THE INNOVATOR

from ANATECH LTD. *"the leader in innovative histology chemicals"* Summer 2008

The Volatile Hematoxylin Market

Since first being used over 100 years ago as a violet-blue nuclear stain, hematoxylin dye has experienced periodic shortages which have had serious impact on the histology and cytology fields. Both World Wars caused curtailment in supply and decrease in quality. Market forces led to significant shortages in the late 1920's, again in the early 1970's and now in 2008. With each event, prices rise, sometimes dramatically, rumors abound and there is a mad scramble for a substitute. Afterwards, prices fall (somewhat), normalcy returns to the lab and substitutes are forgotten. The stage is then set for an exact repeat the next time the dye is unavailable. And it *will* become in short supply again in the future for reasons discussed below.

Why shortages occur

Hematoxylin is a natural product, extracted in a multistep process from the heartwood of logwood trees. Originally found only on the Yucatan Peninsula, logwood now grows around the world on small plantations or in naturalized settings from former plantations. It was one of the most valuable exports to Europe during Colonial times (16th through 19th Centuries), where it was processed and then used to dye textiles black (using an iron mordant). Less desirable methods to create black involved over-dyeing with several colors from other natural dyes, none of which were color fast, so as the garment was subjected to sunlight, wear and washing, the color would change to unpleasant shades of blue, green, red or yellow. Logwood dye kept its black color much longer, so it remained in high demand for hundreds of years until synthetic dyes were invented in the late 1800's. Since then, hematoxylin has become a minor component of the worldwide dye industry. Now the chief uses for logwood dye are to color silk surgical sutures, to dye specialty silk fabrics for Asian markets, to satisfy a small craft textile dying industry, and to stain nuclei in biomedical specimens. None of these constitutes a sizable niche, thus the dye is highly vulnerable to global economies and unrelated market pressures.

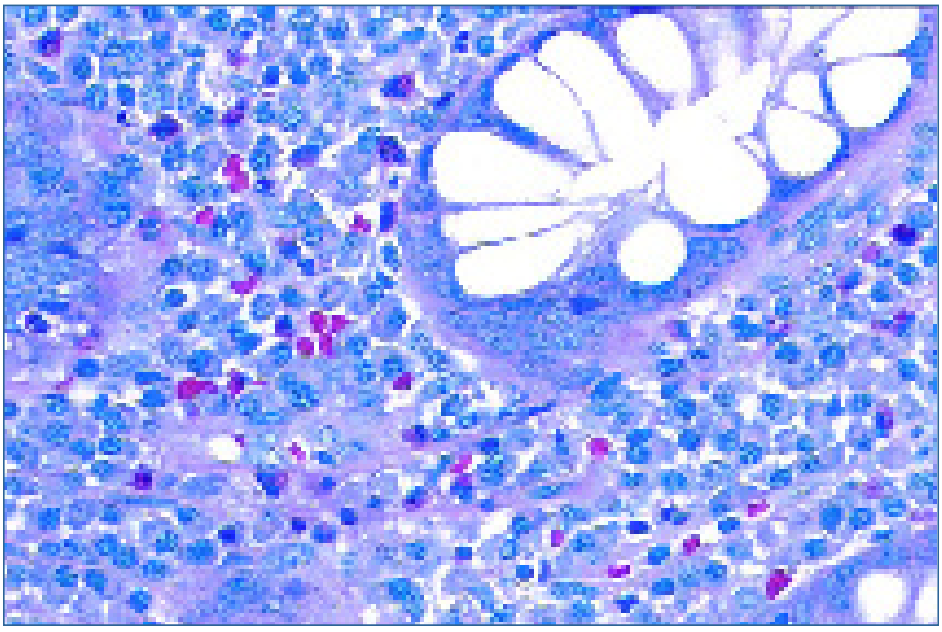

Figure 1.

Colon stained with Anatech's Tango and Eosin-Y. 40x.
Note how well the fine chromatin patterns are shown.

