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Dear Members;

I would like to say a great big thank you to all of those who attended the spring state meeting at Callaway Gardens. It was a wonderful meeting with good lectures, and workshops and lots of vendors. For those of you, who did not get the renovated rooms, let me apologize to you. Our event manager was very upset as well, since all of you were supposed to have the new rooms.

I was especially pleased with the turnout for the membership meeting and on behalf of the entire Board of Directors we would like to say a great big thank you for your suggestions, your input, and your enthusiasm. As you may or may not know by now we are hosting Region III next year and the membership voted by a very large majority to return to Callaway Gardens for this event. The dates of the event are April 13-15th, 2012.

Callaway Gardens’ renovations will be complete by the end of the summer, and they are very excited to have us coming back and about some great events that are being planned. Please go ahead and make your reservations now so that you can make sure you get a room, since the hotel is already filling up fairly fast. Your credit card will not be charged until you check in and if you have any problems getting reservations please let one of the officers know. The speaker list and program are being worked on at this time. If you know of speakers or workshops that you would like to see, have those individuals sending their information to Carl ASAP.

At last count the membership was somewhere around 150 people and growing daily, so if you know of people who are not members encourage them to join since it is free. The person who brings in the most members in will receive a nice monetary award at next year's meeting. My goal for membership is 300 or more people by this time next year, so let’s get it done.

We have a number of new additional board members and you will be introduced to them hopefully by the next issue of the Microtime. I am so grateful to have all of these enthusiastic folks on board helping us to create a better society and organization that can serve you not only now but for years to come. There are some great ideas underway, with a Facebook page and additions to the website. Maybe even Twitter (whatever that is).

I hope that each one of you will be as excited as we are for what lies ahead for us as a state society. We don't really understand what a tremendous shortage there is in our profession, until you try to hire someone. I understand that it took almost 7 months to find someone to fill my job, and I still get daily calls and e-mails from people searching for qualified Histotechnologists.

I hope each of you have a wonderful summer of fun and relaxation with your families. For those of you who are planning to attend NSH in the fall, make your plans early. Have a great summer!

Mike Ayers
President GSH
GSH Annual Meeting

It was a Hail of a Meeting!

Callaway Gardens

April 13-15th, 2012
What a Great Meeting!

GSH or Hell’s Angels?
Dewaxing and Coverslipping Sections Without Xylene or It’s Substitutes

by René J. Buesa, B.Sc. HTL (ASCP) (Ret.) [ rjbuesa@yahoo.com ]

The efforts to eliminate xylene from histology procedures have always concentrated in its substitution from tissue processing but the histology personnel is more exposed to xylene while staining than while processing.

Dewaxing and coverslipping are the two steps in section staining where xylene remains prevalent constituting a major source of exposure that is seldom addressed when trying to get rid of it.

In the sequence of steps leading to the finished slides the tissues are first dehydrated, then infiltrated with paraffin wax to allow producing the sections that in their turn have to be rehydrated before staining. This requires eliminating the paraffin wax first, hydrating and staining the sections afterwards to finally reverse again the whole sequence dehydrating and clearing them before coverslipping. This is the standard procedure and xylene is almost universally used to dewax and clear as first and final steps, respectively.

Some histolabs using alkanes as xylene substitutes also use them to dewax and clear but sometimes the results are not satisfactory which leads to a simple question: is there a better way to dewax and prepare the stained section to coverslip? The short answer is yes, but it is better to review the standard procedure first.

Preparing the sections to stain is done in a sequence of 2 or 3 xylene baths, 4 or 5 baths of pure ethanol followed by water before staining. If we assign to each paraffin section and area of 1 square inch (6.45 cm²) and an average thickness of 5 µm, with a density of 0.9 g/cm³ there will be a total of 0.58 grams of paraffin to be dissolved every 200 slides requiring to change 4 liters of ethanol and 2 liters of xylene at a cost of almost $30 per 100 slides.

There has to be a more efficient way of dewaxing and hydrating the sections before staining and using a dishwasher detergent solution is that way. Only a few years ago nobody subjected sections to hot solutions but nowadays immuno-histochemical (IHC) procedures usually start with a heat induced epitope retrieval (HIER) step where the sections are heated at near the water boiling temperature for at least one hour. This procedure is common practice and nobody now thinks that heating a section will have any detrimental effect on it so dewaxing the sections with a hot solution of dishwasher detergent cannot be considered extreme at all.

The procedure is as follows: prepare a 2% (volume to volume) dishwasher solution (DWS) in distilled water. We have used several brands in our validating tests (Fairy, Persil and Spree) but any reputable commercial brand can be used. Fill two containers with DWS at 90ºC and place the slides 1 minute in each, followed by hot running water for 30 seconds and finally in distilled water at room temperature before proceeding to stain with any routine or special procedure.

