

Cooking with Karen: the New Diva of EM Culinary Cuisine. The Use of Microwave Technology in a Pharmaceutical EM Laboratory

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Remember the first time you popped that hot dog into your new home microwave oven and cooked it in only one minute? A new era of food preparation was born. Adapting this common household appliance for everyday use in a pharmaceutical laboratory is revolutionary. Not since the introduction of the automatic tissue processor has there been such innovation in the world of transmission electron microscopy tissue processing!

Microwave (MW) assisted processing offers a new recipe for success using existing ingredients and new efficient methods. By combining conventional electron microscopy (EM) embedding with MW processing, samples can be collected at necropsy in the morning, processed in the afternoon, and embedded by day's end. The MW assisted processing procedures of Rick Giberson (Ted Pella, Inc) and others [1-3] can be adapted to the pharmaceutical EM laboratory. Routine preparation of multiple samples is as simple as following a recipe – short and sweet!

Must Have “Appliances”

- PELCO BioWave™ with variable wattage, (model 34700)- eliminates ‘cooked’ tissue.
- PELCO ColdSpot™ - reduces MW hot spots.
- MW vacuum chamber (PELCO 3435)- speeds up resin infiltration to just minutes.
- PELCO Prep-Eze™ specimen holders- each holds six samples; can use more than one at a time.

Secret to Success Tips

- Prepare all ingredients, reagents, chemicals and supplies in advance. Microwave processing is fast; the time between steps is too short to use for prep time.
- Determine the quantities of reagents actually used per sample batch. Make recipe batch cards for quick reference. This saves time and reduces waste.
- Use the fixation chart and processing schedule on the next page as guides. Plug fixation time sequence into processing schedule. Check off each step as you go, and take notes to improve your process for the future runs.

The manufacturer's instructions, technical notes, scientific papers, and advice were invaluable in setting up a recipe for success in this pharmaceutical environment [4].

References

- [1] Ted Pella Inc, Microwave-assisted Rapid Processing Protocol for Electron Microscopy, 9/1997.
- [2] RT Giberson, et al., J Vet Diagn Invest, 9:61-67, 1997.
- [3] RT Giberson & RS Demaree Jr., Microwave Techniques and Protocols, Humana Press, Inc 2001.
- [4] PELCO Technical Notes, A New Era in Microwave Processing, MWI TN 10/11/01.

Fixation Chart

Fixative (cool < 20°C) Temp restriction <37°C	Routine tissues with vacuum on
Mod. Karnovsky's or paraformaldehyde containing fixatives	1 min off (HOLD), 70-250W 40-80s on, 3 mins off, 350-450W 40s on
Glutaraldehyde	1 min off, 70-250W 40-80s on, 3 mins off
Osmium tetroxide (as 1° or 2° fixative)	1 min off, 250W 40-80s on, 3 mins off
Repeat indicated time sequence 2-6X for difficult to fix tissues. W=watts	



Suggested Microwave Tissue Processing Checklist

Project _____

Species: _____ 1°Fixative: _____
 Sample(s): _____ Buffer: _____
 Compound: _____ 2° Fixative: _____

date	MW Temperature Restriction Set Point	Processing Time In Microwave	Notes
1. Aldehyde Fixation _____ (from fixation chart) _____	Probe is placed in temperature port of The ColdSpot. 37°C	_____ (from fixation chart) _____ _____	[Start with step 3 for direct osmium fixation]
2. Buffer Rinse			~1min _____ ~5 min _____
3. Osmium/Buffer Post Fixation or 1°Fixation _____	Probe is placed in temperature port of The ColdSpot. 37°C	_____ (from fixation chart) _____ _____	
4. Distilled Water Rinse			Brief ~1 minute _____
5. 2% uranyl acetate, aqueous -cool to < 20°C between steps. (250 watts, with vacuum)	37°C	40 seconds _____ re-cool _____ 40 seconds _____	(Optional step)
6. Distilled water rinse	(only if <i>en bloc</i> UA included)		Brief ~1 minute _____
7. Acetone Dehydration 50% 70% 90% 100% 100%	Temp probe and vacuum not needed for dehydration	40 seconds _____ 40 seconds _____ 40 seconds _____ 40 seconds _____ 40 seconds _____	
8. Infiltration – 1:1 Acetone: epoxy resin ratio	Probe tip is placed into petri dish directly in liquid. 43°C	15 minutes _____ (omit 1:1 if using vacuum)	
100% Resin (750 watts) Use fresh resin for each step	43°C	15 min _____ 15 min _____ Or, if vacuum used then, 2 min _____ 2 min _____ 2min _____	
9. Embedding- transfer tissue to pre-filled, labeled, flat embedding molds or Beem capsules. _____ Polymerize by conventional methods as per manufacturer's instructions. _____			