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Milk an Alternative Clearing Agent- A Novel Pilot Study

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Abstract

Introduction

Clearing of tissues is the most delicate step in diagnostic pathology. An ideal clearing agent should induce rapid removal of dehydrant, it should be easily replaced with paraffin wax and should have low toxicity. It acts as a transitional procedure from dehydration to infiltration. Clearing process plays a crucial role by ensuring maximum removal of the dehydrant(alcohol) from the tissues, making the tissues transparent which in turn enables high levels of penetration of paraffin wax. Xylene is an aromatic hydrocarbon widely used in medical field and in dentistry, xylene is traditionally a popularly used tissue clearing agent due to its rapid clearing capacity and miscibility with most organic solvents. Xylene also has excellent dewaxing and

clearing properties in staining procedures and remains as a gold standard procedure for clearing in tissue processing procedure.

Xylene used in laboratories are mainly composed of composed of m-xylene (40–65%), p-xylene (20%), o-xylene (20%) and ethylbenzene (6-20%) and traces of toluene, trimethyl benzene, phenol, thiophene, pyridine and hydrogen sulfide. Ensuring compliance with OSHA's Occupational safety and health administration permissible exposure limit for xylene is critical to safeguarding the health of workers. Histopathology technicians and oral pathologists, who are frequently exposed to xylene vapors in their workplace, may face elevated risks of kidney, lung, eye, and central nervous system disorders as a result. It is a flammable liquid and may cause over hardening of the tissues when exposed for longer durations. As a result of increasing concerns about the potential carcinogenicity of the chemical agents used in routine tissue processing, implementing eco-friendly chemical agents is necessary. Substitution of an element means replacing or finding a substance that can perform the same function which may lessen the hazards. Milk that originated in a India. It is commonly used in middle-eastern cuisine, Indian Cuisine, as a traditional medicine and in religious rituals. Cow milk is the main source for milk. Milk is obtained from cow contains hundreds of fatty acids. The main composition of Cow milk includes Fats, Saturated fats, Proteins, Mono unsaturated fatty acid, polyunsaturated fatty acid and transfat. Milk is a complex structure of lipids (mainly triglycerides), free fatty acids, phospholipids, fat soluble vitamins, hydrocarbons and carotenoids. This high fat content of milk helps in clearing of tissues without altering the morphology or histological structure of the tissue.In oral and maxillofacial histopathology, a unique approach involves using milk as a clearing agent for certain tissue specimens. This method, known as milk clearing, is particularly beneficial when dealing with delicate or small tissue samples. The milk clearing process entails immersing the tissue in milk, which acts as a gentle clearing agent. The milk gradually replaces the fluid within the tissue, resulting in enhanced transparency. This allows for better visualization of cellular structures and pathological features under the microscope. Milk clearing is advantageous for preserving tissue integrity and minimizing distortion, making it a valuable technique in oral and maxillofacial histopathology, especially for specimens requiring meticulous examination. In an effort to improve working conditions in the histopathologic laboratory, we have investigated whether less toxic substance like milk. Milk may be substituted for toxic organic solvent like xylene, which is currently in use.

Keywords: Diagnostic, Hypothesis, Specimens, Tissue

Aim:

The aim of the study is to evaluate the efficacy of milk as xylene substitute in routine histopathological tissue processing procedure.

Objectives:

1. To determine the effectiveness of milk as a clearing agent
2. To compare and evaluate the effectiveness of milk with the xylene in the histopathological tissue processing procedure

Hypothesis:

Null /Alternate Hypothesis: Milk as a alternative clearing agent used in histopathological tissue processing procedures.

