STANDARD OPERATING PROCEDURE

Title:	**Heart Archive**		
Procedure:	BB.003.01	**Supercedes:**	none
Originator and Date:	Lise Matzke 23OCT2008	**Effective Date:**	23OCT2008
Review Frequency:	annually	**Approved By:**	The iCAPTURE Centre Privacy Team
Total Number of Pages: 14			

Revision History		
Date	**Reviewer**	**Summary of revision**
20Apr2009	Crystal Leung	Reformatted to iCAPTURE format

Purpose

The purpose of this document is to outline standardized procedures for iCAPTURE Biobank personnel to follow when archiving heart specimens from transplant cardectomy or autopsy.

Samples are collected from patients that have been through the informed consent process and agreed to participate in the tissue banking program. Tissue is only suitable for specific research studies if preserved appropriately. This SOP and accompanying protocol set out a well recognized method for effectively archiving heart specimens and preserving their RNA, DNA and protein constituents utilizing several different fixatives for optimal preservation.

Responsibilities

This procedure is applicable to the following:

1. Biobank personnel
2. Others who are responsible for archiving specimen

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".

Definitions

Cardectomy	A surgical procedure to remove the heart from thorax at the time of transplant
LVFW	Left Ventricular Freewall
RVFW	Right Ventricular Freewall
VS	Ventricular Septum
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
LA	Left Atrium
RA	Right Atrium
Ao	Aorta
PA	Pulmonary Artery
MV	Mitral Valve
AV	Aortic Valve
LAD	Left Anterior Descending Artery
RCA	Right Coronary Artery

Materials and Equipment

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

Markers, ink and pens	Aluminum foil (3x3 inch square)
Clean Forceps	Formalin pen
Clean Scalpels for trimming tissue	Permanent marker (i.e. Sharpie)
Containers for fixing tissue	Biohazardous bags
Labels for tissue cassettes, containers	Bucket

Tissue cassettes	Tissue cassettes
Needle/sharps disposal unit	RNAlater
Clean underpads for covering bench surface	Large Knife
Neutral pH Phosphate buffered 10 % Formalin	Biohazard gown
Alcohol (ethanol)	Eye protection
Large and small styrofoam boxes	Gloves
Heart tissue collection buffer	Surgical mask
Dry ice	OCT molds
Liquid nitrogen	Freezer boxes

Procedures

This procedure is intended to ensure that tissue samples collected from consented participants will be preserved in a safe and efficient manner while eliminating the risks of contamination and loss of molecular and structural integrity. Banked tissue that has been adequately preserved is useful for a greater variety of studies. Consistency in procedure is important for obtaining comparable and reliable test results.

These procedures are relevant to heart specimens coming from transplant cardioectomies from the operating room (OR) or from autopsy.

1) Morgue Collection Procedure:

a. For hearts coming from autopsy, the morgue will call for heart pickup from relevant Pathology personnel or Biobank personnel.

b. Heart tissue is sampled in the morgue then placed on ice and taken to Anatomic Pathology to be frozen and/or processed.

c. Enter specimen details into Biobank database and obtain a Biobank identification (ID) number or enter the patient information into the Surgical Log-in book (**see BB.011.01**) and generate the next CRS number.

d. Complete a Autopsy Specimen manila folder. Place the OR requisition form and LVAD core archive form in this folder when finished.

e. Using the Biobank ID or CRS number, label all tissue cassettes, OCT molds, RNA later vials and pieces of tin foil that will be needed. **Ensure that you use the solvent resistant pen (white body with a black cap) to label the tissue**

cassettes. The OCT molds and foil pieces may be labeled with the black permanent marker (does not have to be with the solvent resistant pen). This number will identify the specimens for later retrieval.

f. Place specimen on black photograph mat, and take adequate number of images.

g. Fill the small white Styrofoam box with a layer of dry ice (dry ice is kept on the bottom shelf of the CV Registry -80C freezer located on the 2nd floor Burrard Blg.). Place a small metal tray on the dry ice and this will be used to freeze the OCT and foil wrapped tissues. All frozen samples should be stored on the dry ice while you are archiving the core tissue.

