



**BIOSPECIMEN CORE**

**PROCESSING MANUAL**

**THE AUSTRALIAN OVARIAN CANCER STUDY**  
**BIOSPECIMEN PROCESSING PROTOCOL**

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## **THE AUSTRALIAN OVARIAN CANCER STUDY** **BIOSPECIMEN PROCESSING PROTOCOL**

**RESEARCH ASSISTANTS AT PMCI WILL PROCESS ALL BIOSPECIMENS AND ENTER THE DATA INTO THE AOCS AND TB DATABASES.**

### **BLOOD PROCESSING**

**COLLECTED:**            1 x 9ml EDTA  
                                 1 x 8ml Plain tube  
                                 1 x 9ml Lithium Heparin tube

Process the EDTA/Lithium Heparin tube as detailed below (EDTA/Lithium Heparin Blood Protocol)

Process the plain tube as detailed below (Serum Protocol)

**PROCESS THE BLOOD WITHIN 72 HRS OF COLLECTION- KEEP BLOOD AT ROOM TEMPERATURE UNTIL PROCESSED**

### **SAFETY**

TREAT ALL BLOOD AS POTENTIALLY INFECTIOUS.

PROCESS ALL BLOOD IN A CLASS II BIOLOGICAL SAFETY CABINET FLOW HOOD AND WEAR GLOVES.

DEPC, DMSO AND FICOLL MUST BE HANDLED IN A FUME HOOD

CHECK YOUR HEPATITIS B TITRE EVERY 2 YEARS

See Appendix VII- Guidelines for the Safe Handling of Blood and other Human Products

See Appendix VIII- Occupational Exposures to Blood & Other Body Fluids

See Appendix IX- Management Occupational Exposures to Blood & Other Body Fluids

All plastic wear (10ml tubes, 50ml tubes, cryovials, pasteur pipettes, 5ml, 10ml pipettes) need to be sterile as these cells may one day be cultured. Use standard sterile techniques.

YOU WILL NEED THE FOLLOWING ITEMS FOR PROCESSING BLOODS:

- Sticky labels (unique adhesive label resistant to dry ice and liquid nitrogen)
- Guthrie cards
- Qiagen DNA Kit
- 2ml labelled cryovials
- 10ml/50ml falcon tubes
- 5ml/10ml sterile pipettes
- Pasteur pipettes
- 70% ethanol
- DMSO
- Tris/EDTA buffer
- RPMI 1640
- Ficoll
- P1000
- Gloves
- Safety glasses
- Liquid nitrogen
- Mr Frosty
- Ice/Ice bucket

See Appendix X - catalogue numbers and suppliers of consumables

See Appendix XI – recipes for blood processing solutions

RECEIPT OF BLOOD- LABELLING

**Tubes and Guthrie cards must always have two labels of identification on them.**

Label all cryovials and Guthrie cards with the TB patient ID#, the TB biospecimen number and the 4-digit biospecimen usage code. Before using any patient ID numbers, contact Justine Biggs to confirm this number has not already been allocated.

See Appendix XII- 4-Digit Code Numbers

Place colour coded inserts into the cryovial lids to help distinguish between the fractions collected. See Table 1.

**TABLE 1: Colour Inserts and Storage Locations**

	<b>INSERT</b>	<b>STORAGE</b>
Blood Pellet	Pink	-70°C
Non-lymphocytes	Grey	-70°C
Plasma	Mauve	Liquid Nitrogen
Serum	Yellow	Liquid Nitrogen
White Blood Cells	No Insert	Liquid Nitrogen
Whole Blood	Orange	-70°C
Guthrie Cards		Room Temperature

Record the following information in the AOCS *Biospecimen database*:

Biospecimen ID#

Patient ID# and DOB

Date blood taken

Amount of blood received (ml)

Date blood processed

Number of Guthrie cards

Number of tubes of Serum, Plasma, White Blood Cells, Non-lymphocytes, Blood Pellet and Whole Blood

Samples are to be sent to QIMR periodically, as per Table 2.

