Dept. of Gynaecological Oncology and Centre for Cancer Research,
Westmead Hospital & The Westmead Institute for Medical Research

Standard Operating Procedures
Version 4 - June 2016

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INTRODUCTION

The Gynaecological Oncology Biobank at Westmead was established to systematically collect high quality gynaecological biospecimens for research.

The main aim of the biobank has been to provide tissue to projects investigating the underlying molecular biology of gynaecological disease and to promote and facilitate collaboration between researchers to benefit gynaecological oncology research. In addition to frozen tissue specimens and a specimen of peripheral blood, comprehensive clinical data is collected so that molecular findings can be correlated with response to treatment and outcome. A key aim is to collect biospecimens in a way that maintains the flexibility required for the application of a range of current and emerging research technologies.

Guiding principles

- Patients are provided with information about sample collection, research and ethical implications and if they agree to participate, sign a Patient Information and Consent Form.

- Only tissue in excess to that required for pathological assessment and diagnosis is collected.

- Protection of privacy of participating patients is paramount. Only the Biobank Managers have access to identifying information.

- The biospecimens are only to be used in projects that have been approved by the Western Sydney Local Health District Human Research Ethics Committee (WSLHD HREC) and the HREC at the Institution where the work will be performed, if relevant.

- Applications by researchers for material and information must be scientifically sound and make efficient use of the resources available.

The Gynaecological Oncology Biobank at Westmead is a member of Australasian Biospecimen Network-Oncology, an NHMRC funded network of Australian Tissue Banks that collect biospecimens for cancer research.

PATIENT CONSENT PROTOCOL

The Gynaecological Oncology Biobank at Westmead complies with the NHMRC National Statement On Ethical Conduct In Human Research 2007 and the NSW Human Tissue Act 1983 and amendments 2006.

Tissue is collected under the project "Molecular Biology of Gynaecologic Disease", WSLHD ethics approval number HREC92/10/4.13.

Current versions of Patient Information Sheet and Consent Form are dated 28 July 2010. WSLHD HREC requires an annual report to be submitted every December for this project. See the Research Office for a proforma annual report document. All annual reports are filed in the "Molecular Biology of Gynaecologic Disease" ethics folder.

Consenting Procedure

All forms are in folder labelled "GYN ONC BIOBANK PROCEDURES"

The Gynaecological Oncology Unit secretary starts all new patient files. The secretary will place the Gynaecological Oncology Biobank at Westmead Consent Form in the file for the initial consultation.

When discussing consent with the patient please ensure she understands each point on the Patient Information and Consent Form, highlighting the following:

- Biospecimens will be used in research projects investigating the underlying molecular biology of gynaecological disease.
- Only tissue excess to diagnostic requirements will be used and all types of gynaecological biospecimens (normal, benign, malignant) are useful in research.
- Your biospecimens will be stored for use in different research projects. Your sample will only be used for research that is approved by a Human Research Ethics Committee.
- In addition, if you are willing we also request that you provide a blood sample.
- Following the collection of a sample and blood, no further effort is required on your part.
- Participation is entirely voluntary and you are free to withdraw your consent at any time.

If the patient agrees, they sign the Consent Form and a copy of the Patient Information Sheet and Consent Form is given to them. The patient's file, with the signed Consent Form, is returned to the Gyn Onc secretary. The patient file will be given to the Gyn Onc Data Manager who will then give the Biobank Officer (BBO) the patient consent forms, and inform the BBO when the patients are booked for surgery.

BBO will then start a patient file on the Gyn Onc Biobank Database (for this procedure see data entry page 24).

SPECIMEN COLLECTION & PRESERVATION PROTOCOL

On the day of surgery, the Gynaecological Oncology Biobank Officer (BBO) will attach a blood collection tube to the patient file for collection during surgery and call pathology to inform them of the name, DOB and MRN of consenting patients from which we are expecting to collect tissue. Blood (1 x 10 ml EDTA tube) will be collected before or during surgery.

Remember to treat all samples as potentially infectious.

Specimen collection procedure is as follows:

- Surgeon will page BBO when biospecimen is ready for collection.
- BBO will take liquid nitrogen canister, tumour collection tubes (50 ml Falcon tubes with small hole in the top) and Tissue Collection Form to theatre to collect tumour and bloods.
- Theatre nurse will record the patient name, sample description and time sample was left in the red "urgent specimens" book at theatre reception. BBO needs to sign, date and record the number of samples taken in the red book.
- Specimens will be taken to Anatomical Pathology by the BBO. On arrival at pathology, the BBO will inform the staff that there is a sample "for frozen section".
- Pathologist will identify suitable tissue and if possible, excise an appropriate amount for immediate freezing in liquid nitrogen. The BBO will record when they were paged, when the specimen was collected and when it was frozen on the Tissue Collection Form (Appendix 2). The BBO will complete all other relevant information as requested on the form.

The tissue samples need to be snap frozen as soon as possible to maximise RNA quality. Request can be made for an adjacent portion to be formalin fixed and embedded in paraffin. This is to be processed in a green cassette and when ready, will be stored in the brown cardboard box labelled "Gyn Onc" in the Anatomical Pathology Processing Area.

Specimen preservation procedure is as follows:

- On returning to the lab, record patient Medical Record Number (MRN), date of birth (DoB) and name in the Gyn Onc Biobank Book.
- Label a 15 ml cryovial or 2 ml cryovial depending on the size of the sample with the next available lab number eg 533A. Patient initials and DoB should be recorded on the container.
- Remove sample from the 50 ml falcon tube and transfers to appropriately labelled cryovial. Cryovial is stored in liquid nitrogen.
- Process blood as per protocol (see page 6).
- Record relevant patient information on Gyn Onc Database and store paperwork in patient file in Gyn Onc office.

If there are any problems or you require further information, please contact:

CATHERINE KENNEDY	9845 7376
YOKE-ENG CHIEW	9845 7306
BIOBANK STAFF	page *2 08952
ANNIE STENLAKE DATA MANAGER	9845 6849

BLOOD PROCESSING

Blood is to be collected from patients who have consented to participate in the study "Molecular Biology of Gynaecologic Disease".

Pre-operative blood sample collected from patient (record time of draw on tube) 1 x EDTA (10 ml)

Page *2 08952 for blood to be collected.

Ensure that the Gyn Onc Blood Collection Form is completely filled in. The form is kept in the folder "Gyn Onc Tissue Bank Procedures"

The following aliquots are required:

1. Blood spots (protein saver cards)
2. EDTA whole blood (4 x 2ml)

Post-operative blood sample collected from patient 1 x ACD tube (10 ml) – at time of medical oncology or gynaecological oncology clinic visits. BBO identifies consented patients from clinic list and supplies tubes to clinic nurse. If amenable, patient has tube filled by blood collectors in clinic and returns sample to nurse or reception. This is an ambient sample. Can be left to the following day to process. Clinic nurse will page BBO at end of clinic for bloods to be picked up.

The following aliquots are required:

1. Blood spots (protein saver cards)
2. ACD whole blood (4 x 2ml)

Disease progression blood sample collected from selected participants and processed for circulating DNA. BBO identifies consented patients from clinic list and supplies tubes to clinic nurse. If amenable, patient has tube filled by blood collectors in clinic and returns sample to nurse or reception. Clinic nurse will page BBO at end of clinic for bloods to be picked up.

Blood is processed and frozen as per the Qiagen circulating, cell free nucleic acid protocol.

The following aliquots are required:

1. Plasma (1ml)
2. Buffy Coat

1. Blood Spot

Blood spot (Guthrie) cards (Whatman 903 protein saver cards) are stored at room temperature. These cards are kept on the bottom left hand side of Cath's shelf, behind the glass door.

Always handle cards wearing gloves and do not touch the circles where blood is deposited.

1. Mix EDTA blood thoroughly by inverting tube several times.
2. Wipe top of vacutainer with ethanol before opening.
3. Using a sterile pipette entirely fill the circles on the card taking care not to saturate the paper (each circle will take approximately 40 µl).
4. Label card with the next sequential tissue bank number (eg 533) and the blood spot suffix (BS). Also attach label from database with TB reference number, date and type of specimen details.

5. Air dry thoroughly in safety cabinet before folding over cover. Drying time will vary according to ambient conditions but will take at least 30 min. Cards may be left overnight to dry.
6. File in numerical order and store in locked drawer at room temperature (RT).
7. Record details on blood tracking form for entry into database.

2. EDTA whole blood, plasma and buffy coat

When labelling cryovials it is important to use the following suffixes to enable the type of sample to be readily identified. First use the tissue bank number allocated and then the suffixes below:

EDTA whole blood	WB
EDTA plasma	PB
Buffy coat	BC

1. Transfer two 1.0 ml aliquots of whole blood into pre labelled cryovials (eg 533WB). **For ACD tubes aliquot four 1.0 ml aliquots.**
2. Centrifuge remainder of EDTA blood tube at 3000 g for 20 min at 4˚C.
3. Print labels for tubes from database with TB ref number, date and sample type.
4. Collect 0.5–1.0ml aliquots of plasma into pre labelled cryovials (eg 533PB). Take care not to disturb white cell layer. Record plasma volumes on blood tracking form.
5. Using a sterile pipette tip, remove the layer of white blood cells and platelets sitting on top of the red blood cells, together with a small amount of residual plasma. Take care not to lift red cells.
6. If it is difficult to cleanly separate this buffy coat layer, transfer to a small Eppendorf tube/micro centrifuge tube and centrifuge at 10,000 rpm for 2 min.
7. Place aliquots into pre labelled (eg 533BC) cryovials (approx 0.5 ml each).
8. Record details on blood tracking form.
9. Snap freeze vials by submersion in liquid nitrogen then transfer whole blood & plasma to storage box in -80°C freezer and buffy coat to liquid nitrogen or vapour phase tank. Record locations on tracking form.
10. The specimen vials should be split into 2 different storage boxes - eg if there are 4 vials containing plasma aliquots, 2 should be stored in one box and 2 in a different box. This is to aid the transfer of duplicate specimens to another collection centre for long term storage, ensuring back up in case of local freezer breakdown.

Discard all residual blood tubes in contaminated waste.

Enter all specimen details into database. The "Pathology" layout has a blood collection field to select a yes/no answer. However a more detailed layout is under "Blood" in the database. This allows for the recording of aliquot numbers and storage location. Fill in both layouts.

ASCITES SEPARATION PROTOCOL

- 1 smear of unpurified ascites – to asses tumour cell content
- Cell pellet fixed in 10% formalin for cytoblocks preparation – for IHC
- 1 ml aliquot of snap frozen concentrated unpurified ascites for DNA/RNA/protein extraction
- Aliquots of concentrated unpurified ascites with 10% DMSO – for cell culture

Method

Ascites is collected from the ward in sterile catheter-bags containing 5000U unfractionated heparin and signed consent forms collected. Record volume and appearance of ascites on tracking log.

Aliquot 4x 250μl of the crude cell suspension into Nunc tubes and snap freeze
Use 100ul of the crude cell suspension and make a smear on a microscope slide. Air dry and H&E stain the following day.

