Wisconsin Veterinary Diagnostic Laboratory UNCONTROLLED Document

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Title	Processing and paraffin infiltration of formalin-fixed tissue using the Tissue-Tek VIP 5 Vacuum Infiltration Processor
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1 Introduction

The Tissue-Tek VIP 5 Vacuum Infiltration Processor is a tissue-processing machine equipped to process up to 300 cassettes simultaneously. After the tissue has been trimmed, it can be processed overnight, over the weekend, over holiday breaks or run on a 2-3 hr "short cycle." Different baths of 10% neutral buffered formalin, xylenes, ethanols, and liquid paraffin are used so the paraffin can infiltrate the tissue. This makes the tissue rigid.

2 Specimen submission

2.1 Type

Any type of formalin-fixed trimmed tissue may be processed.

2.2 Special requirements for collection

Trimmed tissues in cassettes are submitted in 10% neutral buffered formalin, 70% Ethanol or Davidson's fixative.

2.3 Handling conditions

Nitrile gloves shall be worn when handling 10% neutral buffered formalin.

Always handle formalin-fixed tissue in an externally vented fume hood.

2.4 Criteria for rejection of sample

- The trimmed tissue shall fit easily into the cassette. If the piece of tissue is too large and/or thick, it will not process properly.
- If, after processing, the tissue appears to be too light in color and is spongy to the touch, it may need to be reprocessed (REF 12.1.1).

3 Materials

3.1 Equipment & Instrumentation

1. Tissue-Tek VIP 5 Vacuum Infiltration Processor
2. TBS paraffin pot
3. Sakura – Tissue Tek TEC Embedding Center
4. Stainless steel jug for transporting liquid paraffin
5. Sakura Tissue-Tek Stainless steel cassette basket
6. Waste containers

3.2 Reagents & Media

1. Ethanol 100%, 95%
2. Infiltration Paraffin
3. Neutral buffered formalin, 10% (NBF)
4. RO/DI H_2O
5. Xylene

3.3 Supplies

1. Sakura Tissue-Tek Activated Carbon Cartridge
2. All purpose wipes
3. Autoclave bags
4. Heat resistant mitt

4 Safety Management

4.1 Required Safety Training:

☒ Chemical fume hood operation
☒ Hazardous chemical use and disposal training

4.2 Required personal protective equipment (PPE):

Minimum: ☒ Lab coat, Safety glasses and closed toe shoes– upon entry of lab
Gloves: ☒ Nitrile
☒ Chemical resistant – when immersing fingers in Xylene

4.3 Hazard Communication

A. Chemical: See MSDS

- **Ethanol** (MSDS-260 and 261)
  - Flammable liquid and vapor
- **Neutral Buffered Formalin** (MSDS-198)
  - Strongly irritating to skin, eyes, respiratory tract, and mucous membranes. Poison. May cause allergic reactions. May cause permanent eye damage. Harmful if inhaled. Harmful if absorbed through skin, causes general tissue damage. Causes eye burns.
  - Ingestion fatal or may cause blindness.
- **Xylene** (MSDS-574)
  - Flammable liquid and vapor. Possible cancer hazard. May cause cancer based on animal data. Harmful if absorbed through skin or if inhaled. Causes eye, skin, and respiratory tract irritation. Inhalation may cause central nervous system effects. Aspiration hazard if swallowed - can enter lungs and cause damage.

B. Biological: ☒ Biosafety Level 2

C. Physical: Use caution when working with molten paraffin – scald hazard.

D. Electrical: N/A

E. Sharps: N/A

F. Ergonomics: N/A

G. Other: N/A

4.4 Waste Disposal

- Xylene is discarded in a designated waste xylene carboy and recycled using the in-house xylene recycler.
- Ethanol, Ethanol contaminated with xylene, histological stains, heavy metal stains and acids must be dumped into a designated waste carboy. Document the type and amount of each solution on the Chemical Inventory Analysis For Waste Solvents In Carboys. Once the carboy is full, transport the carboy with the completed documentation to room 1101 for safe storage until it can be picked up by UW-Safety.
- All broken glassware and/or slides are disposed in the broken glass box located in Room 1124.
- The fume water and cleaning water can be disposed of down the drain.
- Waste paraffin should be poured into a biohazard bag and incinerated. Double bagging may be necessary.