h. Fill a second Styrofoam box with ice chips. Take the box and a plastic 4L specimen container inside. Pour the heart collection buffer into the plastic container. Place the container into the Styrofoam box of ice and when ready, place heart into this bucket.

i. Follow the archive protocol as outlined: see Appendix I: Heart Archive Protocol for Fresh Hearts. *Perform all archive of tissues that are to be tissue cultured first as these are to be placed in culture media as soon as possible*:

   For each tissue section you will take a section for formalin fixation, OCT embedding, flash freezing and RNAlater submersion.

   a. Take section the tissue and place in cassette and place into formalin. Each piece should represent the full thickness of the myocardium (epicardium – endocardium).

   b. Fill up the labeled OCT mold ½ with OCT mold gel. Take section the tissue and place in OCT mold – fill the remainder of the OCT mold with OCT gel. Be sure not to make any bubbles in the OCT gel. Place the OCT mold in the surgical tray on dry ice. Each piece should represent the full thickness of the myocardium (epicardium – endocardium). **See BB.HIST_003.01.**

   c. Take a section of the tissue and wrap it into the labeled 3x3 inch piece of foil. Wrap the ends such that the tissue will not fall out. With a long handled pair of forceps, place the foil into liquid nitrogen for at least 10 seconds. Place the foil in the surgical tray on dry ice. Each piece should

represent the full thickness of the myocardium (epicardium – endocardium). **See BB.HIST_006.01.**

d. Take a section of tissue and submerge into a labeled vial of RNAlater.

j. Once you have finished the protocol, place the metal tray in the -80C freezer.

k. Take the small container of formalin and submit to the iCAPTURE Histology department for processing and embedding.

l. Put the labeled RNAlater vial into the small 4C fridge overnight

m. Remove all images off the digital camera card and store them on the secure P: drive **See BB.002.01.**

n. The following day, take all frozen sections and RNAlater vial and place into the next sequential freezer box and store in -80C fridge. Record the CRS number in the "Freezer Log-in" book. **See BB.013.01**

2) For Heart Transplants

a. The OR will page the Biobank in advance of any heart transplant surgeries. When paged, call the OR and ask:
   a. Find out from the OR when they expect to receive the donor heart.
   b. OR to page you once the recipient's heart is ready for pickup.

o. Using the Biobank ID or CRS number, label all tissue cassettes, OCT molds, RNA later vials and pieces of tin foil that will be needed. **Ensure that you use the solvent resistant pen (white body with a black cap) to label the tissue cassettes.** The OCT molds and foil pieces may be labeled with the black permanent marker (does not have to be with the solvent resistant pen). This number will identify the specimens for later retrieval.

p. Fill the small white Styrofoam box with a layer of dry ice (dry ice is kept on the bottom shelf of the CV Registry -80C freezer located on the 2nd floor Burrard Blg.). Place a small metal tray on the dry ice and this will be used to freeze the OCT and foil wrapped tissues. All frozen samples should be stored on the dry ice while you are archiving the core tissue.

q. Fill a second Styrofoam box with ice chips. Take the box and a plastic 4L specimen container inside. Pour the heart collection buffer into the plastic container. Place the container into the Styrofoam box of ice and when ready, place heart into this bucket.

b. The explanted heart should be placed in the container with buffer and they will page you when the box and specimen is ready for pickup. Ensure the specimen container is properly labeled with the patient's name and a surgical requisition accompanies the specimen.

c. To gain entry to the OR: call the OR using the same pager number they paged you with.

d. Return to the Registry with the specimen and surgical requisition. Place the surgical numbered sticker on the top right corner of the requisition.

e. Place specimen on black photograph mat, and take adequate number of images.

f. Follow the archive protocol as outlined: see **Appendix 1 - Heart Archive Protocol for Fresh. Follow steps i – n in above part 1.**

g. Repeat the archive procedure with the donor tissues and notify the OR to page you when they are ready for pickup.

h. If a Ventricular Assist Device is also sent down, fill the specimen with formalin and leave it in the Registry sink.