You have to realize that some IHC autostainers manufactured by Dako, Leica, and Ventana where you introduce the undewaxed sections use soap solutions to dewax them, as is the case of Bond-Dewax® by Leica.
Using a DWS not only eliminates xylene and ethanol, but is much cheaper because replacing the used DWS will represent a cost of only $0.04 per 100 slides, which is 750 times cheaper and 4 times faster than following the conventional xylene and ethanol sequence.

So far you have been able to dewax your sections without xylene (and ethanol) but, what about once you have stained the sections?

The standard procedure is dehydrating and clearing the stained sections but there is a better way. After staining you dry the sections in an oven at 60ºC for 5 minutes but before that you can either dehydrate them with ethanol (as usual), or wash them in distilled water, or in acetone depending on the staining you use, or your preference. The fundamental change being that you substitute xylene with the oven drying step.

Drying the sections before coverslipping can be considered by many as “anathema” but it can be done (3) and you just have to make sure that the mounting medium is fluid enough, with a consistency similar to that of “baby oil” that is just low density mineral oil.

Coverslipping can be manual but if you use an automated glass coverslipper just make sure that the mounting medium is more diluted than usual. For film coverslippers increase the amount of xylene delivered to attach the film and reduce the film speed by 25 percent.

The only remaining step in the histolab requiring xylene is for cleaning the tissue processor, and that can be eliminated also if you use a 2% aqueous solution of a strong laboratory detergent, one of those used to clean glassware, instead of your used xylene.

Now consider the following: if you use the mixtures of 2-propanol and mineral oil to process tissues, the 2% DWS to dewax, oven dry the stained sections before coverslipping, and clean your tissue processor with a 2% solution of strong glassware detergent, you have a xylene free laboratory greatly improving the health safety in your histolab.

Try all these approaches and assure a much healthier environment for you and your staff!

References:

(2) Henwood, AF: The application of heated detergent Dewaxing and rehydration to immunohistochemistry. Biotechnic & Histochemistry 09/21/2010; pp5
(3) Buesa, RJ: Coverslipping without xylene. Biotechnic & Histochemistry 2010; 85(4): 269-270
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Tissue processing without xylene - Part 2: Using 2-propanol and mineral oil

by René J. Buesa B.Sc., HTL (ASCP) (Ret.)

In Part 1 it was shown that “Alkane” and “Terpene” based substitutes either cause health problems also, or produce results less than desirable making them unacceptable substitutes. Other options have to be explored but before doing so it is necessary to review one fundamental aspect of tissue processing.

It seems safe to assume that a tissue fixed correctly can structurally withstand dehydration and infiltration and this is correct up to a certain point. The general cellular structure will be able to remain in place during tissue processing, but dehydration of intracellular spaces and cell components can alter so slightly the relative positions of mitochondria, Golgi apparatus, or endoplasmic reticulum. The spatial dislocations of these organelles are especially evident in transmitted electron microscopy (TEM) work and explain why it is crucial to have a perfect fixation followed by a gradual dehydration and plastic infiltration in TEM work. But, why? What is the physical explanation for this requirement?

All processing reagents have specific solubility coefficients flowing through the tissues at a rate inverse to it: liquids with low solubility (like melted paraffin) flow more slowly than those more soluble (like alcohols). Also low grade alcohols, like 70% ethanol (70EthOL), contains more water than pure alcohol and therefore has greater solubility than 100EthOL which flows more slowly.

Finally there is the gradient effect resulting from exposing cell structures to changing reagents. The gradient is maximal and affects the structures the most when solubility differences are also maximal. If you place fixed tissues directly in 100EthOL it will shrink because the gradient between the water in the tissue (around 80%) and that in 100Ethol (0%) is very large. The gradient is also greater when any two pure substances mix as is the case between 100EthOL and xylene with a solubility gradient of 9 MPa (Mega Pascal) between them.

Reagent flow and solubility gradient are the physical reasons why optimal infiltration is obtained using chemicals with increasing purity during enough time, especially during infiltration. It is necessary to remember that melted paraffin has to enter between all the tissue components to hold them in place when solid, making infiltration the culmination of all previous steps.

Consequently the “ideal” tissue processing method should have small gradients between successive miscible chemicals. The miscibility issue was the xylene initial alleged advantage when introduced because it mixes with both 100EthOL and melted paraffin.

Following the miscibility requirement a new method was developed using 2-propanol, also called iso-propanol (IP), which is able to mix with water and melted paraffin. The gradient between pure IP and melted paraffin of high molecular weight is of 9 MPa (Mega Pascal), so intermediate steps with paraffin of low molecular weight were included. Such type of paraffin is called “liquid paraffin” or simply “mineral oil” (MO) and is used in two increasing proportions. The first contains 5 parts of IP and 1 part of MO, with a solubility gradient of only
2 MPa between pure IP and this solution. The second step is IP:MO at 2:1 (2 parts of IP and 1 of MO) with a gradient of only 1 MPa with the precedent step.

