

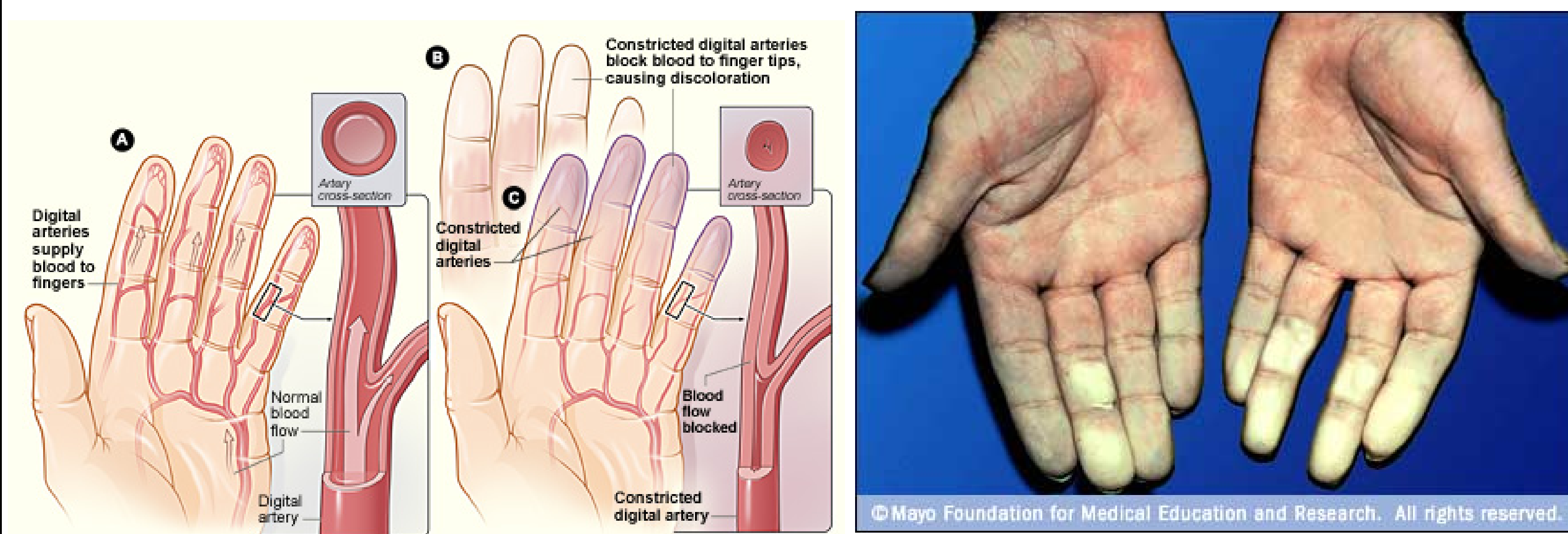
Introduction:

Histotechnology involves the process of fixing and embedding tissue specimens for the purpose of pathological examination. This process involves a number of steps and different chemicals. Multiple advancements have happened since the start of processing tissue, but the methods currently being used have posed issues for personnel who work in the laboratories as well as environmental hazards. Ongoing research has been put in effect to find new innovative ways of getting the same results of tissues with keeping a low carbon footprint and protecting it's employees personal health. The major classes of solvents that pose a threat are the clearing agent, xylene, and the fixative, formaldehyde. They both cause health issues in the people who are exposed, as well as environmental effects.

Background and Current technology:

The most effective techniques used in histology labs today include a multitude of deleterious solvents. Although they are still harmful to the environment and health, they are a great improvement from the resources used in the laboratory 30-40 years ago. A solvent that poses a large threat to health via exposure is xylene. Xylene is used in the current processing method as a "clearing agent." A clearing agent is needed because paraffin is not miscible with the ethanol alcohol used to previously dehydrate the tissue sample. Previous to the use of xylene, dioxane was the standard. Dioxane has been identified by the U.S. Environmental Protection Agency as a probable human carcinogen with numerous cases of death reported. The introduction of xylene made a big impact on decreasing health issues, but excessive exposure to xylene can still affect the brain, digestive system, ear, eye, heart, kidney, liver, and other systems.

