for OsO_4 and 180 exchanges for each resin infiltration step. mPrep/s capsules with 100% resin infiltrated specimens were then removed from the ASP for overnight polymerization at 60°C. Following polymerization, capsules were directly mounted in the microtome, thus reducing effort, preserving orientation and maintaining specimen labeling. 70 nm sections were UA and lead stained for imaging at 80 KeV with a Philips CM120.

Kidney was processed in 45 minutes prior to resin curing (Figure 1), while skeletal muscle, liver, brain and heart (Figures 2-5) were processed in 133 minutes. Tissues were well-preserved with no evidence of incomplete processing as seen with TEM or light microscopy (not shown). Consistency was demonstrated with 24 well-prepared liver specimens prepared in 3 ASP processing batches (Figure 3b).

ASP sample preparation speed was comparable to manual microwave preparation but was much easier since processing was entirely automatic. Further, since specimens were never removed from mPrep/s capsules, this eliminated messy, time-consuming handling in resin and the potential for mix-up errors. Although overnight resin polymerization was used in this study, high temperature thermal or microwave curing could further reduce total preparation time. Rapid aldehyde initial fixation can also be done with the ASP when specimens are not provided to the lab already immersed in a primary fixative.

References

[1] Giberson RT *et al*, Ultrastructural Pathology **27** (2003) p. 187.

[2] Schroeder JA *et al*, Micron **37** (2006) p. 577.

