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***WARTN LABORATORY MANUAL***

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## **PART 1: REGISTRATION**

1. To open database, click on green cross icon on the computer desktop.
2. To login enter username (supervisor) and password (superman). NOTE: Case sensitive!
3. On the main menu select 'Patient procedures' to open the main data entry form.
4. Enter Patient demographic details which are displayed on the top light green section of the screen. The following information is almost always available:
  - Patient's first name
  - Middle name
  - Last name
  - UMRN
  - Sex
  - Date of Birth
  - Address (multiple addresses for one patient can be entered)
  - Fill in the rest of the information if and when available.(e.g. by obtaining Pathology Report where relevant)

NOTE: Information above is not available for the patients on clinical trial (Refer to Clinical Trial Protocols and also to Registration part for each study for specific requirements).

5. Enter Procedure details which are located on the bottom half of the main data entries form. The following information should always be available.
  - Record Type
  - Procedure ID-allocate Tissue Network number
  - Procedure date
  - Hospital
  - Disease Type
  - Patient Consent
  - Fill in the rest of the information if and when available.(e.g. by obtaining Pathology Report where relevant)

NOTE: As soon as patient is registered allocate storage card. If Action Sheet is not available allocate appropriate action sheet as well with the comment on it of why it wasn't available.

6. Enter Sample processing details which are displayed in the bottom part of main data entry form. The following information should always be available:
  - Origin
  - Sample class
  - Processing
  - Processed time
  - Sample locations
  - Fill in the rest of the information if and when available.(e.g. by obtaining Pathology Report where relevant)

NOTE: Information is obtained from the WARTN Action Sheets.

## **PART 2: LABELLING**

### ***PRINTING OF LABELS***

- 1. On the main menu select Administration/Maintenance.**
- 2. Select Print Barcode labels.**
- 3. Predict number of labels required.**

The following table is a guideline as to how many barcode labels to print per procedure and also, how to allocate sub numbers.

<b>Tube type</b>	<b>Quantity of blood (mls)</b>	<b>Sample type</b>	<b>Number of labels per tube</b>
<b>EDTA</b>	5-9	Whole Blood (WBE) Plasma (PE) Buffy Coat (BCE)	2 2-4 1
<b>Lithium Heparin</b>	5-9	Plasma (PLH) Buffy Coat (BCLH)	2-4 1
<b>Serum</b>	5-9	Serum (S)	2-4
<b>ACD A</b>	5-9	Plasma (P-ACDA) Buffy (B-ACDA)	2-4 1

**Note 1:** If there is more than one blood tube processed at the same time, processed aliquots are separated in the following order:

1. WBE
2. PE
3. BCE
4. PLH
5. BCLH
6. S

First sub number (A0) is allocated to WBE and the rest of sub numbers are allocated in consecutive order to the aliquots as they are separated.

**Note 2:** ACD A tubes are replacing EDTA and LiHep tubes. Whole blood is NOT taken from ACD A tubes. Processed aliquots are separated in the following order:

1. P-ACDA

2. B-ACDA
3. S

First sub number (A0) is allocated to P-ACDA and the rest of sub numbers are allocated in consecutive order to the aliquots as they are separated.

**Note 3:** barcode label without sub number (e.g. 00234-) is printed for the Action Sheet.

**4. To generate batch of labels:**

- enter label start, which is TN number (e.g. 00234)
- enter labels end, which is the same as above (e.g. 00234)
- enter required number of labels

**5. To print extra labels in cases where not enough labels were printed initially:**

- enter last label, which is TN number, ( e.g. 00234, database remembers which part has been printed last)
- enter number of extra labels required

**6. Labels can be printed manually if required**

- For example if 00234A3 is required, that is what needs to be entered
- If this label was printed before a pop up message will appear and ask “Barcode has already been printed do you want to print again?” Click “yes”
- If more than one barcode was printed (e.g. 4), it will come up with this message for each barcode that has been printed.
- Last message will ask: “This will print 4 labels?” Click “yes”

## ***LABELLING OF SPECIMEN***

### **CRYOVIALS**

- BLOOD: WARTN No (printed barcode label), UMRN, Patients Initials and Sample Type.
- FROZEN TISSUE: WARTN No (printed barcode label), UMRN, Patient’s Initials and Sample Type.

