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Reducing Reagent Consumption and Improving Efficiency of Specimen Fixation & Embedding, Grid Staining and Archiving using mPrep™ Capsule Processing

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Fixation and embedding of specimens, and handling of TEM grids for staining and archiving have not fundamentally changed in decades. Almost all specimens in small labs are processed manually using commodity containers such as scintillation vials, where each reagent is tediously added and removed manually. With vial processing, typically 2 ml of each reagent is required per exchange to fully immerse a ~1 mm specimen. Since the volume of such a specimen is only 1 µl, reagent consumption is wasteful since even 1 ml is much greater than 7-10x the specimen volume necessary for sufficient reaction [1]. While automatic processers ease tedium, these are too expensive for many labs, still consume substantial reagent, and reduce protocol flexibility compared to manual processing. Grid handling is at least as tedious as specimen handling and is very error prone as grids are manually transferred into and out of grid boxes, stain droplets, or staining apparatuses. Grid handling often damages fragile grids, and can lead to mix-ups since individual grids are not labeled.

The mPrep™ System provides an adaptable and efficient end-to-end solution for processing, handling and archiving specimens and grids that enables any lab to achieve high process productivity while reducing reagent consumption, waste, and costs. The mPrep System entraps specimens or grids within precision-engineered micro-molded capsules that connect to common laboratory pipetters (e.g. Pipetmen) to efficiently deliver reagents directly to the specimen or grid, and enable precise timing of fixation/staining steps. The capsules also greatly reduce manual specimen and grid handling thus reducing potential damage and mix-ups. The system is adaptable to most protocols.

Figure 1a shows 8 mPrep/s capsules attached to an 8-channel pipette (FIG 1a) to simultaneously deliver reagents from fix through resin. The mPrep/s capsules are removed for oven polymerization of resin or microwave processing (not shown). The mPrep/s capsules also function as the embedding mold and provide integral specimen labeling (FIG 1b). Note that blocks may be faced and sectioned right through the capsule (FIG 1c) thus reducing preparation time. Not shown is that mPrep/s capsules incorporate means to internally align specimens to obtain proper orientation for sectioning. Since specimens remain in one labeled capsule from fixation through sectioning, specimen tracking becomes trivial. Precise reagent timing and accurate delivery is enabled with mPrep/s capsules and pipettes so that as little as 10 µl may be used for fixatives, rinses and solvent exchanges, and ~100 µl for resin, compared to 2 ml for vials, thus reducing reagent costs from about $8/block to $0.10/block.

Grids are placed in labeled mPrep/g capsules as they are prepared (e.g. microtomy) and then may never require removal from their labeled capsules (FIG 2a) except for electron microscopy. Grids are tightly held in mPrep/g capsules, yet are inserted/removed as easily as from a grid box. Stains are delivered by pipetting (FIG 2b) enabling precise delivery to the 1 or 2 grids held in the two labeled slots in each capsule. By using multi-channel pipettes (e.g. FIG 1a) or by stacking several mPrep/s capsules together (not shown) dozens of grids may be simultaneously stained. Only 35 µl is required

to stain 2 grids/capsule, thus reducing reagent consumption ~10x compared to droplet methods that typically use 100 µl per grid. This can reduce the cost of a typical immunogold label study from $8/grid to $1/grid. Since grids are not removed from their labeled mPrep/g capsules prior to staining, during staining, and for long-term storage, there is essentially no risk of loss, damage or mix-ups. Since grid exposure to air is minimal, and stain timing is precise, excellent uniform staining quality is achieved, and lead citrate stains do not require NaOH pellets (FIG 3).

Reference

[1] J.J. Bozzola, L.D. Russell (1999) *Electron Microscopy*, 2nd Ed, Jones and Bartlett. Boston. p 19.