Logwood is chipped and extracted with water (usually) to produce a concentrated solution of dye. This is then exported to companies that purify it and generally render it into a dry powder (although there is some use for the liquid extract itself). For all practical purposes, there is only one extractor in business, not counting tiny facilities operating as cottage industries. Only two purifiers of any size exist, one in the United States, the other in France. Obviously, any-

one depending upon a steady supply of hematoxylin is in a precarious position.

Today's shortage was caused not by hoarding, not by loss of all the logwood trees, nor by any of the other rumors in wide circulatißon. The extractor simply took logwood out of its production schedule because it was more profitable to concentrate on other botanicals used in the huge cosmetic and skincare industries. Standing inventories at the purifier plants were not sufficient to tide us over until the next scheduled extraction. Fortunately, negotiations with the extractor led to a reinstatement of production and new supplies of dye should become available during the summer of 2008.

An important point here is that shortages are a predictable outcome of dealing with such a restricted natural commodity. Trying to place blame is futile and unwarranted, as it hardly matters which link in the supply chain breaks. You the laboratory customer may be able to obtain hematoxylin during the period while supply chains are reforged, almost certainly at a price much higher than you are happy to pay. Or, you may not be able to get it at any price. Either way, we have something of interest for you. We can cover you during the outage, or provide a long-term solution, free of market fluctuations at a reasonable price.

Anatech's solution

Several years ago, in anticipation of a time when either price, availability or both would justify a substitute for hematoxylin, we developed a stain made with Mordant blue 3 (CI 43820). Our reasons for choosing this dye included several criteria. Obviously, it had to resemble hematoxylin when paired with eosin, with the same specificity and as close to the same color as possible. But that is not enough. It would do little good to choose a dye likely to have fluctuations in availability. That meant it had to be in large-scale production for a stable industry. Mordant blue 3 is a widely used textile and printing dye manufactured in several countries by independent chemical companies. That ensures long-term availability. As it is a relatively easy dye to synthesize, its cost is reasonable and likely to remain so.

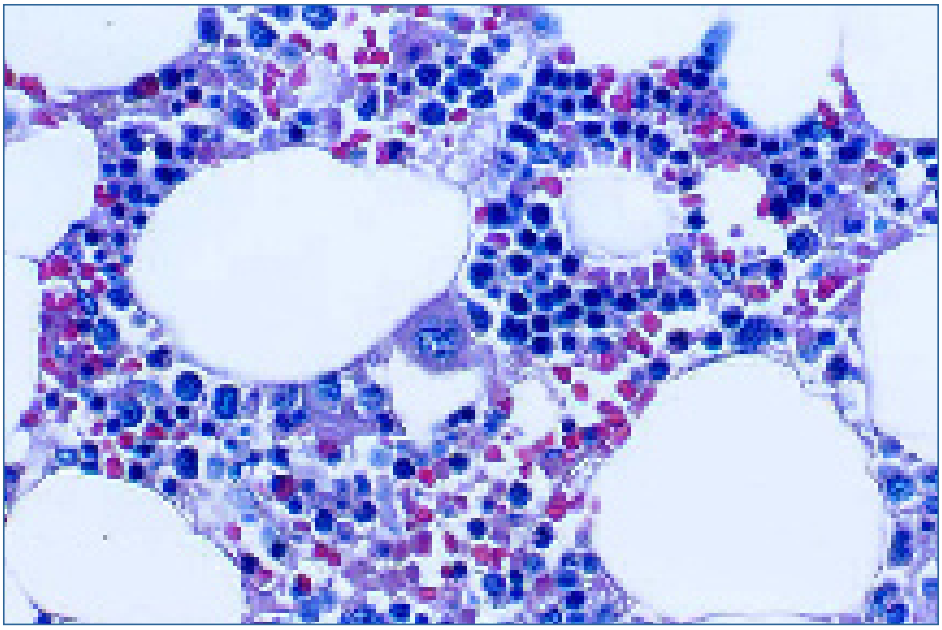

Figure 2.
Bone marrow stained with Anatech's Tango and Eosin-Y. 40x.

