



# *Wisconsin Veterinary Diagnostic Laboratory UNCONTROLLED Document*

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| <i>Number</i>              | PEMBEDDING   |
| <i>Title</i>               | Embedding of processed tissues for histological staining |
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# **Wisconsin Veterinary Diagnostic Laboratory**

## **Standard Operating Procedure**

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### **1 Introduction**

Embedding is a procedure in which fixed, processed tissue is embedded in a paraffin block for tissue sectioning. Paraffin is a substance that will immobilize the tissue for sectioning. Correctly embedded tissues are properly oriented and are able to be faced on a common horizontal plane.

### **2 Specimen submission**

#### **2.1 Type**

Any type of processed tissue.

#### **2.2 Special requirements for collection**

Tissue cassettes are transported in a stainless steel cassette basket on a collection tray, from the tissue processor to the paraffin well of the embedding center.

#### **2.3 Handling conditions**

Although the tissue is fairly stable, it will be stored in liquid paraffin (55-65°C).

#### **2.4 Criteria for rejection of sample**

Spongy and/or fatty tissue samples must be re-processed until adequately processed (PPROCESSVIP5).

### **3 Materials**

#### **3.1 Equipment & Instrumentation**

1. Sakura Tissue-Tek TEC embedding center
2. TBS Paraffin dispenser
3. Incubator
4. Thermo Shandon Para-trimmers
5. Fume Hood

#### **3.2 Reagents & Media**

1. Surgipath Embedding Media
2. Xylene
3. 70% Ethanol

#### **3.3 Supplies**

1. Metal pitcher
2. Heat protective mitt
3. Trays
4. Forceps
5. Embedding/scraping knife
6. Sakura Tissue-Tek stainless steel embedding molds
7. Plastic paraffin scrapers

8. Tissue-Tek small and large tissue tampers
9. Alcohol Lamp/Burner

## 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

##### 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:

- The molten paraffin is hot (65C) and can cause minor burns.
- The alcohol lamp must be used carefully as it is an ignition source and very hot.

#### D. Electrical: N/A

#### E. Sharps: N/A

#### F. Ergonomics: N/A

### 4.4 Disposal of waste

- Liquid paraffin will be dumped in an autoclave bag, tied shut and then sent for incineration.
- The xylene that is used to clean the metal cassette lids will be dumped in a designated waste carboy or be recycled using the in-house recycler.

## 5 Preparation for procedure

### 5.1 Equipment and instrumentation preparation

- Turn on the cryo module of the Sakura Tissue-Tek embedding center by making sure the main power switch is on and pressing the "CRYO" key. The

cooling plate will reach its programmed temperature approximately 15 minutes after the unit is turned on.

- The paraffin wells are filled with infiltration media as needed.
- The paraffin reservoir is filled with embedding media as needed.
- Thermo Shandon Para-trimmers reach programmed temperature within 5 minutes of being switched on.
- Fill alcohol lamp with 70% Ethanol as needed.

## **5.2 Reagents and media preparation**

- To ensure that there is enough excess liquid paraffin; fill the reservoir of the embedding center with Surgipath embedding paraffin pellets or liquid prior to embedding.

## **5.3 Standards/controls preparation - N/A**

## **5.4 Specimen preparation - PPROCESSVIP5**

# **6 Performance of procedure**

1. Using forceps, take a cassette from the paraffin well, remove and set aside the metal lid.
2. Obtain a mold of the appropriate size and fill it with liquid paraffin by pressing the mold against the paraffin dispenser plate.
3. Transfer the tissue from the cassette to the mold with forceps. Orient the tissue as needed to fill the cassette, while keeping the cut side of the tissue facing the bottom of the mold and not allowing tissues to overlap.
4. Move the mold quickly, but carefully, onto the small cold plate to immobilize the tissue.
5. Use a tissue tamper to press down on all areas, of all tissues, to align them on a common horizontal plane.
6. Place the original plastic cassette on top of the mold with the accession number facing to the left.
7. Quickly fill the mold with paraffin.
8. Carefully place the mold on the cryo module and allow it to solidify.
9. Remove each block from the mold with the embedding knife, and remove excess wax from the edges with either a para-trimmer or an embedding knife.
10. Organize the blocks in numerical order.
11. Place blocks on trays and set on shelves by the microtomes until they are sectioned.
12. Collect the metal lids in a glass container along with the stainless steel basket and place in the incubator to melt off excess paraffin wax.
13. Remove the glass container and the stainless steel basket from the incubator and dump excess molten paraffin into the wax collection container.

14. Add metal lids to the stainless steel basket and place in the stainless steel tub in the fume hood and fill with xylene.
15. The lids and stainless steel basket can be removed from the xylene after a couple of hours or once all the paraffin has dissolved.
16. Place the stainless steel basket and metal lids in the tissue processor.
17. Run the cleaning 1 program to remove the residual xylene.
18. When run is completed, place stainless steel basket in fume hood to dry.
19. Turn the cryo module off at the end of the day or when finished with all embedding for the day, whichever comes first.

## **7 Interpretation of results - N/A**

## **8 Report of results - N/A**

## **9 Procedure notes**

### **9.1 Details and helpful hints**

- To ensure the tissue is as close to the face of the block as possible, hold the tissue down with a tissue tamper while the mold is still on the hot plate.
- The tray must be moved QUICKLY from the small cold plate once the tissue has been pressed into place, and the addition of more paraffin must be fast so the paraffin does not cool in layers.
- If the cold plates become covered with paraffin, use the plastic scraper to remove it.
- 70% alcohol lamp/burner can be used to quickly heat forceps to remove solidified paraffin. Carefully wave forceps through flame until excess paraffin is removed. Be sure not to overheat forceps. Use CAUTION when working with an open flame.

### **9.2 Limitations of procedure**

- The size of the mold determines the amount of tissue that can fit in one block.

## **10 References**

- Sakura Finetek U.S.A., Inc. Torrance, CA. Sakura Tissue-Tek VIP 5 Tissue Embedding Console System Operating Manual. 2001;1.1-8.4.

## **11 Summary of Current Revisions**

1. Changed all indirect suggestions like should and may to a more direct wording like will.

## **12 Supplemental Information**

### **12.1 Quick Procedure Reference - N/A**

### **12.2 Flow Diagram - N/A**

### **12.3 Manufacturer's Information - N/A**