**TABLE 2: Sample Storage at PMCI and QIMR**

	<b>PMCI</b>	<b>QIMR</b>
Guthrie Cards	1	1
Blood Pellet	1	1
White Blood Cells	2	1
Non-Lymphocytes	1	1
Serum	2	2
Plasma	6	6

Samples for QIMR will be couriered on dry ice using Blue Circle's overnight delivery service.

## SHIPPING INSTRUCTIONS FOR SAMPLES TO BE COURIERED TO QIMR

### Blue Circle Couriers

Pack the samples in the esky and cover with dry ice. Place a few little holes in the esky to avoid any air pressure build up from the dry ice.

The “Promega enzyme eskies” (extra insulation) are ideal for shipping tissue- Nadia Traficante has these eskies.

Attach the black/white miscellaneous goods sticker on the outside of the esky.

Attach a pre-printed consignment note addressed to:

Georgia Chenevix Trench  
QIMR  
300 Herston Rd  
Herston QLD 4029

Label the consignment note with:

- Georgia Chenevix Trench’s telephone number (07) 3362 0390
- Under description of goods, write “Dry Ice UN1845/ Hazardous”

Call Blue Circle on (03) 9258 6042 and organise collection of your esky. Request that the shipment be charged to the Peter MacCallum Cancer Institute Melbourne, Research Division. The account number is MP114 and the cost centre is P30612.

Blue Circle will pick up from your designated site in any Australian capital city.

Mention that you have an esky packed with dry ice and less than 3kg.

Book a pick up for early afternoon at your designated site for delivery to QIMR by 9am the following day.

Blue Circle will ring you the next day to confirm what time the shipment was delivered at QIMR and the name of the person who signed for the delivery.

Email [georgiaT@qimr.edu.au](mailto:georgiaT@qimr.edu.au) the booking number and ask for confirmation of its arrival.

Note: Contact Nadia Traficante by email or phone (03) 9656 1937 before shipping samples. If AOCS Investigators are travelling from Melbourne to Brisbane, the samples can be brought with them instead of being sent via Blue Circle.

IATA Guidelines- 2kg (maximum) of Dry Ice can be taken on the aircraft as part of the passenger’s carry-on luggage.

## **EDTA/LITHIUM HEPARIN BLOOD PROTOCOL (2 x 9ml tubes)**

The protocol aliquot's 18ml blood into:

### **EDTA TUBE**

- 3 cards of Guthrie spots (from 1.5ml) for direct PCR (Storage at RT)
- 3 freezes of white blood cells (WBC) for RNA and transformation (from 9ml) in LN<sub>2</sub>
- 2 freezes of non-lymphocytes (NL, from same 9ml) for RNA (Storage in -70°C)
- 6 freezes of plasma (Storage in LN<sub>2</sub>)

### **LITHIUM HEPARIN TUBE**

- 2 blood pellets (BP), for DNA isolation (Storage in -70°C)
- 6 freezes of plasma (Storage in LN<sub>2</sub>)

The following is the protocol for processing 26ml blood- 9ml from the EDTA and Lithium Heparin tubes and 8ml from the Serum tube.

#### **A) MAKING GUTHRIE SPOTS- EDTA TUBE**

*Always make Guthrie cards, regardless of volume of blood received.*

- 1) Mix blood thoroughly by inversion before starting.
- 2) Wipe top of vacutainers with ETOH before opening.
- 3) Use the EDTA tube to make two full Guthrie cards by placing a drop of blood in the circles using a P1000 pipette. Use 500ul blood per card. Wipe the base of the pipette with 70% alcohol after each blood.
- 4) Air dry thoroughly (a few days) in the back of the safety cabinet.
- 5) Store in paper envelopes (not plastic) in a filing cabinet. Keep 1 card in a separate envelope for QIMR.