Xylene has also been known to cause Raynaud's phenomenon. Raynaud's phenomenon is the numbness and pain associated with exposed areas, usually the hands. If exposed to both xylene and acetone for long periods of time, necrosis or gangrene of the tissue can occur. The first picture illustrates what is happening to the blood vessels during this disease. The second image is the effects from the disease.



National Heart, Lung and Blood Institute. <http://www.nhlbi.nih.gov/health/health-topics/topics/raynaud/>

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Not only can xylene lead to health risks, it also has been linked to some environmental effects. When xylene is disposed of, it can be released into the environment and has a tendency to add to runoff of near by bodies of water. It has both an acute and chronic toxicity to aquatic life, and causes injury to agriculture and ornamental crops.

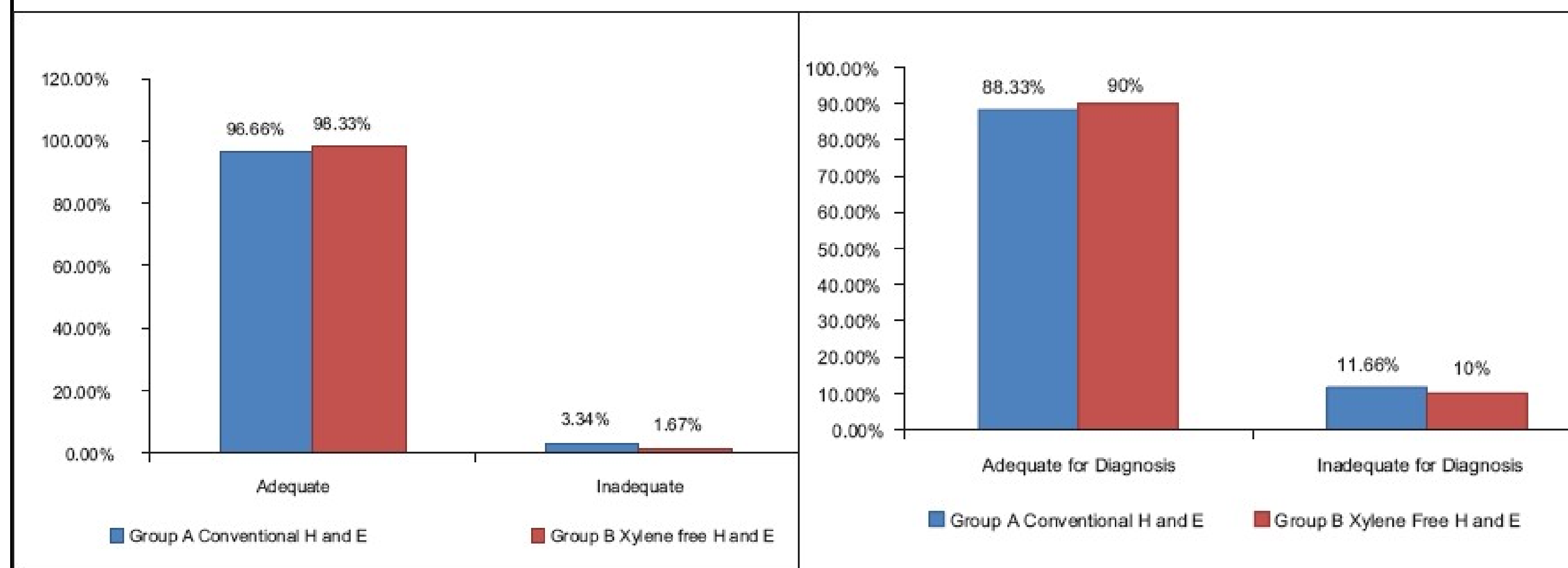
Another solvent prevalent in most grossing labs is formaldehyde. It can serve as a preservative in medical laboratories, as an embalming fluid, and as a sterilizer. Formaldehyde, mixed with a buffer solution to make formalin, is currently the most used fixative in laboratories. Explosive reactions can occur if formaldehyde is in contact with various solutions such as peroxide, nitrogen oxide, and performic acid. Effects of long term exposure to formaldehyde have been tested and in 2011, the National Toxicology Program, an interagency program of the Department of Health and Human Services, published that it is known to be a human carcinogen. With prolonged exposure it also acts as a neurotoxin.

