

## 1.0 PURPOSE

- 1.1 This standard operating procedure (SOP) provides detailed instructions for paraffin processing of research tissue biospecimens that have been collected under the BPV Phase II protocol and fixed with formalin. The tissue processing equipment used for this procedure is the Leica PELORIS™ Rapid Tissue Processor. This SOP also includes a summary of the procedures to be followed for reagent management and maintenance. All operating and maintenance procedures related to the Leica PELORIS™ Rapid Tissue Processor will be governed by the equipment user manual (*Leica PELORIS™ Rapid Tissue Processor User Manual*, Revision J, Leica Biosystems, Melbourne Pty Ltd., 2011) unless otherwise noted by the biospecimen source site (BSS) technical project manager.

## 2.0 SCOPE

- 2.1 This procedure is applicable to all formalin-fixed biospecimens to be processed for paraffin embedding as part of the BPV Phase II Study or as directed by the Study Management Group.

## 3.0 RESPONSIBILITY

- 3.1 It is the responsibility of the principal investigator at each BSS to ensure that this procedure is followed.
- 3.2 It is the responsibility of the local program supervisor to ensure that all personnel were trained in accordance with this SOP and the operation of the tissue processing equipment and that both training events are documented.
- 3.3 It is the responsibility of all laboratory personnel to ensure that he/she has read, understands, and follows this SOP.

## 4.0 DEFINITIONS

### 4.1 Definitions

- 4.1.1 **Case ID:** Identifies study subjects.
- 4.1.2 **Specimen ID:** Identifies each blood and tissue biospecimen from a study subject and is used on all tissue cassettes and other specimen containers.
- 4.1.3 **Experimental Key ID:** Identifies the randomized configuration of experimental conditions (for example, delay to fixation and time in fixative) that are used during processing of study biospecimens.

### 4.2 Acronyms

CDR	Comprehensive Data Resource
BPV	Biospecimen Pre-Analytical Variables
BSS	Biospecimen Source Site
SOP	Standard Operating Procedure

## 5.0 ENVIRONMENTAL HEALTH AND SAFETY

- 5.1 Wear appropriate personal protective equipment at all times.
- 5.2 Dispose of all contaminated supplies in the appropriate biohazard and sharps containers.
- 5.3 Handle all chemicals appropriately according to material safety data sheets.

## 6.0 MATERIALS AND EQUIPMENT

### 6.1 Materials

Item Number	Description	Vendor	Catalog Number
1	10% neutral buffered formalin	Fisher Diagnostics	23-427-098
2	Xylene	StatLab	8400-1
3	100% absolute ethanol	PHARMCO-AAPER	CAS 64-17-5
		KOPTEC	CAS 64-17-5
4	Paraffin (low melting temperature)	Fisher	23-021-401 or 23-021-752
5	95% reagent alcohol	StatLab	9500-1
6	70% reagent alcohol (dilution of 100% ethanol)	as above	n/a

### 6.2 Equipment

Item Number	Description
1	Leica PELORIS™ Rapid Tissue Processor

## 7.0 PROCEDURE

- 7.1 Data Entry I Into the Required Case Report Forms in BBRB's Comprehensive Data Resource (CDR) Database
  - 7.1.1 Time frame for completing the form in the CDR: **OP-0004-F1\_BPV FFPE Tissue Processing/Embedding Form** must be completed within 72 hours of completing the embedding.

### 7.2 Requirements for Standardized Processing Reagents

#### 7.2.1 General Requirements

It is crucial to the success of the BPV Study that key tissue processing reagents, including formalin, ethanol, xylene, and paraffin, be standardized throughout the entire study for all cases and at all participating BSS. Please refer to the sponsor-provided list of standardized study supplies and reagents.

#### 7.2.2 Requirements for Ethanols

##### 7.2.2.1 100% Ethanol

**This SOP requires the use of absolute ethanol (100%)** for processing the tissue in the Leica PELORIS™ tissue processor. Do not use histological-grade (95% ethanol) or reagent-grade ethanol, isopropyl alcohol, or denatured ethanol, despite recommendations to the contrary in the 2011 Leica User Manual.

### 7.3 Equipment Specifications

- 7.3.1 It is mandatory that before study protocol initiation, a qualified technical service representative make a site visit to ensure that the tissue processor is correctly installed and set up.
- 7.3.2 All equipment must be configured according to this SOP and all relevant staff are to be properly trained on correct usage.
- 7.3.3 The tissue processor provided for this study must be dedicated for use with BPV biospecimens only and not for any other research or clinical specimens.

