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Meeting-report

An Efficient Clinical TEM Workflow Using Automated Specimen Processing

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ARUP Laboratories, a CAP, ISO-15189, and CLIA-certified national reference diagnostic lab processes about 1,300 renal biopsy TEM (transmission electron microscopy) specimens per year, plus a variable number of liver, gastrointestinal, cilia, and skeletal and heart muscle biopsies. Here we describe ARUP's workflow using an mPrep™ ASP-1000 Automated Specimen Processor (Microscopy Innovations) to improve consistency and reduce labor [1-3].

The ARUP EM lab prepares 4 to 15 patient renal specimens daily. Since other specimen types are handled in smaller numbers, we illustrate the workflow with renal specimens. Renal specimens arrive (Fig. 1A) as one or more 18-gauge needle biopsy cores fixed overnight in glutaraldehyde-paraformaldehyde (GA-PA). EM staff remove the needle cores and cut them into 1-2 mm long segments (Fig. 1B). Up to 8 of these segments are then placed into a single mPrep/s specimen capsule for each patient. This capsule is capped with a barcode-labeled second mPrep/s capsule to entrap and identify the patient specimen (Fig. 1C-D). On days with more than 8 patient specimens, two capsules are stacked to double the processing capacity (Fig. 1D). For TEM processing, capsules are attached to the ASP-1000 pipette head: On the day shown here, there were 5 patients, so 5 single stacked capsules were placed on the ASP head (Fig. 1E). On days with 9 to 16 patient specimens, capsules would be double stacked (Fig. 1D). Thus, the ASP can process 1 to 16 patients at a time with up to 8 tissue pieces per patient, providing a single preparation run capacity of 1 to 128 tissue segments.

At each reagent-protocol step, the ASP draws reagent into the capsules (aspirates) from 12-channel microplates to fully immerse the specimens (Fig. 1E). The ASP then gently and repeatedly aspirates and dispenses additional reagent in-out while keeping specimens continuously immersed. This rapidly infiltrates reagents through each tissue piece. Each reagent protocol step uses ~5 ml (in each microplate column), thus reagent consumption is ~40 µl/step when processing 128 tissue pieces (for 16 patient specimens) and ~80 µl/step for 64 tissue pieces (8 patient specimens). The conservative ARUP protocol uses 3 buffer GA-PA rinse-outs, OsO_4, uranyl acetate, 3 water rinses, graded ethanols, 6 100% ethanols, and 6 100% acetones, graded resin infiltration in 1:1 and 3:1 epoxy:acetone, then 3 100% 812-epoxy formulation steps.

The operator starts the ASP protocol and then adds the OsO_4, uranyl acetate, and water rinses while the ASP performs the GA-PA buffer rinse-outs. The ASP later alerts lab staff for the timely addition of acetone and epoxy resin but otherwise operates without intervention. Protocol duration for 1 to 8 patients takes 3 hours, while for 9-16 patients takes 3.5 hours with the longer time needed to fill and empty the taller double-stacked capsules. A "Protocol Completed" alert signals staff when the tissue is 100% resin infiltrated. The patient-labeled capsules are then transferred from the ASP to a dish containing an embedding mold with the same number of labeled and resin-filled wells as there are biopsy segments for each patient (Fig. 1F). The biopsy segments are then removed from the capsule, orientated in the wells, and polymerized overnight at 70C.

Block facing, microtomy, semi-thin and thin sectioning, grid staining (uranyl acetate and lead citrate), and imaging are done the next morning. Every patient block is sampled with 0.4 µm toluidine blue stained sections on bar-coded glass slides (Fig 1G). Three grids are prepared per block with a minimum of 2 sections per grid. Imaging uses a JEOL 1400 Flash TEM, with a Gatan Rio camera. JPG images are stored on a server for pathologists and client access. Time from sample receipt to images on the server is ~24 hrs. Figure 2 shows TEM biopsy examples.

