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Review Article

BIOSAFE SUBSTITUTES TO XYLENE: A REVIEW

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ABSTRACT

Tissues have to undergo through series of 'tissue processing' procedures before they are ready to be diagnosed under the microscope. The various steps of tissue processing include fixing, dehydration, clearing and infiltration. Clearing refers to the process of replacing the dehydrant with a substance that is miscible with the embedding medium. It is one of the most critical steps of tissue processing and largely affects the clarity of the final section and hence the precision of diagnosis. Since time immemorial, xylene has been the non-substitutable clearing agent used in histology laboratories. However, off late, xylene has been found to have many toxic effects. This review aims to discusses the properties and toxic effects of xylene, merits and demerits of the other commonly used clearing agents and also critically analyses the various suggested substitutes of xylene.

INTRODUCTION

'DIAGNOSIS' [Greek: *'dia'*- through or by means of and*'gnosis'* - knowledge] is the process of identifying and determining the nature and cause of a disease through complete evaluation of patient & review of the lab findings (Rai, 2016). Though the attempts to establish a confirmed diagnosis began since the days of Hippocrates, establishing a confirmed diagnosis became possible only after the advent of histotechniques (Rai, 2016). 'Histotechniques' refer to the series of chemical procedures through which the tissues have to undergo before they are ready to be microscopically examined and diagnosed (Bancroft and Gamble, 2002). It is important that at every stage of the histotechniques, the integrity of tissue is maintained. The tissue must be fixed and processed in such a manner that when microscopically examined all the structures can be differentiated leading to a correct diagnosis. The aim of tissue processing is to embed the tissue in a solid medium firm enough to support the tissue and give it sufficient rigidity to enable thin sections to be cut and yet soft enough not to damage the knife or tissue. The whole process of tissue processing is divided into four major steps:

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(a) Fixation: It is the first or foundation step which is done immediately after biopsy. During this process, the semi fluid state of the cell is converted into a semisolid state thus maintaining, the morphology and structural details of the tissue. (Culling, 1985).

(b) Dehydration: It refers to the removal of fixative and water from the tissues. There are a number of dehydrating agents. This is usually done by subjecting the tissue to increasing concentration of alcohols like 70% to 95% to 100%. (Bancroft and Gamble, 2002)

(c) Clearing: It is the process of replacing the dehydrant with a substance that is miscible with the embedding medium (paraffin). The term "clearing" comes from the fact that the clearing agents often have the same refractive index as proteins. As a result when the tissue is completely infiltrated with the clearing agent, it becomes translucent. This change in appearance is often used as an indication of the effectiveness or completeness of the clearing process the chemical solution most commonly used is xylene. (Ofusori *et al.,* 2009)

(d) Infiltration: The fourth and final step in the tissue sample treatment is infiltrating the sample, usually with paraffin wax. In this step the cleared tissue samples are placed into paraffin heated to a few degrees above its melting temperature.

Once all these have been done, it is then sectioned on a microtome. (Ofusori *et al.,* 2009). Most histology.cal processing laboratories use xylene as a clearing agent on a daily basis which indeed offers innumerable advantages but unfortunately is considered to be highly toxic making it a potential occupational hazard for the histopathological technicians. This review aims to discusses the merits and demerits of the commonly used clearing agents and also critically analyses the various suggested substitutes of xylene.

Ideal Requisites of a Clearing Agent (Culling, 1985)

- Speedily removes the dehydrating agent.
- Easily removes the molten wax
- Causes minimum tissue damage
- Minimum Toxicity
- Cost effective

Commonly Used Clearing Agents (Bancroft and Gamble, 2002)

- **Xylene:** It has a rapid and excellent clearing action. Biopsy specimens of 3-4 mm are cleared in 4 hours with xylene. Unfortunately, it has been found to be extremely toxic.
- **Toluene and Benzene:** They are similar in properties to xylene and are less damaging to tissues on prolonged exposure. Unfortunately, it has also been found to be extremely toxic.
- **Chloroform:** It is slower in action but it causes less brittleness. It is expensive and inflammable.
- **Cedar Wood Oil:** It is recommended for treatment of delicate tissues as it has the least hardening effect. It is very slow in action and expensive.
- **Methyl Benzoate and Methyl Salicylate**: These are slow acting clearing agents and can be used when double embedding is required.

In 1950s, considering the merits and demerits of the various clearing agents, , Xylene was considered as the best clearing agent technically and also the safest alternative to dangerous chemicals like aniline oil, benzene, chlorofoam , toluene, dioxane etc. But till 1970s, there were great concerns about its safety.