Transfer fluid to 4 x 250 ml centrifuge flasks and spin down at 400 g for 5 min at RT.
The supernatant is discarded and the soft pellet is resuspended in an equal volume of complete medium (10N). This is divided into 2 aliquots and processed as follows:

A.
DMSO is added to one of the resuspended pellet to a final concentration of 10% (i.e to 9 mls of ascites pellet add 1 ml of sterile DMSO). 1 ml aliquots of ascites/DMSO are transferred to Nunc tubes and stored at –80°C overnight, and then transferred to liquid nitrogen the following day.

B.
10% Formalin is added to the other resusupended pellet (approx 4X volume of pellet, up to 50 ml) and then transferred to a 50 ml falcon tube.
Store overnight at 4°C and then pellet at 1500rpm for 10 min. Discard supernatant and process as for paraffin embedded cytoblock preparation. (See Gyn Onc Methods folder for this protocol).

Note:
Cytospins are air-dried and then stored at 4°C overnight and Giemsa stained the following day. For other staining protocols alternative fixatives may be required.

STORAGE OF BIOSPECIMENS

Tissue samples are initially stored in the liquid nitrogen tanks in the Gyn Onc lab.

Samples are periodically moved to Westmead Millennium Institute Cryostorage facility on Level 1.

On the day of the move collect dry ice pellets from the Clinical Sciences wash up room and place into 2 eskies. The boxes for the isothermal are available from Ces Nast in the WMI Store room. Label the box with the next available isothermal shelf number eg 12L. Take the samples that are to be moved and place them in the new boxes on dry ice. Check the lab number eg 533 off against the location that the sample has come from eg 3E5 and write the new location in the lab book eg 12L. It is useful if there are 2 people performing this as it is quicker and allows someone to double check the storage location.

Move the samples as quickly as possible to the WMI facility. The key for the isothermal is kept in tissue culture in the co-ordinators top drawer.

Once the samples are in their new location, update the storage details in the Gyn Onc database.

Blood samples are stored in the box labelled "Gyn Onc Blood" on the 3rd shelf of the -80˚C freezer in the Clinical Sciences Corridor. The key to the freezer is in the top drawer of Catherine E's bench.

Buffy Coat is stored in liquid nitrogen.

Guthrie cards are stored in a box labelled "Blood Spot Cards" on Catherine K's bench in the Gyn Onc lab.

MAINTENANCE OF TISSUE BANK

Liquid nitrogen is filled every Tuesday by BOC, and every Friday afternoon each container is topped up by the BBO with approx 10 litres of liquid nitrogen from the dewars stored in the lab. After topping up, use the ruler on the bench behind the tanks to measure the level. It should be over 40 cm. If not, more liquid nitrogen is available from the Fertility Centre, room 2178. Ensure that the lids of the containers are pushed down firmly.

If the alarm goes off, check cause of alarm. If low level light is flashing, top up liquid nitrogen. The only other reason that the light will flash is if the power source to the alarm is interrupted.

CRYOSECTIONING OF FROZEN TISSUE

Materials:

- Super frost slides
- OMI #10 Disposable safety scalpel
- Forceps
- Paint brush
- Nunc vials
- Slide rack
- Kimwipes
- S35 Microtome blade
- OCT compound (Tissue-Tek)
- Hammer
- Safety glasses
- Canister of liquid nitrogen with tumours
- Microm HM505E Cryostat (Rm 3031, Level 3, WMI)

Procedure:

* Microtome training with WMI Histology is compulsory. *

1. Reset temperature of Microtome to -30°C (normally its on at -20°C)
2. Spray paper towel/tissue with 70% ethanol & clean the stage of microtome & surrounding areas (do not spray ethanol directly)
3. Ethanol clean the forceps & paint brush. Place them in the -30C microtome.
4. Label 4 nunc vials & slides with:
    - tumour number
    - date
    - the corresponding number 1,2,3,4
5. Wrap labelled nunc vials in aluminium foil & place them in the Liquid nitrogen canister to freeze.
6. Place labelled slides on top of glass door of Microtome.
7. When ready to cut, place a kimwipe underneath the cutter to collect unwanted tissue shavings (makes cleaning after a lot easier).
8. Carefully insert the blade using forceps, secure the blade & flip back the metal piece to cover the blade.
9. Squeeze some OCT compound into the round mounting piece & place the tissue on it.
10. Leave the mounted tissue in the -30C microtome to set.
11. Insert the mould into the holder & when ready to cut, flip open the metal piece that covers the blade.
12. Cut 10 um section on to the slide for H&E – Slide 1.
13. Cut subsequent 4 X 100 um sections into the labelled Nunc vial 1. Place into liquid nitrogen canister.
14. Cut another 10 um section on to the slide for H&E – Slide 2.
15. Cut subsequent 4 X 100 um sections into the labelled Nunc vial 2. Place into liquid nitrogen canister.
16. Repeat H&E & cryosections for Slide 3, 4 & Vial 3,4 respectively.
17. Store cryosections in liquid nitrogen tank.

H&E staining of frozen sections

1. 10-15s in Harris haemotoxylin
2. Rinse in water
3. 10s in Scots Bluing solution (3.5g sodium bicarbonate, 20 g magnesium sulphate in 1 L water)
4. Rinse in water
5. 10-15s in Eosin/Erythrosin B solution(ICPMR) # AEYWEST – specially prepared for ICPMR from Australian Biostain.
6. 2 x 10s Abs EtOH to wash & dehydrate
7. Dry in fumehood
8. 20s Histolene in fume hood
9. Mount.

RNA extraction from cryosections

Materials:

- Absolute RNA microprep kit (Stratagene #40085)
- Canister of liquid nitrogen with cryosection vials
- Disposable scalpel (Feather Safety razor Co Ltd)
- Hand held mini homogeniser
- Plastic pestles (treated with RNase-out soln, cleaned & autoclaved)
- Heating block at 60C with an eppendorf of Elution buffer
- 100% & 70% AR grade Ethanol
- 1.5 ml eppendorf tubes

Preparing reagents from the kit:

- Add 290 ul DNase buffer to the lyophilised RNase-free DNase. Swirl to mix but try not to introduce bubbles. Store at -20°C
- Add 16 ml 100% ethanol to High Salt wash buffer bottle (HSB).
- Add 68 ml 100% ethanol to Low salt buffer bottle (LSB)
- Label date on bottles after addition.
- Store at RT

In the fume hood:

- Prepare fresh mixture of Lysis buffer & B-ME before use: 1.4 ul B-ME /200 ul
- Add 200 ul Lysis buffer-BME to the eppendorf tubes. Cap the lids before removing from fume hood.

On the bench:

1. Remove disposable scalpel from packaging & place them in the liquid nitrogen canister.
2. Insert a plastic pestle into the Hand held mini homogeniser.
3. Remove a cryosection from the vial using a frozen scalpel as quickly as possible & drop it into the eppendorf containing 200 ul of lysis-BME buffer.
4. Homogenized it for a couple of seconds.
5. Add equal vol of 70% ethanol. Vortex 5s.
6. Place RNA binding spin cup into 2 ml eppendorfs.
7. Spin 14,000 rpm, 1 min.
8. Remove & retain spin cup but discard filtrate.
9. Replace spin cup into eppendorf & add 600 ul LSN.
10. Spin 14,000 rpm, 1 min.
11. Discard filtrate & respin 14,000 rpm X 2 min.

12. Prepare DNase solution – gently mix 5 ul DNase + 25 ul Digest buffer.
13. Add DNase solution directly into the fiber matrix inside spin cup. Incubate at 37°C, 15 min.
14. Add 500 ul HSB into spin cup.
15. Spin 14,000 rpm, 1 min.
16. Discard filtrate & add 600 ul LSB into spin cup.
17. Spin 14,000 rpm, 1 min.
18. Discard filtrate & add 300 ul LSB into spin cup.
19. Spin 14,000 rpm, 2 min.
20. Transfer spin cup into 1.5 ml eppendorfs.
21. Add 30 ul Elution buffer (prewarmed to 60°C) directly into the fiber matrix inside spin cup.
22. Incubate RT, 2 mins.
23. Spin 14,000 rpm, 1 min.
24. Quantitate RNA using Quantagene Pro.
25. Run samples on Agilent 2100 Bioanalyser to determine RNA quality.

RNA analysis on Agilent Bioanalyser

Book a time with by Gandhi Kaushal.
Email him at kaushal_gandhi@wmi.usyd.edu.au

Fill the Agilent BioAnalyser usage form & email this to Kaushal

Sample preparation:
* avoid using powdered gloves, ART tips & arturus amplication kit elution buffer as they interfere with process. *

- Samples must be 25-500 ng/ul & dissolved in 2 ul sterile water (water for irrigation) or low salt/EDTA buffer in 0.5 ml PCR tubes
- Take samples in esky of ice to Kaushal (Rm 3033, level 2 WMI)
- Results will be emailed following completion of run (usually within 2 hrs)

DNA extraction from cryosections

Materials:

- DNeasy Tissue kit (Qiagen #69504)
- 100% ethanol
- Waterbath at 55°C
- Heating block at 70˚C

Preparing reagents from the kit:

- Before using for the first time, add the appropriate amounts of ethanol (96-100%) to buffers AW1 and AW2 as indicated on the bottles.

On the bench:

1. Pipette 180 ul Buffer ATL into eppendorf tubes.
2. Remove a cryosection from storage.
3. Add 20 ul PK, vortex 5-10s, & incubate at 55˚C until tissue is completely lysed. Vortex occasionally during incubation to disperse the sample. Lysis is usually 1-3 hr but over night is convenient. (will not affect them adversely)
4. Vortex for 15s. Add 200 ul Buffer AL & **immediately** vortex 5-10s. Incubate at 70°C, 10 min.
5. Add 200 ul 100% ethanol to the sample & mix thoroughly to yield homogenous solution.
6. Pipette the mixture to DNeasy spin column placed in a 2 ml collection tube provided.
7. Spin 8000 rpm, 1 min. Discard flow-through & collection tube.
8. Place the spin column in a new collection tube, add 500 ul Buffer AW1.
9. Spin 8000 rpm, 1 min. Discard flow-through & collection tube.
10. Place the spin column in another collection tube, add 500 ul Buffer AW2.
11. Spin 14000 rpm, 3 min. Discard flow-through & collection tube (ensure that no carry over ethanol by carefully removing column without contact with flow-through)
12. Place spin column in a clean 1.5 ml eppendorf & pipette 100 ul Buffer AE onto the DNeasy membrane.
13. Incubate RT, 1 min.
14. Spin 8000 rpm, 1 min to eluate.
15. Repeat elution with a new eppendorf tube to prevent dilution of first eluate.
16. Quantitate DNA using Qubit flourometer.

DNA extraction from EDTA/ heparin/ citrate blood samples

Materials:

- FlexiGene DNA kit (Qiagen #51204)
- 100% Ethanol
- Waterbath at 37˚C, then 65˚C.
- Heating block at 65˚C

Preparing reagents from the kit:

- Resuspend the lyophilised Protease with 0.3 ml Buffer FG3
- Store at 4˚C

Prepare Buffer FG2/Qiagen Protease mixture (Buffer FG2P) no more than 1 hr before use:

- For every 4 ml blood, need 2 ml Buffer FG2 & 20 ul Protease.
- Hence for 8 X 4 ml blood, need 16 ml Buffer FG2 & 160 ul Protease.