Methods And Methodology:

Materials

1. Pasteurized milk – clearing agent

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2. Biopsy tissues: oral and maxillofacial tissue sample (Site: i) mucosa ii) adipose tissue iii) muscle and iv) salivary gland)
3. Fixative (e.g., formalin)
4. Dehydrating agents (e.g., alcohol series- 70%, 95%, 100%)
5. Clearing agent –(xylene)
6. Embedding medium for impregnation (e.g., paraffin wax)
7. Microtome
8. Staining reagents (e.g., hematoxylin and eosin)

Sample selection:

We prepared 2 groups of soft tissue specimens distributed for routine histopathological evaluation using xylene (Group A) and Milk (Group B) as Clearing agents. The specimens fall under the 4categories of i) mucosa ii) adipose tissue iii) muscle and iv) salivary gland

Procedure:

1. Fixation:

- Submerge each biopsy tissue sample in fixative (e.g., formalin) immediately after collection. Allow fixation to proceed for the recommended time based on tissue size and type. Duration:24 hours

2. Dehydration:

- Transfer the fixed biopsy tissues through a series of increasing concentrations of alcohol (e.g., 70%, 80%, 95%, 100%) to dehydrate the tissues thoroughly. Ensure adequate time in each alcohol concentration to remove water from the tissues.
  Duration :30 minutes

3. Clearing with Milk:

- Prepare fresh whole milk for use as the clearing agent. Optionally, dilute the milk with distilled water to achieve the desired concentration.
- Submerge each dehydrated biopsy tissue sample in the milk clearing agent. Allow the tissues to soak in the milk for a 30 minutes to make them transparent. The duration may vary depending on tissue thickness and type. Concentration of xylene are m-xylene (40%- 65%), p-xylene (20%), o-xylene (20%). Concentration of milk -100%

4. Embedding:

- Once the tissues have become transparent, remove them from the milk clearing agent and transfer them to the embedding medium (e.g., paraffin wax). Ensure proper orientation of the tissues for sectioning. Duration: 1 overnight

5. Sectioning:

- Use a microtome to cut thin sections (typically 4-5 micrometers thick) of the embedded tissues. Collect the sections on glass slides for further processing.

6. Staining:

- Perform staining of the tissue sections using appropriate histological stains (e.g., hematoxin and eosin) to visualize cellular structures and tissue morphology.

7. Microscopic Examination:

- Examine the stained tissue sections under a microscope to assess cellular details, tissue architecture, and any pathological changes. Interobserver calculation will be made.

8. Documentation:

- Record observations, including tissue characteristics, staining patterns, and any abnormalities observed during microscopic examination.

9. Interpretation:

- Interpret the histological features of each biopsy tissue sample based on microscopic findings, correlating with clinical information to provide a comprehensive diagnostic report.

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10. Quality Control:

- Ensure adherence to standard laboratory protocols and quality assurance measures throughout the procedure to maintain the integrity and reliability of the histopathological analysis.
- **Subject selection**

Inclusion criteria

All freshly taken oral soft tissues obtained during diagnostic and operative procedures before fixed with any other fixatives.

Exclusion criteria

- Archival tissues
- Extra oral lesion tissues
- Hard tissues
- Any oral tissues already fixed with fixatives

- **Study design:** Invitro
- **Study setting:** (Department of Oral Pathology and Microbiology) Rajas Dental College and Hospital
- **Study period:** 3 months
- **Study groups:** 2 Groups

Group A- clearing by xylene

Group B – clearing by Pasteurized milk

20 sample- from oral mucosa during minor oral surgeries

Sample size estimation: tests –

- **Sample size:** 10 per Group
- **Statistical Analysis**

Software: SPSS V.22 IBM Statistical USA

Statistical test:

- Descriptive analysis: Mean & standard deviation
- Independent-t-Test

Technique used:

- Specimen collection
- Staining technique
- Scoring criteria:

It was evaluated with Histomorphologic criteria such as nuclear staining, cytoplasmic Staining, background staining and artifacts by two independent Oral Pathologists. The histomorphologic criteria were graded with a score of 1-4 for nuclear staining and cytoplasmic staining, 0-3 for background staining and 0-1 for artefacts

Histomorphologic criteria	Scoring
i) Nuclear staining	1- Poor
	2- fair
ii) Cytoplasmic staining	3- good
	4- excellent
iii) Background staining	0-nil
	1-mild
	2-moderate
	3-severe
iv) Artefacts	0-no
	1-yes

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