Validating a xylene-free processing application for the Shandon Excelsior™ ES

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Imagine, if you will, a safer, faster, and cheaper way of processing tissue. Just when you thought it could not be done, I am here to tell you say good bye to xylene, xylene substitutes, hazardous disposal, recycling (reclaiming) xylene not to mention the fumes and expenses of purchasing both xylene and alcohol.

If you were to take a look at xylene and some of its pros and cons, you would find a rather dismal chemical without many benefactors. The Environmental Protection Agency (EPA) website (http://www.epa.gov/ttn/atw/hlthef/xylenes.html) has an update on xylene, revised in January 2000. The summary paragraph reads:

"... Acute (short-term) inhalation exposure to mixed xylenes in humans results in irritation of the eyes, nose, and throat, gastrointestinal effects, eye irritation, and neurological effects. Chronic (long-term) inhalation exposure of humans to mixed xylenes results primarily in central nervous system (CNS) effects, such as headache, dizziness, fatigue, tremors, and in coordination; respiratory, cardiovascular, and kidney effects have also been reported. EPA has classified mixed xylenes as a Group D, not classifiable as to human carcinogenicity. "

I know at this point you are saying this is nothing new. We know how bad it is. That is why we switched years ago to a substitute. Those that have not switched argue the other point; those substitutes are no where as effective as xylene and some smell like an orange processing plant that gives you a headache.

So, just where does this leave us? I am happy to report that there has been a method developed to process tissue without xylene or a xylene substitute. In fact, the only xylene substitute used is in the flush (cleaning) cycle. Xylene- free processing has been developed and performed in microwave processing but not on a standard tissue processor. With the flexibility of the Shandon Excelsior™ ES, it has made xylene-free processing a reality.

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In our laboratory, over 40 types of benign and malignant tissues ranging from gallbladder to bone and from thyroid to breast tissue were parallel tested (a section for routine and a section for xylene free). From fresh tissue (less than 1 hour fixation) to autopsy tissue that had been in 10 % formalin for a year (yes we suffer from separation anxiety) parallel testing was performed. The staining results for xylene-free were comparable to standard processing.
Another benefit is for those that process more than one run in a day. This protocol was able to cut 2 + hours off the overall processing time. We also noted that there was no significant shrinkage of the tissue and the tissue did not shrink in the block during storage.

Okay, not everything is perfect and yes, even xylene-free has some drawbacks. The first problem encountered was during grossing, the standard 3mm section worked fine for all tissue but having a cassette crammed (a.k.a. a blivet),

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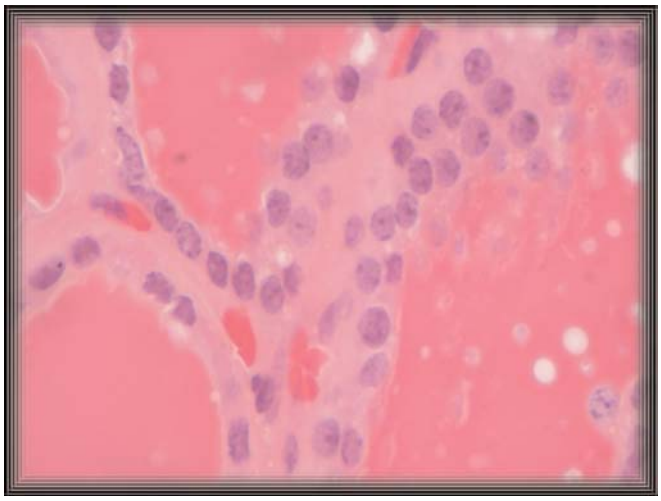
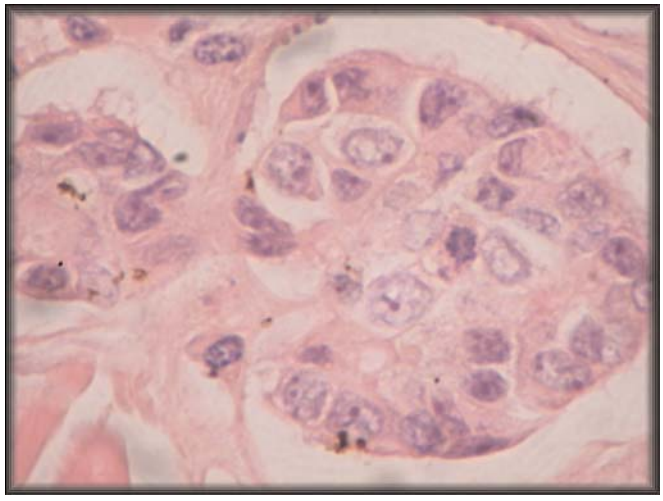
yielded the same as current processing methods.

The next problem that I encountered was with some of the staining times. The specific problem I encountered was a lack of eosin staining within the center of the tissue section. The solution was just adding a little time in the deparaffinizing steps and extending the time in the eosin. The hematoxylin on the other hand, needed no adjustment. Actually, the nuclear chromatin staining was crisp and well defined. This was especially noted in the thyroid tissue and in all the colon sections, benign and malignant.



Thyroid adenoma processed xylene-free on the Excelsior™ ES

As far as immunohistochemistry (IHC) staining, a section of melanoma was stained with the following markers; cytokeratin AE1/AE3, HMB45, PNL-2, and S100. The stained results were the same. No adjustments were needed for antigen retrieval or incubation times. There were two different detection methods used. The AE1/AE3 and PNL-2 used a polymer with an enhanced DAB and the HMB45 and S100 used an enhanced labeled streptavidin biotin complex (HRP) with DAB.



Breast carcinoma processed xylene-free on the Excelsior™ ES

I would like to take this space and add a personal note. This experience has been, at the least, a refresher course in some of the most basic steps of histology. The things that I had forgotten about right after taking the boards some 15 years ago, had to have the cobwebs brushed off them for me to use. To work with Thermo on this was also something that needs noted. When they approached me to do beta testing for them, I was hesitant at first. With the daily schedule and personnel issues I did not think I had the time nor did we have the case load that it would take to make a valid study. I work in a small lab in a rural setting. Validation studies are for large facilities with tens of thousands of cases and plenty of man power. Or so I thought. After sitting down with them and going over everything, I found that what they were asking was not much more than I am doing now. They handled the setup and installation and since we have Excelsior, training was minimal. They were there for any questions or concerns that came about. Since the completion of this testing, I have learned that Stafford General hospital in the UK, along with a hospital in Rotterdam, Holland has started testing this protocol. I would also like to say if anyone that would ever have the opportunity to be a beta site for this study or another, I say go for it. You never know what snowball you may start rolling.