

# Rapid Robotic Parallel Processing of Liver Tissue for TEM using the mPrep™ ASP-1000

mPrep™  
System

Applications Note #603

## Introduction

To examine automated processing repeatability, the mPrep ASP-1000 Automated Specimen Processor and mPrep/s™ capsules (Figure 1) were used to prepare 24 parallel tissue specimens from the same liver for TEM. Specimens were prepared in three batches of eight capsules. Each batch was attached to the mPrep ASP-1000 for reagent processing from primary fixative through 100% resin infiltration, a process which took only 133 minutes. Handling by forceps was required only once, when the specimens were placed into barcode-labeled mPrep/s capsules. The specimens remained in labeled capsules throughout the parallel automated processing steps (post-fixation rinse, osmication, dehydration, resin infiltration), as well as throughout embedding (which eliminated a time-consuming, messy transfer into an embedding mold) and sectioning (Figure 2).

## Methods and Materials

Liver tissue from an adult rat was perfusion-fixed, excised, immersed in fixative (2% glutaraldehyde, 4% paraformaldehyde) and refrigerated in storage fixative (0.2% glutaraldehyde, 0.4% paraformaldehyde) until subsequent processing.

Twenty-four specimens were prepared from the aldehyde-fixed tissue to test the repeatability of the ASP-1000 system. The tissue was prepared by first cutting with a vibratome to a 500- $\mu\text{m}$  (0.5-mm) thickness and then with a 2-mm circular punch to create thin disks. Specimen disks were placed in mPrep/s capsules using the mPrep/s workstation (Figure 3), sandwiched flat between the bottom of the capsule and the top screen in the orientation as shown in Figure 2.

All reagents, except osmium tetroxide ( $\text{OsO}_4$ ), were pre-dispensed into two 12-column polypropylene reagent reservoirs (Figure 3). For safety and ease of disposal,  $\text{OsO}_4$  was dispensed into a separate reservoir. The filled reagent reservoirs and an empty mPrep/bench™ 96-well silicone rack (to receive capsules for polymerization) were placed on the ASP-1000 deck (Figure 4). The ASP-1000 was operated in an enclosure ventilated into a fume cabinet to control hazardous fumes.

Three batches of eight specimens each were processed as described below to resin curing in less than 8 hours. Eight mPrep/s capsules containing perfusion-fixed liver specimens were mounted on the ASP-1000 before the 133-minute processing program was initiated (Figure 4). Fluid exchanges were performed at two-second intervals to constantly deliver fresh reagent to the specimens. Osmium was added to and removed from the specified reservoir shortly before and after Steps 4, 5, and 6 in the automation protocol to ensure operator safety.



Figure 1: ASP-1000 setup with mPrep/s capsules and reagent plates.



Figure 2: mPrep/s capsule containing a resin-embedded osmicated liver sample.



Figure 3: mPrep/s Workstation. The mPrep Workstation was used to orient and entrap the aldehyde-fixed tissue in mPrep/s capsules.



When all reagent steps were completed, the ASP-1000 automatically moved into position above the mPrep/bench silicone rack and prompted the operator to eject capsules into the rack. The operator then filled each capsule with a small volume of resin and placed the rack into a 60°C oven for overnight polymerization.

Reagent cleanup consisted simply of moving the solvent and resin reservoirs (Plates 5 and 6, respectively) to the back of the fume hood and allowing the acetone and alcohol to evaporate. The osmium reservoir was emptied into the appropriate waste container after being depleted with corn oil. Plate 5 was rinsed and dried for reuse. Plate 6 was polymerized at 60°C overnight and discarded into general lab waste.

Capsules containing polymerized specimens were mounted directly in the ultramicrotome chuck (Figure 5). The block face was trimmed and cut into 1-µm thick sections for examination by light microscopy before obtaining 70-nm thin sections for TEM imaging.