#### **B) PLASMA (mauve insert)- EDTA AND LITHIUM HEPARIN TUBES**

- 1) Spin both vacutainers (about 8ml) at 1500 rpm for 15min to separate plasma
- 2) After wiping each tube with alcohol, remove about 3 ml plasma (not the white cells in the buffy coat)
- 3) Aliquot plasma into 0.5ml labelled cryovials (8-12 aliquots). Label tube with either EDTA or Lithium Heparin.
- 4) Place the vials into two different storage containers- 4-6 plasma vials into one storage container for PMCI, 4-6 plasma vial into the other (for QIMR).
- 5) Store the aliquots in LN<sub>2</sub>

D) BLOOD PELLETT (pink insert)- LITHIUM HEPARIN TUBE

Lyse red blood cells to make blood pellet (BP) for DNA isolation:

- 1) Transfer blood remaining in the Lithium Heparin tube to a labelled 50ml tube
- 2) Fill tube with Tris-EDTA buffer and mix vigorously. Place on ice for 2-5 minutes.
- 3) Spin ASAP at 2500 rpm for 10 min
- 4) Carefully pour off the supernatant into a beaker containing 5 grams of Diversol (may need to suction off- pellet can be slippery). Briefly vortex the pellet and add 50ml Tris-EDTA buffer. Shake vigorously.
- 5) Pour 25ml of sample into another falcon tube
- 6) Spin both tubes at 2500 rpm for 10 min
- 7) Repeat washing if red cells persist
- 8) Carefully pour off supernatant
- 9) Using a swirling motion remove the pellets (and a small volume of supernatant) with a pipette and transfer to 2 labelled cryovials.
- 10) Place the cryovials into two different storage containers- 1 vial into one storage container for PMCI, 1 vial into the other (for QIMR).
- 11) Store in -70°C until DNA required

DNA ISOLATION:

Use the QIAGEN "QIAamp DNA Blood Mini Kit" (Catalogue # 51106)

Follow the Blood and Body Fluid Spin Protocol, page 27 of the Instruction Manual

SEND 10ug OF DNA TO QIMR (GCT) VIA EXPRESS POST AS REQUESTED (~MONTHLY).

Record this information in the AOCS Biospecimen database

E) WHITE BLOOD CELLS (no insert)- EDTA TUBE

Separate White Blood Cells (WBC) by Ficoll gradient:

- 1) Transfer the remaining blood in the EDTA tube to a labelled 50ml falcon tube containing 10ml RPMI 1640
- 2) Aliquot 3ml Ficoll, after swabbing lid/forceps with alcohol, into 2 labelled 10mls centrifuge tubes
- 3) Carefully layer the diluted blood from step 1 onto each tube of Ficoll. Treat gently, do not mix
- 4) Spin immediately at 1500 rpm for 30 mins, WITHOUT A BRAKE
- 5) Remove the top layer (RPMI 1640) and discard (~ 3-4ml)
- 6) Collect 1ml WBCs (second layer) with a sterile pipette and place into a 50ml tubes that has 10 ml RPMI. Use a swirling motion to "vacuum up " the WBCs. Do not take to much Ficoll (third layer) from below the cells as it is toxic to the cells.
- 7) The Ficoll tubes are kept for the red non-lymphocyte (NL) fraction detailed below
- 8) Spin WBC at 1500 rpm for 10 minutes. Brake can be used here.
- 9) Pour off the supernatant from the WBCs carefully into a waste container containing 5 grams of Diversol. Add 3 ml of freezing mix to cells and resuspend by flicking the bottom of the tube.

- 10) Dispense the WBCs into 3 x 1ml freezes in labelled cryovials.
- 11) Place WBCs and on ice for a few minutes, no more than 10mins. Place the vials in "Mr Frosty"
- 12) Place the 3 WBCs vials into the -70 freezer using two different storage containers, 2WBCs vials into one storage container, 1 WBC vial into the other (for QIMR). Transfer on a weekly basis to LN<sub>2</sub>, again into 2 different storage tanks.

Ship to QIMR in small batches?? OR Store at Melb Uni/shared space?