Xylene

Xylene is an aromatic hydrocarbon widely used in industry and medical technology as a solvent. It is a colorless, sweet-smelling liquid or gas occurring naturally in petroleum, coal and wood tar, and is so named because it is found in crude wood spirit (Gr. xy`lon- wood). It has a chemical formula of C6 H4 (CH 3)2 and is referred to as "dimethyl benzene" because it consists of a six-carbon ring to which two methyl groups are bound. It exists in three isomeric forms: ortho-, meta- andpara-xylene (Kandyala *et al.,* 2010). Xylene is used as a solvent in the printing, rubber, paint and leather industries. It is found in small amounts in airplane fuel, gasoline and cigarette smoke. In dentistry, xylene is used in histological laboratories for tissue processing, staining and cover slipping and also in endodontic retreatment as a guttapercha solvent. Its high solvency factor allows maximum displacement of alcohol and renders the tissue transparent, enhancing paraffin infiltration.

In staining procedures, its excellent dewaxing and clearing capabilities contribute to brilliantly stained slides. Laboratory-grade xylene is composed of m-xylene (40-65%), p-xylene (20%), o-xylene (20%) and ethyl benzene (6-20%) and traces of toluene, trimethyl benzene, phenol, thiophene, pyridine and hydrogen sulfide. Histopathological technicians who routinely come in contact with xylene-contaminated solvents in the workplace are the population most likely to be exposed to high levels of xylene (Kandyala R et al, 2010). The current Occupational Safety and Health Administration permissible exposure limit for xylene is 100 ppm as an 8-h time-weighted average (TWA) concentration (Kandyala *et al.,* 2010). The National Institute for Occupational Safety and Health recommended exposure limits for xylene at 100 ppm as a TWA for up to a 10-h work shift and a 40-h work week and 200 ppm for 10 min as a short-term limit. Besides occupational exposure, the principal pathway of human contact is via soil contamination from leaking underground storage tanks containing petroleum products. Xylene can leak into the soil, surface water or ground water where it may remain for months or more before it breaks down into other chemicals. However, as it evaporates easily, most of it goes into the air and gets broken down by sunlight into other less-harmful chemicals. Most people begin to smell xylene in air at 0.08-3.7 ppm (parts per million) and begin to taste it in water at 0.53-1.8 ppm.

Toxic Effects of Xylene

The histopathological laboratory technicians are routinely exposed to xylene during procedures like tissue processing, clearing, staining, placing a cover slip and cleaning tissue processors. The exposure and handling of xylene is maximum during dewaxing of sections. Exposure to xylene can occur via inhalation, ingestion, eye or skin contact (Negi *et al.,* 2013).It is primarily metabolized in the liver by oxidation of a methyl group and conjugation with glycine to yield methyl hippuric acid, which is excreted in the urine. Xylene causes health effects from both acute (<14 days) and also chronic (>365 days) exposure. The type and severity of health effects depends on several factors, including the amount of chemical you are exposed to and the length of time you are exposed for. Individuals also react differently to different levels of exposure. All these effects are caused by depletion of mitochondrial adenosine triphosphate (ATP) in the affected cells.

Nervous System

Effect of xylene on the central nervous system is attributed to the liposolubility of xylene in the neuronal membrane. It has been suggested that xylene disturbs the action of proteins essential to normal neuronal function either by disruption of the lipid environment in which the membrane proteins function or by direct interaction with the proteins in the membranes (Savoleinen and Pfaffli, 1980). Long-term exposure may lead to headaches, irritability, depression, insomnia, agitation, extreme tiredness, tremors, impaired concentration and short-term memory. This condition is sometimes generally referred to as "organic solvent syndrome" (Honma *et al.,* 1983).

Lungs

Exposure to xylene at levels of 200 ppm or greater can irritate the lungs, causing chest pain and shortness of breath. Extreme overexposure (*e.g.*, in a confined space) can result in pulmonary edema, a potentially life-threatening condition in which the lungs fill with fluid.

Ear, Nose and Throat

Irritation of the nose and throat can occur at approximately 200 ppm after 3-5 min. Accidental splash in the eye may damage the surface of the eye, which will heal within a few days.

Liver and Kidney

At very high levels of exposure, xylene can injure the liver and kidneys, but this is extremely unlikely to happen without noticeable effects on the nervous system. Generally, such damage is reversible. Low-level occupational exposure does not affect the liver and the kidneys.

