James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research

STANDARD OPERATING PROCEDURE

Title:	**Processing and Sectioning of JB-4 Glycol Methacrylate Embedded Tissues**		
Procedure:	BB_HIST.008.01	**Supercedes:**	none
Originator and Date:	Crystal Leung 19NOV2008	**Effective Date:**	19NOV2008
Review Frequency:	annually	**Approved By:**	The iCAPTURE Centre Privacy Team
Total Number of Pages: 4			

Revision History		
Date	**Reviewer**	**Summary of revision**
21Apr2009	Crystal Leung	Reformatted to iCAPTURE format

Purpose

The standard operating procedure (SOP) establishes the standard for processing and sectioning of JB-4 glycol methacrylate embedded tissues performed by Biobank personnel who have been trained in the use of the manual JB-4 rotary microtomes.

Responsibilities

This procedure is applicable to the following personnel:

- Biobank personnel who are responsible for sectioning JB-4 glycol methacrylate embedded tissues such as stented arteries and bones.

Safety

For safe handling of human biological material refer to BB.001.01, “Handling Biohazardous Materials”. Wear PPE (lab coat, gloves, goggles, etc.) when working with biohazardous materials and chemical substances. Refer to Material Safety Data Sheets (MSDS) for safe handling, disposal and storage of chemical substances.

For microtome use, Biobank personnel must complete safety and practical training on the site-specific model(s) of microtome. Follow equipment manual or site procedures for maintenance, use and safety.

Definitions

JB-4 Glycol Methacrylate	A plastic polymer embedding medium to support hard tissues such as bone and stented arteries for sectioning.
Stented Artery	A metal mesh-like device (stent) is surgically placed into a narrowed artery to hold it open.
SOP	Standard Operating Procedure. Document used to control the method and requirements by which personnel will perform their activities.
PPE	Personal Protective Equipment
Microtome	An instrument used to cut a specimen, as of organic tissue, into thin sections for microscopic examination.
MSDS	Materials Safety Data Sheets

Materials and Equipments

The materials, equipments and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site procedure.

JB-4 Plus Embedding Kit (Polysciences)	JB-4 Microtome (Sorvall)
Vacuum Chamber	JB-4 Microtome Specimen Holders
70%, 80%, 90%, and absolute Isopropanol	JB-4 Microtome Specimen Holder Affixation Tray
Polypropylene Tubes	Hot Plate
PPE – lab coats, gloves	Water Bath
High-speed rotary saw	Paraffin Wax
Surgical blade for tissue trimming	Laboratory tape for labeling

Procedures

- **Note:** Use the JB-4 Plus Embedding Kit under a fume hood with appropriate gloves.

1) Fixation

a) Obtain hard tissues such as bones or stented arteries.
b) If specimens are large, cut them into smaller pieces.
c) Fix tissues in 10% Neutral Buffered Formalin in room temperature overnight.
d) Trim away excess tissues with a surgical blade after formalin fixation.

2) Dehydration

a) Place each tissue specimen in to a polypropylene tube and dehydrate them in room temperature.
b) Fill each polypropylene tube with 70% isopropanol to dehydrate tissues for one hour and then empty the tubes.
c) Fill each polypropylene tube with 80% isopropanol to dehydrate tissues for one hour and then empty the tubes.
d) Fill each polypropylene tube with 90% isopropanol to dehydrate tissues for one hour and then empty the tubes.
e) Fill each polypropylene tube with absolute isopropanol to dehydrate tissues in for one hour and then empty the tubes. Repeat this step two more times

3) Infiltration

a) From the JB-4 Plus Embedding Kit, carefully weigh out 1.25g of plasticized benzoyl peroxide (catalyst).
b) Measure out 100mL of JB-4 Plus Solution A (monomer) and add the catalyst to the monomer while stirring on a magnetic stirrer to create the infiltration solution.
c) Mix both components until completely dissolved, which takes approximately 10 to 20 minutes. This infiltration solution can be stored for up to two weeks in a dark cool area or in the refrigerator at 4°C.
d) Carefully pour the infiltration solution in the polypropylene tubes containing the tissues and ensure that the solution completely covers the tissues.
e) Place all the polypropylene tubes in a vacuum chamber at room temperature and allow the tissues to be infiltrated overnight.
f) Remove all the infiltration solution from the tubes the next day and repeat steps d) to f) two more times.
g) Do not expose the tissues to heat or direct light during infiltration.

4) Embedding

a) From the JB-4 Plus Embedding Kit, carefully measure out 1.6mL (must be exact measurement) of JB-4 Plus Solution B (accelerator).
b) Make up 25mL of **fresh** infiltration solution (as per above) and mix it with JB-4 Plus Solution B thoroughly to create the embedding solution and begin embedding immediately.

c) Carefully but quickly pour the embedding solution in the polypropylene tubes containing the tissues and ensure that the solution completely covers the tissues.
d) Then add a few drops of paraffin wax to cover the top of the embedding solution and then tightly cap the tube to create an anaerobic environment.

5) Cutting

a) Once the embedding solution has polymerized and hardened (takes approximately one week), remove the embedded tissue from the polypropylene tube by breaking the tube with a hammer. The tissues are now embedded in a plastic medium in the shape of the tube.
b) If there appears to have a liquid film, it can be removed by draining.
c) Use a high-speed rotary saw to cut the tissues into 5mm plastic blocks.

6) Sectioning

a) The 5mm plastics blocks are affixed to the specimen holders using the embedding solution.
b) Place the plastic blocks in the specimen holder affixation tray along with a label.
c) Fill half of the tray up with the embedding solution.
d) Place the specimen holder into the tray.
e) Add a few more drops of the embedding solution into the tray via the hole in the specimen holder until solution reaches the top of the tray.
f) Seal the gap between the tray and the holder with paraffin wax and allow for affixation (takes approximately one week).
g) Once affixed, proceed to sectioning using the JB-4 microtome (refer to BB_HIST.002.01 “Sectioning of Paraffin Embedded Tissue” for general guidelines).
h) Heat slides on hot plate (60°C) to promote adhesion to the slide.

7) Precautions and Disposal of JB-4 Plus Embedding Kit

a) Store the kit in a cool dark place such as the flammable cabinet.
b) To dispose the infiltration and embedding solutions, mix all solutions together in a plastic container and let polymerize in the fume hood.
c) Dispose in accordance with local hazardous waste regulations.