

A Comparative Study of Clearing Agents

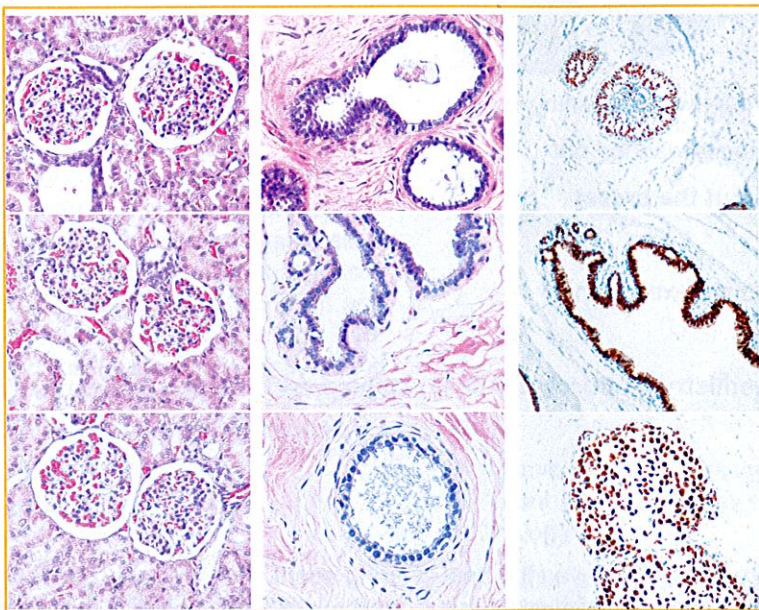
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Processing with xylene can be an unnecessary health risk but can be easily avoided by using xylene replacements, which pose much less of a hazard. Xylene replacements are relatively new, so data regarding their processing, staining, and coverslipping capabilities are still being collected. Labs are hesitant to replace xylene because it has proven to work well in all procedures. This study compares the processing quality of xylene and two different xylene replacements, explicitly aliphatic hydrocarbons. The following steps were taken: processing using the three clearing agents, embedding, sectioning and staining of tissue from a normal kidney and an abnormal breast. Pathologists from the University of Michigan Hospital then proceeded to rate the slides stained with hematoxylin and eosin and the ER 6F11 antibody for: clarity of tissue structures, staining uniformity, artifacts, and most importantly nuclear and cytoplasmic detail.

Aliphatic hydrocarbon A and B both have a NFPA code of: health hazard: 2, flammability: 1 and reactivity: 0. They also both have a threshold limit value of 300 ppm, a flashpoint less than 200°F, are combustible, moderately evaporative, nearly odorless, possess selective solvent power, and are very gentle on specimens. Xylene has a health risk of 2, flammability of 3, reactivity of 0, and special hazard consideration as a carcinogen. Xylene has a threshold limit value of 100 ppm, has neurological effects, and is an irritant. Xylene also has a flashpoint below 100°F, is flammable,

moderately evaporative, has moderate odor, strong solvent power, and harsh effects on specimen. All three are able to be recycled or must be disposed of via licensed waste hauler. The prices for a four gallon case of each clearing product ranged from \$373.79 (xylene) to \$74.42 (aliphatic hydrocarbon B).



KIDNEY, BREAST-H&E, BREAST-ER (LEFT TO RIGHT) PROCESSED USING CLEARING AGENTS A, B, C (TOP TO BOTTOM)

A whole kidney from Pfizer and a whole abnormal breast from Sparrow Hospital were each cut into three separate pieces, for a total of six. Using a Sakura Tissue Tek VIP, one piece of kidney and one of abnormal breast were cleared using aliphatic hydrocarbon A, the second set used aliphatic hydrocarbon B, and the third set used xylene. The processing programs for all specimens prior to the clearing was as follows: (1) Prefer, (1) 80% ethanol, (3) 95% ethanol, (2) 100% ethanol, (3) clearing agent, paraffin, cleaning xylene and alcohol. All stations were programmed for one hour.

Once the tissue was processed, embedded, and sectioned it was stained with hematoxylin and eosin to demonstrate the nuclear and cytoplasmic detail. Then the abnormal breast tissue was stained at the University of Michigan Hospital with the Estrogen Receptor Clone 6F11 (ER 6F11) antibody, an indicator of breast cancer. The stained slides were then reviewed and rated by the UM Hospital pathologists for processing quality, clarity of tissue structures, staining uniformity, artifacts, and most importantly nuclear and cytoplasmic detail according to ASCP standards.

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The rating scale used for the microscopy was 4 = Excellent, 3 = Acceptable, 2 = Fair, 1 = Poor.

The overall rating for most of the sections was "excellent", regardless of clearing agent. One observer rated clearants (A) and (B) with a 3, but deemed all "diagnosable". Individual comments that were noted were:

- (A) was the best because the cytoplasm stood out more
- (C) color seemed washed out
- (C) kidney and breast - Excellent
- (A) Color more vibrant, (B) and (C) there was little difference
- (A) and (B) kidney and breast: Acceptable
- Staining not uniform
- None of them were too bad
- Nuclear detail pretty equivalent
- Preferred xylene because the nuclear detail seemed crisper
- No differences
- All diagnosable

Comments specifically about the kidney were:

- (A) No complaints
- (B) Cytoplasm preserved; redder; staining crisper
- (C) Not horrible; color looks washed out

Comments specifically about the breast

- (A) Beautiful
- (B) Little less stain; nothing extraordinary
- (C) Not much difference

All of the Immunohistochemistry (antibody ER 6F11) stained sections were given a rating of 4. Comments were:

- No differences
- All look good
- Easily diagnosable

In conclusion, aliphatic hydrocarbon B won as the best clearing agent. This was only because it was the most economical of the 3 clearants tested and scored well for processing quality. However, overall it didn't seem to matter which clearing agent was used during processing because the quality was found to be excellent in all three. 

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References:

- Carson, Frieda L. and Christa Hladik. *Histotechnology, A Self-Instructional Text*. 3rd Edition. Chicago: ASCP Press, 2009: 35-37.
- Product MSDS for aliphatic hydrocarbon A and B; MSDS xylene.