Blood

There is no evidence that exposure to xylene affects the blood cells in humans. Earlier reports of low red blood cell counts (anemia) may have been due to contamination of xylene with benzene.

Gastro Intestinal Tract

Symptoms of nausea, vomiting and gastric discomfort were observed in workers exposed to xylene vapors (unspecified concentration), which were reversible.

Skin

Xylene, like other organic solvents, can dissolve the skin's natural protective oils. Frequent or prolonged skin contact can cause irritation and dermatitis, dryness, flaking and cracking of the skin. Damaged skin may allow greater absorption of chemicals. Xylene easily penetrates most ordinary clothing and can become trapped in ordinary gloves and boots. Xylene trapped in the clothing can cause burns and blistering.

DISCUSSION

Though there are a large number of clearing agents available but still over the last century, anatomists and pathologists have used xylene by choice. Xylene offers a huge number of advantages: it is a stable fluid, speedily removes the dehydrating agent, easily removes the molten wax, causes minimum tissue damage and minimum brittleness and is cost effective. Moreover the pathologists are trained to look at sections cleared with Xylene and are therefore reluctant to change the microscopic appearance of diagnostic tissues by using a different clearing agent. However, many substitute chemicals like limonene reagents, aliphatic and aromatic hydrocarbons, and mineral oil mixtures are being used to substitute xylene as a clearing agent during tissue processing. Limonene reagents are composed of ‘d- limonene’ , which is a hydrocarbon.it is the major component of citrus peel oils. It is prepared by steam distillation of orange peels. It has a strong citrus smell. It has been used in the Food and Cosmetic industry and has been regarded as safe but in recent years, it has been used in some pathological labs and head aches, nausea and effects on CNS have been reported on its use. Taneeru S et al (2013) used Limonene oil & Sesame oil as a substitute for xylene to deparaffinize tissue sections during hematoxylin and eosin (H&E) staining and compared them with conventionally deparaffinized H&E sections.

The study revealed better results with sesame oil in tissue processing and suggested its use as an alternative to xylene. Mineral (paraffin) oil mixtures look promising in eliminating xylene from most of the procedures. Isopropanol alone or mixed with molten paraffin is a technically acceptable and cost-effective substitute for xylene for tissue processing. It has been demonstrated that the best clearing agents from the sectioning quality and diagnostic value point of view, with automated or manual protocols, are mixtures of 5:1 and 2:1 isopropanol and mineral oil, followed by undiluted mineral oil, all at 50 degree C, making them a safer and cheaper substitute than xylene (Beusa RJ. 2000). The term aliphatic means that these hydrocarbons are arranged in the form of a "chain" instead of being arranged in a "ring" (aromatic). Because of their aliphatic structure, the substitutes generally need more time to exact the same effect on the tissue as does their aromatic counterpart. Some high-boiling aromatic hydrocarbon mixtures having lower volatility than xylenes have been manufactured. These are not so popular because they are just as toxic as xylene. The various substitutes to Xylene discussed above (including limonene reagents, aliphatic hydrocarbons, vegetable oils and mineral oils) were found to be less effective and more expensive. So, many novel reagents have been experimented upon by various researches to find a suitable substitute.

A study by Andre *et al.,* (1994) substituted xylene with a mixture of peanut oil, soyabean oil, coconut oil and cotton oil and concluded that it was a poor alternative, as the quality of sections cleared with Xylene were better. Sermadi W et al (2014) compared the efficacy of coconut oil with that of xylene, as a clearant. The results proved coconut oil to be an efficient substitute for xylene, as it is non-hazardous, less expensive and caused less shrinkage of the tissue. It was concluded that coconut oil can be used as a de-alcoholization agent in the histopathological laboratory, without losing the quality of the histological details. Esan *et al.,* (2015) did a study by using groundnut oil as an alternative clearing agent to xylene in histological tissue processing. The tissue samples were processed with groundnut oil as a clearing agent and the other tissue samples were processed along with xylene as a clearing agent for a period of 4 hours to 24 hours using a manual tissue processing method. All the tissue samples processed with both groundnut oil and with xylene as a clearing agents were sectioned with a rotary microtome and stained with Masson’s Trichrome and Haematoxylin and Eosin methods. The results showed that there was no difference between tissue sections cleared in Groundnut oil and those tissue sections cleared in Xylene.