The ARUP ASP specimen prep workflow requires only 1 person-hour of hands-on effort, divided between 2 persons working together for ~30 minutes each to ensure no mix-ups when patient specimens are moved, when barcode or human-readable labels are changed, and for documentation. This total one-person-hour effort is a substantial reduction from ARUP's prior microwave workflow requiring 6-7 person-hours, which demanded nearly nonstop manual reagent exchanges and considerable care to ensure specimens were not accidentally pipetted, damaged, or lost during reagent exchanges. Reproducible process timing was especially difficult to achieve on days with higher numbers of specimens. By contrast, the ASP provides precise reagent timing and reduced handling of toxic reagents while consuming much less reagent; just ~5 mls at each reagent step for 1 to 16 patient specimens consisting of up to 128 tissue pieces.

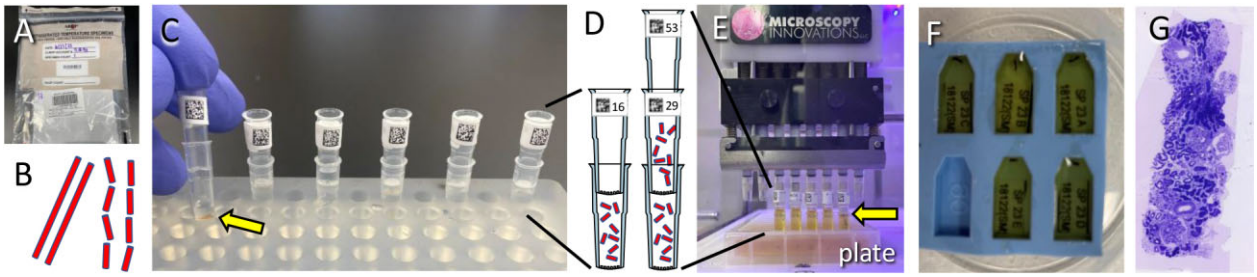

Fig. 1. Workflow. A) Specimen package. B) Cut 18-G renal cores into 1-2 mm segments. C-D) Insert up to 8 segments per mPrep/s capsule (arrow), and cap with labeled capsule. D) Single and double stack capsule diagram with 8 renal segments/capsule. E) Five single-stack capsules on ASP 8-channel head for processing. Reagents in microplate (plate), reagent level in capsules (arrow). F) Renal segments labeled and flat embedded. G) Toluidine blue renal slide.

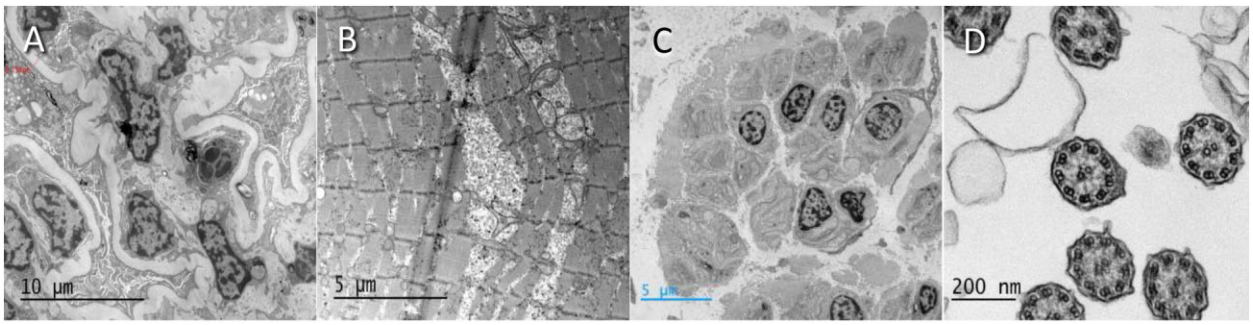

Fig. 2. TEM biopsy examples of A) Renal, B) Skeletal muscle, C) Nerve, and D) Cilia specimens.

