1.0 PURPOSE

This standard operating procedure (SOP) describes the process to be followed for the paraffin embedding of formalin-fixed and processed tissues as well as the maintenance of tissue embedding equipment.

2.0 SCOPE

This procedure will be applicable to all fixed tissue samples to be paraffin embedded for the BPV Phase II study.

3.0 RESPONSIBILITY

It is the responsibility of the principal investigator at each biospecimen source site (BSS) to ensure that this procedure is followed.

It is the responsibility of the local program supervisor to ensure that histology laboratory personnel have been trained in accordance with this SOP and that the training is documented.

It is the responsibility of the histology laboratory personnel to ensure that he/she has read, understands and follows the SOP.

4.0 DEFINITIONS

4.1 Definitions

Case ID	Identifies study subjects.
Specimen ID	Identifies each blood and tissue biospecimen from a study subject and is used on all tissue cassettes and other containers.
Experimental Key ID	Identifies the randomized configuration of experimental conditions (delay to fixation and fixation time) that are used during processing of the study biospecimens.

4.2 **Acronyms**

CDR	Comprehensive Data Resource
BPV	Biospecimen Pre-Analytical Variables
BSS	Biospecimen Source Site
SOP	Standard Operating Procedure
FFPE	Formalin-fixed Paraffin-embedded
QC	Quality Control

5.0 ENVIRONMENTAL HEALTH AND SAFETY

5.1 Follow universal precautions (CDC-1987) and 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/EQUIPMENT

6.1 **Materials**

Item Number	Description	Quantity Needed per Subject	Vendor	Catalog Number
1	Paraffin (low melting temperature)	As needed	Fisher	23-021-401 or 23-021-752

6.2 **Equipment**

Item Number	Description
1	Tissue embedding center
2	Reusable metal base molds

7.0 PROCEDURE

7.1 Data entry into the required case report forms in the CDR database:

7.1.1 Time frame for completing the form in the CDR: The **OP-0004-F1_BPV FFPE Tissue Processing-Embedding Form** must be completed within 72 hours of tissue embedding, and the **OP-0004-F3_BPV FFPE Section-Stain Form** should be completed within 48 hours of completion of staining.

7.2 **Important Considerations**

7.2.1 During paraffin embedding, mount paraffin specimen blocks on the same tissue cassette in which it was collected and fixed. It is essential to preserve the linkage of paraffin block to the original cassette label (ID).

7.2.2 On completion of paraffin embedding work, histology laboratory personnel should record all pertinent details regarding the Formalin-fixed Paraffin-embedded (FFPE) blocks prepared in the OP-0004-F1_BPV FFPE Tissue Processing-Embedding Form in the CDR database or the corresponding paper form.

7.2.3 Although BSSs are required to utilize their own standard workflows and SOPs for the preparation of FFPE sections from the quality control (QC) FFPE block and for hematoxylin and eosin staining of the sections prepared from QC FFPE block, they are required to record all the information requested in the OP-0004-F3_BPV FFPE Section-Stain Form in the CDR database and/or into the appropriate paper form.

7.3 **Paraffin Embedding of Tissue Samples**

7.3.1 Use a standardized size of embedding base mold which most efficiently accommodates each experimental sample.

7.3.2 To start the tissue embedding process, turn on the embedding center and allow at least 30 minutes before the embedding of samples for the unit to reach the set temperature.

7.3.3 The device should be set to a standardized temperature of 58°C (±3°C) for the paraffin reservoir.

7.3.4 Before use, verify the temperatures of the paraffin reservoir, the tissue holding tank, and the cold plate and record on the **OP-0004-F2, BPV Paraffin Embedding Center Maintenance Chart**.

7.3.5 Select a processed specimen for embedding, open the cassette lid and carefully inspect for and remove any adherent tissue fragments, then discard the lid in the biohazard waste disposal. Do not discard the labeled cassette.

7.3.6 Place a pre-warmed embedding base mold under the dispensing nozzle and fill with melted paraffin.

7.3.7 Transfer the tissue specimen(s) to the embedding base mold by using a heated forceps; orient it appropriately by using the cold spot to start the solidification of the paraffin and help hold the tissue in its appropriate orientation as described below. Use an appropriate tool or forceps to ensure that the specimen is embedded flat/even. Inspect the forceps to make sure that no tissue remains stuck to it.

7.3.8 During the paraffin embedding process, the technician should standardize the orientation of each tissue sample in its individual paraffin block.

7.3.9 Each sample should be oriented in the final paraffin block with the long axis parallel to the surface of the block to be cut on the microtome (such that resulting paraffin sections will yield maximum cross-sectional tissue area). Ensure that the side of the specimen that was face down in the cassette is also face down within the block.

7.3.10 Place the cassette on top of the base mold, pick up the base mold with cassette, hold it under the dispensing nozzle, and fill it with melted paraffin to completely cover the bottom of the cassette.

7.3.11 Place the base mold with cassette on the cold plate. Allow it to completely cool and solidify for 30 minutes, or until the block releases easily, before attempting to pull the paraffin block/cassette away from the base mold. The paraffin block should release easily when cool enough. If it does not release easily, replace it on the cold plate for 10 minutes and try to release the paraffin block from the base mold again.

7.3.12 Complete **OP-0004-F1_BPV FFPE Tissue Processing-Embedding Form** in the CDR database or the corresponding paper form for each sample/case.

7.3.13 Repeat steps above for each cassette transferred from the tissue processor.

7.3.14 Handle only one cassette at a time to prevent potential mix-ups of Experimental Samples.

7.3.15 Clean up the embedding area and perform all maintenance on the embedding center, as specified in the embedding center maintenance protocol. Document maintenance by using the **OP-0004-F2_BPV Paraffin Embedding Center Maintenance Chart.**

8.0 ATTACHMENTS

8.1 BPV Tissue Processing-Embedding Form, OP-0004-F1

8.2 BPV Paraffin Embedding Center Maintenance Chart, OP-0004-F2

8.3 BPV FFPE Section-Stain Form, OP-0004-F3