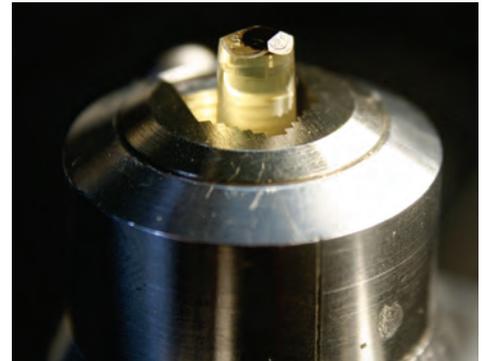


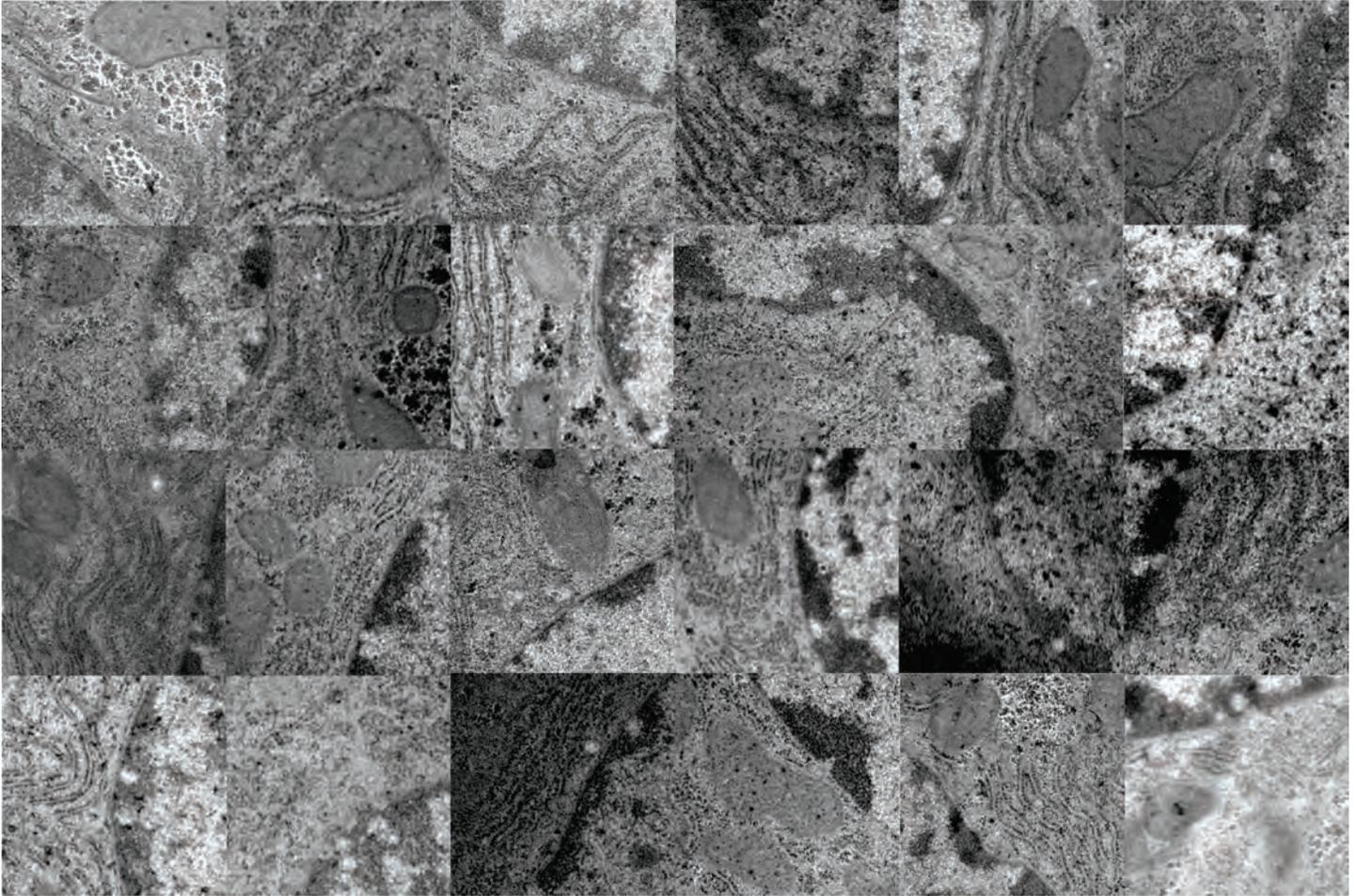
Figure 5: Specimens inside capsule mounted in ultramicrotome chuck.