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An Efficient Clinical TEM Workflow Using Automated Specimen Preparation

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Summary

ARUP Laboratories (Associated Regional and University Pathologists) is one of the USA's largest clinical pathology labs with over 35 years of experience. ARUP is a CAP, ISO-15189, and CLIA-certified national reference diagnostic lab. ARUP's electron microscopy (EM) unit prepares about 1300 patient renal specimens per year, plus a variable number of liver, gastrointestinal, cilia, and skeletal and heart muscle biopsies.

Problem

ARUP previously prepared EM pathology specimens using microwave processing. While this enabled specimen prep in about 3.5 hours (from buffered aldehyde fixative rinse-out to 100% resin infiltration) there were several problems:

- Microwave processing was highly labor-intensive
- Two persons were required for all 3.5 hours
- Nearly nonstop manual reagent exchanges
- Toxic reagent handling
- High reagent consumption
- Process timing was not entirely reproducible, especially on days with greater numbers of specimens
- Great care was needed to ensure specimens were not accidentally pipetted, damaged, or lost during reagent exchanges.
- **Total labor: 6-7 person-hours, 2 persons throughout all processing.**

Solution

mPrep™ ASP™-1000 Automated Specimen Processor (ASP) workflow:

- Automated processing: 3 hours for up to 8 patients (up to 64 biopsy segments). 3.5 hours for 9-16 patients (up to 128 biopsy segments)
- 100% process timing consistency
- Minimal handling of toxic reagents – only at set up and clean up
- Low reagent consumption: 40-80 µl per protocol step per specimen
- Encapsulated & labeled specimens for near-zero loss or damage risk
- 2 persons only when verifying specimen identification.
- **Total labor: 1 person-hour** [1-3].

TEM Biopsy Workflow

Specimen receipt and capsule loading

The ARUP EM unit typically prepares 4 to 15 patient renal specimens daily, plus muscles and other specimens. We illustrate here the workflow with renal specimens (Fig. 1).

Refrigerated renal specimens arrive in labeled pouches (Fig. 1A), with one or more 18-gauge needle biopsy cores in vials (Fig. 1B) after overnight fixation in glutaraldehyde-paraformaldehyde (GA-PA).

Needle cores are removed and cut into 2-3 mm long segments (Fig. 1C). Up to 8 segments are placed into each mPrep/s specimen capsule held in a mPrep/bench, a silicone 96-well plate that seals capsule bottoms to keep specimens GA-PA immersed. Each patient's specimen capsule is labeled and capped with a barcode-labeled capsule to entrap and ID the biopsy segments (Fig. 1D).

On days with 8 or fewer patient specimens, capsules are "single-stacked". On days with 8 or more patient specimens, capsules are "double-stacked", which doubles the ASP processing capacity (Fig. 1E).