5 Preparation for procedure

5.1 Equipment and instrumentation preparation

- Ensure the reagents have not been used more than 10 times.

5.2 Reagents and media preparation

As needed, the following reagents are prepared by combining the listed ingredients. A larger amount of reagent can also be prepared by using the 20 L carboys. Be sure to label all secondary containers with the contents, date, initials and hazards of the chemical.

70% ethanol
RO/DI H_2O …….. 1.05 L
95% ethanol…….. 2.95 L

80% ethanol
RO/DI H_2O …….. 0.63 L
95% ethanol…….. 3.37 L

5.3 Standards/controls preparation - NA

5.4 Specimen preparation

- All tissue must be fixed in formalin or alcohol, trimmed into cassettes, and appropriately placed into the stainless steel baskets.

6 Performance of procedure

1. Load all cassettes into the processing basket at a trim station equipped with laminar flow. Rinse gloves with water prior to leaving the protection of the laminar flow hood.
2. Seal lid on the transport container while at trim station.
3. Carry sealed transport container to tissue processor.
4. Unlock the retort by sliding the locking mechanism to the left and then flip the lever up.
5. Silence any alarm by pressing the “Alarm Off” button.
6. Under the canopy exhaust hood, drain the formalin from the basket and place the cassettes into the retort.
7. Once all tissues are loaded, place the metal cover over the basket in the retort.
8. Close the lid and lock the retort by sliding the lock mechanism to the right.
9. Rinse gloves immediately with water and dry.
10. Press the “Tissue Processing” button.
11. Select the appropriate cycle.
12. Select either “Delay Start” or “Immediate Start” depending on when the cassettes were placed in the retort.
    a. Select “Delay Start” if the tissues were placed in the retort before 4:30 PM.
    b. Select “Immediate Start” if the tissues are placed in the retort at or after 4:30 PM or if the 2 or 3 hour short cycle process for biopsies is being run.
13. Confirm estimated end time/date at the bottom of the screen.

14. Record number of cassettes loaded and tasks performed on the processor maintenance production log (FM-P-22).
15. The processing times for each of the cycles are as follows:
    a. Overnight, Weekend, Holiday 1 Day, Holiday 3 Day, Holiday 4 Day, Holiday 5 Day cycles:
        - 10% NBF – 30 min
        - 70% Ethanol – 45 min
        - 80% Ethanol – 45 min
        - 95% Ethanol – 45 min
        - 95% Ethanol – 60 min
        - 100% Ethanol – 60 min
        - 100% Ethanol – 60 min
        - 100% Ethanol – 60 min
        - Xylene – 60 min
        - Xylene – 60 min
        - Liquid Paraffin – 60 min
        - Liquid Paraffin – 60 min
        - Liquid Paraffin – 60 min
        - Liquid Paraffin – 60 min
    b. Short Cycle 3 HR:
        - 10% NBF – 0 min
        - 70% Ethanol – 15 min
        - 80% Ethanol – 15 min
        - 95% Ethanol – 15 min
        - 95% Ethanol – 15 min
        - 100% Ethanol – 15 min
        - 100% Ethanol – 15 min
        - 100% Ethanol – 15 min
        - Xylene – 15 min
        - Xylene – 15 min
        - Liquid Paraffin – 0 min
        - Liquid Paraffin – 15 min
        - Liquid Paraffin – 15 min
        - Liquid Paraffin – 15 min
    c. Short Cycle 2 HR:
        - 10% NBF – 0 min
        - 10% NBF – 0 min
        - 70% Ethanol – 10 min
        - 80% Ethanol – 10 min
        - 95% Ethanol – 10 min
        - 95% Ethanol – 10 min
        - 100% Ethanol – 10 min
        - 100% Ethanol – 10 min
        - 100% Ethanol – 10 min
        - Xylene – 10 min
        - Xylene – 10 min
        - Liquid Paraffin – 0 min
        - Liquid Paraffin – 0 min

