

CLEARING

CLEARING (DEALCOHOLIZATION)

INTRODUCTION

- This is the step between dehydration and paraffin infiltration.
- The purpose of this step is **removal** of the **alcohol** and **replacement** with a **fluid that will mix with paraffin**.
- The tissue is placed in two or three changes of clearing agents (volume 10 times the tissue).

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INTRODUCTION

- Good clearing agents should replace alcohol quickly and not over harden the tissue.
- This step is called **clearing** because they penetrate, make the tissue **transparent** and also **harden** it.
- Too long in the clearing agent hardens the tissue and the tissue become difficult to section.

CLEARING AGENTS

- Most clearing agents are highly toxic and inflammable and should be handled with care.



CLEARING AGENTS

- **Xylene** is the most commonly used clearing agent.
- If dehydration is incomplete the **xylene** becomes opaque or milky as water comes out of the tissue. If this occurs, put the tissue back into absolute alcohol and continue dehydration.



Xylene

DANGER

Flammable liquid and vapor. Causes skin irritation. May be fatal if swallowed and enters airways. May cause drowsiness or dizziness.

PREVENTION

Keep away from heat, sparks, and open flames. — No smoking. Keep container tightly closed.

Avoid breathing vapors or mist. Wash hands and any other contaminated skin thoroughly after handling. Wear protective gloves and eye protection. Use only outdoors or in a well-ventilated area.

RESPONSE

If swallowed: Immediately call a poison center or doctor. Do NOT induce vomiting. **If inhaled:** Remove person to fresh air and keep comfortable for breathing. Call a poison center or doctor if you feel unwell. **If on skin:** Take off all contaminated clothing. Wash with plenty of soap and water or shower. Wash contaminated clothing before reuse. If skin irritation occurs: Get medical attention.

In case of fire: Use foam, water spray or fog. Dry chemical, carbon dioxide or sand may be used for small fires only. Do NOT use water in a jet.

CLEARING AGENTS

- **Benzene** penetrates and clears tissue very rapidly, produces a minimum of shrinkage.
- **Cedarwood oil and chloroform** are also clearing reagents.

CLEARING AGENTS

- **Tert-Butanol (TBA)** and **n-butanol** are also good clearing agents **TBA** is preferred over **n-butanol** because it has less of a tissue hardening effect.

CLEARING AGENTS

- **Isopropanol** is used in the microwave technique

CLEARING AGENTS

- **Histo-Clear** is adopted as a xylene substitute because it is considered non toxic.

CLEARING AGENTS...CON

- Choice of clearing agents depend upon the following:
 - 1- The type of tissue to be processed.
 - 2- Speedy removal of the dehydrating agent.
 - 3- Ease of removal by molten paraffin wax.
 - 3- Minimal tissue damage.

Paraffin/TBA Method

Step	95%EtOH	100%EtOH	Water	TBA	Paraffin Oil	Note
1	50		40	10		
2	50		30	20		
3	50		15	35		
4	50			50		
5		25		75		Use dye in step 5 And incubate 1-2h
6		25		75		
7				100		
8				100		
9				67	33	

Proceed with paraffin/TBA infiltration

Paraffin/Xylene Method

Step	EtOH:Xylene	Time
1	3:1	1h
2	1:1	1h
3	1:3	1h
4	Xylene	1h
5	Xylene	1h
6	Proceed with paraffin/xylene infiltration	