



Microwave Tissue Processing

From Donna Willis, Harris Methodist Hospital, Ft. Worth, Texas

PURPOSE:

To provide rapid tissue processing that will decrease turn-around-time.

PRINCIPLE:

Microwave exposure is used to dehydrate, clear and impregnate tissue samples. Fixation of tissue samples is achieved prior to processing in the microwave oven. processing schedules are developed using tissue thickness as a determining factor. Dehydration is accomplished using Ethyl Alcohol. Isopropanol is substituted for xylene as a clearing agent. The isopropanol is "boiled out" of the tissue during the impregnation process by heating paraffin above the boiling point of isopropanol.

EQUIPMENT:

1. Rotator
2. Commercial Microwave operating at approx. 600 watts.
3. Paraffin pot. Temperature set at 84°C. This instrument is used to recycle paraffin.
4. Paraffin pot. Temperature set at 60°C.

QUALITY ASSURANCE:

Follow departmental Microwave Tissue processing Quality Assurance procedure.

REAGENTS:

1. Ethyl Alcohol:

Safety Data: Health hazards of Flammable and Irritant should be considered when using this chemical. NFPA rating of 2,3,0 should be followed. Target organs are nerves and liver. Personal Protective Equipment to be used: impervious lab coat, gloves, eye protection, respiratory protection if exposure is above threshold limits. First Aid procedures: Swallowing: If victim is conscious and able to swallow, have victim drink water or milk to dilute. Never give anything by

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mouth if victim is unconscious or having convulsions. CALL A PHYSICIAN OR CONTROL CENTER IMMEDIATELY. Induce vomiting only if advised by physician or Poison Control Center. Inhalation: Immediately remove victim to fresh air. If victim has stopped breathing, give artificial respiration, preferable mouth-to-mouth. GET MEDICAL ATTENTION IMMEDIATELY. Contact with eyes or skin: Immediately flush affected area with plenty of cool water. Eyes should be flushed for at least 15 minutes. Remove and wash contaminated clothing before reuse. GET MEDICAL ATTENTION IMMEDIATELY. All splashes to the eyes must be reported to Employee Health. Disposal: Do not flush to drain. Distill and reuse or collect and dispose at an approved hazardous waste site.

2. Isopropanol: Purchased from Allegiance Scientific Products.

Safety Data: Health hazards of Flammable, Teratogen, Irritant should be considered when using this chemical. NFPA rating of 1.3.0.0 should be followed. Target organs are eyes, nose, throat, skin, CNS, respiratory tract and GI tract. Personal Protective Equipment to be used: lab coat, gloves, eye protection. First Aid procedures- Inhalation: Remove from exposure area to fresh air immediately. If breathing has stopped, give artificial respiration. If breathing is difficult give oxygen. Get medical attention immediately. Eye Contact: Flush eyes immediately with large amounts of water, occasionally lifting upper and lower lids for at least 15-20 minutes. If irritation persists, seek medical attention. All splashes to the eyes must be reported to Employee Health. Skin Contact: Remove contaminated clothing and shoes immediately. Wash effected area with soap and mild detergent and large amounts of water. If irritation persists, seek medical attention. Ingestion: If swallowed, induce vomiting by giving two glasses of water and sticking fingers down throat. Keep victims head lower than hips to prevent aspiration into lungs. Never give anything by mouth to an unconscious person. Call a physician immediately. Disposal: Do not flush to drain. Collect and dispose at an approved hazardous waste site.

