ANATECH LTD

INNOVATOR

Clearing Agents

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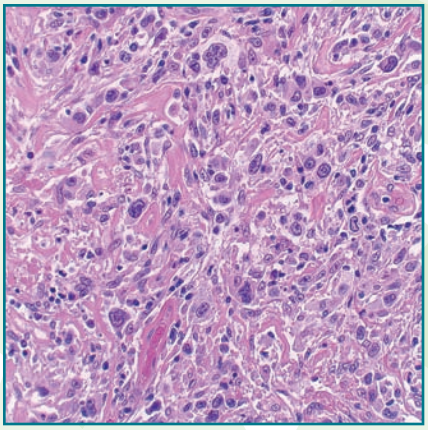
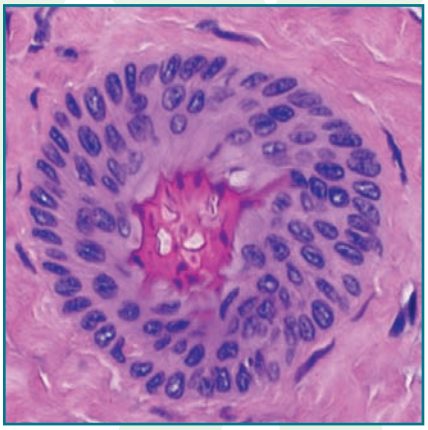
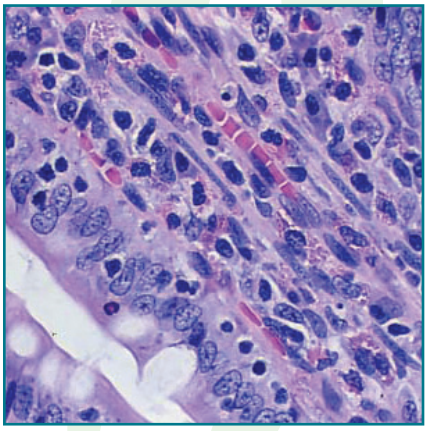






The three H&E sections of (top to bottom) lymph node (Hodgkins Lymphoma), normal skin and normal colon, were processed and coverslipped with Pro-Par Clearant.

THE DECISION TO USE A XYLENE SUBSTITUTE IS CRYSTAL CLEAR!

Despite the availability of safer alternatives and xylene's known hazards, xylene is still the most commonly used clearant in histology laboratories today. Many fallacies concerning the use of xylene substitutes contribute to this continuing trend. This Innovator will dispel these myths and provide the information necessary for an informed decision regarding the clearing in both processing and staining protocols.

WHY SHOULD YOU BE USING A XYLENE SUBSTITUTE?

Xylene substitutes are technically better chemicals for tissue processing than xylene. Let's explore the chemistry of tissue processing and clearants to better understand why this is true. Tissue processing involves four major steps: fixation, dehydration, clearing and paraffin infiltration.

FIXATION

Fixation is the most important, and most difficult, processing step to achieve. It's important because fixation prevents autolysis and preserves cellular morphology. Difficulty arises when tissue sections are too large, fixation time too short, or both. Without adequate fixation the tissues cannot withstand the exposure to the remaining processing chemicals.

DEHYDRATION

As the name states, dehydration removes water. However, water is found in the tissue in two forms, free water and bound water. Free water, which acts as a solvent for salts, proteins and sugars, can be easily extracted. To better visualize this, imagine squeezing a piece of raw meat (muscle tissue). Despite the fact that meat contains 65% water, only a few drops of water will trickle out. This is the free water component. The unextractable portion, known as bound water, is an integral part of the macromolecule. Bound water is so tightly held that heat (cooking) or chemicals are required to remove it. Correct dehydration during tissue processing extracts only free water. Excessive tissue dehydration resulting from long exposure to anhydrous alcohols, or heat, removes the bound water. The removal of bound water alters the molecular structure. Then, characteristics of overprocessing: shrinkage, dry tissues, parched earth effect, abnormal staining, are observed.

CLEARING & INFILTRATION

The primary role of the clearant is to displace the anhydrous alcohols and prepare the tissue for paraffin infiltration. A second function for clearing agents is to assist in dissolving lipids.