1. Thaw frozen blood tubes quickly in 37˚C waterbath & store on ice. (change temp of bath to 65˚C for later use)
2. Pipette 10 ml Buffer FG1 into a 50 ml centrifuge tube. Can do 8 at a time.
3. In fumehood: Add 4 ml blood & mix by inverting the tubes 5X.
4. Spin tubes at 2000g, 5 min on swing out rotor in 2 lots of 4
5. Discard supernatant carefully as the pellet may be loose. Invert tube onto daylee towel to drain, taking care the pellet remains in the tube.
6. Add 2 ml Buffer FG2P, close the tube & vortex immediately until pellet is completely homogenized. **Important** to vortex each tube immediately after addition of buffer.

7. Invert tube 3x, incubate in 65˚C waterbath, 10 min (colour changes from red to olive green, indicating protein digestion)
8. Add 2 ml isopropanol & thoroughly mix by inversion until DNA precipitant becomes visible as threads or clump.
9. Centrifuge for 3 min at 2,000g.
10. Discard supernatant carefully as the pellet may be loose. Invert tube onto daylee towel to drain, taking care the pellet remains in the tube.
11. Add 2 ml 70% Ethanol & vortex for 5 seconds.
12. Centrifuge for 3 min at 2,000g.
13. Discard supernatant carefully as the pellet may be loose. Invert tube onto daylee towel to drain, taking care the pellet remains in the tube.
14. Air dry the DNA pellet for 5 min.
15. Add 100 ul Buffer FG3, vortex for 5 seconds at low speed & dissolve the DNA by incubating for 1 hr a 65˚C in the heating block.
16. Quantitate DNA using Qubit flourometer.

DNA Quantitation

Quant-iT™dsDNA HS (10 pg/ul – 1000 ng/ul) Assay

Critical Assay Parameters

Assay Temperature

The Quant-iT™ dsDNA HS assay for the Qubit™ fluorometer delivers optimal performance when all solutions are at RT (22–28˚C). The Quant-iT™ assays were designed to be performed at RT, as temperature fluctuations can influence the accuracy of the assay. To minimize temperature fluctuations, store the Quant-iT™dsDNA HS reagent and the Quant-iT™ dsDNA HS buffer at room temperature and insert all assay tubes into the Qubit™ fluorometer only for as much time as it takes for the instrument to measure the fluorescence, as the Qubit™ fluorometer can raise the temperature of the assay solution significantly, even over a period of a few minutes. Do not hold the assay tubes in your hand before reading, as this will warm the solution and result in a low reading.

Incubation Time

In order to allow the Quant-iT™ assay to reach maximum fluorescence, incubate the tubes for the DNA and RNA assays for 2 minutes after mixing the sample or standard with the working solution. After this incubation period, the fluorescence signal is stable for 3 hours at RT.

Photobleaching of the Quant-iT™ Reagent

The Quant-iT™ reagents exhibit high photostability in the Qubit™ fluorometer, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings. It is important to remember, however, that if the assay tube remains in the Qubit™ fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature. (The temperature inside the Qubit™ fluorometer may be as much as 3˚C above room temperature after 1 hour.) For this reason, if you want to perform multiple readings of a single tube, you should remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

Experimental Protocol

1. Set up the number of 0.5 mL tubes you will need for standards and samples. The Quant-iT™ dsDNA HS assay requires 2 standards (Std 1 & 2)
   Note: Use only thin-wall, clear 0.5 mL PCR tubes. Acceptable tubes include Qubit™ assay tubes (500 tubes, Invitrogen Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part number 10011-830).

2. Label the tube lids.

3. Make the Quant-iT™ working solution by diluting the Quant-iT™ dsDNA HS reagent 1:200 in Quant-iT™ dsDNA BR buffer. Do not mix the working solution in a glass container.

   Note: The final volume in each assay tube must be 200 µL. Each standard tube will require 190 µL of Quant-iT™ working solution, and each sample tube will require anywhere from 180 µL to 199 µL. Prepare sufficient Quant-iT™ working solution to accommodate all standards and samples. For example, for 8 samples, prepare enough working solution for the samples and 2 standards: ~200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Quant-iT™ reagent plus 1,990 µL of Quant-iT™ buffer).

4. Load 190 µL of Quant-iT™ working solution into Std 1 & Std 2 tubes.

5. Pipette 10 µL of Quant-iT™ standard 1 & 2 into the appropriate tube and mix by vortexing 2–3 seconds, being careful not to create bubbles.

6. Load Quant-iT™ working solution into individual assay tubes so that the final volume in each tube after adding sample is 200 µL.
   Note: Your sample can be anywhere between 1 µL and 20 µL, therefore, load each assay tube with a volume of Quant-iT™ working solution anywhere between 180 µL and 199 µL.

7. Add each of your samples to assay tubes containing the correct volume of Quant-iT™ working solution (prepared in step 6) and mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µL.

8. Allow all tubes to incubate at room temperature for 2 minutes.

9. Calibrate the standards & measure concentrations of assay tubes on the Qubit™ fluorometer.

10. Record the reading given by the Qubit™ fluorometer.

PATHOLOGY REVIEW PROCEDURE

AIMS

1. To review histopathology and confirm that the frozen specimen held in the Gynaecological Oncology Biobank at Westmead contains tissue of the same type and histology as the primary tumour described in the diagnostic pathology report.

2. To determine the quantity and quality of tumour tissue within the specimen held in the Gynaecological Oncology Biobank at Westmead.

3. To re-grade the case using a consistent, pre-defined grading system on diagnostic H&E slides using the Silverberg grading system.

4. To check for and record any interesting co-existing pathology.

5. To select a representative block that may be cored (1.0 mm diameter) for tissue microarray (TMA) construction and to mark 3 selected areas on the corresponding H&E.

FLOW CHART FOR PATH REGRADING, CRYOSECTION COMPARISON AND TISSUE MICROARRAY CORE SELECTION

1. Relevant H&E cryosection from biobank specimen will be selected by the Gynaecological Oncology Biobank at Westmead team.

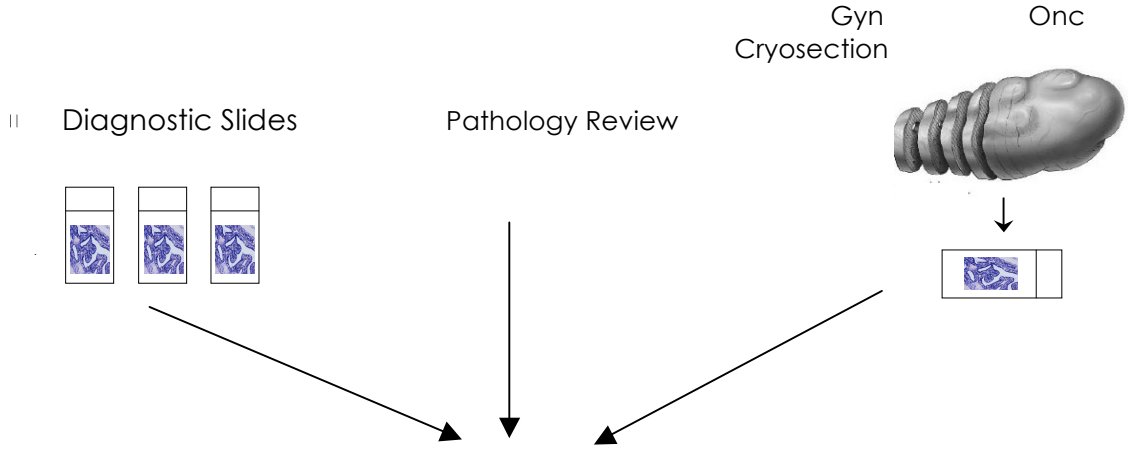
2. Tissue from the Gynaecological Oncology Biobank at Westmead will be matched to specimen code on pathology report. 3x H&E's will be selected from blocks/slides held in Anatomical Pathology (eg A1-A3).

3. The Gynaecological Oncology Biobank at Westmead Path Review Form, along with diagnostic H&Es selected above, a copy of the pathology report and a H&E of the cryosection, will be given to the pathologist.

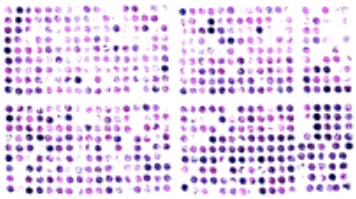
    - **Diagnostic slides will be used to:**
        1. Review and re-grade the case using the Silverberg criteria.

        2. Pathologist will then select the most suitable section/block that concurs with the pathology report for tissue microarray (TMA). Pathologist then circles two appropriate areas on the slide corresponding to the block to be cored (0.6 mm diameter) for TMA construction.

    - **Cryosection** from frozen tissue specimen will be reviewed for tissue content and concordance with diagnostic Pathology Report



- Path Review
- Regrading
- Tissue Microarray
- QC of tissue bank specimen



TISSUE ARRAY PROTOCOL

On completion of the pathology review process a mud map is made from each slide.

The selected blocks that correspond to the chosen slides are collected from Anatomical Pathology. When blocks or slides are collected from pathology ensure that you record this in the black research book in the administrative area. Record the Westmead Hospital Accession number eg SW-08012345, the patients name, amount taken, your name, department and extension number.

The block and selected slide are compared and the area on the block that corresponds to the area chosen by the pathologist during the path review is circled.

The blocks that have been chosen are then ready for use in a tissue microarray.

PATHOLOGY REVIEW

GRADING OF OVARIAN CARCINOMA (Rosemary Balleine)

Ref: Silverberg S G, Histopathologic grading of ovarian carcinoma: a review and proposal International Journal of Gynaecological Pathology (2000) 19: 7-15

ARCHITECTURE

- Decide on the histological pattern displayed by the majority of the fields of a given tumour

1. Predominantly glandular (note: must be true round glands of uniform or variable size)
2. Papillary (includes slit like spaces)
3. Solid

NUCLEAR GRADE

- Chose area with greatest pleomorphism in half a low power field

1. Regular uniform vesicular nuclei
   Variation in diameter no more than 2:1
   Low n:c
   No chromatin clumping or prominent nucleoli

2. Variation in size from 2:1 to 4:1
   Intermediate variation in shape
   Nucleoli recognisable but small
   Some chromatin clumping
   No bizarre cells

3. Marked variation in nuclear size (>4:1) and shape
   High n:c
   Prominent chromatin clumping
   Thick nuclear membrane
   Large eosinophilic nucleoli
   Bizarre cells often present

MITOTIC ACTIVITY

Assess most active area (usually the periphery)
Assess 30 high power fields and nominate highest score per 10 hpf.