Reminders

- take fresh weight of heart
- mark surgical number on each specimen container and mark what containers you have received
- wear appropriate safety gear i.e. – goggles, gloves, face masks, yellow gowns
- fill in % Lumen Narrowing. If patent denote with "0"
- comment on anything you feel is unique or of interest to the pathologist e.g.) bypass graft, ventricular assist device, coronary bypass graft, inserted pacemaker etc.

James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research

Appendix I

Heart Archive Protocol for Fresh Hearts

Date: _____ **BIOBANK #:** _____

Name of Archiver(s): _____ **Fresh Heart Weight:** _____

Check off all tissue containers received:

Heart: ☐ **Aortic Tissue:** ☐ **Pulmonary Artery:** ☐ **Other:** _____

Archive of Fresh Heart Tissue

Freezer Box #: _____ (OCT blocks, tissue frozen for DNA extraction, RNA-later vials)

Note:
1. **Obtain sections that have an X inside the box.**
2. **If the section is not taken, please draw a line and cross out the entire row.**
3. **Describe amount of luminal narrowing as % LN.**

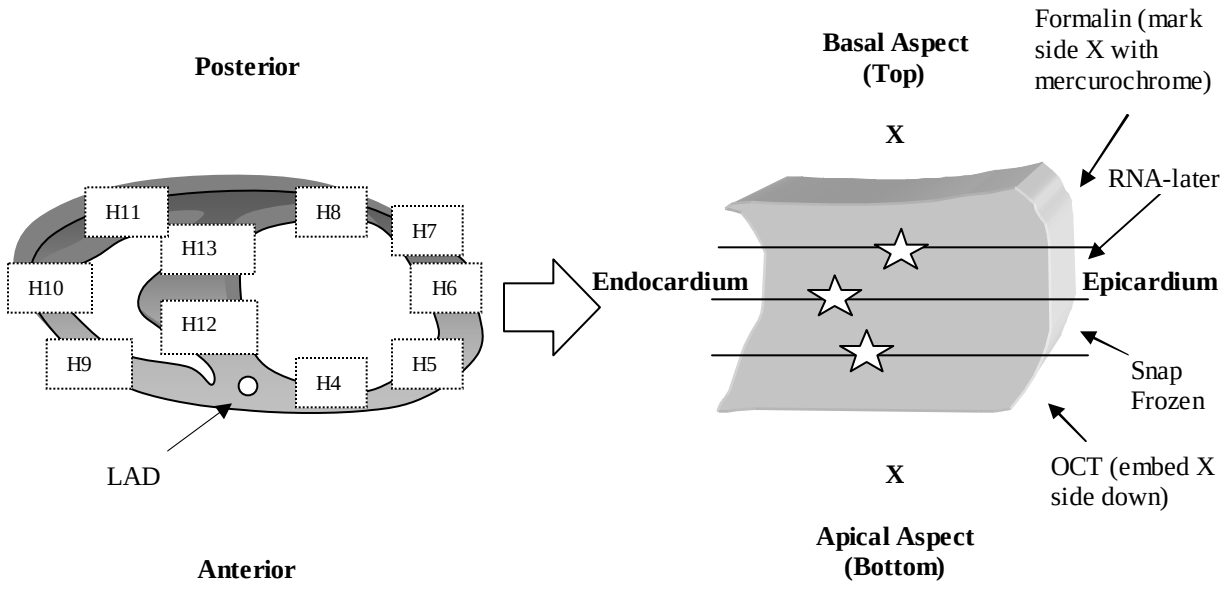
Section description	Section #	Formalin	OCT-Frozen	Snap Frozen (approx. 0.5g)	RNA-later (Approx. 3mm^3 cube)	Perform for Autopsy	Perform for Heart Transplant
Left Main Artery	H19 (prox)	X ___%LN	X ___%LN	X _____g	X	X	X
RCA	H19 (prox)	X ___%LN	X ___%LN	X _____g	X	X	X
	H21 (mid)	X ___%LN	X ___%LN	X _____g	X	X	
Left Circumflex Artery	H22 (prox)	X ___%LN	X ___%LN	X _____g	X	X	X
LAD	H23 (prox)	X ___%LN	X ___%LN	X _____g	X	X	X
	H24 (mid)	X ___%LN	X ___%LN	X _____g	X	X	
	H25 (distal)	X ___%LN	X ___%LN	X _____g	X		