Plate Map					
Deck Position/Labware/Reagents					
	<table border="1"> <tr> <th>Position 1</th> <th>Position 2</th> </tr> <tr> <td>mPrep/bench rack</td> <td>Single Reservoir</td> </tr> </table>	Position 1	Position 2	mPrep/bench rack	Single Reservoir
Position 1	Position 2				
mPrep/bench rack	Single Reservoir				
Col. 6	<table border="1"> <tr> <td>empty</td> <td>Osmium</td> </tr> </table>	empty	Osmium		
empty	Osmium				
	<table border="1"> <tr> <th>Position 3</th> <th>Position 4</th> </tr> <tr> <td>not used</td> <td>not used</td> </tr> </table>	Position 3	Position 4	not used	not used
Position 3	Position 4				
not used	not used				
Col. n/a					
	<table border="1"> <tr> <th>Position 5</th> <th>Position 6</th> </tr> <tr> <td>12-Column Reservoir</td> <td>12-Column Reservoir</td> </tr> </table>	Position 5	Position 6	12-Column Reservoir	12-Column Reservoir
Position 5	Position 6				
12-Column Reservoir	12-Column Reservoir				
Col. 1	Fix rinse 1- 0.1M PB      25% resin				
2	Fix rinse 2- 0.1M PB      50% resin				
3	Fix rinse 3- 0.1M PB      75% resin				
4	Os rinse 1 (H2O)          100% resin				
5	Os rinse 2 (H2O)          100% resin				
6	50% ethanol                  100% resin				
7	70% ethanol				
8	90% ethanol				
9	95% ethanol				
10	100% ethanol				
11	Acetone				
12	Acetone				

Automation Protocol						
Fast Processing/mPrep™ ASP-1000						
Step	Reagent	Time (min)	Plate	Column	Fluid exchanges	Delay (ms)
1	Fix Rinse 1	2.3	5	1	60	0
2	Fix Rinse 2	2.3	5	2	60	0
3	Fix Rinse 3	2.3	5	3	60	0
4	Osmium	35	2	6	600	500
5	Os Rinse 1	2.3	5	4	60	0
6	Os Rinse 2	2.3	5	5	60	0
7	50% Ethanol	2.3	5	6	60	0
8	70% Ethanol	2.3	5	7	60	0
9	90% Ethanol	2.3	5	8	60	0
10	95% Ethanol	2.3	5	9	60	0
11	100% Ehtanol	4.6	5	10	120	0
12	Acetone 1	4.6	5	11	120	0
13	Acetone 2	4.6	5	12	120	0
14	25% Resin	10.5	6	1	180	500
15	50% Resin	10.5	6	2	180	500
16	75% Resin	10.5	6	3	180	500
17	100% Resin	10.5	6	4	180	500
18	100% Resin	10.5	6	5	180	500
19	100% Resin	10.5	6	6	180	500
Total Time		132.5	2 hours 12.5 minutes			

**PROTOCOL MUST BE RUN IN VENTILATED ENCLOSURE OR HOOD!**

Figure 4: Reagent layout on auto-processor deck (left) and automation protocol (right)



**Figure 6: Reproducibility Study.** These 24 micrographs consistently show well-preserved, fully embedded tissue from a rat liver sample processed with the ASP-1000 in 3 separate batches of 8 capsules over the course of a day. To view larger micrographs, view our poster [Fast, Walk-away, Automated Processing of Mammalian Tissue for LM and TEM](#).