Nikon Optiphot microscope with field diameter of 0.663 and field area of 0.345 mm^2

1. <9
2. 10-24
3. >25

Rosemary Balleine microscope details: Olympus BX51 field diameter of 0.545, area 0.233 mm^2
Raghwa Sharma microscope details: Olympus BX40, field diameter of 0.545, area 0.233 mm^2

1. <6
2. 7-16
3. >17

FINAL GRADE

Total score 3 to 5 = grade 1
6 to 7 = grade 2
8 or 9 = grade 3

TISSUE BANK OVARIAN PATHOLOGY SLIDE REVIEW

PATHOLOGIST _____ **DATE** _____

1. ANATOMICAL PATHOLOGY DIAGNOSTIC H&E SLIDES

BLOCK IDs _____ _____ _____

SLIDE IDs _____ _____ _____

PATIENT INITIALS _____ **MICROSCOPE USED** _____

TUMOUR TYPE ☐ Benign ☐ LMP ☐ Malignant

HISTOLOGY ☐ Serous ☐ Mucinous ☐ Endometrioid ☐ Clear Cell

☐ Mixed (specify)_____

☐ Other (specify**)** _____

GRADE (please refer to grading notes)	**1**	**2**	**3**
ARCHITECTURAL PATTERN	☐ Glandular	☐ Papillary	☐ Solid
NUCLEAR ANAPLASIA	☐ Mild	☐ Moderate	☐ Severe
MITOTIC COUNTS	☐ ≤6	☐ 7-16	☐ ≥17
	1 (3-5)	2 (6-7)	3 (8-9)
OVERALL GRADE	☐	☐	☐

COMMENT (necrosis, vessels, other?) _____

CO-EXISTING OVARIAN PATHOLOGY IN SECTION (eg adjacent LMP, cystadenoma, endometriosis)

Please specify (including subtype if applicable**)**_____

Does the section appear representative of the lesion as described in the pathology report ?
___**YES**___**NO**

COMMENT

_____

2. GYN ONC TISSUE BANK H&E CRYOSECTION

SLIDE ID _____ Tissue Bank Specimen Received _____

Does cryosection concur with the path report and above slide? ___YES___NO

TUMOUR COMPONENT

☐ 0 – 25% ☐ 25 – 50% ☐ 50 – 75% ☐ 75 – 100%

NORMAL TISSUE COMPONENT

TYPE(S) eg Fallopian tube _____

☐ 0 – 25% ☐ 25 – 50% ☐ 50 – 75% ☐ 75 – 100%

PROPORTION NECROTIC

☐ 0 – 25% ☐ 25 – 50% ☐ 50 – 75% ☐ 75 – 100%

COMMENT

_____

SELECTION OF BLOCK FOR TISSUE ARRAY CORING

SLIDE ID _____ **PATIENT INITIALS** _____

BLOCK ID _____

Please select two areas for tissue array coring that are representative of the main pathology. Please circle selected areas with pen.

Areas selected **YES** _____ **NO** _____

Biobank Officer will photocopy slide and keep a hard copy in the tissue bank file.

COMMENTS _____

_____

PATHOLOGY REVIEW TRACKING LOG

PATIENT INITIALS _____ GYN ONC TUMOUR ID _____

GYN ONC TISSUE BANK H&E CRYOSECTION SLIDE ID _____

PATHOLOGY REPORT COPIED DATE _____ PATH ID _____

POTENTIAL BLOCKS FOR SLIDE SELECTION FROM PATH REPORT

_____

ID OF 3 BLOCKS/SLIDES SELECTED FOR PATH REVIEW

_____

CORRESPONDING H&E SECTIONS OBTAINED FROM ANATOMICAL PATHOLOGY

SLIDE NUMBER	DATE OBTAINED	DATE RETURNED

- SLIDES, PATH REPORT & REVIEW FORM DELIVERED TO PATHOLOGIST

  PATHOLOGIST NAME _____ DATE _____

- SLIDES, PATH REPORT & REVIEW FORM RETURNED DATE _____

- INFORMATION FROM REGRADING ENTERED INTO DATABASE DATE _____

- MUD MAP MADE OF CHOSEN SECTION/SLIDE DATE _____

- BLOCK FOR TMA OBTAINED FROM ANATOMICAL PATHOLOGY

  BLOCK ID _____ DATE TAKEN _____ DATE RETURNED _____

- BLOCK SENT FOR ARRAYING DATE SENT _____ DATE RETURNED _____

DATABASE, DATA ENTRY AND BACK-UP PROCEDURES

It is critical to the operation of the tissue bank that the database is up to date. As soon as any patient information is received it should be entered into the database. The greater the collection of information for a particular sample, the more valuable it is.

The database is password protected and only accessible by the BBO.

DATA ENTRY

Consent
When a patient consent form is collected, a patient file is started by opening the database, selecting the "Pathology" Layout, then selecting "New Record" from the "Record" window.
The minimum information that needs to be recorded is:

- Name
- Date of Birth
- Medical Record Number (MRN)
- Select the "Yes" tick box for the Consent field

When this is done, place the consent form in a plastic sleeve and print out a label using the "Label" layout. The form is then placed in the "signed consent forms" folder ready for the additional information that will follow when the patient has surgery.

Sample Collection
When a sample has been collected (eg tissue, blood, ascites) ensure that the appropriate form has been filled in (see Appendix 2). On return to the lab record the following in the Red Gyn Onc Biobank Book in room 2204:

- Next available Tissue Bank Number (eg 533)
- Name
- Date of Birth
- MRN
- Anatomical site of sample (eg right ovary)
- Sample code (eg 533A)
- Storage location
- Date of surgery and surgeon

If a sample from more than one anatomical site is collected from a single patient then additional letters need to be used, eg 533A ovary, 533B omentum. Blood is recorded using the codes in the Blood Processing protocol on pages 6 & 7.

Using the information in the Red Biobank book and the white "Tissue Collection Form" enter the following information into the "Pathology" Layout in the database:

- Tumour Number
- Tumour Availability eg "Fresh frozen tissue"
- Primary site
- Collection Date
- Age at surgery
- Surgeon

Then go to the "tumour location" layout and fill in the storage location and the time the specimen was collected and frozen.

If Blood was collected, tick "Yes" in the Blood field in the Pathology Layout, then enter specific information such as volume and amount of tubes frozen in the "Blood" layout.

Take the patient's plastic sleeve from the "signed consent forms" folder, add the white "Tissue Collection Form and move it to the "files awaiting path reports" folder.

Tumour Board
Every Wednesday the Gynaecological Oncology Multidisciplinary Team Tumour Board meeting is held in the Anatomical Pathology Department from 8.30 till 10 am.
At the meeting, notes are taken relevant to patients from whom samples have been obtained, in particular, primary site, FIGO stage, largest diameter of residual disease, grade and co-existing pathology.

On return from the meeting enter the information into the "Pathology" Layout in the database:
Tick "yes" in the "tumour board report" field
Primary Site
Stage (eg 3C)
Grade
Any other relevant information (such as previous cancer or synchronous tumour) in the "Comments" field.

Then go to the "Clinical Review" layout and fill in the "Residual Disease" field.

Patients that have benign disease will not usually be discussed at tumour board.

Pathology Report
The pathology report is available on the Cerner database about a week after the patient's surgery. The Biobank staff have access to this database. Alternatively, the pathology report can be photocopied from the patients Gyn Onc file located in the secretary's office. Also copy and file the Operation Report. There is a field in the "Pathology" layout to record this.

Information needed from the Pathology report:
Tumour Type (eg serous adenocarcinoma)
Grade
Silverberg grade (if applicable)
Coded Nomenclature (ICD-O code)

The pathology report may also contain immunohistochemical staining results. These are to be recorded in the "Tumour markers/Immuno" layout. Occasionally, the report will mention that an additional report will be issued (eg if mismatch repair staining is requested) ensure that this is copied.

BACK-UP PROCEDURE

Every day the server automatically creates a back up file of all the Gyn Onc Filemaker Pro databases. The last 7 days of back up files may be accessed through the server if there is a problem with the current file.

Every Friday the server is backed up using an external hard drive. Two copies are kept in separate areas of the hospital. One is in room 2218 and the other in the SW-TCRC offices in the Crown Princess Mary Cancer Care Centre Administrative Area.

Back up procedure:
Take the external hard drive from the top drawer of the filing cabinet in room 2218. Attach hard drive to the server using the USB cable.
Copy the server onto the back up hard drive.
When complete, label the hard drive box with the date of back up and take the hard drive to SW-TCRC office, where it is kept in a locked filing cabinet (currently it is kept in the SW-TCRC Manager's filing cabinet in CPMCC, Clinical Support 1).

Then take the hard drive that is in Pam's filing cabinet (this will have the server back up from the previous week) and take it to room 2218 and place it in the filing cabinet ready to be backed up the following week.

Using this protocol, there are always 2 back up copies of the server in 2 separate areas of the hospital.

APPENDIX 1

Clinical Data Collection Form A

Clinical Data Collection Form B

Treatment Form

CA125 Form

Gynaecological Oncology biobank at Westmead

FORM A - Clinical Data

Primary Ovary and Peritoneal Cases

MRN | **Initials** | **Date of Birth** *(dd/mm/yy)* | **GynBiobank Sample ID**

Site Name | **Form Completed by** | **Date Form Completed** *dd/mm/yy*

Information obtained from (tick all that apply):

Central Medical Records ☐ | Gyn Onc Records ☐ | Gyn Onc Database (CRS) ☐

Medical Oncology Records ☐ | Medical Oncology Database ☐ | PIMS ☐

Other ☐ Specify: _____

GENERAL HISTORY

Height _____ **Weight** _____ **kg** *(at diagnosis)*

Date height/weight taken *dd/mm/yy* .....

Age at Menarche .....

Nunber of Pregnancies *(gravida)* .....

Number of Births *(parity)* .....

Age at Menopause

Contraception Use

1=Oral
2=Tubal Ligation
3=IUD
4=Barrier
5=None
6=Other

Any other details *(ie length of use, brand name etc)* .....

Hormone Replacement Therapy

1=prior use
2=current use
3=never used

Any other details *(ie length of use, brand name etc)* .....

CANCER HISTORY

Histopathological diagnosis .....
Please attach copy of the histology report (if more than one, attach all –ie. initial biopsy + cytology + debulking surgery)

Date of histopathological diagnosis *dd/mm/yy* .....

Basis of diagnosis Surgery Biopsy Cytology/FNA

Initials | MRN | W M

Final Histopathological diagnosis

(a) Primary site: Ovary ☐ Fallopian tube ☐ Peritoneum ☐ Other ☐ *Specify* .....

(b) Sub-type: *Mark X all that apply*
Serous ☐ Mucinous ☐ Endometrioid ☐ MMMT ☐ Clear Cell ☐
Other ☐ → *Specify* .....

(c) Behaviour: *(if applicable)* **Invasive** ☐ → Grade: Silverberg *(1,2 or 3)* ☐ Description: 1 =Well differentiated; 2 =Moderately differentiated; 3 =Poorly differentiated; 4 =Undifferentiated ☐ *(ie. can be 1 + 2, overall grade =2)*

Architecture: *(if applicable)* Score *(1,2 or 3)* ☐ Nuclear grade ☐ Mitotic Activity: *(circle)* 1 = <6; 2 = 7-16; 3 = >17 ☐ *(ie. Per 10 HPF)*

(d) Behaviour: *(if applicable)* **Borderline** ☐ → Grade: High (H) or Low (L) ☐

	Yes	No
Microinvasion	☐	☐
Micropapillary	☐	☐

(e) Behaviour: **Benign** ☐

Comments: .....

.....

Has the patient been treated previously for this malignancy?

☐ ☐ → 1=prior chemotherapy; 2=prior hormonal; 3=prior radiation; 4=prior immunological; 5=prior surgery ☐

Specify: .....