Comments for coronary artery sections (Please describe amount of calcification, presence of bypass grafts, stents, or other unusual findings):

Section Description	Section #	Formalin	OCT-Frozen	Snap Frozen (approx. 0.5g)	RNA-later (approx. 3mm^3 cube)	Other	Perform for Autopsy	Perform for Heart Transplant
LVFW anterior	H4	X	X	X _____g	X	Place 0.5g portion of LVFW in glutar-aldehyde	X	X
LVFW anterior-lateral	H5	X	X	X _____g	X			
LVFW lateral	H6	X	X	X _____g	X			
LVFW posterior-lateral	H7	X	X	X _____g	X			
LVFW posterior	H8	X	X	X _____g	X			
RVFW anterior	H9	X	X	X _____g	X			
RVFW lateral	H10	X	X	X _____g	X			
RVFW posterior	H11	X	X	X _____g	X			

VS anterior	**H12**	**X**	**X**	**X** _____**g**	**X**			

VS posterior	**H13**	**X**	**X**	**X** _____**g**	**X**			

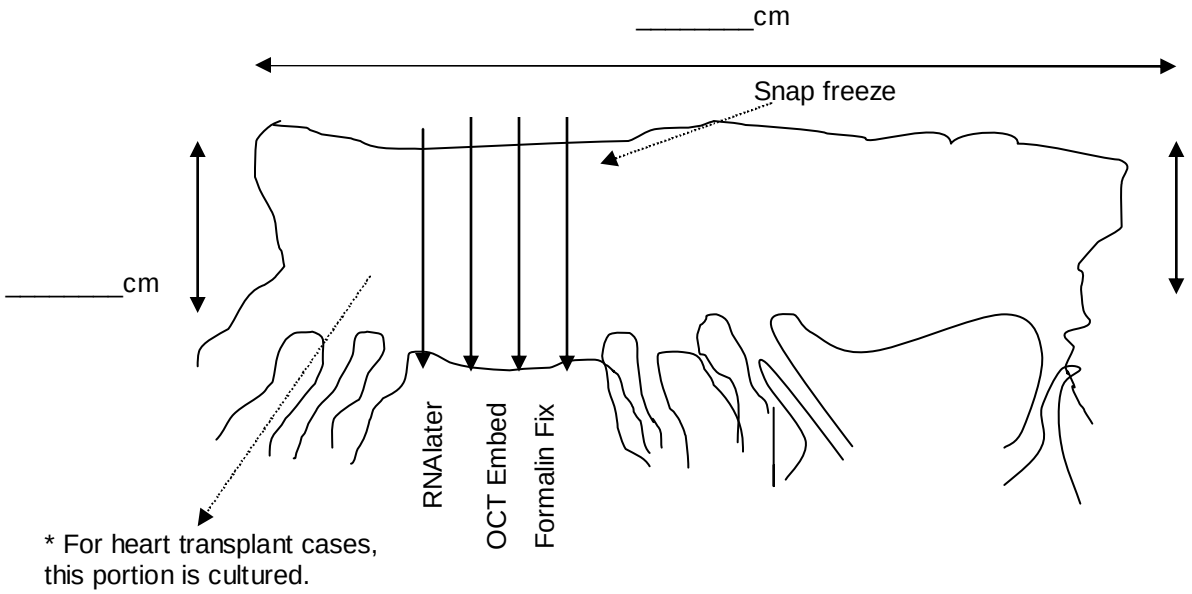


Section Description	Section #	Formalin	OCT-Frozen	Snap Frozen (approx. 0.5g)	RNA-later (approx. 3mm^3 cube)	Other	Perform for Autopsy	Perform for Heart Transplant
LA	**H14**	**X**	**X**	**X _____g**	**X**			
RA	**H15**	**X**	**X**	**X _____g**	**X**			
Ao Root	**H16**	**X**	**X**	**X _____g**	**X**			
PA Root	**H17**	**X**	**X**	**X _____g**	**X**			
MV-anterior leaflet	**H18**	**X**	**X**	**X**		**Archive ½ of anterior leaflet. See diagram.**	**X**	**X**