Current Advancements in Technology:

The main solutions that cause problems for people in contact with them are xylene and formaldehyde. This is because of the multiple purposes each has in the lab, and the amount of exposure time. Once exposed via the respiratory system, the xylene and formaldehyde have the ability to store in adipose cells. Exposure can be as short as 1.5 years to cause many health issues. Currently, many substitutes for xylene have been found that range from synthetic derivatives to natural resources. The introduction of vegetable oil as a clearing substitute resulted from the synthetically developed substitutes being less effective as well as more expensive than xylene. It allows tissue to be cleared faster and gives a quicker transition from dehydration to infiltration with paraffin.

Figure 1. shows the first series of graphs as comparing adequate vs. inadequate staining. The blue column represents normal hematoxylin and eosin staining using xylene as a clearing agent. The red column represents hematoxylin and eosin staining that did not use xylene, and instead used mineral oils as a substitute. From comparing just on the stance of adequacy, staining without xylene yields more adequate slides and less inadequate slides than xylene. In regards to using those slides for proper diagnosis, the slides using xylene substitutes are more adequate for coming to a diagnosis.

Figure 1.



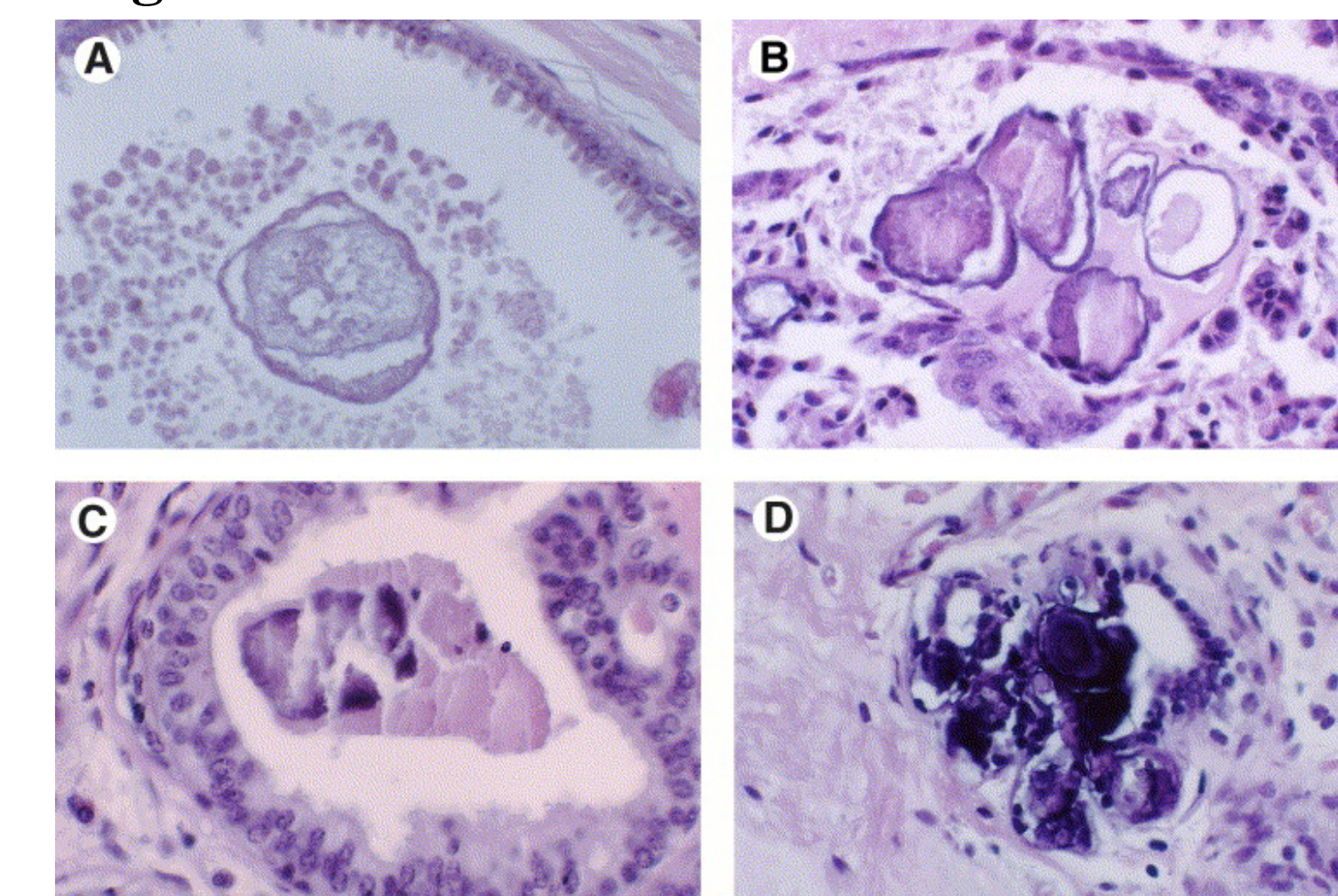
Graph 1: Comparison of adequacy of nuclear staining in group A and B. Graph 6: Comparison of staining for adequacy of diagnosis in group A and B