.....

Has the patient been enrolled into a clinical trial?

NO ☐ **YES** ☐ → **Specify Trial/Protocol No:**

.....

Has the patient been referred to a Family Cancer Clinic (FCC) for genetic testing or been enrolled in KConFab?

NO ☐ **YES** ☐

Genes being tested - documented in medical record:
(Mark X all that apply)

BRCA1 ☐ HNPCC ☐ Other ☐ Specify:
BRCA2 ☐ Li-Fraumeni/P53 ☐ .....

Are the results known and in the medical record?

NO ☐ **YES** ☐ → Please attach a de-identified copy of the results to this form *(patient name deleted and replaced with ID number)*

Initials

W M

GynBiobank Sample ID

Other malignancies

Has the patient had a previous or concurrent malignancy?
(excluding non-melanoma skin cancers and pre-invasive lesions of the cervix)

NO ☐ YES ☐

Previous ☐ → **Date of diagnosis:** *dd/mm/yy*

Specify site: .....

Concurrent ☐ → **Date of diagnosis:** *dd/mm/yy*

Specify site: .....

Has the patient had cancer therapy for a previous malignancy?

NO ☐ YES ☐ **Brief Description:** →

.....

PRIMARY SURGERY

Institution at which surgery performed .....

Name of surgeon completing procedure .....
(+Consultant if performed by Fellow/Resident)

Prior gynaecological surgery
1=No
2=Yes, hysterectomy
3=Yes, other - specify .....

Date of surgery for ovarian cancer .....
dd/mm/yy

Type of surgery
1=Primary Surgery (no prior therapy)
2=Interval Debulk (primary surgery during chemotherapy)
3=Second Look Laparotomy
4=other (eg surgical staging, FNA) .....

Residual disease after surgery

No residual disease	☐		
Macroscopic	☐ →	≤ 1 cm residual	☐
		> 1 and ≤ 2 cm	☐
Tumour not resected	☐	> 2 cm residual	☐
Unknown	☐	size not recorded	☐

Record max dimension: ☐ **mm / cm** *Circle*

Also record any text comments *e.g. optimally debulked, suboptimally debulked, bulky, minimal etc*

.....

Extensive milliary disease NO ☐ YES ☐

Initials	MRN	GynBiobank Sample ID
		W M

Surgical Procedure

In the boxes below please enter '1' if data is derived from the Path report and '2' if derived from Operation Report – please attach copy.

	Surgical Procedure			Involved by Cancer		Comments
	YES	NO	PREVIOUS	YES	NO	
Left ovary	☐	☐	☐	☐	☐	
Left fallopian tube	☐	☐	☐	☐	☐	
Right Ovary	☐	☐	☐	☐	☐	
Right fallopian tube	☐	☐	☐	☐	☐	
Hysterectomy (uterus)	☐	☐	☐	☐	☐	
fertility sparing surgery	☐	☐	☐			
omentectomy	☐	☐	☐	☐	☐	Note if biopsy, partial or full (Circle)_____
tumour removal (check path report)	☐	☐	☐			
peritoneal biopsies	☐	☐	☐	☐	☐	
peritoneal washings	☐	☐	☐	☐	☐	or equivocal or unknown (Circle)_____
retroperitoneal lymph node sampling	☐	☐	☐	☐	☐	
complete lymphadenectomy*	☐	☐	☐			
appendicectomy	☐	☐	☐	☐	☐	
Other-Specify _____ *(eg. colostomy/ileostomy)*	☐	☐	☐	☐	☐	
External ovarian surface				☐	☐	
Ascites	☐	☐	☐	☐	☐	Enter amount in mls _____ ☐
Abdominal implants >2 cm				☐		
Distant metastases				☐		

Tumour capsule Intact ☐ spontaneous rupture ☐ rupture during surgery ☐

EXTENT OF STAGING (FOR STAGE I AND II ONLY)

☐ ***Full staging procedure:*** Total abdominal hysterectomy and bilateral salpingo-oophorectomy with removal of tumour, peritoneal biopsies, omentectomy, peritoneal washings, retroperitoneal lymph node sampling or complete lymphadenectomy (*including pelvic and para-aortic nodes)

☐ ***Full staging procedure with fertility sparing surgery:*** as above but without hysterectomy, LSO and/or BSO

☐ ***Staging procedure without lymphadenectomy:*** as above but without retroperitoneal (pelvic and para-aortic) lymph node dissection or sampling.

☐ ***Incomplete staging:*** Any primary surgery that departs from the above stated standards

☐ ***Inoperable***: no tumour debulking/removal

Comments: .....

.....

Initials	MRN	GynBiobank Sample ID
		W M

FIGO STAGE **Determined by:** ☐ Medical Record ☐ Evaluation of op and histo reports

IA ☐ IB ☐ IC ☐

0=tumour on the surface of one or both ovaries
1=malignant cells detected in washings
2=malignant cells detected in ascites
3=spontaneous capsule rupture
4=capsule rupture during surgery

☐

IIA ☐ IIB ☐ IIC ☐

0=tumour on the surface of one or both ovaries
1=malignant cells detected in washings
2=malignant cells detected in ascites
3=spontaneous capsule rupture
4=capsule rupture during surgery

☐

IIIA ☐ IIIB ☐ IIIC ☐

5= abdominal implants >2cm in diameter
6= positive retroperitoneal or inguinal lymph nodes

☐

IV ☐ Other ☐

NS= Not Staged
NK= Not Known

☐

Comments: .....

FIGO (1988) STAGING FOR OVARIAN AND PRIMARY PERITONEAL CANCER: (Peddersson, 1994) – can be found in the GUIDELINES

☐

PRIMARY INVESTIGATIONS

CA125 Levels

	NO	YES	
CA125 Levels	☐	☐ →	*Please complete "CA125 Assays" Form (and/or attach printout of CA125 history)*

PRIMARY TREATMENT

☐ ☐ → *For Neoadjuvant & Primary Treatment or Treatment for recurrence, please fill out separate Form (T)*

Has the patient been referred to another centre for further treatment?

NO	YES	
☐	☐ →	*Please complete details below*

Dr Name: .....

Centre: .....

Address: .....

Phone: ..... Fax: ..... Email: .....

Initials	MRN	GynBiobank Sample ID
		W M

DISEASE PROGRESSION

Date of last Dr assessment
dd/mm/yy .....

Disease progression

NO ☐ **YES** ☐

Date of first progression
dd/mm/yy

How determined:
1=Histologically
2=Radiologically
3=Physically
4=CA125 Marker

Specify Site/Other details (ie imaging):

.....

.....

Additional Surgery

Date of surgery	Procedure(s)	Residual Disease *1=none or microscopic* *2=macroscopic disease(enter max diameter)* *3=extensive milliary disease*

PATIENT STATUS

Date patient last seen
dd/mm/yy .....

Alive ☐

Discharged
(from specialist care,
GP care only) ☐

GP Name: .....

Address: .....

Phone: ..... Fax: .....

Email: .....

Lost to follow up ☐

Date of last contact
dd/mm/yy

Comments: .....

(eg. Presumed dead but unconfirmed, moved, missed appointments)

Dead ☐

Date of death:
dd/mm/yy

Main cause of death:

1=progression of disease (ovarian ca)
2=treatment related
3=other, specify
4=cause(s) unknown .....

Autopsy performed: **NO** ☐ **YES** ☐

Gynaecological Oncology biobank at Westmead

FORM B – Clinical Follow Up

Primary Ovary, Fallopian Tube and Peritoneal Cases

MRN	Initials	Date of Birth *(dd/mm/yy)*	GynBiobank Sample ID
			W M

Follow Up Form Number B

Information obtained from (tick all that apply):

Central Medical Records	☐	Gyn Onc Records	☐	Gyn Onc Database (CRS)	☐
Medical Oncology Records	☐	Medical Oncology Database	☐	PIMS	☐
Other	☐	Specify: _____			

Form completed by

Date form completed

PATIENT STATUS

Date patient last seen *dd/mm/yy* .....

Alive ☐

Discharged (from specialist care, *GP care only)* ☐

GP Name: .....

Address: .....

Phone: ..... Fax: .....

Email: .....

Lost to follow up ☐

▼ Date of last contact *dd/mm/yy*

Comments: .....

(eg. Presumed dead but unconfirmed, moved, missed appointments)

Dead ☐

▼ Date of death: *dd/mm/yy*

Main cause of death:

1=progression of disease (ovarian ca)
2=treatment related
3=other, specify
4=cause(s) unknown .....

Autopsy performed: **NO** ☐ **YES** ☐

CA125 LEVELS SINCE LAST FOLLOW-UP

	NO	YES	
CA125 Levels	☐	☐ →	*Please complete "CA125 Assays" Form (and/or attach printout of CA125 history)*

Has the patient been enrolled into a clinical trial since last form was completed?

NO	YES	
☐	☐ →	**Specify Trial/Protocol No:** .....

Initials	MRN	GynBiobank Sample ID
		W M

DISEASE PROGRESSION SINCE LAST FOLLOW UP

Date of last Dr assessment
dd/mm/yy .....

Disease progression

(For multiple recurrences, pls copy additional pages and document 1 per page)

NO ☐ **YES** ☐

Date of assessment
dd/mm/yy

Disease progression/recurrence
1=No
2=Yes

Date of progression/recurrence
dd/mm/yy

How determined:
1=Histologically 3=Physically
2=Radiologically 4=Marker

Specify Site/Other details (ie imaging):

.....

.....

Has the patient been referred to a Family Cancer Clinic (FCC) for genetic testing or been enrolled in KConFab since last form?

NO ☐ **YES** ☐

Genes being tested - documented in medical record:
(Mark X all that apply)

BRCA1 ☐ HNPCC ☐ Other ☐ Specify:
BRCA2 ☐ Li-Fraumeni/P53 ☐ .....

Are the results known and in the medical record?

NO ☐ **YES** ☐ → Please attach a de-identified copy of the results to this form *(patient name deleted and replaced with ID number)*

ADDITIONAL SURGERY

Date of surgery	Procedure(s)	Residual Disease *1=none or microscopic* *2=macroscopic disease(enter max diameter)* *3=extensive milliary disease*

ADDITIONAL TREATMENT

Has the patient had treatment since the last form?

NO ☐ **YES** ☐ → Please complete *Form (T)*

Gynaecological Oncology biobank at Westmead

FORM T - Treatment

MRN | **Initials** | **Date of Birth** *(dd/mm/yy)* | **GynBiobank Sample ID** (W M)

Site Name | **Form completed by** | **Date Form Completed** *dd/mm/yy*

Information obtained from (tick all that apply):

Central Medical Records ☐ | Gyn Onc Records ☐ | Gyn Onc Database (CRS) ☐

Medical Oncology Records ☐ | Medical Oncology Database ☐ | PIMS ☐

Other ☐ Specify: _____

TREATMENT INFORMATION

		NO	YES
Primary treatment	Neoadjuvant treatment	☐	☐
	Adjuvant treatment	☐	☐
	Treatment for Progression	☐	☐

Did the patient have any of the following as part of her treatment?