The study proved that groundnut oil, which is a cheap, easily available and non-toxic agent is suitable and can be used as a clearing agent in histological tissue processing. Many researches have even tried liquid dishwashing soap as a clearing agent. Its merits are that it is readily available and is much cheaper as compared to xylene. It is composed of sodium lauryl sulfate, sodium dodecyl benzene sulfonate, cocamidopropyl betaine and nonionic surfactants, all of which have been found to be safe. Falkeholm *et al.,* (2001) and Buesa *et al.,* (2009) have shown the advantages of hot dishwashing soap (DWS) solution for deparaffinization of tissue sections for H and E staining and some special staining procedures like periodic acid-Schiff (PAS) staining and Van Gieson staining.

Negi *et al.,* (2013) evaluated the efficacy of 1.7% dishwashing soap (DWS) solution as a deparaffinizing agent for hematoxylin and eosin (H and E) staining and compared it with xylene. 1.7% DWS was found to be an effective alternative deparaffinizing agent to xylene and meanwhile facilitating as less biohazardous, economical and a faster deparaffinizing agent. Thus we see that clearing agents have their own merits and demerits.

Although all chemicals discussed in the present manuscript have to be dealt with carefully but unfortunately, xylene has been reported to have maximum hazardous effects. The OSHA (Occupational Safety and Health Administration) regulation standard has declared Xylene as hazardous and is advocating its substitution with less dangerous chemicals. Some studies have proposed the use of natural and eco-friendly solutions as alternatives to xylene. Research is being done to search for safer and technically good alternatives.

Conclusion

Clearing is a vital part of histotechniques. No clearing agent is ideal. But unfortunately, xylene is a corroborated biohazard, its routine use as a clearing agent is a major health and safety concern. Considering the toxicity of xylene, it is desirable to minimize its use in histopathology laboratory without compromising the staining quality and hence the appropriate diagnosis. Thus the quest for safer alternatives is envisaged.

REFERENCES

Andre GG, Wenger JB, Rebolloso D, Arrington JB, Mehm WJ. 1994. Evaluation of clearing and infiltration mixtures (CIMs) as xylene substitutes for tissue processing. J *Histotechnol.* 17:137–42.

Bancroft, J.D, Gamble, M. 2002. Theory and Practice of Histological Techniques 5th edition. Churchill Livingstone Publication pp: 63-108.

Buesa RJ, Peshkov MV. 2009. Histology without xylene. *Ann Diagn Pathol*. 13:246–56.

Buesa, RJ. 2000. Mineral oil: The best xylene substitute for tissue processing yet? *J Histotechnol*. 23:143-8.

Culling, C.F.A. 1985. A Handbook of Histopathological and Histochemical Techniques 4th edition Butterworth Publication pp: 27-77.

Esan, E.O., Ajileye, A. B., Fasogbon, S. A. 2015. Groundnut oil as an alternative clearing agent to xylene in histological tissue processing. *Adv Lab Med Int*. 5(3): 24-31.

Falkeholm, L, Grant, CA, Magnusson, A, Mollur, E. 2001. Xylene-free method for histological preparation: A Multicentre Evaluation. *Lab Invest*. 81:1213–21.

Honma, T, Sudo, A, Miyagawa, M, Sato M, Hasegawa, H. 1983. Significant changes in the amounts of neurotransmitter and related substances in rat brain induced by subacute exposure to low levels of toluene and xylene. *Ind Health,* 21:143-51.

Kandyala, R., Raghavendra, S. C., Rajasekharan, S. T. 2010. Xylene: An overview of its health hazards and preventive measures. *J Oral Maxillofac Pathol*., 14:1-5

Negi, A., Puri, A., Gupta, R., Chauhan, I., Nangia, R., Sachdeva, A. 2013. Biosafe alternative to xylene: A comparative study. *J Oral Maxillofac Pathol*., 17:363-6

Ofusori, D., Ayoka, A.O., Adeeyo, O.A., Stephen, O. and Adewole, S.O. 2009. *Int. J. Morphol*., 27: 211-218.

Rai, R. 2016. Tissue Fixatives: A Review. *International Journal of Pharmaceutics and Drug Analysis*., 2016; 4: 183-187.

Savoleinen, H., Pfaffli, P. 1980. Dose-dependent neurochemical changes during short-term inhalation exposure to m-xylene. *Arch Toxicol*. 45:117-22.

Sermadi, W., Prabhu, S., Acharya, S., Javali, S. 2014. Comparing the efficacy of coconut oil and xylene as a clearing agent in the histopathology laboratory. *Journal of Oral and Maxillofacial Pathology, JOMFP*. 18: 49-53.

Taneeru, S., Guttikonda, V.R., Vanajakshi, C.N., Korlepara, R. 2013. *Health Sciences,* 23: JS004.

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