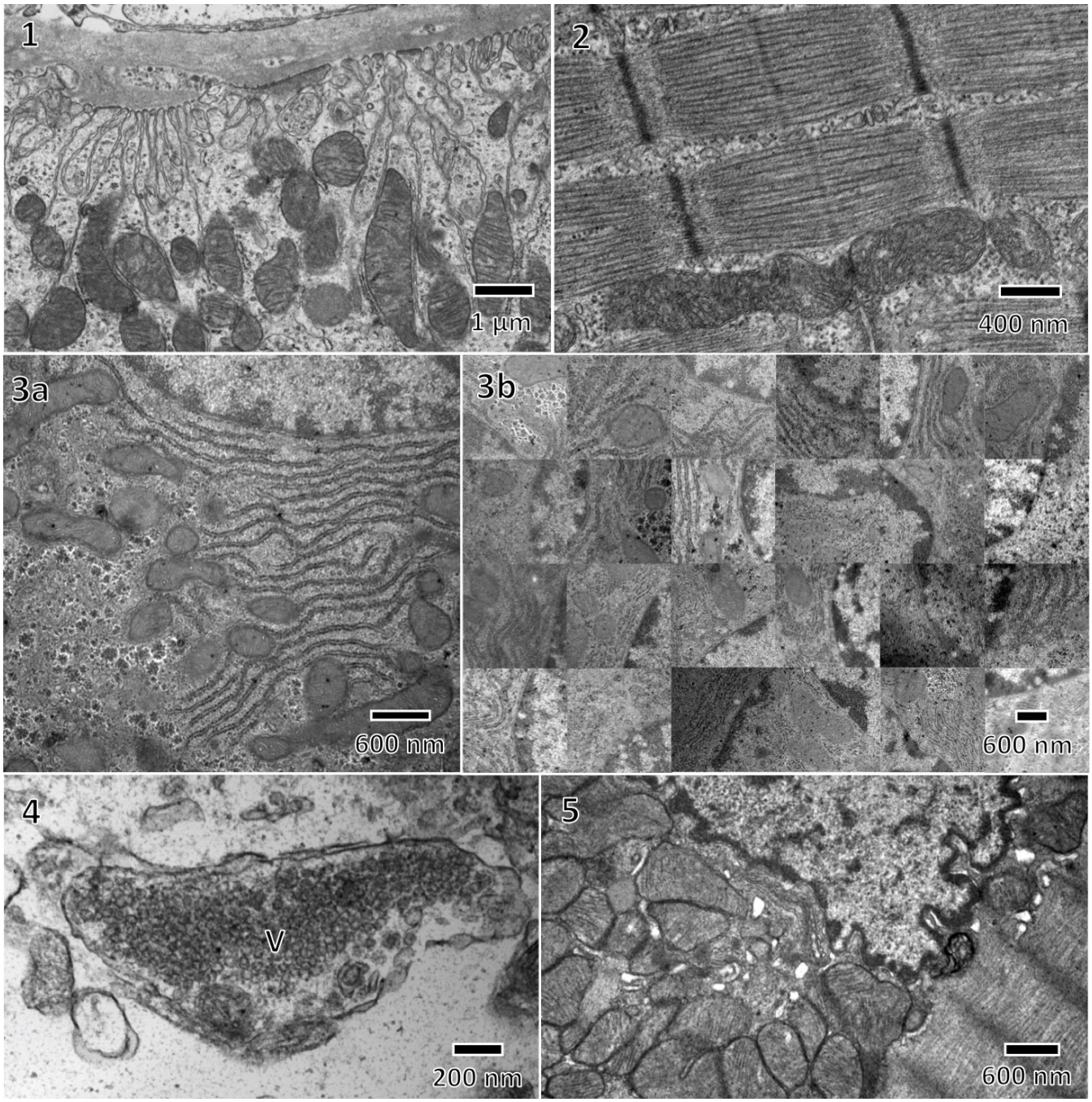

Figure 1: Kidney (rat) proximal convoluted tubule with invaginating membrane folds on basolateral side. **Figure 2:** Gastrocnemius skeletal muscle (rat) myofibril with sarcomeres, mitochondria sarcoplasmic reticulum, Z-lines, and M-lines. **Figure 3:** Liver hepatocyte (rat) with clear rough endoplasmic reticulum, glycogen particles, mitochondria and a nucleus (a). Reproducibility shown with 24 liver samples (b). **Figure 4:** Brain synapse (mouse) with synaptic vesicles (V). **Figure 5:** Cardiac muscle (mouse).

Automated Rapid Preparation of Tissue Specimens for TEM Pathology

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Introduction

Pathology specimens can never be prepared quickly enough. Most tissues are prepared for TEM pathology using chemical fixation, dehydration, and resin embedding, followed by microtomy and grid preparation. Historical reagent processing techniques are:

1) Manual reagent exchanges in vials, typically requiring 1-3 days,
2) Automated immersion processing, typically requiring 1-2 days,
3) Microwave processing, typically requiring several hours [1].

Although microwave processing is very fast, it requires manual reagent exchanges every few minutes. Historical methods also require the manual transfer of unlabeled resin-infiltrated specimens into embedding molds, which adds substantial preparation time [2].

We demonstrate fully automated reagent processing in 1-2 hours. We also demonstrate the elimination of handling specimens in resin while providing specimen tracking from receipt through microtomy.

Experimental

Rats were perfusion-fixed with buffered 4% paraformaldehyde and 1% glutaraldehyde. Tissues were then excised, immersed in fix, and stored at 5°C. To demonstrate clinical prep where perfusion is not possible, tissues were excised from mice, cut into ~2 mm pieces, immersed in fix and stored. In the electron microscopy lab (Figure 1), tissues were removed from vials, cut as needed, and entrapped and oriented in labeled mPrep/s capsules. Capsules were mounted on an mPrep ASP-1000 and processed with conventional chemicals using rapid repeated reagent exchanges (Table below).

Step	Reagent	Reagent exchanges	Time (kidney)	Time (other tissues)
1	Buffer rinse 1	60	0.5 min	2.3 min
2	Buffer rinse 2	60	0.5	2.3
3	Buffer rinse 3	60	0.5	2.3
4	1% buffered OsO4	600	5	35
5	OsO4 H20 rinse 1	60	0.5	2.3
6	OsO4 H20 rinse 2	60	0.5	2.3
7	50% Ethanol	60	0.5	2.3
8	70% Ethanol	60	0.5	2.3
9	90% Ethanol	60	0.5	2.3
10	95% Ethanol	60	0.5	2.3
11	100% Ethanol	120	2	4.6
12	Acetone 1	120	2	4.6
13	Acetone 2	120	2	4.6
14	25% Embed 812	180	5	10.5
15	50% Embed 812	180	5	10.5
16	75% Embed 812	180	5	10.5
17	100% Embed 812	180	5	10.5
18	100% Embed 812	180	5	10.5
19	100% Embed 812	180	5	10.5
		Total time:	**45.5 min**	**132.5 min**

Capsules with resin-infiltrated specimens were removed from the ASP-1000 for 60°C overnight polymerization. 70 nm sections were cut from capsule-blocks. Grids were stained with 8% uranyl acetate in 50% ethanol and Reynolds lead citrate. Images were obtained at 80 KeV with a Philips CM120 and a BioSprint camera.

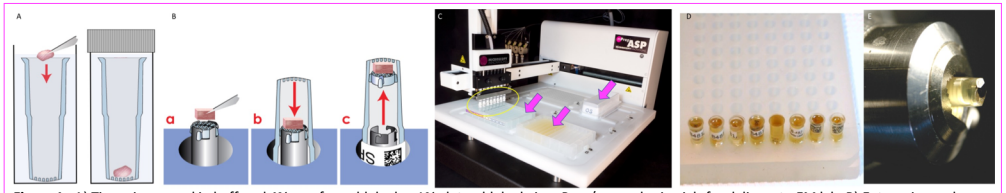

Figure 1: A) Tissue immersed in buffered 4% paraformaldehyde - 1% glutaraldehyde in mPrep/s capsules in vials for delivery to EM lab. B) Entrapping and orienting tissue for sectioning in capsules using mPrep/s Workstation. C) Eight capsules (circled) on ASP-1000 with processing reagents in microwell plates (arrows). D) Capsules filled with 100% epoxy resin held in mPrep Bench for 60°C polymerization. E) Capsule clamped directly in microtome chuck for sectioning.

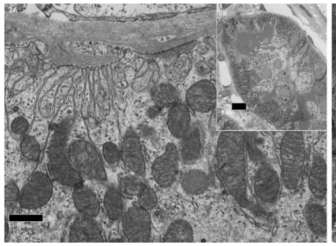

Figure 2: Kidney (rat) processed in 46 minutes. A) Proximal convoluted tubule. Note invaginating membranes, mitochondria, cristae. B) Lower resolution showing most of a glomerulus.