NOTE: UMRN, Patient’s initials and Sample Type are written by permanent waterproof marker.

Patient’s initials - Christian name then surname abbreviation.

### **PARAFFIN BLOCKS (CASSETTES)**

- WARTN No, UMRN and Tissue Type on the cassette as well as ticket.

NOTE: All the details are written by 2B pencil or pacer pencil.

### **H&E SLIDES**

- WARTN No, UMRN and Tissue Type

## **PART 3: BLOOD PROCESSING**

### ***STERILE TECHNIQUE***

It is important to keep the working area sterile to avoid any contamination. All work must be carried out in a Biological Safety Cabinet Class II.

Before use, the Cabinet must be sterilized using ultraviolet radiation. Ensure that the cabinet is empty and clean. Place the steel shield in place then switch on the UV light and run for 20 minutes. Turn off the UV light and press “run” to turn on the HEPA filter and air curtain. Spray each item that is to be placed into the cabinet including blood tubes with 70% ethanol. Spray gloved hands each time you re-enter the cabinet. If gloves are contaminated with blood, discard and replace them.

When taking lids off tubes/bottles ensure that your hand does not pass over the opening, as air coming down from the filter will blow potential contaminants into the containers. Keep hands to the side and place all lids out of the way and on their tops, inside-up.

### ***LABORATORY EQUIPMENT TO USE IN SAFETY CABINET***

- Large test tube rack,
- Small test tube rack,
- Sterile cryovials (Number estimated from the quantity of blood),
- Automatic pipette, set at 50µl and filter pipette tips,
- Automatic pipette, set at 450µl and filter pipette tips,
- DMSO,
- Individually wrapped disposable pipettes,
- Biohazard bag placed in plastic bucket, set up, for waste,
- Black marker pen,
- Storage card and pen.
- Printed labels.

### ***PROCESSING***

#### **WHOLE BLOOD (EDTA)**

1. Dispense 50µl DMSO into a 1ml sterile cryovial. X 2  
(If patient has two 9ml EDTA tubes, do four cryovials).
2. Invert EDTA tube twice then add 450µl of blood to each cryovial.

3. Invert cryovial to mix the whole blood with the DMSO.  
NOTE: DMSO is cytotoxic at Room Temperature, therefore as soon as it is mixed with blood it should be placed in Mr. Frosty, which is filled up with 200 ml of isopropanol.
4. Place into “Mr. Frosty” rate limiting freezer. Transfer to  $-80^{\circ}\text{C}$  ASAP.  
Note: After minimum time in Mr. Frosty, Thaw Mr. Frosty for next use.

### PLASMA (LH/EDTA/ACD A)

1. Spin vacutainer (about 9ml) at 1500rpm for 15 min to separate plasma.  
NOTE: Ensure rotor is balanced.
2. After carefully wiping each tube with alcohol, remove about 3ml plasma.  
NOTE: Do not suck up white cells in buffy coat.
3. Aliquot 1 ml of plasma into labeled cryovials (3 aliquots)
4. Place into cryoshipper to snap freeze.
5. Transfer to  $-80^{\circ}\text{C}$  freezer.

### BUFFY COAT (LH/EDTA/ACD A)

1. Take buffy coat off with about 100 $\mu\text{l}$ s of plasma using a disposable sterile paster pipette.  
NOTE: Be careful not to lift red cells (if possible).
2. Aliquot as appropriate into labeled cryovials
3. Place in to cryoshipper to snap freeze.
4. Transfer to  $-80^{\circ}\text{C}$  freezer.

### SERUM

1. Spin blood at 2500 rpm for 10 minutes
2. Aliquot 1ml into labeled cryovials.
3. Place in to cryoshipper to snap freeze.
4. Transfer to  $-80^{\circ}\text{C}$  freezer.