3. Paraffin:

Safety Data: Health hazards: Under normal working conditions, the use of this product is deemed non-hazardous. According to OSHA, paraffin fume may be considered to be hazardous if the workplace airborne concentration exceeds 2mg/m³. Skin Contact: Exposure to hot product may cause thermal burns. Eye contact: Exposure to hot products may cause thermal burns.. Ingestion: If large quantities are consumed, nausea and vomiting may occur. NFPA rating of 0,1,0 should be considered when using this product. Personal Protective Equipment: Ventilation; Provide general ventilation to insure fume concentrations do no exceed 3mg/m³. Gloves: Use protective gloves when handling hot material. Eye Protection: Safety glasses should be worn as good laboratory practices. First Aid: Inhalation: Remove from exposure area to fresh air immediately. If breathing has stopped, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately. Skin Contact: In case of skin contact with product under normal conditions, wash thoroughly with mild detergent and large amounts of water. Immediately contact a physician for treatment to thermal burns. Eye Contact: If hot product is splashed into eyes, flush immediately with clear water and contact a physician. Ingestion: If very large amounts are swallowed seek medical advice. Disposal: Ensure compliance with local, state and federal regulations.

PROCEDURE:

Microwave Biopsy (1mm thick) procedure (process time 45 minutes including fixation.)

1. Tissue samples are placed in plastic prelabeled cassettes and allowed a fixation time of 30 minutes for fresh tissue. (Most samples are fixed adequately upon receipt.) Fresh samples should be placed on a rotomixer agitator to enhance fixation.
2. Rinse cassettes with water to eliminate the possibility of a salt precipitation when the cassettes are placed in the Ethyl Alcohol.
3. Place cassettes into the Teflon processing rack. 20 cassettes will fit in 1 rack leaving the top level empty to allow for evaporation. 80 cassettes can be processed at a time using 4 racks and 4 plastic processing containers. (If you are processing less than 8 cassettes you should place at least 8 empty cassettes in the bottom of the rack. This will regulate the temperature and ensure proper processing.
4. Place processing rack in plastic container and fill with 100% Ethyl Alcohol to rinse off water. Discard alcohol and fill with 400 mL. of fresh 100% Ethyl Alcohol.
5. Place the plastic container in the microwave oven and place the temperature probe centrally into the container. Make sure the probe does not touch the cassettes.
6. When starting the microwave follow these steps:
 - a. Depress the power switch and observe that after a brief period, during which the vent system comes up to speed, the stop switch indicator extinguishes.
 - b. Depress the lamp switch and observe that the chamber light is illuminated.
 - c. The turntable is not used therefore the switch should not be depressed.
 - d. The airflow bubbler is not used therefore the switch should not be depressed
 - e. Set the timer select pushbutton to the minutes selection.
 - f. Set the time-at temperature mode using the timer mode select switch.
 - g. Set the time pause position (out) of timer/door button.
7. Microwave at a temperature of 67°C. for 5 minutes.
8. Take the plastic container and processing rack from the microwave and remove the rack from the container draining the rack on a paper towel. Dump the Ethyl Alcohol.
9. Place the processing rack back into the plastic container and fill with Isopropyl Alcohol.
10. Place the plastic container in the microwave. Add the temperature probe centrally into the bath and microwave at a temperature of 74°C. for 3 minutes.
11. Remove the plastic container and rack from the microwave. Place the processing rack into an empty container and fill with paraffin that is at a temperature setting of 60°C. Agitate the rack

so that the excess Isopropanol will mix with the Paraffin. Pour this paraffin into a paraffin pot that is at a temperature of 84°C. Refill the container with fresh Paraffin that is 60°C.

12. Place the container in the microwave. Add the temperature probe centrally into the bath and microwave at a temperature of 65°C. for 2 minutes.
13. Open the microwave door and agitate the rack several times to ensure temperature consistency. Adjust the temperature setting to 80°C. for 5 minutes.
14. Remove the container from the microwave and dump paraffin into the paraffin pot that is set at a temperature of 84°C.
15. Remove the processing rack from the plastic container and place cassettes at embedding center.
16. Place processing rack into xylene to remove excess paraffin. Both plastic containers can be reused for the next run.

PROCEDURE NOTES:

1. The microwave must be vented just like a chemical fume hood. Do not vent to regular return air vents.
2. Cassettes should be equally distributed in cassette rack.
3. When processing less than 8 cassettes use a minimum of 8 empty cassettes on the bottom of the rack to regulate temperature.

REFERENCE:

H2800 Microwave processor User's Manual.