Xylene has an additional characteristic that we need to consider: its reactivity with water. Yes, water! Xylene is thought to be insoluble in water but "practically insoluble" is the accurate description. Xylene actually does have the ability to hold some water. What does this mean to tissue processing? It means that xylene can pull small amounts of water from the tissue. But, by the time the tissue has reached the xylene processing stations, the alcohols have already removed the free water. This leaves bound water susceptible to removal by the xylene, and is why xylene can dry out the tissue specimens.

Everyone, particularly in labs with only one processor, has experienced dry tissue during microtomy – those blocks that require soaking in water to get a section. It is difficult to process small biopsies, dense uterus and fatty breast tissues together and have them all come out perfect. The fatty specimens and large/dense specimens need longer times in the processing reagents while the smaller/delicate specimens need shorter times. The removal of bound water is partially controlled by processing in graded percentages of alcohol. However, xylene must be used at full strength to be effective. The xylene time required for the average tissue is just too long for the smaller sized/delicate specimens and sets the stage for overprocessed, dried tissues.

The Solution to a Dilemma

So, the dilemma is how to allow sufficient time for clearing and to assist with the fatty tissues without risking the removal of the bound water. The answer is to choose the right clearing agent. Select a clearing agent that can perform xylene's desired functions without stripping tissues of the bound water necessary to maintain molecular structure.

Anatech understands the chemistry of clearants, especially the pros and cons of water solubility, and used this knowledge to develop the perfect xylene substitute. We needed a chemical that would not remove bound water from the tissue, yet would be able to handle moisture contamination that can happen in staining. We also wanted a clearant that was safer than xylene. For decades papers have been published in major medical journals with titles such as:

"Symptoms and signs in workers exposed predominantly to xylenes."

"Conversely exposure-related effects between atmospheric m-xylene concentrations and human body sense of balance."

"Neurobehavioral effects of long-term exposure to xylene and mixed organic solvents in shipyard spray painters."

"Cytogenetic effects of low level exposure to toluene, xylene and their mixture on human blood lymphocytes."

"Neurobehavioral and respiratory symptoms of formaldehyde and xylene exposure in histology technicians."

"Prenatal toxicity of xylene."

Anatech's extensive research produced a clearant that uses a paraffinic solvent (aliphatic hydrocarbon) as its active ingredient. Aliphatic hydrocarbons are used as solvents for agricultural chemical formulations and are known to be safer than xylene. There are numerous xylene substitutes on the market that are aliphatic hydrocarbons. These differ by hydrocarbon chain length, which determines the solution's degree of flammability and evaporation rate. Anatech selected a blend of aliphatic hydrocarbons that mimics what we like about xylene: it's soluble with alcohol and lipids, can dissolve wax and has a rapid evaporation rate. It's true that most aliphatic hydrocarbons have no tolerance for water, but an additive distinctive to Anatech's Pro-Par Clearant allows it to absorb excess moisture. The result is a clearant with the following characteristics:

- Gentle – leaves bound water during processing
- Moisture tolerant – prevents eosin bleeding and haziness
- Recyclable – reduces costs
- Fast drying – optimal formula for coverslipping
- Low toxicity – safer for the environment

Anatech's xylene substitute, Pro-Par Clearant, is the perfect solution!

Pro-Par Clearant in Processing

Pro-Par Clearant is capable of dissolving tissue lipids and is completely soluble with anhydrous alcohol, displacing it from the tissue specimen. However, Pro-Par Clearant

cannot overprocess and dry out your specimens because it does not have the power to remove bound water. In fact Pro-Par Clearant is so gentle that we recommend using three stations of it on the tissue processor. This additional clearing station assures that the extra large fatty specimens are properly processed; knowing the extra station/time cannot dry the specimen!

What about using Pro-Par Clearant in staining?

No problem here either. The solubility with paraffin and alcohols that we depend upon in processing holds true in staining. Pro-Par Clearant effectively dissolves paraffin and also displaces the alcohol in dehydration, preparing the slides for coverslipping.

To deparaffinize slides we recommend three stations of clearant, three minutes each. It is just good practice to use three clearant stations; the first station will always be contaminated since every slide entering it has paraffin on it. The second station will likely have residual paraffin carryover. A third station of clearant assures complete removal of the paraffin.