Our new product is called Tango Stain Set, a kit with two components: Tango Mordant and Tango Stain. While its use differs slightly from hematoxylin, Tango will fit right into your automatic stainer. Like hematoxylin, the dye in Tango Stain requires a metal mordant, but we have separated the mordant from the stain, much like you do when staining with iron hematoxylin. Metals mixed with mordant dyes don't always make for stable solutions, and can make selective differentiation difficult.

A review of our staining procedure will explain the theory behind staining with Tango (see sidebar on page 4). Notice that there is no acid differentiation and no bluing agent. We have added a step with Tango Mordant, but the net effect will be to eliminate one station for most laboratories.

After hydration, Tango Mordant impregnates sections with a metallic mordant. Excess mordant is then rinsed off. You may be surprised to see that Tango Stain is reddish-orange, not purple. When sections come out of Tango Stain, they too will be reddish-orange, but will

quickly change to blue (nucleic acids) and pink (other tissue elements) in tap water. Further exposure to tap water removes the weakly bound red dye selectively. The 70% alcohol slows differentiation and prepares the section for the counterstain. After eosin, use the 95% alcohols to remove unbound dye and differentiate the pink and red colors.

Results

With Tango, nuclei are blue to violet blue. Time in the tap water rinse after staining is critical to achieving the correct intensity and shade. If too short, eosin colors will be off (dense and dull), and if too long, nuclei will lose their violet hue. Different fixatives, fixation histories and section thickness may affect final nuclear and cytoplasmic colors, but these are controlled by the tap water rinse after staining. Do not reduce staining time in eosin. If this schedule produces eosin colors that are too intense, simply add a second station of 95% alcohol. In short, this is a very easy, readily controlled procedure. The photomicrographs speak for themselves.

Explanation of figures

Perhaps the best evidence for Tango's specificity and selectivity for DNA, RNA and their associated proteins is seen in Fig. 1. Here, a variety of cells populate the loose connective tissue of the colon. Chromatin patterns are strikingly clear. Eosinophils and bright red blood cells are obvious.

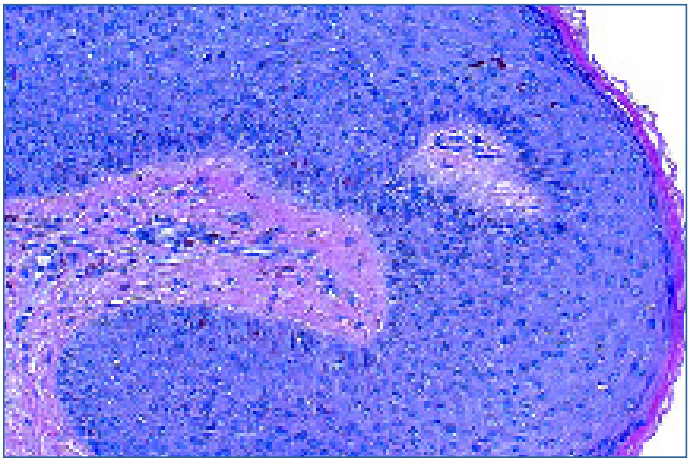

Figure 3.

Skin stained with Anatech's Tango and Eosin-Y. 40x.

Small round lymphocytes and the elongated nuclei of reticular cells are easily identified. But plasma cells are the best feature of this tissue, as they show the delicate differentiation Tango can achieve. These large cells have small nuclei characteristically pushed to one side of the cell. Their cytoplasm is rich in cytoplasmic RNA, so most of it stains dark violet-blue. A lighter perinuclear area represents the Golgi body which does not contain RNA and appears pink.

Another exquisite example of what Tango can do is the bone marrow shown in Fig. 2. Small, dark round nuclei belong to lymphocytes. Note the thin rim of lighter blue cytoplasm, clearly differentiated. A variety of other hematopoietic cells present a spectrum of hues.