### 7.4 Paraffin Processing of Tissue Biospecimens

- 7.4.1 Standard paraffin processing cycles (described below) will be used for all biospecimens in this study and should be held constant.
- 7.4.2 It is optimal to perform fixation of biospecimens in formalin jars before placement in the tissue processor. Under optimal circumstances, the tissue processor should be loaded immediately before required use; however, due to the specialized timing requirements of this study, tissue fixation may be carried out in the processor before the start of a processing cycle.
- 7.4.3 Scan each cassette to record the time at which the biospecimen was placed in the tissue processor. Load biospecimens into the tissue processor per the instructions in the user manual.
- 7.4.4 Several programs can be used to allow maximum processing of biospecimens. The BSS will need to preprogram the Leica processor to the specific settings as outlined in **Table 1** at the end of this document. The BSS will fix the tissues in a jar on the bench top, in the processor, or any combination of the two; however, the tissues must be in fixative on the Leica processor for no less than 60 minutes, as outlined in **Table 1**. The BSS will create and use any processor cycle necessary to optimally process tissues for this experiment.
- 7.4.5 The specific delay to fixation and time in fixative time points have been designed to optimize processing cycles to reduce the number of runs and maximize the number of cassettes included in each run.
- 7.4.6 All processing must be done within 10 minutes (module I) or 30 minutes (module II) of each experimental time point.

- 7.4.7 The cassettes must not remain in the tissue processor longer than 60 minutes after the processing cycle is complete.
- 7.4.8 All relevant details associated with each tissue processing run should be recorded in the CDR database per the **OP-0004-F1\_BPV FFPE Tissue Processing/Embedding Form**. The **OP-0004-F1\_BPV FFPE Tissue Processing/Embedding Form** must be completed and submitted within 72 hours of completion of embedding.
- 7.5 Readiness for Paraffin Embedding
- 7.5.1 Upon completion of tissue processing, samples permeated with liquid (melted) paraffin are ready for paraffin embedding.
- 7.5.2 The process of paraffin embedding is covered by **BPV FFPE Tissue Embedding, OP-0004**.
- 7.6 Reagent Management
- 7.6.1 Refer to the user manual for processor reagent management and changes.
- 7.7 Summary of Cleaning and Maintenance Schedule
- 7.7.1 Refer to the user manual for the recommended cleaning and maintenance schedule:
- Daily (when in use)
    - Clean lids and seals
    - Clean retorts
    - Check reagent levels
    - Clean top surface
    - Clean touch screen
  - Weekly
    - Empty condensate bottle
    - Check reagent bottles
    - Clean wax bath
    - Check bottle connectors
    - Clean exterior surfaces
  - 60–90 days
    - Change carbon filter
    - Check lid seals
  - As required
    - Retort acid clean
- 7.8 Maintenance and Reagent Records

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- 7.8.1 Record equipment maintenance in the maintenance record provided in the user manual (p. 114).
- 7.8.2 Record the date of change and manufacturer details associated with the addition of new reagents to the processor in Attachment 1 to this SOP (**BPV FFPE Tissue Processor Reagent Record, OP-0003-F1**).

## 8.0 METRICS

None

## 9.0 REFERENCES

- 9.1 *Leica PELORIS™ Rapid Tissue Processor User Manual*, Revision J, Leica Biosystems, Melbourne Pty Ltd., 2011
- 9.2 Sakura Tissue-Tek® VIP 1000 Document #/version: S09-148-OP-L-001\01

## 10.0 ATTACHMENTS

- 10.1 BPV FFPE Tissue Processor Reagent Record, OP-0003-F1

**Biospecimen Pre-Analytical Variables  
 (BPV) Program  
 FFPE Tissue Processing**

**Table 1: BPV Phase II Leica Processor Settings**

**Please note:** Table 1 shows the minimum amount of time for formalin fixation. The time of step 1 can be altered to optimize tissue processing and minimize burden on the BSS staff as long as step 1 is at least 60 minutes.

Step	Reagent Type	Reagent Group	Time (min)	Temp (°C)	P/V	Stirrer	Drip Time (sec)
1	Formalin	Fixatives	60	35	Ambient	Low	30
2	70% Ethanol	Dehydrants	30	35	Ambient	Low	30
3	95% Ethanol	Dehydrants	30	35	Ambient	Low	30
4	95% Ethanol	Dehydrants	30	35	Ambient	Low	30
5	100% Ethanol	Dehydrants	30	35	Ambient	Low	30
6	100% Ethanol	Dehydrants	40	35	Ambient	Low	30
7	100% Ethanol	Dehydrants	40	35	Ambient	Low	30
8	Xylene	Clearers	30	35	Ambient	Low	30
9	Xylene	Clearers	30	35	Ambient	Low	30
10	Xylene	Clearers	40	35	Ambient	Low	30
11	Paraffin Wax	Wax	40	58	Vacuum	Low	30
12	Paraffin Wax	Wax	40	58	Vacuum	Low	30
13	Paraffin Wax	Wax	40	58	Vacuum	Low	30

**PROCESSING TIME: 8 hours**