## PROCESSING OF 2 BLOOD TUBES OR MORE

If more than one blood tube is received (E.G. EDTA, LiHep and Serum tube) processing is performed as follows:

1. Serum tube is placed in centrifuge for 10 min at 2500rpm. (DO NOT aliquot serum as soon as it is centrifuged).
2. While serum is spinning fume hood is set up for processing.
3. Whole blood is separated from EDTA tubes (i.e. 2 cryovials per tube). Tubes are labeled and placed in Mr. Frosty and -80°C freezer at WAIMR. “Change of iso-propanol” book is filled in.
4. EDTA and LiHep tubes are placed in centrifuge for 15 min at 1500 rpm.
5. From the quantity of blood received the number of aliquots (cryovials) can be estimated and labeled while EDTA and LiHep tubes are spinning.
6. Once EDTA and LiHep tubes are centrifuged, aliquot plasma, buffy coat and serum in the following order:

Plasma (EDTA)	-PE
Buffy Coat (EDTA)	-BCE
Plasma (LiHep)	-PLH
Buffy Coat (LiHep)	-BCLH
Serum	-S
7. Snap freeze plasma and serum.  
DO NOT snap freeze more than one case at the time. Keep cryovials within LN canister for at least 2 min.
8. Organize plasma and serum in their numerical order straight away within one of the preparation boxes. This will make storing away easier and reduce time of handling of specimens.

NOTE: Aliquots of WBE are recorded in ‘Change of isopropanol’ book because isopropanol should be changed after 5<sup>th</sup> time of its usage.

## **PART 4: TISSUE PROCESSING**

### ***FIXATION***

- 10% Neutral Buffered Formalin (10% NBF) is used for the fixation of tissue.
- Tissue is taken for processing within 24 hours.  
Note: Fixation time is dependent on tissue size. Small tissue pieces (10x10x3 mm) fixed in 10% NBF for 6-24 hours will generally show good cytological preservation and immunolocalization, with a minimum of antigen masking. (Farmilo and Stead, 2001).

### ***PROCESSING***

All tissue processing is done in the UWA Pathology lab, 1<sup>st</sup> floor M block.

In the processing room you will need:

Cutting board  
Forceps  
Scalpel blade  
White cassette  
Record card  
Ticket  
Pencil

1. Place piece of tissue per block.
2. Label cassette and ticket with a 2B pencil or pacer pencil.
  - WARTN No, UMRN and Tissue type.
3. Place the ticket inside cassette with tissue and place it into a 10% NBF container. Leave it on back bench and notify Slavica (Laboratory Technician).
4. Fill out card with details of what services you are using from the Pathology Dept.:
  - Who/dept...your name/WARTN...,
  - Dr...Nik Zeps...,
  - Date,
  - Number of blocks for processing and embedding.

NOTE: Processing may not be planned that night therefore ask Pathology staff.

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## ***EMBEDDING***

When processing is complete embedding is done the next morning.

The block needs to be embedded into a mould with wax for microtomy in the future.

1. Blocks are taken from the processor into embedding centre wax tray by pathology staff.
2. Choose the mould size appropriate to the tissue.
3. Sit the mould underneath the wax dispensing nozzle leave for a few seconds to warm up.
4. Dispense enough wax to fill the bottom of the mould.
5. Take out cassette and drain off wax for a few seconds
6. Place onto embedding platform where it is warm, break off lid (throw away) with forceps and take out tissue.
7. Place tissue into mould with warmed forceps, orientate it, hold it down and slide mould over to the small cold plate for quick setting.
8. Flatten tissue with forceps to make a leveled surface.
9. Place the cassette, with ticket inside, on top of mould with the number facing to the right, fill with wax.
10. Place onto  $-5^{\circ}\text{C}$  cold plate to set.
11. Once set scrap off excess wax from sides.
12. File block away (Where, needs to be decided) .

## **PART 5: CUTTING AND STAINING OF FROZEN TISSUE**

### Cutting

- The cryostat can only be used by a trained technical assistant.
- Depending on the clients needs, the standard thickness of sections cut is 4µm.

### Staining

- Haematoxylin and Eosin (H&E)

The differences in technique when staining unfixed frozen section are the following:

- Tendency to lift from the slide more easily during staining. To overcome this tendency adhesive slides are used.
- Eosin staining is depressed.
- Nuclear staining is enhanced.