Finally the tissues go from (IP:MO at 2:1) to pure MO followed by melted paraffin, with a final gradient of only 1 MPa. This new method using MO between dehydration and the paraffin wax infiltration was tested with 52 types of tissues, followed by 64 histochemical methods, and 115 antibodies with no statistical differences with parallel sections processed with ethanol-xylene (1; 2) making this IP and MO alternate method completely reliable and an ideal xylene substitute.

After the tissues have been fixed, the general protocol with tissue processors is the following:

<table>
<thead>
<tr>
<th>Station</th>
<th>Reagent</th>
<th>minutes</th>
<th>temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70% 2-propanol (70IP)</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>80% 2-propanol (80IP)</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>90% 2-propanol (90IP)</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>95% 2-propanol (95IP)</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>5</td>
<td>Pure 2-propanol (100IP)</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>6</td>
<td>2-propanol:MO (IP:MO at 5:1)</td>
<td>90</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>2-propanol:MO (IP:MO at 2:1)</td>
<td>90</td>
<td>50</td>
</tr>
<tr>
<td>8</td>
<td>Pure MO</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td>9</td>
<td>Paraffin wax</td>
<td>45</td>
<td>58</td>
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<tr>
<td>10</td>
<td>Paraffin wax</td>
<td>20</td>
<td>58</td>
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<tr>
<td>11</td>
<td>Paraffin wax</td>
<td>20</td>
<td>58</td>
</tr>
<tr>
<td>12</td>
<td>Paraffin wax</td>
<td>50</td>
<td>58</td>
</tr>
</tbody>
</table>

Note that dehydration with increasing IP concentrations takes 75 minutes only because the "clearing" intermediate solutions (IP:MO at 5:1 and IP:MO at 2:1) complete the dehydration while starting the infiltration with mineral oil or "liquid paraffin". Heat at 50°C in stations 6 and 7 is required to allow IP and MO mixing completely.

Clearing and infiltration (stations 6 thru 12) represent 83% of the whole protocol time assuring complete dehydration and perfect infiltration. Blocks cut excellently regardless of the tissue and any following special procedure can be completed as usual. The method is especially good for fat and hard connective containing tissues like breast, skin, and uterus. Besides that, MO cost is 56% that of xylene.

Mineral oil, the main ingredient in "baby oil" formulas, is absolutely safe and it is very likely you have already used it in skin, and sun blocking lotions. Try this method and you will never abandon it!

THE GEORGIA SOCIETY FOR HISTOTECHNOLOGY

When

April 13-15, 2012

Where

Calloway Gardens

See You There!
REGION III & FLORIDA MEETINGS

What an exciting time to be interim VP/vendor liaison of our society as our membership climbs. I challenge everyone to recruit at least ONE new member and tell everyone about our website and FREE membership. We need every member's input as we prepare to host next year's NSH Region III meeting back at Callaway Gardens. Every member can contribute by doing two things:

Send us your ideas and "save the date" for April 13-15, 2012.

Since our state meeting at Callaway in March I attended the Region III meeting in Nashville and the Florida State meeting in Tampa.

Southern hospitality at its finest awaited me in Nashville. The Tennessee members presented excellent speakers and made us comfortable on the ninth floor of the Hilton Gardens. I personally enjoyed workshops given by our own Lamar Jones and was able to implement some new ideas in my own lab. Tennessee board officers, including new president Michelle Foster, shared their experience planning a Region meeting.

Thank you to Michelle for your continued guidance and to Jennifer Burch for guidance & suggestions with vendors. Vendor support was evident at Region III and at the FSH meeting. We can't thank our vendors enough for their continued generosity during hard economic times. This makes a state meeting all the more appealing. Where else can we get continuing education, meet with our peers, and have fun?

With Florida being a tourist destination you can't go wrong with sunshine and a large group of histotechs. I personally wanted to hear Skip Brown speak and not only sat in his workshop, but enjoyed his company at the awards luncheon. And what an impressive event as they recognized achievements in histotechnology for the past year. As I sat and listened the one phrase that came to mind was "dedication to education". And that is what we all are working toward. So thank you to Tennessee and Florida for delivering southern hospitality, as the members of GSH look forward to hosting Region III next April.

Wanda K. Simons

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GSH recommends that you also consider joining NSH in order to be eligible to serve as a representative of GSH in the House of Delegates at the annual Symposium and actively participate in your society. Only NSH members who are also GSH members can serve in the HOD.

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Membership is free to anyone working in histology in Georgia but you must fill out a form each year to be a member. If you do not live in but work in Georgia, you qualify. If you do not work in Georgia you may still be a non-voting member with other member privileges such as discount rates at meetings.

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