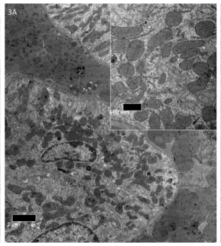

Figure 3: Kidney (mouse) prep in 133 minutes. A) Portion of glomerulus. B) higher resolution of dense mitochondria region.

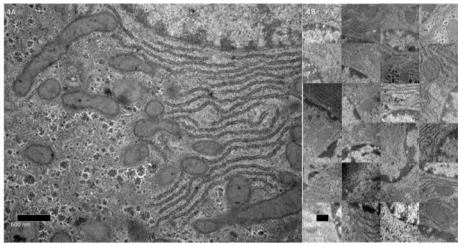

Figure 4: Liver (rat) prep in 133 minutes. A) Hepatocyte in sinusoidal region. Note rough ER, glycogen alpha particles, nuclear envelope, mitochondria and cristae. B) 24 liver samples processed in 3 separate ASP batches. Note consistently well preserved, fully embedded tissue.

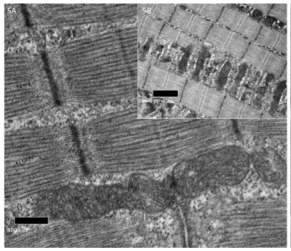

Figure 5: Gastrocnemius skeletal muscle (rat) prep in 133 minutes. Higher (A) and lower resolution (B). Note myofibrils, sarcomeres, mitochondria with cristae, sarcoplasmic reticulum, Z and M-lines.

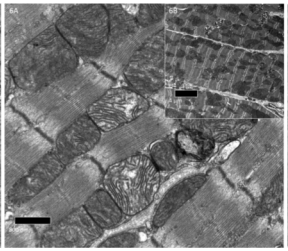

Figure 6: Cardiac muscle (rat) processed in 133 minutes at higher (A) and lower resolution (B). Note myofibril organization, sarcomeres, mitochondria with clear cristae and sarcoplasmic reticulum.

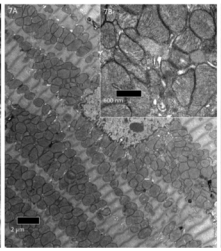

Figure 7: Cardiac muscle (mouse) prep in 133 minutes. Lower (A) and higher resolution (B). Note well-preserved muscle morphology.

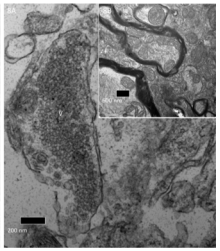

Figure 8: Brain (mouse) prep in 133 minutes. A) Synapse at higher resolution with synaptic vesicles (V). B) Myelinated axons at lower resolution.

Results

Tissues were automatically and rapidly prepared for TEM with the mPrep ASP-1000 from immersion in aldehyde fixative through 100% resin infiltration:

- Kidney was processed in 46 minutes prior to resin curing
- Muscle, liver, brain, heart, and others were processed in 133 minutes prior to resin curing
- Tissue samples were directly handled only once – when cut and oriented in mPrep/s capsules
- The transfer of resin-infiltrated specimens into embedding molds was eliminated
- All tissues were well-preserved with no evidence of incomplete processing as observed with TEM or LM (not shown)
- Consistent processing also demonstrated with 24 liver specimens prepared in 3 separate ASP-1000 processing batches.

Discussion

Automated ASP-1000 processing provided high-quality preparation in less time and with much less effort than historical methods:

- Reagent processing speed was comparable to microwaves, but with fully automated reagent exchanges
- Process time was much less than manual vial or automated immersion processing
- Messy transfers of resin-infiltrated specimens into embedding molds were eliminated
- Encapsulated and labeled specimens cut handling and mix-ups

The ASP-1000 can be programmed for other specimen protocols including aldehyde fixation, en bloc staining, immuno-labeling, acrylic embedding, serial block face SEM, and even grid prep.

How is the process speed so fast?

Chemical fixation and embedding is diffusion limited. With the ASP-1000, rapid processing was achieved by accelerating diffusion into specimens with repeated directed fluid flow: Kidney was OsO_4-fixed with 600 reagent exchanges repeated every ½ second, and the tissue was infiltrated with 100% epoxy with 180 exchanges in 5 minutes.

Summary

Rapid and consistent tissue preparation is highly desired for diagnostic pathology and research. We demonstrated rapid automated hands-off preparation of multiple mammalian tissues:

- Kidney prepared in 46 minutes and 133 minutes
- Liver, brain, skeletal and cardiac muscles in 133 minutes
- No messy transfer of specimens into embedding molds
- Direct specimen handling eliminated once inserted into capsules
- Encapsulated & labeled specimens provides traceability
- Consistent and reproducible results.

References

[1] Giberson RT, et. al, Ultrastructural Pathology 27 (2003) 187–196.
[2] Schroeder JA, et al, Micron 37 (2006) 577–590.