### **1. Staining**

10% formalin	1-2	minutes
Water	10-20	seconds
Harris Haematoxylin	1	minute
Water	10-20	seconds
Acid Alcohol (1%)	1-2	seconds
Water	10-20	seconds
Scotts Tap water	10-20	seconds
Water	10-20	seconds
Eosin (1%)	30	seconds

Wash briefly, dehydrate, clear and mount.

### **Results**

Nuclei	blue
Background	shades of pink to red

## **PART 6: CUTTING AND STAINING OF FORMALIN FIXED TISSUE**

### Cutting

- The microtome can only be used by a trained technical assistant.
- Depending on the clients needs, the standard thickness of sections cut is 4µm.

### Staining

- Haematoxylin and Eosin (H&E)

H&E staining is staining of nuclei by oxidizing haematoxylin through mordant bonds of metals such as aluminum, followed by counterstaining by the xanthene dye eosin, which colours in varying shades the different fibres and cytoplasm.

### **2. Dewaxing**

Xylene	3 minutes with occasional agitation
Xylene	3 minutes with occasional agitation
Alcohol (100%)	10 dips
Alcohol (100%)	10 dips
Alcohol (95%)	10 dips
Alcohol (70%)	10 dips
Water	10 dips

### **3. Staining**

Harris Haematoxylin	3 minutes
Running water	1 minute with gentle agitation
Acid Alcohol (1%)	5 dips or 10 seconds
Running water	1 minute with gentle agitation
Scotts Tap water	1 minute
Running water	2 minutes with gentle agitation
Alcohol (70%)	10 dips
Alcohol (95%)	10 dips
Eosin (1%)	30 seconds

### **4. Dehydration**

Alcohol (100%)	10 dips
Alcohol (100%)	10 dips
Alcohol (100%)	10 dips
Alcohol (100%)	10 dips
Xylene	10 dips
Xylene	10 dips and leave

Slides are now ready to coverslip with DPX.

### **Results**

Keratohyalin, nuclei, cytoplasmic RNA, some calcium salts, urates, bacteria (weakly)	blue
Muscle, keratin, coarse elastic fibres, fibrin, fibrinoid	bright red
Collagen, reticulin, myelinated nerve fibres, amyloid	pink
Red blood cells	orange

## Part 7: DNA EXTRACTION

(Not done at this stage at WARTN)

## **PART 8: STORAGE**

### ***LOCATION***

The WARTN cryofacility is located in the basement of E block, accessible by stairs or lifts near the shopping precinct. The room comprises approximately 140m<sup>2</sup> of purpose built storage space with room for 28 freezer units.

Access to the room is by electronic swipe card.

An inventory of freezers is provided in the Part 9 with details of ownership, those responsible for their maintenance and their relevant contact details.

### ***BLOOD***

1. Whole Blood EDTA is stored in -150 °C Freezer.

- Allocate positions for separated WBE in the Freezer map/file (red file) and store WBE according to the allocations.
- Record WARTN No, UMRN, Patient's initials and sample type.
- Each study is stored separately.

2. Plasma EDTA, Plasma LiHep. and Buffy coat are stored in -80 ° C Freezer.

- Allocate positions in the Freezer map/file (blue file) and store plasma according to the allocations.
- Record WARTN No, UMRN, Patient's initials and sample type.
- Each study is stored separately.

### ***FROZEN TISSUE***

- Frozen Tissue is stored in -150°C Freezer.
- Allocate positions in the Freezer map/file and store the tissue according to the allocations.

### ***PARAFFIN BLOCKS***

- Paraffin blocks are stored in the plastic storage drawers, in a numerical order.
- Drawers are located in the basement

### ***H&E SLIDES***

- H&E slides are stored in the stainless steel drawers, in numerical order.
- Drawers are located in the basement.