F) NON-LYMPHOCYTES (grey insert)

- 1) Collect 1 ml of red Non-Lymphocytes (NL) fraction from both of the tubes of blood remaining after the WBC Protocol. Use a swirling motion and place into a labelled 10ml tube containing 5ml RPMI
- 2) Spin at 1500 rpm for 10 min. Brake can be used here.
- 3) Discard the supernatant into a waste bottle containing 5 grams of Diversol.
- 4) Add 300ul of Freezing Solution to 2 labelled cryovials and place the tubes on ice.  
Note: Freezing Solution should be made fresh each time. Keep on ice at all times.
- 5) Add half of the NL layer from step 3 to one of the cryovials containing the 300ul of Freezing Solution and the remaining half to the other cryovial.
- 6) Gently mix with a pipette.
- 7) Place tubes on ice. Place vials in "Mr Frosty". Transfer at -70°C freezer as soon as possible as DMSO is toxic at RT. Vials can be stored in "Mr Frosty" for up to 4 days before transferring them to the Liquid Nitrogen storage tanks. Place the NL vials into two different storage containers- 1 NL vial into one storage container for PMCI, 1 NL vial into the other (for QIMR).

If less than 30 ml but more than 10 ml of blood is collected the Lithium Heparin and Serum tubes will not be full. These tubes can still be processed as described above, but if there is less than 2mls of blood in the Lithium Heparin tube, 2 ml should be removed from the EDTA tube to make a Blood Pellet before the blood is separated on Ficoll.

If 10mls of blood is collected, there will only be one EDTA tube. Process this blood in the following way:

- 1) Use 1ml to make 2 Guthrie cards
- 2) Remove 2ml for the blood pellet
- 3) Use the rest of the blood for plasma, 3 WBC freezes plus 2 NL freeze

If less than 6 ml blood received:

- 1) Use 0.5ml to make 1 Guthrie card
- 2) Use the rest of the blood for plasma, 3 WBC freezes and 2 NL freezes

## **SERUM PROTOCOL**

- 1) Spin blood in the plain tube at 2500 rpm for 10 minutes
- 2) Transfer the serum to labelled cryovials in 1ml aliquots. Serum aliquots will have yellow inserts.
- 3) Store samples in Liquid Nitrogen

## **MOUTHWASH PROTOCOL**

- 1) Add 35ml TE to the mouthwash sample and spin at 1500rpm for 5 minutes
- 2) Wash cells twice, each time with 45ml TE
- 3) Resuspend cell pellet in 500ul TE and transfer to a 2ml cryovials
- 4) Store sample at -70°C

### **DNA ISOLATION:**

Use the QIAGEN “QIAamp DNA Blood Mini Kit” (Catalogue # 51106)

Follow the Blood and Body Fluid Spin Protocol, page 27 of the Instruction Manual

**SEND 10ug OF DNA TO QIMR (GCT) VIA EXPRESS POST AS REQUESTED.**

Record this information in the AOCS Biospecimen database

## **URINE**

### **YOU WILL NEED THE FOLLOWING ITEMS FOR PROCESSING URINE:**

- Sticky labels (unique adhesive label resistant to dry ice and liquid nitrogen)
- 2ml labelled cryovials
- Gloves
- Liquid Nitrogen

See Appendix X for catalogue numbers and suppliers of consumables

Vapour-Phase Liquid Nitrogen storage is recommended. Proteins, hormones and metabolites will all be stable if stored at -70°C or VP-LN<sub>2</sub>.

#### **Tubes must always have two labels of identification on them.**

Label all cryovials with the participants initials, DOB, AOCS ID# and AOCS Biospecimen#.

In addition, label all cryovials with the TB patient ID# and the 4-digit usage code number. Lisa Devereux or Justine Biggs will generate the TB numbers on request when an AOCS ID# is given to them.

Thaw 10ml vacuette tube and aliquot urine into 5 x 2ml labelled nunc cryovials. Place the cryovials into two different storage containers- 3 vials into one storage container for PMCI, 2 vials into the other (for QIMR).