	Unknown	NO	YES	
Chemotherapy	☐	☐	☐	→ Complete 'A' Chemo section of this form
Radiation Therapy	☐	☐	☐	→ Complete 'B' Rad section of this form
Hormonal Therapy	☐	☐	☐	→ Complete 'C' Hormone Therapy Table
Other Therapy	☐	☐	☐	→ Complete 'D' Other Therapy Table

Primary Treatment Response ☐

1=Complete response
2=Partial response
3=Stable disease
4=Increasing disease
5= Symptomatic deterioration
6= Not Applicable
7= Non measurable disease at baseline

If "4" Increasing disease, specify - A = Rising CA125, B = Clinical, C = Radiological ☐ *Note all that apply*

If " 2 or 3", was response based on - D = Tumour volume/size, E = CA125 levels ☐

						W	M				

Initials **MRN** **GynBiobank Sample ID**

A. CHEMOTHERAPY

Height cms **Weight** kgs **BSA** **Date taken**

Serum creatinine µmol/l **Date taken**
(first result prior to chemo, if reported)

Cycle Number	Drug Name(s)	Dose (mg/m2 or AUC)	Dose administered (mg)	Route *1=IV 2=IP 3=PO*	Date Given *dd/mm/yy*	Reason for change/cessation *See key below*

Reason for change/cessation key: 1=CR; 2=PR; 3=No Change; 4=PD; 5=Excessive Toxicity*; 6=Patient Refusal; 7=Death; 8=Other – Please specify (ie Drug reaction)

Please copy more pages if needed to complete line/s of treatment

		W M
Initials	**MRN**	**GynBiobank Sample ID**

B. RADIOTHERAPY

Start Date *dd/mm/yy*	**Stop Date** *dd/mm/yy*	**Anatomical Site**	**Total Dose (Gy)**	**Number of Fractions**

C. HORMONAL THERAPY

Start date *dd/mm/yy*	**Stop date** *dd/mm/yy*	**Drug Name**	**Dose**	**Schedule** *ie how many times a day or week etc*

D. OTHER THERAPY

Start date *dd/mm/yy*	**Stop date** *dd/mm/yy*	**Therapy Description or Drug Name**	**Dose** *(if applicable)*	**Other Comment**

Gynaecological Oncology biobank at Westmead

FORM C – CA125 Assays

MRN	Initials	Date of Birth *(dd/mm/yy)*	GynBiobank Sample ID
			W M

Site Name	Form completed by	Date Form Completed *dd/mm/yy*

Information obtained from (tick all that apply):

Central Medical Records ☐ Gyn Onc Records ☐ Gyn Onc Database (CRS) ☐

Medical Oncology Records ☐ Medical Oncology Database ☐ PIMS ☐

Other ☐ Specify: _____

Preoperative and post operative CA125 levels (please clearly specify pre-operative measurement with an asterisk*)

Date of collection *dd/mm/yy*	**CA125 level** *(U/ml)*	**Normal Range**	**CA125 Assay/Kit** *(eg Abbott Axsym, DPC Immulite)*

Initials	MRN	GynBiobank Sample ID

Date of collection *dd/mm/yy*	**CA125 level** *(U/ml)*	**Normal Range**	**CA125 Assay/Kit** *(eg Abbott Axsym, DPC Immulite)*

APPENDIX 2

Tissue Collection Form

Recurrent Tissue Collection Form

Blood Collection Form

GYN ONCOLOGY TISSUE COLLECTION FORM

PLEASE FILL IN THIS FORM EACH TIME A TISSUE SAMPLE IS COLLECTED

GYN ONC TUMOUR # _____ PATIENT INITIALS _____

PATIENT DOB _____ DATE COLLECTED _____

SURGEON _____ PATHOLOGIST _____

PROVISIONAL DIAGNOSIS (IF KNOWN)

☐ Primary Ovarian
☐ Benign Ovarian
☐ Secondary Ovarian
☐ Other _____

ANATOMICAL SITE OF SPECIMEN

☐ **Left** Ovarian Mass
Number Of Sample Tubes
- Frozen _____
- 10% Formalin _____
- Blocks _____

☐ **Right** Ovarian Mass
Number Of Sample Tubes
Frozen _____
10% Formalin _____
- Blocks _____

☐ Uterus
Number Of Sample Tubes
- Frozen _____
- 10% Formalin _____
- Blocks _____

☐ Omentum
Number Of Sample Tubes
- Frozen _____
- 10% Formalin _____
- Blocks _____

☐ Other _____
Number Of Sample Tubes
- Frozen _____
- 10% Formalin _____
- Blocks _____

TIME ELAPSED BEFORE FREEZING/FIXING TISSUE Time Paged _____

Time Collected _____

Time Frozen _____

TIME SAMPLE WAS IN FORMALIN _____

OTHER COMMENTS _____

_____

_____

_____

_____

GYN ONCOLOGY RECURRENT DISEASE COLLECTION FORM

PLEASE FILL IN THIS FORM EACH TIME A SAMPLE IS COLLECTED

GYN ONC TUMOUR # _____ PATIENT INITIALS _____

PATIENT DOB _____ DATE COLLECTED _____

SURGEON _____ PATHOLOGIST _____

TISSUE COLLECTED ☐ YES ☐ NO

IF YES, ANATOMICAL SITE OF SPECIMEN

☐ **Left** Ovarian Mass
Number Of Sample Tubes
- Frozen _____
- 10% Formalin _____
- Blocks _____

☐ **Right** Ovarian Mass
Number Of Sample Tubes
Frozen _____
10% Formalin _____
- Blocks _____

☐ Uterus
Number Of Sample Tubes
- Frozen _____
- 10% Formalin _____
- Blocks _____

☐ Omentum
Number Of Sample Tubes
- Frozen _____
- 10% Formalin _____
- Blocks _____

☐ Other _____
Number Of Sample Tubes
- Frozen _____
- 10% Formalin _____
- Blocks _____

TIME ELAPSED BEFORE FREEZING/FIXING TISSUE Time Paged _____

Time Collected _____

Time Frozen _____

TIME SAMPLE WAS IN FORMALIN _____

ASCITES COLLECTED ☐ YES ☐ NO

IF YES, RECORD VOLUME _____

OTHER COMMENTS _____

_____

_____

_____

_____

GYN ONCOLOGY BLOOD COLLECTION FORM

PLEASE FILL IN THIS FORM EACH TIME A BLOOD SAMPLE IS COLLECTED

PATIENT TUMOUR ID# _____

PATIENT DOB _____ PATIENT INITIALS _____

TIME BLOOD COLLECTED

- ☐ Pre-admission clinic
- ☐ Pre- surgery
- ☐ During surgery- as close to the start of surgery as possible
- ☐ Immediate post-surgery
- ☐ Post-surgery, during chemotherapy, just prior to Cycle 2
- ☐ Post-surgery, post-chemotherapy

DATE COLLECTED _____

TIME OF DAY COLLECTED _____

DATE AND TIME OF LAST MEAL _____

DATE OF LAST MENSTRUAL PERIOD _____

AMOUNT COLLECTED (ML)

- ☐ EDTA _____
- ☐ ACD _____
- ☐ Serum _____

DATE AND TIME OF PROCESSING_____

OTHER COMMENTS _____

_____

_____

_____

APPENDIX 3

Consent Forms and Participant Information Sheet

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Study Title: Molecular Biology of Gynaecologic Disease

Name of Researchers: Dr Gerard Wain, Dr Alison Brand, A/Prof Russell Hogg and A/Prof Paul Harnett

Request

We ask that you consider giving your permission for storage of a sample of your tissue in the Westmead Gynaecologic Tissue Bank for possible use in future research. This form provides you with information to help you decide whether you will allow this.
Please take the time to read the following information carefully and discuss it with others if you wish.

What kind of tissue will be taken, and how?

The collection of a biopsy for tissue banking involves no additional procedures and will not affect your treatment in any way. During surgery tissue samples are routinely taken and examined by a pathologist. If we have your permission, and if there is remaining tissue, the pathologist will allocate a small sample for tissue banking. On average, the sample allocated will be smaller than the size of a 20 cent piece. If you agree, a blood sample, approximately 10 ml (2 teaspoons), will also be collected. This blood sample is used to compare the genes in your blood with the genes in your biopsy.

Will the tissue sample be identifiable as mine after it is stored?

The stored tissue sample will be identifiable as yours.
As the sample will be identifiable as yours, we will maintain your confidentiality by keeping any clinical information in a locked filing cabinet and password-protected database. We allocate your sample a unique code, so your name and any other identifying information is never revealed to third parties. Information will be obtained from your medical records, however all the information gathered during the course of this research is completely confidential and only accessible to the researchers involved.

What will happen to my tissue sample?

We wish to store (or 'bank') the sample for potential, and as yet unspecified, research in the future. Not all potentially beneficial future research can be known at any one time, as the need for future research is determined by ongoing developments in the field.

How will I know if my samples are being used in the future?

If you agree to your tissue sample/s being stored for future research, they may be used for research projects in the future, with the approval of a Human Research Ethics Committee. The Human Research Ethics Committee will determine whether, or not, your consent should be obtained at that time for a particular research project.

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Study Title: Molecular Biology of Gynaecologic Disease

It will be possible to provide you with feedback about the findings of potential future research however we will not be able to give you the individual results from your samples.

Who will have access to my tissue sample once it has been stored?
The custodians charged with ensuring appropriate standards are met in storing and managing the tissue bank will have access to your sample.
Researchers involved in research approved by a Human Research Ethics Committee may also have access to your sample.

Will drug or biotechnology companies be able to use my sample for profit in the future?'
There is the possibility that research involving your blood or tissue sample may result in commercially viable technology or treatments. You will not however be able to claim financial benefit from any discoveries arising from the use of your tissue sample.

How long will my tissue sample be stored?'
Your tissue sample will be stored indefinitely until required for specific research projects.

Will I be able to get my sample back if I change my mind once it has been stored in the 'tissue bank'?'
It may not be possible to return your sample because it may have already been used for research. Also, it may not be appropriate to return your sample, for example where this may pose an infectious risk. However, you may contact your study doctor at any time and request that your sample be destroyed.

Complaints
This project has been approved by the Sydney West Area Health Service Human Research Ethics Committee. Any person with concerns or complaints about the conduct of this study should contact Ms Jillian Gwynne Lewis who is the person nominated to receive complaints from research participants. You should contact her on 9845 7014 and quote the protocol reference number HS.sb/HREC92/10/4.13.

If you have any concerns about the conduct of the study, or your rights as a study participant, you may contact:

Westmead Hospital Patient Representative, Ms Jillian Gwynne Lewis,
Telephone No 9845 7014 or email jillian.lewis@swahs.health.nsw.gov.au

Contact details
When you have read this information, the researchers will discuss it with you and any queries you may have. If you would like to know more at any stage, or have any

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Study Title: Molecular Biology of Gynaecologic Disease

problems while on the study, please do not hesitate to contact them on the numbers below.

Dr Gerard Wain
Working Hours Phone No – (02) 9 845 6801
After Hours Phone No – (02) 9 845 5555 (and ask to page Dr Wain)

OR

A/Prof Paul Harnett
Working Hours Phone No – (02) 9 845 6954
After Hours Phone No - (02) 9 845 5555 (and ask to page A/Prof Harnett)

Thank you for taking the time to consider this study.
If you wish to take part in it, please sign the attached consent form.
This information sheet is for you to keep.