## **PART 9: CRYOFACILITY**

### ***MAPS OF FREEZERS AND THEIR CONTENTS***

#### REVCO -80°C (DEVICE NO.14, DATALOGGER 2828)

Shelf 1	No Inventory
Shelf 2	No Inventory
Shelf 3	Boxes 1-80 WARTN- Plasma - Serum
Shelf 4	Boxes 81-160 WARTN- Plasma - Serum
Shelf 5	No Inventory

#### **WARTN Specimens (Boxes allocated for each study):**

**AOCS/Ovarian Collection: 1 – 36, 38, 40, 41, 43, 45, 47, 49, 50, 51, 52, 54, 57, 60, 63**

**IORT: 62, 64, 65,**

**RADAR: 37, 39, 42, 46, 48, 53, 56, 59, 61, 66**

**FES: 44, 55**

**CRC Toxicity: 8, 9, 10, 11, 12**

**COPD Dose Ranging: 58,**

**Radiation/Oncology collection: 67,**

SANYO -80°C (DEVICE 10, DATALOGGER 2827)

Shelf 1	HIMS- Health and Maine Study Paul Norman, Barry Iacopetta & Lyle Palmer Serum and DNA Full shelf
Shelf 2	Intermittent Androgen Blockade (IAB) Nigel Spry Boxes with Sera (30 Boxes full) Boxes 1-24 & 25-48 ½ full shelf
Shelf 3	LSMA- Lottery State Micro Facility Nigel Swanson DNA in 384 well plates = Total ~ 40 ½ full shelf
Shelf 4	No inventory

**REVCO -150°C (BIRDS EYE VIEW FREEZER MAP)**

TOP

<b>13</b> ↓ <b>18</b>		<b>115</b> ↓ <b>D1-D6 (7TM)</b>	
<b>19</b> ↓ <b>24</b>	<b>25</b> ↓ <b>30</b>	<b>109</b> ↓ <b>H9-H13 (7TM)</b>	<b>43</b> ↓ <b>H1-H6 (7TM)</b>
<b>55</b> ↓ <b>G1/G2/G3</b> <b>A8/G7/G8</b>	<b>31</b> ↓ <b>G12/G13</b>	<b>49</b> ↓ <b>H7/H8 (7TM)</b>	<b>37</b> ↓ <b>G9/G10/G11</b> <b>MD/Cells/?</b>

BOTTOM

<b>73</b> ↓ <b>78</b>	<b>91</b> ↓ <b>96</b>	<b>121</b> ↓ <b>D8-D13 (7TM)</b>	
<b>67</b> ↓ <b>72</b>	<b>85</b> ↓ <b>90</b>	<b>103</b> ↓ <b>108</b>	<b>7</b> ↓ <b>12</b>
<b>61</b> ↓ <b>66</b>	<b>79</b> ↓ <b>84</b>	<b>97</b> ↓ <b>102</b>	<b>1</b> ↓ <b>6</b>

**WARTN Specimens (Boxes allocated for each study):**

- AOCS (WBE): 8, 9, 11, 19, 22, 24, 26, 28, 30, 31,**
- AOCS (Tissue): 1, 2, 3, 4, 5, 6, 7, 10, 12, 13, 15, 16, 17, 18, 23, 27,**
- IORT (WBE): 33**
- IORT (Tissue): 14,**
- RADAR (WBE): 25, 29, 32,**
- Breast collection (Tissue): 34**
- CRC Toxicity: 8, 22**

## **FREEZER FAILURE AND BACK UP PLAN**

Refer to the manual for the boot up and shutdown for each freezer.

In event of breakdown contact Jon Braine, from Unimed Australia Pty Ltd, on 0407 99 56 35.

### **ALARMS**

Each freezer has two set points

“WARM” is the highest temperature cabinet may achieve before alarming  
“COLD” is the lowest temperature cabinet may achieve before alarming

In the event of freezer failure both the internal alarm and the external BMS alarm will be activated. The internal alarm is linked to the controller for liquid refrigerant and will activate this automatically at the warm set point.

Refer to operating manuals for controls of each freezer (also a flow chart is affixed to freezer door)

### **CO2 BACKUP**

Each freezer has its own CO2 backup regulator linked to a cylinder. Refer to manufacturers instructions on how to assemble, maintain and set these for each model.

Cylinders are Liquid Withdraw Food Grade g size CO aligal supplied by **Air Liquide (08) 9330 7422**

### **LIQUID N2 BACKUP**

This is provided by WAIMR and is a 240litre storage tank supplying the liquid nitrogen dewar. The level indicator is a yellow ring in a glass tube on the manifold of the tank that falls according to how full the tank is. **CHECK** the level each time you enter the facility and report any sudden drops in level to the WARTN Manager.