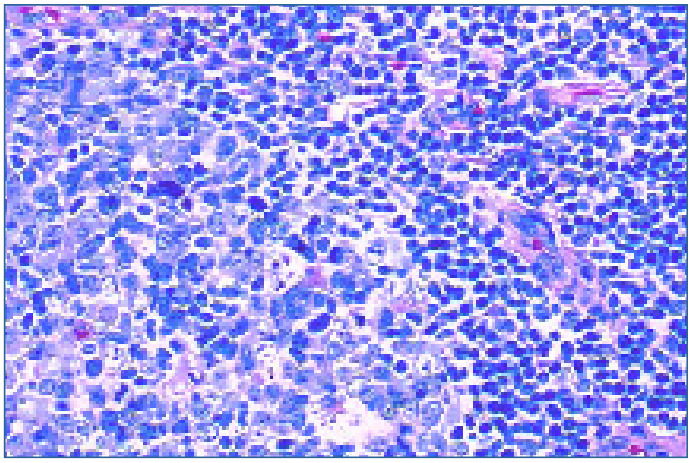

Figure 4.

Node with reactive lymphoid hyperplasia, stained with Anatech's Tango and Eosin-Y. 40x.

Cornified skin (Fig. 3) stained with Tango closely resembles the familiar color patterns of a section stained with hematoxylin and eosin. Note the granular layer of epithelial cells, characterized by dark blue cytoplasm, lying just beneath the violet-pink stratum corneum.

Tango produces delicate staining of the closely packed cells of a lymph node (Fig. 4). As in the other photomicrographs, chromatin patterns, nucleoli and nuclear membranes show distincly against delicately colored cytoplasm.

Packaging

We've made purchasing the kit as easy as using it. Tango is packaged as a pair, 1 gallon each of mordant and stain. No need to purchase one or the other alone. Simply order Tango. When stain intensity starts to lighten, replace both components. Tango Mordant may appear slightly cloudy, but that is normal as it is a colloidal suspension.

The aftermath

The hematoxylin shortage will eventually end, again. The dye will be in limited supply for a while as the distribution pipelines are filled. Wholesale prices for the dye have quadrupled between September 2007 and June 2008. Stain prices are sure to follow and they will not fall back to 2007 levels for a long time, if at all.

Staining Protocol

1. Hydrate slides to water
2. Tango Mordant..... 2 minutes
3. Tap water..... 2 x 2 minutes each
4. Tango Stain ..... 2 minutes
5. Tap water..... 2 x 2 minutes each
6. 70% alcohol .....1 minute
7. Anatech eosin.....1 minute
8. 95% alcohol.....1 minute
9. 100% alcohol..... 3 x 1 minutes each
10. Clearant..... 3 x 1 minutes each
11. Coverslip

You have two choices if you cannot obtain hematoxylin: buy a substitute and switch back to hematoxylin when it becomes available again; or, stay with a substitute if it is priced favorably and you like the results. Anatech will still be in the hematoxylin business after this is over, but we are suggesting that staying with a good, reasonably priced substitute would have a strategic advantage. You would be free of the steep price hikes and inevitable shortages inherent in a geopolitically limited natural dye subject to the vagaries of fluctuating economic conditions.

Other companies will probably start to sell substitutes, most likely based on celestine blue B (CI 51050). We studied that dye and rejected it. It is expensive (in part because it has no large-scale use). It is difficult (and thus expensive) to prepare solutions from it. And it tends to be more of a sky blue than a hematoxylin-like blue. It is not a replacement product for the long haul.

For availability, price, convenience and technical expertise behind the product, look to Anatech Ltd. when your workflow is threatened with disruption and your budget is about to collapse.

References

For the most scholarly and comprehensive account of logwood and its dye, see pages 263-274 in Cardon, D (2007). *Natural dyes.* Archetype Publications, London, 778 pages.

Product	Cat.#	Unit
Tango	852	2 x 1 gallon