- Liquid Paraffin – 15 min
- Liquid Paraffin – 15 min

d. Overnight 70% and Weekend 70%
   - 10% NBF – 0 min
   - 70% Ethanol – 60 min
   - 80% Ethanol – 60 min
   - 95% Ethanol – 60 min
   - 95% Ethanol – 60 min
   - 100% Ethanol – 60 min
   - 100% Ethanol – 60 min
   - 100% Ethanol – 60 min
   - Xylene – 60 min
   - Xylene – 60 min
   - Liquid Paraffin – 60 min
   - Liquid Paraffin – 60 min
   - Liquid Paraffin – 60 min
   - Liquid Paraffin – 60 min

16. The alarm will sound when processing is complete.
17. Press the “Alarm Off” button to silence any alarm.
18. Press the “Drain Retort” button and allow approximately 5 minutes for this process to complete.
19. Remove the basket from the retort and carry to the embedding center. Place the cassettes into the paraffin holding tray in the embedding center (PEMBEDDING).
20. Remove excess paraffin from retort with an absobant wipe and discard.
21. Place deparaffinized baskets and cassette lids in retort.
22. Close and lock the lid.
23. Press “Start cleaning 1” or “Start cleaning 2”.
24. A brief alarm will sound when cleaning is complete.
25. Remove the cleaned basket and lids from the retort and wipe out with an absorbant wipe and discard.
26. The processor is then ready for use again.
27. The processors are used on a regular basis and need routine maintenance.
    a. Every 10 run cycles the following steps need to be taken:
       i. Remove the reagent bottles from the processor and empty the bottles, within a chemical fumehood, according to instructions in Section 4.4
       ii. Fill the first four bottles with warm water and place in the first four stations of the processor.
       iii. Press “Cleaning”→ “Clean Retort” → “Warm Water Rinse”.
       iv. While the warm water rinse is proceeding, refill all bottles, within a chemical fumehood with the appropriate fresh solutions.
       v. Securely replace all bottles, except the first four, in the corresponding station.

vi. Once the warm water rinse is complete, remove the four water bottles and dump the water down the drain.
vii. Replace the appropriate refilled bottles in the first four stations of the processor.
viii. Open the paraffin oven door, and empty paraffin containers into a trash can lined with autoclave bags.
ix. Allow the paraffin to cool for several hours, then remove it from the can.
x. Place the bagged paraffin into a large secondary containment unit until solidified.
xi. Place solidified paraffin into a Stericycle bin for disposal.
xii. Refill the containers with fresh liquid paraffin from the TBS paraffin warmer and replace the container in the appropriate location.
xiii. Once all containers have been replaced, close the paraffin door.
xiv. Refill the TBS paraffin warmer with solid infiltration paraffin pellets.
xv. Document that completion of the reagents change and warm water rinse on the processor maintenance production log.

b. Annually:
i. Preventative maintenance by service technician.

7 Interpretation of results

1. If an error occurs during the processing cycle then a RAW will be initiated and also noted on the Processor Maintenance / Production Log. If no error occurs, the cycle is valid and the proper function has been verified.

8 Report of results

1. Ensure the processor maintenance/production log is completed after use.

9 Procedure notes

9.1 Details and helpful hints

1. It may be helpful to keep a small cardboard collection tray next to the processor for transporting the steel basket to the embedding center.
2. After loading multiple mesh cassettes place the metal cover over the basket in the retort as usual, then add white plastic weight to prevent cassettes from floating and/or under processing.

9.2 Limitations of procedure - NA

10 References - NA

11 Summary of Current Revisions

1. Section 6: Added safety measures to process.

12 Supplemental Information

12.1 Quick Procedure Reference

1. De-processing/re-processing procedure
   - Two 30 minute periods in xylene.
   - Two 30 minute periods in 100% ethanol.
   - One 30 minute period in 95% ethanol.
   - Rinse with tap water and store in 10% NBF until next available run.

12.2 Flow Diagram - NA

12.3 Manufacturer's Information

Sakura Finetek U.S.A., Inc., 1-800-725-8723, Torrance, CA 90501 (MAN-P-39)