The tank is filled by Air Liquide at 6.30am every Friday. Contact is Gerry 0412940480

WA Research Tissue Network Cryofacility  
In the event of alarming

Freezer No	Type	Locked (y/n)	Key location	Backup	Owner	Principal Contact	Phone number	Second Contact	Phone number 2
1	Revco -80°C	Y	Keogh	No	Keogh Institute	Terry Burgess	2786	93328631 (home)	
2	Sanyo -80°C	N		No	NGL	S. Schwab	2711	0408944746	
3									
4									
5									
6									
7									
8	Sanyo -40°C	N		CO2	WARTN	Nik Zeps	0408 069 377	Sanela Bilic	0431 99 2546
9									
10	Sanyo -80°C	N		CO2	WARTN	Nik Zeps	0408 069 377	Sanela Bilic	0431 99 2546
11	Sanyo -80°C	Y	Path Centre	No	PATHCENTRE	Christine Chin	X 2363 A/H 93891794	Caroline Chapman	X 2363 A/H 0416350830
12	Sanyo -80°C Two Door	Y	Path Centre	No	PATHCENTRE	Christine Chin	X 2363 A/H 93891794		
13									
14	REVCO -80°C	N		CO2	WARTN	Nik Zeps	0408 069 377	Sanela Bilic	0431 99 2546
15									
16									
17									
18									
19	Revco -150°C	N		T-W XL 240L LN2	WARTN	Nik Zeps	0408 069 377	Sanela Bilic	0431 99 2546
20	Taylor- Wharton -170°C	Y	WAIMR	T-W XL 240L LN2	WAIMR	Kerin Eidne	0414 651 133	Sanela Bilic	0431 99 2546
21									
22									
23									
24									
25									
26									
27									
28									

## **PART10: EQUIPMENT SERVICES AND MAINTENANCE**

### ***FREEZERS***

Revco -80°C & Sanyo -80°C

Maintenance: Freezers are cleaned every 10 days approximately.

While cleaning, freezers should not be open for longer than one minute as temperature will increase.

100% ethanol can be used as it melts ice instantly. The floor around freezer should not be left wet after cleaning. (Use towels available from WAIMR cleaning room).

Service: Annual service performed

### ***CENTRIFUGE***

Maintenance:

Service: Annual service performed

### ***MICROTOME***

Maintenance:

## **PART 12: ORDERING**

### ***STATIONARY***

1. Stationary is chosen from the BOISE catalogue.
2. Fill in the “OTHER ITEMS FAX ORDER FORM”  
Refer to *Template 12*.
3. Fax the order form on 9277 9189.
4. Delivery docket is received with the items. Delivery docket has to be photocopied and sent to Fremantle Supply. Original copy is placed in “Account 2437” file – Part 2.

### ***LABORATORY EQUIPMENT AND REAGENTS***

1. Have a written quote of the items to be ordered.
2. Fill out Hospital Purchase Requisition form as follows:
  - ORDER COST CENTRE NBR: 2437
  - PAYING COST CENTRE NBR: 2437
  - SUGGESTED VENDOR: Name of the company from which items are ordered
  - VENDOR TEL AND FAX NBRS: are provided with the quote
  - ASSET NEW OR REPLACEMENT: Tick appropriate box
  - PURPOSE OF REQUISITION: Indicate the purpose
  - DATE GOODS REQ'D BY: ASAP
  - REQUESTOR: Dr Nik Zeps
  - EXT NBR: 3223
  - DEPARTMENT: Radiation/Oncology
  - AUTHORISED REQUISITIONING OFFICER: DR NIK ZEPS (Obtain signature)
  - HIGHER APPROVAL OFFICER: DR DAVID JOSEPH (Obtain signature)
3. To obtain Dr Joseph's signature give Purchase Requisition Form to Sheila Murphy-McGuire (Dr Joseph's Administration Assistant) at Radiation/Oncology.
4. Attach the quote and white copy of Purchase Requisition form and send to Fremantle Supply.

NOTE: All orders will go to Radiation Oncology unless specified.

Also, if something is urgent it needs to be indicated on the Purchase Requisition form.