James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research

STANDARD OPERATING PROCEDURE

Title:	**Lung Archive**		
Procedure:	BB.004.01	**Supercedes:**	none
Originator and Date:	Lise Matzke 21OCT2008	**Effective Date:**	21OCT2008
Review Frequency:	annually	**Approved By:**	The iCAPTURE Centre Privacy Team
Total Number of Pages: 3			

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 Biobank personnel to follow when archiving lung specimens from transplant 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. Other personnel who are responsible for archiving biospecimens

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 or hazardous chemicals. Refer to BB.001.01 (Handling Biohazardous Materials).

Definitions

Archive	The physical process of harvesting tissue for indefinite storage
Fixation	To preserve tissues in an as life-like a state as possible.
PPE	Personal protective gear including and not limited to: gloves, biohazard gown, lab coat, eye protection, surgical mask, etc.
SOP	Standard Operating Procedure. Document used to control the method and requirements by which personnel will perform their activities.

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.

Liquid nitrogen	Clamps
Formalin	White plastic tubs to hold formalin
Yellow gown	Biohazardous bags (8” x 12”)
Latex gloves	Yellow plastic waste bags
Freezer boxes	Buffered neutral formalin
Tissue cassettes	Cryomatrix/NaCl 1:1 mixture
Tissue cassette pens/pencil	Phenokill
Permanent marker	Biohazard gown
Scalpels	Eye protection
Bucket	Gloves
Aluminum foil	Surgical mask
Straight dissecting forceps	

Procedures

1) OR will page Biobank personnel.
2) Pick up lung directly from determined location as per OR guidelines.
3) Take the specimen to Pathology Department for processing.

4) Take lung to processing room (insert room location).
5) Weigh the lung and the bucket then remove lung from bucket and weigh empty bucket to get fresh weight of lung. Record weights in appropriate location as per site guidelines.
6) In the Biological safety cabinet, remove samples required for pathology (resection margin, nodes, tumor samples, anything else abnormal), place in labeled cassettes and fix in formalin. Then remove any samples required for research purposes requiring living tissues (airways, normal lung, lavage). Also take sample of tumor and wrap in labeled piece of tin foil labeled with case number and snap freeze it in liquid nitrogen. In the Frozen Lung book, record length of bronchus attached, length of any suture/staple lines, size and location of tumor, size and location of any pleural pucker, distance of tumor from resection margin, and any other pertinent information (i.e. any other abnormalities).
7) To lavage the lung, put piece of tubing into bronchus of segment you want to lavage (away from the tumor). Connect this to a 60 ml syringe filled with saline. Push in the saline and then slowly draw back on the plunger. You may have to inflate with saline a number of times to get back enough saline/cells for your experiment. You may find you need to gently squeeze the lung as you are drawing back in order to draw any liquid out.
8) After lavage, inflate the lung with Cryomatrix diluted 1:1 with saline. Place one end of the tube into the reservoir of Cryomatrix and the other end with a small nipple on it into the bronchus. The tube is connected to an old dialysis pump to aid in pumping the Cryomatrix into the lung. Inflate until lung looks fully inflated.
9) Disconnect the tubes and the lung is placed on a large piece of aluminum foil and weighed-record weight in Frozen Lung book. Suspend lung over, but not in, liquid nitrogen until frozen. Depending on size this could take 30 min. or more. **Do not clamp the airways** as this will cause lung to crack as it freezes-the Cryomatrix/saline mixture will expand as it freezes and needs a way to escape. If you put the lung directly into liquid nitrogen it will also crack/shatter.
10) Once frozen, transfer the lung to a -70 freezer for a few hours to let it warm up a bit otherwise it is too brittle to handle.
11) Cut into slices 1-1.5 cm thick using a meat saw and then core with a drill press with a 1.0 cm radius hole saw. The cores are wrapped in labeled tin foil and stored at -70. The remaining pieces of frozen lung are placed in a bucket with formalin and left to thaw/fix overnight or until fixed.
12) Clean the meat saw and drill press with Phenokill.