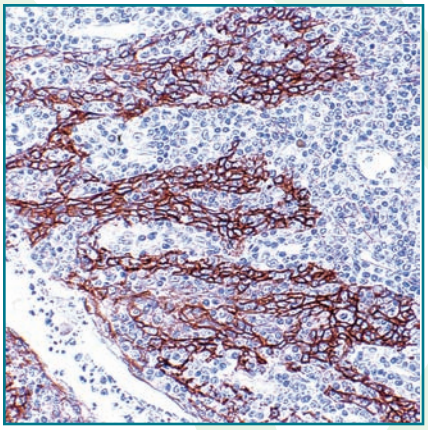
Similarly, three stations is good practice for clearing slides prior to coverslipping. This, along with proper dehydration, will help prevent eosin bleeding and/or hazy slides. These artifacts, which can also occur with xylene, have given substitutes a bad reputation! The reality is that inadequate dehydration during staining is often the primary source of these problems.

Obviously, the use of anhydrous alcohol is essential for effective dehydration. However, anhydrous alcohol does not stay anhydrous because water carryover from previous stations is a constant source of contamination. The problem is exacerbated in high humidity environments where alcohol can pull moisture directly from the air. While xylene's "practically insoluble" water characteristic and Pro-Par Clearant's unique formula allow them to hold some of this excess moisture, other xylene substitutes have no tolerance at all.

As moisture levels increase, all clearants will eventually exceed their water capacity. Therefore, moisture contamination of the anhydrous alcohols will cause hazy slides and eosin bleeding. So, another good practice is to assure complete dehydration by using three stations of anhydrous alcohol, one minute each, prior to the clearant. Follow this with three stations, one minute each of clearant. Just as the right product can prevent dry tissues in processing, the proper product and protocol can eliminate staining problems.

What about Pro-Par and IHC?

The binding of an antibody is dependent upon the correct conformation of the tissue antigen's epitope. Anything that changes the shape of the antigen will reduce immunoreactivity. During tissue processing the capability to remove bound water from the tissue changes the shape (denaturation) of the tissue. Fixatives and alcohol denature tissues the most, clearants that leave bound water intact do not have this type of influence.



Tonsil fixed in Anatech's Prefer Fixative. Processed with Pro-Par Clearant. Stained with EGFR (clone 2-18C9), no antigen retrieval. Coverslipped with Pro-Par Clearant & Refrax Mounting Medium.

Coverslipping with Pro-Par Clearant

Mounting media resins are dissolved in either xylene or toluene. Both of these solvents are compatible with aliphatic hydrocarbons, so that does not pose a coverslipping problem. Resins vary by manufacturer and can be the old slow drying, yellow colored pinenes or fast drying, colorless acrylics. Some of the acrylic resins are not compatible with aliphatic hydrocarbons. So, obtaining a mounting media brand that has a compatible resin is crucial to successfully using a xylene substitute. Anatech has developed Refrax Mounting Medium with a fast drying acrylic resin that is non-oxidizing (stains won't fade). Refrax Mounting Medium has all the characteristics needed for a modern mountant.

Cost Analysis

When xylene substitutes were introduced in the early 1980's they were more expensive than xylene. Technical and safety advantages were promoted as the reasons to switch. The cost of petroleum products continues to soar yearly (gasoline is a prime example). Today, the list price of Anatech's Pro-Par Clearant is less than xylene! Pro-Par Clearant is now better for the bottom line as well as the tissues and your safety! And, yes, it can also be recycled!

Anatech Innovator

So, why haven't you tried a xylene substitute?

Is it simply a fear of change? Change is very difficult for most people, especially when the change could fail and have potentially disastrous results. Admittedly, risking anything but perfect processing and staining is not acceptable when patient welfare is at stake. Keep in mind that alternate reagents like xylene substitutes have gone through extensive testing to assure that they are capable of performing the necessary function. Of course, all chemicals have limitations. It's not uncommon to obtain an under-processed fatty specimen from a processing run that uses xylene as the clearant. But, it is easy to forget those failures when comparing results after making a change.

Chemistry has already given us improved quality and safety in the histology lab. Chloroform and benzene were once commonly used clearants. Their health and physical hazards caused them to be left behind. It is time to get away from xylene and use a chemical that is better suited to our technical needs and is even safer.

Now that you know the real facts behind xylene substitutes you can make that change with confidence!

Pro-Par Clearant

Cat #510 ..... 1 gallon

Cat #511 .....Case/4 x 1 gallon

Cat #515 ..... 5 gallon drum

Cat #519 ..... 55 gallon drum

Refrax Mounting Medium

Cat #711 ..... 1 pint

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