						Culture ½ of anterior leaflet. See diagram. _____g		**X**

Gross Description (total weight, measurements):

Anterior and Posterior MV = _____g

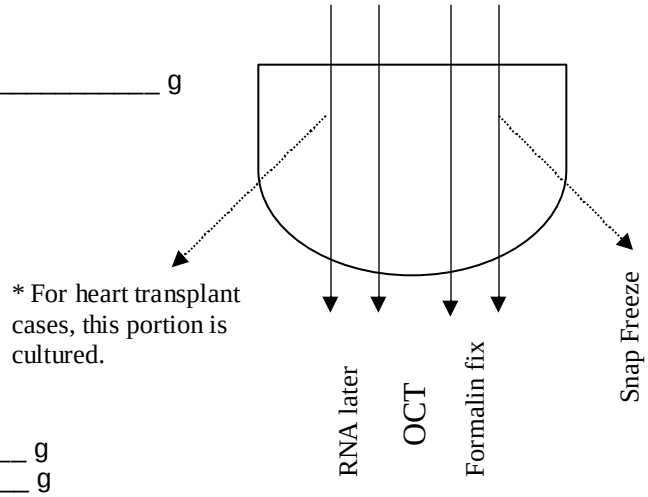


Section description	Section #	Formalin	OCT-Frozen	Snap Frozen (approx. 0.5g)	RNA-later (Approx. 3mm^3 cube)	Perform for Autopsy	Perform for Heart Transplant
Donor Ao Root	**H26**	**X**	**X**	**X _____g**	**X**	**X**	**X**
	Gross description (total weight and measurements):						
Donor PA Root	**H27**	**X**	**X**	**X _____g**	**X**	**X**	**X**
	Gross description (total weight and measurements):						
AV-Left Cusp	**H28**	**X**	**X**	**X**	**X**		**X**

AV-Right Cusp	**H30**	**X**	**X**	**X**	**X**		**X**

Gross Description (total weight, measurements):

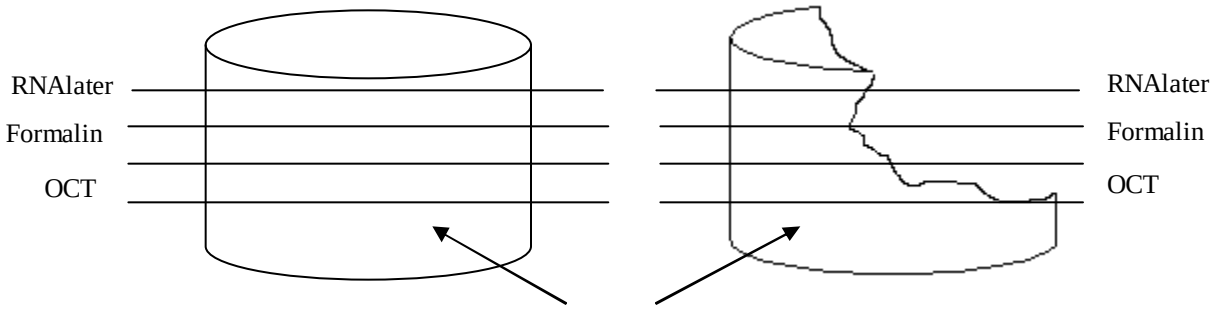
Total weight of Left, Posterior, Right AV Cusps = _____ g



* For heart transplant cases, this portion is cultured.

Donor Root

Total weight of Donor Ao Root = _____ g
Total weight of Donor PA Root = _____ g



Additional comments: