James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research

STANDARD OPERATING PROCEDURE

Title:	**General Archiving for Intra Mammary Arteries, Saphenous Veins, Radial Arteries**		
Procedure:	**BB.016.01**	**Supercedes:**	**none**
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Revision History		
Date	**Reviewer**	**Summary of revision**
20Apr2009	Crystal Leung	Reformatted to iCAPTURE format

Purpose

This is a procedure for the archiving of discarded lengths intra mammary arteries (IMA) saphenous veins (SV) and radial arteries (RA) collected from the Operating Room (OR) during a coronary artery bypass graft.

Responsibilities

This procedure is applicable to:

- Biobank personnel
- Other Biobank personnel who may be responsible for archiving vessels

Safety

Universal precautions are a method of infection control in which all human tissue, blood and body fluids are treated as if they are infectious. Be sure to wear appropriate personal protective equipment (gloves, yellow gown, eye protection etc.). This SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals. Refer to BB.001.01 “Handling Biohazardous Materials”.

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Definitions

SV	Saphenous vein
IMA	Internal mammary artery (L=left; R= right)
RA	Radial artery
PPE	Personal protective gear including and not limited to: gloves, biohazard gown, lab coat, eye protection, surgical mask, etc.
Archive	The physical process of harvesting tissue for indefinite storage
Fixation	To preserve tissues in an as life-like a state as possible.
Prox	Proximal
Mid	Middle
Biobank identification number	Unique identifier that links a specific piece of data or sample to a patient in an un-identified form. This includes CR(S) and PRL numbers
Biohazardous materials	Human tissue, cells, body fluids, or culture materials that may contain infectious or other hazardous materials
Biospecimens (wet data)	Any tissue, blood, blood product, urine, DNA or RNA extraction or product.
Dry Data	Data as it exists on paper or in a database. They may include patient data.
SOP	Standard Operating Procedure. Document used to control the methods and requirements by which personnel will perform their activities.

Materials and Equipment

Silica based petri dish	tissue cassettes
white plastic cup	microcentrifuge tubes, flat topped, siliconized
Kimwipes™	freezer cannisters
freezer boxes	white plastic tubs to hold formalin (from Histo)
OCT molds	latex gloves
yellow gown	biohazardous bags (8” x 12
micro scissors	scalpel
buffered neutral formalin	Presept™

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sodium thiosulphate	

Procedures

Specimen Collection:

OR runners will collect from the OR from 0830 to 1630. Any samples collected by the OR at the end of the day will be stored in the mini fridge in the OR core for collection the next morning.

1. When a specimen has been collected by the OR the pager will beep and **631XX** will appear in the window. The **XX** corresponds to the operating theatre where the surgery is taking place. eg. 63114 indicates that the surgery is in OR 14. Turn the pager off.

2. Proceed to the OR located on the third floor of the Providence Building. Before entering the OR you must be dressed in OR greens. To obtain OR greens, ask the receptionist at the OR desk for the dressing room swipe card. In the dressing room are shelves of greens in various sizes. Keep the greens on for the rest of your shift. A surgical cap must also be worn before entering the OR core and can be found on a cart in the OR. The specimen will be located in the Human Blood Vessel Laboratory (HBVL) fridge in the OR core by Operating Theatre 5 and should be in a biohazard bag with a specimen information sheet.

3. If there is no specimen in the fridge check the theatre in which the surgery is occurring to see if someone is waiting for you.

4. Once the sample has been retrieved, match the name and the patient hospital number on the vial with that on the specimen information sheet. If it does not match speak to a nurse to determine which is the correct name and patient hospital number.

5. Proceed to the Human Blood Vessel Laboratory located in room B52F of the Burrard Building basement.

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Accessioning:

1. Remove the specimen information sheet and place it on the accessioning bench in the HBVL. Place the sample, still in the biohazard bag, on the archiving bench. Once again make sure the name and hospital number on the tube match those on the requisition. If they do not, contact the OR.

2. Check the Registry database to see if the research subject has been previously entered into the database.
3. If not, enter the research subject's addressograph information into the database. The database will generate an iCAPTURE ID for this research subject. Write this ID onto the tissue cassettes for the tissue to be archived. Take a manila folder and in the box marked "Surgical File" write the research subject's iCAPTURE ID number. Write this number on the tissue requisition as well as on top of the tissue archive form.

NOTE: The folder and sheet are to be filled out on the non-biohazardous countertop of the HBVL and is not to be handled while wearing gloves.

4. Fill out the front of the folder with following information from the specimen information sheet and patient addressograph (Figure 1):

    - date of surgery
    - iCAPTURE ID
    - patient's sex and age
    - surgeon's name (Refer to Appendix for a list of surgeons)
    - physician's name
    - name of hospital

Please refer to Figure 1 for a copy of an addressograph label.

Patient Hospital Number
Patient last name, first and middle name
Surgeon/Family Physician
Admission Date Sex Age Date of Birth
Patient Street Address
Patient Address City Postal Code
Personal Health Care Number

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Figure 1 Addressograph Label

5. On the archive sheet fill out the following:
    - the same as above
    - diabetes status (yes, no, or not indicated)

Please refer to Figure 2 for a diagram of the archiving sheet.

6. Under the appropriate letter on the archiving sheet record the following information:
    - type of specimen collected (please refer to Appendix for coding)
    - time specimen was placed in vial
    - time specimen was collected from the OR
    - time the specimen was archived
    - record RPMI in notes section

Archiving Procedure:

1. Retrieve the styrofoam box containing dry ice from -80^{o}C freezer and place it in a **non-biohazardous** area of the archiving bench.

2. Put on yellow gown and gloves

3. In the biohazardous area fill a tub with formalin and place a lid on it. Get silica based petri dish and plastic cup and bring all these items to the archiving bench.

4. Remove and discard gloves in a biohazardous container.

5. Get the HEPES archiving solution from the refrigerator. Pour the archiving solution to a depth of approximately 5 mm into the silica based petri dish. Return the archiving solution to the refrigerator.

6. Put a new pair of gloves on and use tweezers to remove the tissue from the sample vial and place into the silica-based petri dish.

7. If the sample cannot be seen then pour the contents of sample vial into the plastic cup. Transfer the tissue from the cup to the silica-based petri dish using tweezers. Pour the contents of the cup back into the sample vial.

8. Place the silica-based petri dish on the dissecting microscope stage and turn on the light.

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9. Adjust the magnification and locate the vessel keeping in mind that arteries have thicker walls than veins. Begin cleaning the vessel by removing the connective tissue from it.

10. Draw a sketch on a piece of paper once the vessel is cleaned noting the relative position of staples, sutures, and cardioplegic tips. Please refer to Figure ## . The sketch will later be transferred to the space provided on the specimen information sheet.

11. Cut a 3 mm segment by pressing down firmly with the scalpel. This is segment H1. Mark on the diagram the placement of the cut, indicating this with a vertical line through the drawing and label H1 above the section. Please refer to Figure ##.

12. Label the front edge of cassette with the iCAPTURE ID number, the section number and H1. Using forceps, place the segment into the cassette. Close the cassette lid and place it in the formalin tub. One tub can hold 15 cassettes.

13. Label the top, bottom and side of an OCT cassette with the iCAPTURE number, specimen number, and H5. Fill the cassette with OCT until it is slightly convex.

14. From the same end as the H1 segment was taken from, cut another 3 mm segment. This is the H5 segment.

15. Transfer the segment to the cassette, placing it in the OCT upright so that the cross section can be seen. If the segment does not stay upright place the entire cassette in the dry ice cooler. Cooling the OCT thickens it and the vessel can be easily manipulated into position. Do not wait too long for the cooling because you do not want the OCT to completely freeze.

16. Using a Kimwipe or an ungloved hand remove the lid from the styrofoam cooler and place the OCT cassette on top of the dry ice. Place the cassette in the cooler of dry ice making sure the cassette is placed horizontally. If not, cassette and its contents will have to be thawed and the freezing repeated which may ruin the segment.

17. Indicate on the sketch where the cut was made for the H5 segment. Do this in the same manner as for the H1 segment.

18. The next sections, H2, H3, H4, are treated in the same manner as H1. Prepare only as many cassettes as required. If H1-H4 segments are small and may possibly fall through the cassette, place the segment into a mesh bag and place the bag into the cassette.

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19. Segments H6, H7, H8 are flash frozen. They are cut as described above with **minimum length of 1 cm**. Sections may be more than 1 cm such that the remaining vessel is used up in these four sections.

20. Weigh the section on the biohazadous scale placing a piece of the filter paper on the balance, taring the balance, placing the specimen on the filter paper and recording the weight on the archiving sheet. Transfer the section into a microcentrifuge tube, label the tube lid and side. Label a microcentrifuge tube for each segment with the VB number, specimen letter and the segment number on the side and lid. Put segment in the white cup containing some HEPES or RPMI. Bring the cup, and tubes to the biohazardous sink area. Turn on the scale and place a piece of low weight nitrogen paper on the balance. Close the door and tare the balance. Place the segment on the paper, close the door and note the weight of the specimen in milligrams (mg). Place the segment in the appropriately labeled tube.

21. Using a Kimwipe to open the cooler box, place the tubes on top of the dry ice. Return the cooler to the –80°C freezer and place the tubes in their appropriate freezer boxes as soon as possible.

22. Put all bits of tissue, sutures and staples into the specimen vial. Transfer the solution from the petri dish into the plastic cup and pour into the specimen vial if there is room. Otherwise pour the rest into the biohazardous waste container. Pour the contents of the cup into the biohazardous decontamination bucket containing Presept.

23. Throw specimen vial into the biohazardous waste container on the archiving bench.

24. Disinfect all utensils by soaking individually in Presept for a minimum of five minutes. Rinse in hot water and place in the drying trays. Soak the silica based petri place and cup in Presept for no longer than 30 seconds. Rinse in hot water and place in drying trays. Replace the Presept if it does not turn purple when HEPES is poured in. **NOTE: Before discarding Presept mix 1:1 with thiosulphate.**

25. Turn scale off. Spray with ethanol and wipe dry.

26. Discard gloves and face mask into biohazardous waste container. Remove goggles and smock and put back in designated space.

27. Using one gloved had return styrofoam cooler to freezer. Place OCT and flash frozen segments into their appropriate boxes. Note the box number.

28. Write box number of OCT and flash frozen segments on appropriate patient folders on bottom right hand corner.

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29. Files are brought to the Cardioavascular Registry Office and entered into the Registry database. Consent for Contact and Consent for Research forms will be added to the manila file folder. The files can then be filed in the filing cabinets located in rm 211, 2nd floor Burrard Building.

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