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Study Title: Molecular Biology of Gynaecologic Disease

Name of Researcher: Dr Gerard Wain, Dr Alison Brand, A/Prof Russell Hogg and A/Prof Paul Harnett

1. I understand that the researcher will conduct this study in a manner conforming to ethical and scientific principles set out by the National Health and Medical Research Council of Australia and the Good Clinical Research Practice Guidelines of the Therapeutic Goods Administration.

2. I acknowledge that I have read, or have had read to me the Participant Information Sheet relating to this study. I acknowledge that I understand the Participant Information Sheet. I acknowledge that the general purposes, methods, demands and possible risks and inconveniences which may occur to me during the study have been explained to me by _____ ("the researcher") and I, being over the age of 16 years or over the age of 14 years but under the age of 16 years (*delete as applicable*), acknowledge that I understand the general purposes, methods, demands and possible risks and inconveniences which may occur during the study.

3. I acknowledge that I have been given time to consider the information and to seek other advice.

4. I acknowledge that refusal to take part in this study will not affect the usual treatment of my condition.

5. I acknowledge that I am volunteering to take part in this study and I may withdraw at any time.

6. I acknowledge that this research has been approved by the Sydney West Area Health Service Human Research Ethics Committee.

7. I acknowledge that I have received a copy of this form and the Participant Information Sheet, which I have signed.

8. I acknowledge any regulatory authorities may have access to my medical records to monitor the research in which I am agreeing to participate. However, I understand my identity will not be disclosed to anyone else or in publications or presentations.

Before signing, please read 'IMPORTANT NOTE' following.

IMPORTANT NOTE

This consent should only be signed as follows:

1. *Where a participant is between the age of 14 and 16 years, it should be signed by the participant and by a parent or person responsible.*
2. *Where a participant is under the age of 14 years, then the parent or person responsible only should sign the consent form.*
3. *Where a participant has impaired capacity, intellectual disability or is unconscious, then specific approval for the process for obtaining consent must be sought from the Human Research Ethics Committee.*

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Study Title: Molecular Biology of Gynaecologic Disease

Name of participant _____ Date of Birth _____

Address of participant _____

Signature of participant _____ Date: _____

Name of parent or person responsible (where applicable) _____

Address of parent or person responsible (where applicable)

_____

Signature of parent or person responsible (where applicable)

_____ Date: _____

Signature of researcher _____ Date: _____

Signature of witness_____ Date: _____

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Study Title: Molecular Biology of Gynaecologic Disease

Name of Researchers: Dr Gerard Wain, Dr Alison Brand, A/Prof Russell Hogg and A/Prof Paul Harnett

Request

We ask that you consider giving your permission for storage of a sample of your tissue in the Westmead Gynaecologic Tissue Bank for possible use in future research. This form provides you with information to help you decide whether you will allow this.
Please take the time to read the following information carefully and discuss it with others if you wish.

What kind of tissue will be taken, and how?

The collection of a biopsy for tissue banking involves no additional procedures and will not affect your treatment in any way. During surgery tissue samples are routinely taken and examined by a pathologist. If we have your permission, and if there is remaining tissue, the pathologist will allocate a small sample for tissue banking. On average, the sample allocated will be smaller than the size of a 20 cent piece. If you agree, a blood sample, approximately 10 ml (2 teaspoons), will also be collected. This blood sample is used to compare the genes in your blood with the genes in your biopsy.

Will the tissue sample be identifiable as mine after it is stored?

The stored tissue sample will be identifiable as yours.
As the sample will be identifiable as yours, we will maintain your confidentiality by keeping any clinical information in a locked filing cabinet and password-protected database. We allocate your sample a unique code, so your name and any other identifying information is never revealed to third parties. Information will be obtained from your medical records, however all the information gathered during the course of this research is completely confidential and only accessible to the researchers involved.

What will happen to my tissue sample?

We wish to store (or 'bank') the sample for potential, and as yet unspecified, research in the future. Not all potentially beneficial future research can be known at any one time, as the need for future research is determined by ongoing developments in the field.

How will I know if my samples are being used in the future?

If you agree to your tissue sample/s being stored for future research, they may be used for research projects in the future, with the approval of a Human Research Ethics Committee. The Human Research Ethics Committee will determine whether, or not, your consent should be obtained at that time for a particular research project.

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Study Title: Molecular Biology of Gynaecologic Disease

It will be possible to provide you with feedback about the findings of potential future research however we will not be able to give you the individual results from your samples.

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Researchers involved in research approved by a Human Research Ethics Committee may also have access to your sample.

Will drug or biotechnology companies be able to use my sample for profit in the future?'
There is the possibility that research involving your blood or tissue sample may result in commercially viable technology or treatments. You will not however be able to claim financial benefit from any discoveries arising from the use of your tissue sample.

How long will my tissue sample be stored?'
Your tissue sample will be stored indefinitely until required for specific research projects.

Will I be able to get my sample back if I change my mind once it has been stored in the 'tissue bank'?'
It may not be possible to return your sample because it may have already been used for research. Also, it may not be appropriate to return your sample, for example where this may pose an infectious risk. However, you may contact your study doctor at any time and request that your sample be destroyed.

Complaints
This project has been approved by the Sydney West Area Health Service Human Research Ethics Committee. Any person with concerns or complaints about the conduct of this study should contact Ms Jillian Gwynne Lewis who is the person nominated to receive complaints from research participants. You should contact her on 9845 7014 and quote the protocol reference number HS.sb/HREC92/10/4.13.

If you have any concerns about the conduct of the study, or your rights as a study participant, you may contact:

Westmead Hospital Patient Representative, Ms Jillian Gwynne Lewis,
Telephone No 9845 7014 or email jillian.lewis@swahs.health.nsw.gov.au

Contact details
When you have read this information, the researchers will discuss it with you and any queries you may have. If you would like to know more at any stage, or have any

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Study Title: Molecular Biology of Gynaecologic Disease

problems while on the study, please do not hesitate to contact them on the numbers below.

Dr Gerard Wain
Working Hours Phone No – (02) 9 845 6801
After Hours Phone No – (02) 9 845 5555 (and ask to page Dr Wain)

OR

A/Prof Paul Harnett
Working Hours Phone No – (02) 9 845 6954
After Hours Phone No - (02) 9 845 5555 (and ask to page A/Prof Harnett)

Thank you for taking the time to consider this study.
If you wish to take part in it, please sign the attached consent form.
This information sheet is for you to keep.

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Study Title: Molecular Biology of Gynaecologic Disease

Name of Researchers: Dr Gerard Wain, Dr Alison Brand, A/Prof Russell Hogg and A/Prof Paul Harnett

1. I understand that the researcher will conduct this study in a manner conforming to ethical and scientific principles set out by the National Health and Medical Research Council of Australia and the Good Clinical Research Practice Guidelines of the Therapeutic Goods Administration.

2. I acknowledge that I have read, or have had read to me the Participant Information Sheet relating to this study. I acknowledge that I understand the Participant Information Sheet. I acknowledge that the general purposes, methods, demands and possible risks and inconveniences which may occur to me during the study have been explained to me by _____ ("the researcher") and I, being over the age of 16 years or over the age of 14 years but under the age of 16 years (*delete as applicable*), acknowledge that I understand the general purposes, methods, demands and possible risks and inconveniences which may occur during the study.

3. I acknowledge that I have been given time to consider the information and to seek other advice.

4. I acknowledge that refusal to take part in this study will not affect the usual treatment of my condition.

5. I acknowledge that I am volunteering to take part in this study and I may withdraw at any time.

6. I acknowledge that this research has been approved by the Sydney West Area Health Service Human Research Ethics Committee.

7. I acknowledge that I have received a copy of this form and the Participant Information Sheet, which I have signed.

8. I acknowledge any regulatory authorities may have access to my medical records to monitor the research in which I am agreeing to participate. However, I understand my identity will not be disclosed to anyone else or in publications or presentations.

Before signing, please read 'IMPORTANT NOTE' following.

IMPORTANT NOTE

This consent should only be signed as follows:

1. *Where a participant is between the age of 14 and 16 years, it should be signed by the participant and by a parent or person responsible.*
2. *Where a participant is under the age of 14 years, then the parent or person responsible only should sign the consent form.*
3. *Where a participant has impaired capacity, intellectual disability or is unconscious, then specific approval for the process for obtaining consent must be sought from the Human Research Ethics Committee.*

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Study Title: Molecular Biology of Gynaecologic Disease

Name of participant _____ Date of Birth _____

Address of participant _____

Signature of participant _____ Date: _____

Name of parent or person responsible_____

Address of parent or person responsible (where applicable) _____

_____

Signature of parent or person responsible (where applicable) _____

_____ Date: _____

Signature of researcher _____ Date: _____

Signature of witness _____ Date: _____

APPENDIX 4

Access Policy for Biological Specimens

Application Form for Biospecimens

POLICY FOR ACCESS TO BIOLOGICAL SPECIMENS

Applications by researchers for material and information must have HREC approval, be scientifically sound and demonstrate that the material will be used efficiently. Applicants must describe the proposed research in detail and demonstrate availability of funds for the proposed research.

The proposed work will be reviewed by the Gynaecological Oncology Biobank at Westmead Committee (GynBiobank) to evaluate whether the application comprises a scientifically justifiable, feasible and high priority use of the material currently available. The project may also undergo independent peer review eg by the Western Sydney Local Health District (WSLHD) Scientific Advisory Committee, unless grant support has been gained through a national peer review process such as NHMRC.

In the event that an application is rejected, an independent appeal process will be implemented based on guidelines developed by the tissue bank network, ABN:Oncology.

Access to material from the biobank will require approval of the GynBiobank Committee, the WSLHD HREC / HRECs of other participating institutions. The applicant is responsible for all ethics applications. Where tissue has been collected for a specific purpose / project and stored in their biobank, permission to use the material in other projects is required from the contributor.

On approval, the Biobank Manager will provide the researcher with the tissue requested. Whilst we take every step to ensure that biobank details are up-to-date we cannot guarantee that every tissue amount requested will be available as we are constrained by the amount of tissue that can be collected. Additional information such as clinical outcome or assistance with data interpretation will be negotiated and will usually be provided via collaboration with the appropriate clinicians and/or researchers. Provision of such data will also be subject to HREC approval.

Researchers are requested to provide an annual research report, including abstracts and publications arising from research utilising the Gynaecological Oncology Biobank at Westmead resource and acknowledge the biobank in all publications and presentations. Authorship on any manuscript utilising tissues from the biobank will be in keeping with the NHMRC/AVCC Statement and Guidelines on Research Practice.

As a main aim of the biobank is to benefit research into gynaecological cancer, it is requested that, once published, data obtained on individual samples is made available, to continuously add value to the biospecimens held in the biobank.

Transfer of materials is subject to terms and conditions of a Materials Transfer Agreement with WSLHD and applicants may be asked to contribute to the costs of preparing and shipping biological materials.