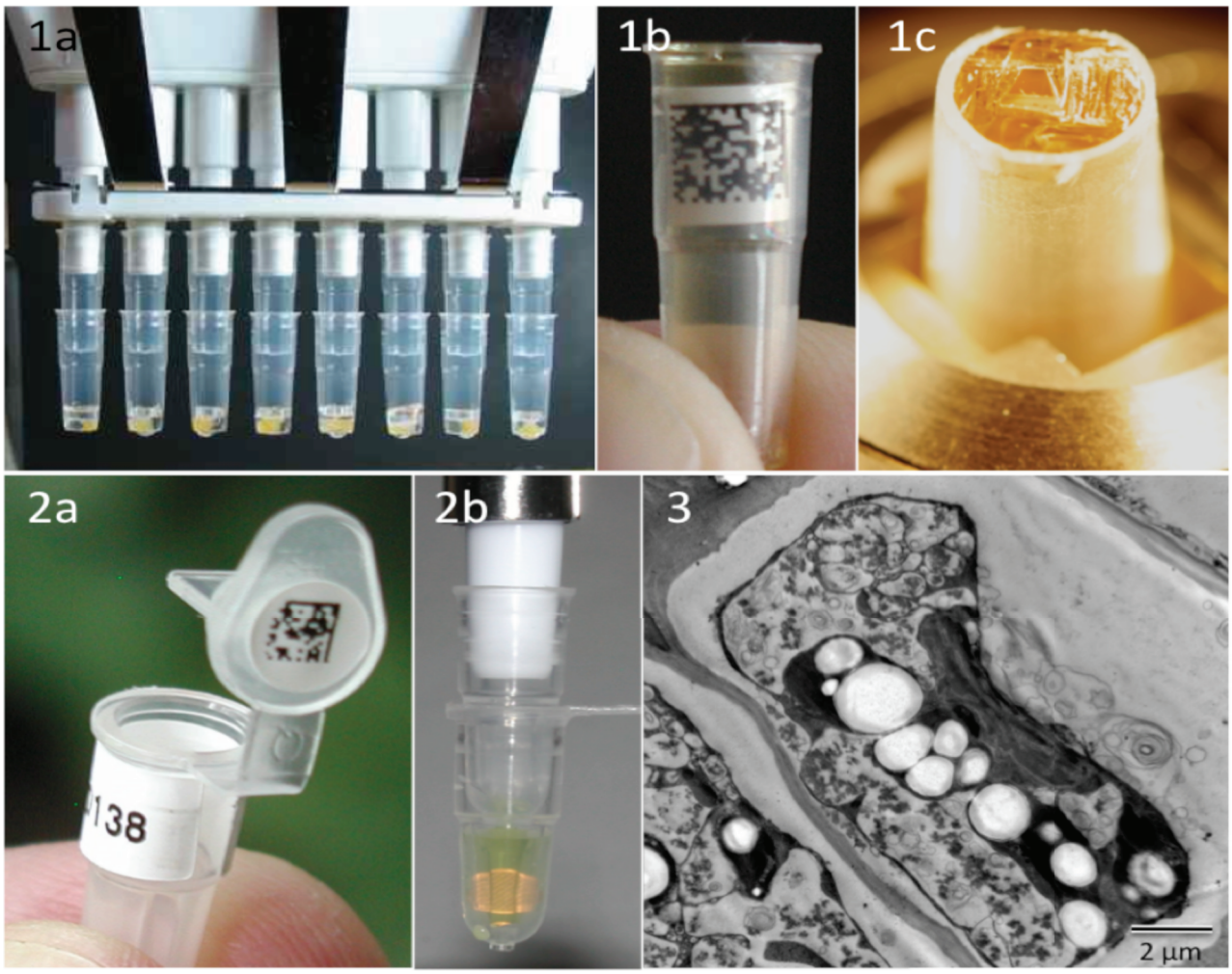

FIG. 1. a) Eight mPrep/s™ capsules attached to 8-channel Pipetman for fix/embed processing. Each capsule contains 1 mm specimen with 25 µl reagent. b) Epoxy filled mPrep/s capsule with embedded 2D barcode label. c) mPrep/s capsule in microtome chuck trimmed through capsule for sectioning.
FIG 2. a) An mPrep/g™ capsule with both 2D barcode and alphanumeric labels. Snap-top lid (shown open) provides archival grid storage. b) mPrep/g capsule attached to 1-channel Pipetman with grid immersed in uranyl acetate stain.
FIG 3. TEM micrograph of Dieffenbachia prepared with Karnovsky's fixative and grid stained with ethanolic uranyl acetate and lead citrate without NaOH pellets. JEOL 1200EX at 80 kV. Note uniform and intense staining without precipitant obtained using mPrep/g capsule staining.

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