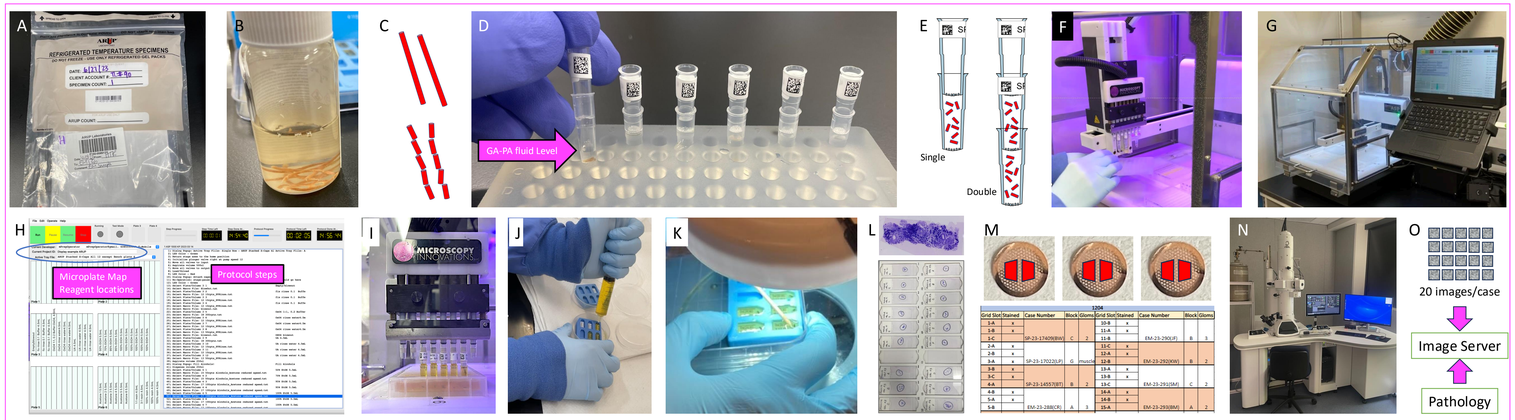

Figure 1: Workflow shown with renal biopsies. **A)** 18Ga renal cores received in barcode-labeled packages **(B)** immersed in GA-PA. **C)** Cores cut to 2-3 mm lengths**. D)** Up to 8 core segments entrapped per mPrep/s capsule capped with a second bar-code labeled capsule, held in mPrep/bench with sufficient GA-PA to immerse specimens (arrow). **E)** Diagram of mPrep/s capsules holding multiple core segments in single and double-stack configurations. **F)** Loading capsules onto ASP. **G)** ASP-1000 and laptop controller at ARUP. **H)** ASP Controller Dashboard, preparation identification field (circled). **I)** Five specimen-containing capsules on ASP after 100% resin infiltration. **J)** Resin and labels into embedding mold using one mold per patient. **K)** Orienting renal core segments before overnight 70C curing. **L)** Glass knife 0.4 um sections are toluidine blue stained for light microscopy to locate regions of interest. **M)** Two diamond knife 100 nm sections on 150 mesh Cu grids, sections oriented in opposition to minimize grid bar interference, 3 grids/patient, 2 grids post-stained saturated with uranyl acetate + Reynold's lead, 1 grid reserved if needed. Electronic grid log. **N)** JEOL 1400 with Gatan Rio Camera, 120 KeV. **O)** About 20 images per case are placed on an Image Server for pathologists.

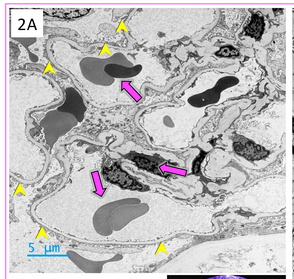

Figure 2: Renal biopsy - Patient with mild glomerulonephritis. A) TEM shows healthy glomerulus with some foot process effacement around basement membranes (arrowheads) red blood cells in capillary space as well as mesangial podocyte nuclei (arrows). B) LM of the glomerulus.

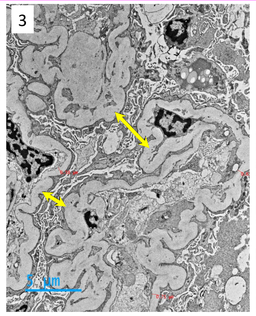

Figure 3: Renal biopsy - Patient with atrial fibrillation, coronary artery disease, bacteremia, diabetes mellitus, hypertension, pancreatic cancer, and pericardial effusion. Measurements confirm the thickened basement membrane that can occur with these conditions (double arrows).

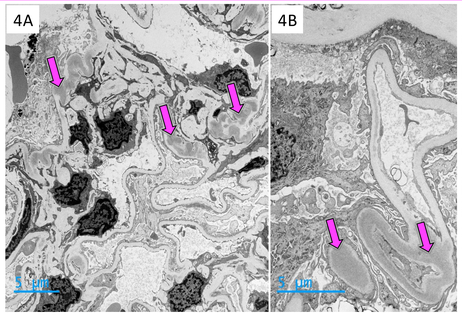

Figure 4: Renal biopsy - Patient with dense deposits disease, focal segmental and global glomerulosclerosis, interstitial fibrosis and tubular atrophy, and atherio and arteriolosclerosis, at lower (A) and higher (B) magnifications Note electron-dense deposits in membranes at different magnifications (arrows). Otherwise, membranes look ok with significant foot process effacement.