Store urine samples in LN<sub>2</sub>

## **TISSUE PROCESSING**

### **YOU WILL NEED THE FOLLOWING ITEMS FOR PROCESSING TISSUE:**

- Sticky labels (unique adhesive label resistant to dry ice and liquid nitrogen)
- 70% ethanol
- 10% formalin
- DEPC treated water
- 2ml labelled cryovials
- 25ml yellow cap specimen containers
- Liquid nitrogen
- Gloves
- Safety glasses

See Appendix X for catalogue numbers and suppliers of consumables

Treat all tissue as potentially infectious.

Tissue must be retained for at least 2 weeks before homogenising or cutting in the event that the pathologist may need the tissue for diagnostic purposes.

#### **Tubes must always have two labels of identification on them.**

Label all cryovials with the participants initials, DOB, AOCS ID# and AOCS Biospecimen#.

In addition, label all cryovials with the TB patient ID# and the 4-digit usage code number. Lisa Devereux or Justine Biggs will generate the TB numbers on request when an AOCS ID# is given to them.

### **SNAP FREEZING PROTOCOL**

- 1) Place the tissue sample (1cm<sup>3</sup>/2000mg piece) in a labelled 25ml specimen bottle. If more than 1 piece of tissue is to be frozen, place each piece into the same specimen container, separated by a piece of parafilm. (Each specimen container will hold 4 pieces of tissue, 1cm<sup>3</sup> each)
- 2) Immerse the specimen tube into the vapour-phase liquid nitrogen cryopak shipper. The sample can be carried in here for several days.
- 3) Store the sample in a cardboard storage box at -70°C. Batch samples and ship to PMCI for processing (see AOCS Biospecimen Collection Protocol)

## PROCESSING AT PMCI

- 1) The tissue will need to be transferred to new specimen tubes (Labels will not stick to the cold tubes)
- 2) Homogenise 1 piece (1cm<sup>3</sup>/2000mg) of tissue (See Homogenising Protocol below) and store this tissue in a labelled 2ml cryovials in LN<sub>2</sub>
- 3) The remaining tissue will be stored whole in LN<sub>2</sub> until required
- 4) Store the backup samples for QIMR (all whole tissue) in a separate cardboard storage box in LN<sub>2</sub>. These samples will be shipped to QIMR in batches.

Samples for QIMR will be couriered on dry ice using Blue Circle's overnight delivery service.

### Shipping Instructions

Pack the samples in the esky and cover with dry ice. Place a few little holes in the esky to avoid any air pressure build up from the dry ice.

Attach the black/white miscellaneous goods sticker on the outside of the esky.

Attach a pre-printed consignment note addressed to:

Georgia Chenevix Trench

QIMR

300 Herston Rd

Herston QLD 4029

Label the consignment note with:

- Georgia Chenevix Trench's telephone number (07) 3362 0390
- Under description of goods, write "Dry Ice UN1845/ Hazardous"

Call Blue Circle on (03) 9258 6042 and organise collection of your esky. Request that the shipment be charged to the Peter MacCallum Cancer Institute Melbourne, Research Division. The account number is MP114 and the cost centre is P30612. Blue Circle will pick up from your designated site in any Australian capital city. Mention that you have an esky packed with dry ice and less than 3kg.

Book a pick up for early afternoon at your designated site for delivery to QIMR by 9am the following day.

Blue Circle will ring you the next day to confirm what time the shipment was delivered at QIMR and the name of the person who signed for the delivery.

Email [georgiaT@qimr.edu.au](mailto:georgiaT@qimr.edu.au) the booking number and ask for confirmation of its arrival.

Note: Contact Nadia Traficante by email or phone (03) 9656 1937 before shipping samples. If AOCS Investigators are travelling from Melbourne to Brisbane, the samples can be brought with them instead of being sent via Blue Circle.

IATA Guidelines- 2kg (maximum) of Dry Ice can be taken on the aircraft as part of the passenger's carry-on luggage.