Ankle, Mahuri R., and Priya S. Joshi. "A Study to Evaluate the Efficacy of Xylene-free Hematoxylin and Eosin Staining Procedure as Compared to the Conventional Hematoxylin and Eosin Staining: An Experimental Study." *Journal of Oral and Maxillofacial Pathology* 15.2 (2011)

Clear-Advantage is also a synthetic substitute for xylene. It is an odorless, colorless solution that is a derivative petrochemical, or derived from natural gases. This substitute also has many advantages in the lab instead of xylene, and is produced in bulk by certain manufacturers.

Formaldehyde substitutions have also been made within labs. One type of substitute is Glyo-Fixx, a glyoxal-based fixative. Glyo-Fixx is a non-carcinogenic form of formaldehyde. It does have some health issues associated with it, including skin irritation and dizziness with high concentrations of inhalation. It has been proven to yield the same fixing results as formaldehyde.

Figure 2.



This figure shows 4 histological sections. Slides A-C are slides that have been fixated with Glyo-Fixx. Slide D is fixed using the conventional way of formaldehyde. The dark precipitate seen in slide D reflected calcification that was not properly able to fix with formaldehyde. From these tissues, it can be determined that the Glyo-Fixx may stain a lighter color, but can be just as clear or more clear than traditional formaldehyde.

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Advantages of Eco-Friendly Solvents:

Experiments have been conducted to test the effectiveness of using vegetable oil and Clear-Advantage as substitute clearing agents for xylene. The conclusion was made that processing tissue with mineral oil was equivalent to processing with xylene. Advantages of using mineral oil in the lab include:

- Less shrinkage of permanent sections from previously frozen sections
- Superior infiltration of lymph nodes
- Tissue sections are softer to cut, especially brain, uterus and decalcified sections
- Slides dry 3 times faster than with xylene.
- Slides clear faster and crisper
- Paraffin dissolves 50% faster than with xylene.

Other advantages of using mineral oil include an easier and more efficient recycling. Mineral oil can be collected with the paraffin and collected for incineration. It has been shown to have a less deleterious effect on the environment, and usually has no negative consequences if a major spill does not occur.