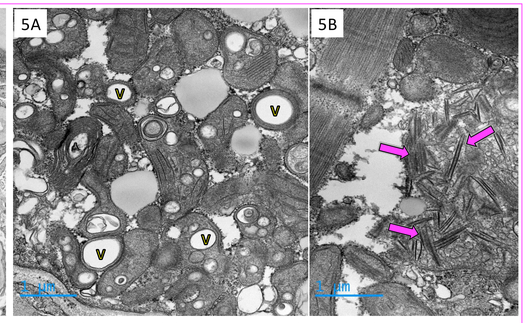

Figure 5: Skeletal muscle biopsy - Patient with mitochondrial cytopathy and active necrotizing myopathy. A) Note increased concentration of mitochondria and vacuolation in the mitochondria (V). B) Muscle with extensive paracrystalline inclusions (arrows).

Specimen loading onto ASP-1000 for automated processing

Capsules with biopsies are loaded onto the ASP-1000 (Fig. 1F). ASPs can process 1 to 8 single-stacked or 9 to 16 double-stacked capsules (Fig. 1E), providing a capacity of 1 to 16 patient capsules, each containing up to 8 tissue pieces, totaling up to 128 biopsy segments (specimens).

ASP-1000 Automated Operation

ASP-1000 at ARUP (Fig. 1G). The ASP Dashboard (Fig. 1H) provides run/stop/pause commands, process timing, operator and specimen info, reagent locations, and protocol sequence (Fig. 1H).

Upon activating "Run", the ASP begins GA-PA buffer rinse-outs. For each step, reagent is aspirated into the capsules to immerse all specimen segments (Fig. 1I). Repeated gentle bi-directional-flow of each reagent provides rapid infiltration: 3 buffer rinses - OsO_4 - UrAc - 3 water rinses - 50%, 70%, 95%, 6×100% ethanols - 6 100% acetones - 1:1 & 3:1 812 epoxy-acetones - 3x100% epoxy. Each step uses ~5 ml, consuming ~40 µl per biopsy segment (specimen) for 128 biopsy segments (16 patients) or ~80 µl for 64 segments (8 patients).

The ASP alerts the operator for the timely addition of acetone and epoxy resin but otherwise operates without user intervention. ASP "runs" take 3 hours for 1-8 patients or 3.5 hours for 9-16 patients. A "Protocol Completed" alert signals staff when tissue is 100% resin infiltrated. Capsules with 100% resin-infiltrated specimens (Fig. 1I) are removed from the ASP to one dish per patient with an embedding mold containing the same number of wells as there are biopsy segments for that patient (Fig. 1J). Segments are removed from the capsules and orientated (Fig. 1K). All specimen transfers are done with two persons to verify specimen ID. Blocks are polymerized overnight at 70C.

Sectioning, Grid Staining, and Imaging

Microtomy, grid staining (uranyl acetate & lead citrate), and imaging are done the next morning. Every patient block is sampled with 0.4 µm toluidine blue stained sections on bar-coded slides (Fig 1L). 3 grids are prepared per block with 2+ sections per grid, and electronically logged (Fig. 1M). Imaging uses a JEOL 1400 Flash TEM with a Gatan Rio camera (Fig. 1N). JPG images are stored on a server for pathologist and client access (Fig. 1J). Time from sample receipt to images is ~24 hrs.

Pathology Examples

Renal tissues (Figs. 2-4) and skeletal muscle (Fig. 5). Additional ASP protocols with CAP, ISO, and CLIA certifications are in process for other tissues and specimens including heart muscle and liver.

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