## **HOMOGENISING PROTOCOL**

### **EQUIPMENT**

Dry ice in an esky

Hammer

Liquid nitrogen Dewars (1L and 2L)

Metal tube block

Greiner screw top tubes

Safety goggles, gloves and facemask

Spatulas

Steel homogenisers

Wood

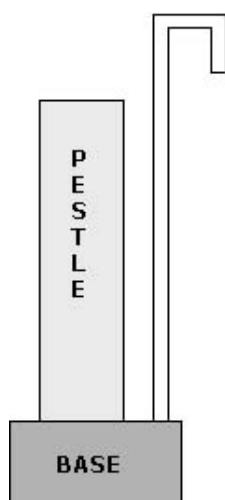


Figure 1. Steel Homogeniser

***This procedure has been written in consultation with Dr Monica Slavin, Infectious Diseases Manager.***

***Follow safety instructions for handling liquid nitrogen in the Peter MacCallum Cancer Institute Safety Policy.***

***Gloves must be worn throughout the procedure. A facemask and safety goggles must be worn when handling the homogeniser containing tissue outside the safety cabinet.***

***Use a fresh homogeniser for each tissue.***

1. The steel homogenisers, as shown in Figure 1, and the metal tube block are stored in the KConFab  $-70^{\circ}\text{C}$  freezer.
2. Place a sheet of bench coat on an open area on the lab floor.
3. Fill the two liquid nitrogen Dewars (1 litre and 2 litre) with liquid nitrogen.
4. Remove two steel homogenisers from the  $-70^{\circ}\text{C}$  and place in the 2 litre Dewar. Wait until the liquid nitrogen ceases bubbling.
5. Place tissue, numbered tubes, and spatulas into 1 litre Dewar.
6. Place metal tube block onto dry ice in an esky.
7. Weigh the tissue in the container to obtain an estimate of the number of 250mg aliquots. The weight of the container is approximately 8.46g.
8. Label the tubes with the specimen number and the patient ID number, and place into the metal tube block on dry ice.
9. Remove the homogeniser from the Dewar and place it in the class II safety cabinet.
10. Place tissue sample into the base of the homogeniser and put pestle on top.
11. Remove from the class II safety cabinet and place on the bench coat on the floor.
12. Place the wood block on top of the pestle as a shock absorber. Using a hammer hit the wood 3-5 times.
13. Return the unopened homogeniser to the class II safety cabinet.
14. Remove the pestle. Break and mix powder with a frozen spatula. If repeat crushing is necessary, replace the pestle and repeat steps 9 and 10.
15. Loosen the lid of a screw top tube but do not remove. Place the tube into liquid nitrogen.
16. Transfer powder to each labelled frozen tubes using a spatula and placed in the metal block with their lids closed and transferred from the safety cabinet to the balance for weighing. Each aliquot is 250mg. The weight of the screw top tube is approximately 1.95g. Place tubes into liquid nitrogen.

17. Allow the used homogenisers to defrost at room temperature for approximately 2 hours in the safety cabinet.
18. In the safety cabinet, soak the homogenisers into a container of 2% Decon for 20 minutes.
19. Wash and scrub the homogenisers in 2% Decon. Rinse well with tap water, followed by distilled water and finally in 70 alcohol.
20. Leave the homogenisers to air dry at room temperature.
21. Return the homogenisers to the -70°C for at least 3 hours or overnight before usage. It is important that homogenisers are completely dry before frozen, otherwise base and pestle cannot be separated.

**70% ALCOHOL FIXATION PROTOCOL**

- 1) Place tissue in DEPC treated water for a minimum of 4 hours
- 2) Transfer the tissue to a cassette labelled with the **AOCS ID#, AOCS Biospecimen #, TB patient ID# and TB biospecimen #.**
- 3) Process the blocks on the Tissue Tek VIP processor using Program 9

**TABLE 1: PROGRAM 9- ALCOHOL FIX**

STATION	SOLUTION	TIME	TEMPERATURE	PRESSURE VACUUM	AGITATION
3	70% alcohol	1:00		ON	ON
4	90% alcohol	1:00		ON	ON
5	100% alcohol	1:00		ON	ON
6	100% alcohol	1:30		ON	ON
7	Clearant	1:30		ON	ON
8	100% alcohol	1:30		ON	ON
9	Xylene	1:30		ON	ON
10	Xylene	1:30		ON	ON
11	Wax	1:30	60°C	ON	ON
12	Wax	1:00	60°C	ON	ON
13	Wax	1:00	60°C	ON	ON
14	Wax	0:00		ON	ON