The use of this substitute in the lab decreases health issues for the personnel exposed to it. There is no concern of it being carcinogenic or toxic. The only exposure issues that occur can be eye irritation if it gets into the eye.

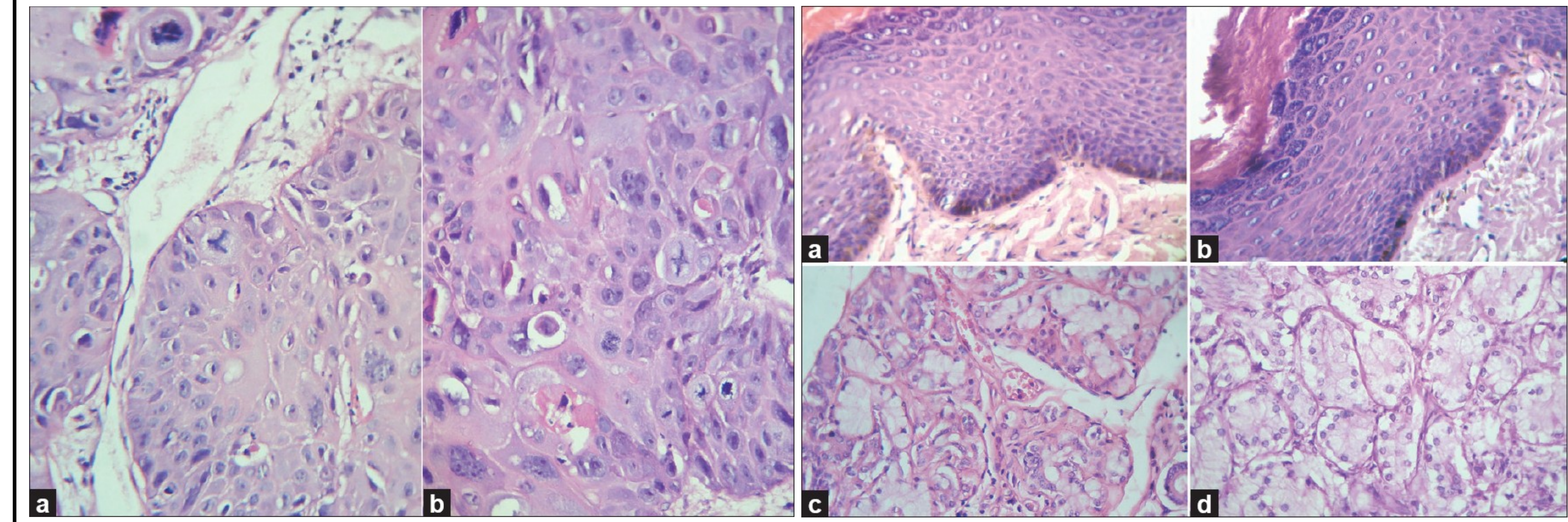


Figure 2: Photomicrograph showing the crisp staining of epithelium, connective tissues, and glandular tissue. (a) Epithelium and connective tissue stained with routine H and E staining, (H and E, 100x), (b) Xylene- and alcohol-free H and E staining, (H and E, 100x), (c) Glandular tissue stained with routine H and E staining, (H and E, 40x), (d) Glandular tissue stained with xylene- and alcohol-free H and E staining, (H and E, 40x)

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These histological views of tissues help draw the conclusion that quality of the stain and ability to diagnosis is not at all impaired with the substitution of xylene for other environmentally safe solvents.

Glyo-Fixx used as a fixative has also shown many advantages. Experiments concluded that this solvent needed less time than formaldehyde to fix and yielded the same level of quality. This may be a better solution than using formaldehyde because it is a non-carcinogen as well as non-sensitizing. It is overall safer for the personnel using it. It also is environmentally safe.

Conclusion:

Although solution protocol has come a long way since what was first being used, there is still ample amount of research and innovative solutions that can start to be integrated into histotechnology labs. This will allow for a lower negative impact on the environment as well as improve the quality of personnel's health.

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