## Results and Discussion

The mPrep ASP-1000 Automated Specimen Processor was used to process 24 mammalian liver specimens in three batches of eight specimens each. Each 8-specimen batch was completed in only 133 minutes, plus a few minutes of changeover time between batches. All 24 samples across the three runs were consistently prepared and showed well-preserved ultrastructure homogeneity within samples that was verified by longitudinal section. There were no preparation failures (Figure 6).

The mPrep ASP-1000 System offered these distinct advantages:

- Walk-away convenience – Robotic system eliminated all manual effort required during reagent processing
- Rapid – Performed post-fix rinse through 100% resin infiltration in 133 minutes

- Easy – Eliminated handling of resin-infiltrated specimens & transfer to embedding mold
- Safe – Specimens handled only once, when placed in labeled mPrep/s capsules
- Enhanced traceability – Barcode-labeled capsules eliminated chance of sample mix up
- Repeatable – Robotic processing provided highest degree of consistency
- Simple – Reagent setup and cleanup are easy and logical with single-use reservoirs.
- Streamlined – Eliminated the frequent manual intervention required by microwave processing methods or the complicated assembly/disassembly steps and specimen transfers that are required for embedding with other automated tissue processors.

## Conclusions

The mPrep ASP-1000 System eliminated hands-on effort required of technicians for reagent processing and saved elapsed time by automatically and rapidly preparing multiple specimens in parallel. Processing in barcode-labeled mPrep/s capsules provided consistent results with minimal effort, and practically zero chance for sample loss, mix-up, or damage.

The mPrep ASP-1000 System produced liver tissue specimens prepared for biological TEM that provided excellent image quality by processing with a fully-automated, walk-away system shown to be rapid, repeatable and robust.

## Ordering Information

Product #	Item Description/Catalog Information
41000	mPrep ASP-1000 Automated Specimen Processor
42100	mPrep/s Workstation
22200	mPrep/s capsules in storage box - 8 capsules, 12 screens, 8 blank label sets
22500	mPrep/s capsules - bulk pack: 96 capsules, screens & blank label sets
31500	mPrep/f30 Standard filter-couplers in capsule storage box, 16/pk
34000	mPrep/bench 96-well rack, silicone
52501	12-channel reagent reservoir, polypropylene, sold by EACH, 25 each/case
52001	R15-50HDPE - 15ml Reagent Reservoirs, non-sterile, HDPE, 50/pk
32010	mPrep/s Insertion Tool

## Benefits of ASP-1000 Processing in mPrep/s Capsules

- Fast processing offers quicker results.
- Simple setup and cleanup saves effort.
- Technicians are more productive when freed from frequent manual intervention or complicated setup/cleanup procedures.
- The simple-to-use ASP-1000 is supplied with many ready-to-use protocols.
- Easily customizable COBRA control software allows an unlimited number of processing steps.
- The PC-based software can send text messages to operators for any step needing operator involvement, such as when processing is complete.
- The ASP-1000 deck holds up to six standard microplates or reservoirs, allowing up to 72 reagent or rinse positions.
- Dispensing reagents from microplates reduces consumption and enables up to eight variable conditions.
- Automation provides precise reagent control and uniform processing times across multiple samples.
- Specimen handling is reduced to “one touch” with mPrep/s capsules.
- Barcode or alphanumeric labeling of capsules reduces error potential, simplifies sample management, and enables GLP compliance.
- Patented mPrep/s capsules allow users to orient a specimen during placement into the capsule without further manipulation.
- Automated processing in mPrep capsules provides scientists with consistent results day-in-and-day-out.