- 4) Embedded blocks in paraffin wax
- 5) Cut a section from each block and stain with Haematoxylin and Eosin
- 6) Image the sections and store on the database

**10% FORMALIN FIXATION PROTOCOL**

- 1) Place tissue in 10% formalin for a minimum of 12 hours
- 7) Transfer the tissue to a cassette labelled with the **AOCS ID#, AOCS Biospecimen #, TB patient ID# and TB biospecimen #.**
- 2) Process the blocks on the Tissue Tek VIP processor using Program 9

**TABLE 2: PROGRAM 9- FORMALIN FIX**

STATION	SOLUTION	TIME	TEMPERATURE	PRESSURE VACUUM	AGITATION
1	50% alcohol	0:00		OFF	OFF
3	70% alcohol	0:00		OFF	OFF
4	90% alcohol	1:00		ON	ON
5	100% alcohol	1:00		ON	ON
6	100% alcohol	1:30		ON	ON
7	Clearant	1:30		ON	ON
8	100% alcohol	1:30		ON	ON
9	Xylene	1:30		ON	ON
10	Xylene	1:30		ON	ON
11	Wax	1:30	60°C	ON	ON
12	Wax	1:00	60°C	ON	ON
13	Wax	1.00	60°C	ON	ON
14	Wax	0:00			

- 3) Embedded blocks in paraffin wax
- 4) Cut a section from each block and stain with Haematoxylin and Eosin
- 5) Image the sections and store on the database

## **DATA ENTRY**

Record the following information in the AOCS Biospecimen database:

### Blood

AOCS ID#  
Specimen ID#- generated at PMCI  
Date collected  
Date sent to PMCI  
Amount collected  
Date processed  
Fractions processed  
Location of fractions  
Mouthwash- date processed  
DNA processed- amount, location

### Urine

AOCS ID#  
Specimen ID#- generated at PMCI  
Date collected  
Date sent to PMCI  
Amount collected  
Location of samples

### Tissue

AOCS ID#  
Specimen ID#- generated at PMCI  
Hospital  
Surgeon  
Pathologist  
Tissue Type  
Consent Form  
Time elapsed before freezing/fixing tissue  
Number of tissue pieces frozen at -70°C  
Number of aliquots of powdered tissue  
Date powdered  
Number of tissue blocks fixed in 70% alcohol  
Number of tissue blocks fixed in 10% formalin  
Date blocks processed  
H&E images- low and high power  
Storage of additional specimens  
Path report requested  
Path report received  
Path report sent to PCMI

The AOCS Research Nurses collecting the biospecimens will have entered some of this information in their study log as well as the tracking database. This information will be linked to the main biospecimen database.

The additional information will need to be recorded on the Tissue Bank database. This will be linked to the AOCS Biospecimen database. Tissue Bank Staff will not be able to access AOCS samples and data.

### Pathology

Each Research Nurse will be responsible for collecting the pathology reports for their patients and sending them to PMCI. Nadia Traficante will follow up any missing reports.

The PMCI Biospecimen processors will enter the information recorded on the pathology reports on the AOCS pathology database. This database will be linked to the AOCS Biospecimen database.

## **APPENDICES**

Appendix I:	Mouthwash Protocol A
Appendix II:	Mouthwash Protocol B
Appendix III:	Approach Letter
Appendix IV:	Blood Pro-forma Fax Sheet
Appendix V:	Tissue Pro-forma Fax Sheet
Appendix VI:	Tissue Collection Flowchart
Appendix VII:	Guidelines for the Safe Handling of Blood and Other Human Products
Appendix VIII:	Occupational Exposures to Blood & Other Body Fluids
Appendix IX:	Management Of Occupational Exposures From Blood And Other Body Fluids
Appendix X:	Reagents/Consumables
Appendix XI:	Recipes for Blood Processing Solutions
Appendix XII:	4-Digit Code Numbers