GYNAECOLOGICAL ONCOLOGY BIOBANK AT WESTMEAD
APPLICATION FORM

APPLICATION FOR WORK INVOLVING USE OF BIOLOGICAL MATERIAL AND/OR DATA

PRINCIPAL INVESTIGATOR		
Name:	Affiliation:	
Address:		
Suburb:	State:	Postcode:
Phone:	Email:	

CO INVESTIGATOR (S)	
Name:	Affiliation:
Name:	Affiliation:

TITLE OF PROJECT:

BRIEF SUMMARY OF STUDY (300 words)

SECTION 1

In order for the committee to determine whether your request is an appropriate use of this valuable resource, please provide:

a) Background and justification of the proposed research

b) Hypotheses and aims

c) Feasibility of the work, including design, statistical power, access to key technologies, experience of the host laboratory, and available staff and funding to support the work

d) Indicate how the work would benefit from the use of Gynaecological Oncology Biobank at Westmead samples.

e) Justify the number and type of samples requested in the context of the proposed research.

f) Indicate whether obtaining samples in batches is feasible and whether there is merit in sending part of the requested material to gain feedback on progress before the complete number of samples is sent

Please make responses as succinct as possible and provide this information in 6 pages or less.

Pilot studies, involving approximately 20-30 samples or less, or for example which provide preliminary information for a grant application, should be described in less than 2 pages.

SECTION 2

In order to assess the impact of your request on the resource, and the workload required to access the material/data, please indicate the number, amount, and type of sample (eg germline DNA, tumour DNA, frozen tumour RNA, blocks, sections), and what data is required (clinical, pathology or other) –

Type of sample _____

_____

Number of samples _____

Amount of sample (if applicable) _____

List the data items requested-

_____

SECTION 3

It is important that your work accords with the ethical standards that govern use of the Gynaecological Oncology Biobank at Westmead resource. Please provide evidence of ethical clearance for the project including copies of approved institutional human research ethics applications and all correspondence with the human research ethics committee. Where applicable this must be provided from each of the participating institutions.

DOES THE PROJECT HAVE ETHICS APPROVAL? YES ☐ NO ☐
(if yes, please provide evidence)

IF NOT, IS AN HREC APPLICATION IN PROGRESS? YES ☐ NO ☐

The following question applies to NSW applicants only

HAS A NSW NATIONAL ETHICS APPLICATION FORM (NEAF) BEEN COMPLETED? YES ☐ NO ☐

SECTION 4

To assist the committee in assessing the merit of the science proposed it is useful know if the work has been peer-reviewed and has successfully obtained grant funding. If so, please provide evidence of peer-reviewed success of the proposed research and wherever possible, provide copies of the referees' reports.

HAS THE PROJECT BEEN PEER REVIEWED? YES ☐ NO ☐

IF YES, BY A GRANTING BODY? YES ☐ NO ☐
(if yes, please provide evidence)

SPECIFY GRANTING BODY & GRANT NUMBER

BY OTHER PARTY? Please Specify

DO YOU HAVE FUNDING FOR THIS STUDY? YES ☐ NO ☐
(if yes, please provide evidence)

IF NOT, FUNDING BEING SOUGHT? YES ☐ NO ☐

PLEASE STATE THE DURATION OF THE PROJECT eg 2009-2012 _____

SECTION 5

Please list publications of the Chief Investigator(s) for the last five years.

SECTION 6

If samples are being requested, please complete the following-

Mode of shipping

Address for shipping

CONTACT NUMBER

Suggested arrangement for payment of shipping

SECTION 7

Collaborations with commercial organizations that involve the use of Gynaecological Oncology Biobank at Westmead material, data or information arising from the work should be listed.

ACKNOWLEDGEMENT

We ask that any publications arising from the use of specimens and data provided for this project acknowledge the Gynaecological Oncology Biobank at Westmead, a member of the Australasian Biospecimen Network-Oncology group, which is funded by the National Health and Medical Research Council Enabling Grants ID 310670 & ID 628903 and the Cancer Institute NSW Grant ID 12/RIG/1-17 & 15/RIG/1-16.

We also request that a copy of all abstracts and publications be sent to Catherine Kennedy, Biobank Manager c.kennedy@sydney.edu.au.

CHECKLIST OF MATERIAL REQUIRED AS PART OF FULL AND PILOT APPLICATIONS FOR BIOSPECIMENS AND/OR DATA

- Scientific proposal submitted. Less than one page is required for a pilot project;
- List of biological material requested (if applicable);
- Evidence of ethical clearance for the project;
- Evidence of approval for a grant application that has already undergone peer review by a funding agency. This is not required for pilot projects;
- Information on the resources available to conduct the research;
- Publications of the Chief Investigator(s) for the last five years;
- Suggested timeline for the project;
- Suggested protocol for shipping;
- An outline of consulting agreements, collaborations and research projects between investigators named on the application and commercial organisations.

CONTACT INFORMATION

Catherine Kennedy, Manager, Gynaecological Oncology Biobank at Westmead

Department of Gynaecological Oncology and Centre for Cancer Research

The Westmead Institute for Medical Research, Westmead Hospital, Westmead NSW 2145, Australia

Telephone: + 61 2 9845 7376 Fax: +61 2 98459681

Email: c.kennedy@sydney.edu.au

APPENDIX 5

Material Transfer Agreement

WESTERN SYDNEY LOCAL HEALTH DISTRICT
MATERIALS TRANSFER AGREEMENT

BETWEEN The **Western Sydney Local Health District**, (WSLHD) ABN 48 702 394 764, and having its office at Institute Road, Westmead NSW 2145; ("**WSLHD**");

AND, **Insert name and address of Institution** (the "**Recipient**")

The Recipient of the materials named in the Schedule (the "Materials") agrees with WSLHD, that the supply of the Materials described in the Schedule will be governed by the following terms and conditions:

A. Definitions

(a) "Agreement" means this Materials Transfer Agreement.

(b) "Commercial Purposes", in relation to any thing shall mean the use of the thing to generate revenue, and specifically includes the sale, lease, license, or other transfer of the thing to a for-profit organization. Commercial Purposes shall also include use of the thing by any person, including The Recipient and WSLHD, to perform contract research, to produce or manufacture products for general sale, to conduct research activities that are subject to consulting or licensing obligations to another party or that result in any sale, lease, license, or transfer of the thing.

(c) "Confidential Information" shall mean the Materials, the Results and any and all information disclosed by one party to the other under the terms of this Agreement. However, Confidential Information shall not include any portion of the information which is Excluded Information.

(d) "Excluded Information" shall mean any information which: (i) at the time of its disclosure hereunder was generally available to the public; (ii) after its disclosure hereunder becomes generally available to the public, except through breach of this Agreement by either party, (iii) was in either party's possession on a non-confidential basis prior to the time of disclosure by or on behalf of a party hereunder as demonstrated by that party's competent written records, and was not required directly or indirectly from the other party; or (iv) becomes available to either party on a non-confidential basis from a third party that is not legally prohibited from disclosing such Confidential Information.

(e) "Inventions" shall mean (a) all inventions, substances, discoveries, developments, designs, improvements, formulae, know-how, techniques or other information of technical or commercial importance created by the use of, or relating to the Materials, (b) new uses for the Materials, or (c) methods for the preparation thereof.

(f) "Materials" means the material, information and data described in the Schedule, and any material, information and data derived from, based on or incorporating any part of the Materials (including progeny), whether modified or unmodified, and includes any sample or any part of the Materials, but does not include the Results.

(g) "Project" means the research to be undertaken by The Recipient using the Materials and which is more particularly described in the Schedule.

(h) "Results" means the statistics, information and data (but not any new substances or Inventions) generated, developed or derived directly from the use or application of the Materials in the Project.

(i) "Publication" has the meaning to publish or publicly disclose or present any Results or Inventions.

(j) "Term" means the period of time starting on the date this Agreement is executed by the last of the parties to execute it and ending on the first of: (a) the date the Project is discontinued; or (b) the date this Agreement is terminated in accordance with clause 22

1. Recipient shall use Materials or any progeny, modification or derivatives of the Materials, or any substances that incorporate any part of the Materials, only for the purposes of research in connection with the project named in the Schedule and subject to any Special Conditions specified in the Schedule. The materials must not be released to any person not under direct supervision of the Recipient Scientist, or to any organization without the prior written permission of the WSLHD. The Materials are to be used with caution and prudence in any experimental work, as not all of their characteristics are known. The Recipient will be responsible for ensuring compliance with all applicable legislation.

2. Any use of the Materials, any progeny or modification of the Materials, or any substances that incorporate any part of the Materials for commercial purposes shall require a separate agreement between Recipient and WSLHD.

3. The Recipient acknowledges that the Materials and any progeny or modification of the Materials, or any

substances that incorporate any part of the Materials will remain the property of WSLHD and that the Recipient has only a non-exclusive right to use the Materials and any progeny or modification within the terms of this Agreement.

4. The Recipient agrees to maintain in confidence any information provided to it by WSLHD that is not already in the public domain. The Information shall only be disclosed to those persons working in connection with the project named in the Schedule. The Recipient shall not have any obligation of confidentiality with respect to information that: (a) is or becomes in the public domain through no fault of the Recipient; or (b) is already in the possession of the Recipient prior to receipt from the WSLHD; or (c) is demonstrably developed independently by the Recipient; or (d) is obtained from a third party who is not under a confidentiality obligation to WSLHD; or (e) is required by law to be disclosed to a competent judicial or administrative body.

5. The Recipient acknowledges that the Material is or may be the subject of a patent application. Except as provided in this Agreement, the Recipient agrees that it has no express or implied license or any other right to any patents, patent applications, trade secrets or other proprietary rights of WSLHD. In particular, no express or implied license or other right is provided to use the Material and any progeny of the Material for commercial purposes.

6. Recipient will keep WSLHD fully informed of the results arising from the research which will be held in confidence. If WSLHD believes that a patentable invention has been made Recipient agrees to discuss this fully with representatives of WSLHD and/or its appointed agent. Recipient must not lodge any patent application or any other application for the statutory protection of the Materials or any progeny, modification or derivatives of the Materials, or any substances that incorporate any part of the Materials, without the prior written consent of WSLHD, which will not be unreasonably withheld. Property rights in inventions arising from Recipient's use of WSLHD Materials shall be determined by the parties taking into account the role and contribution of the Recipient and WSLHD in making such invention.

7. In the event that Recipient generates any new or commercially valuable knowledge from the use of the Materials, WSLHD shall be entitled to a royalty-free, non-exclusive license to use any such knowledge in research at WSLHD.

8. If the Recipient wishes to publish any results from the research which utilises the Materials and any progeny or modification, or any substances that incorporate any part of the Materials, such publication shall require the prior written consent of WSLHD. Such publication should acknowledge WSLHD as well as the scientist supplying the materials and in the case of an arranged collaboration, should include authorship of WSLHD scientists.

9. WSLHD may make the Materials available to others.

10. The Recipient will return to WSLHD or, at WSLHD's request, arrange the disposal or destruction of all unused Materials and any progeny or modification of the Materials once the Recipient's project for which has been supplied discontinues or there is no further need of the Materials in connection with that project.

11. The Recipient warrants they have any regulatory approval, license or consent necessary or required by law, or any government agency or other body, relating to the collection and possession of the Materials.

12. The Recipient warrants they have any ethics and regulatory approval, license or consent necessary or required by law, or any government agency or other body, relating to the use of the Material for the purposes of the project named in the Schedule.

13. WSLHD gives no warranty that use of the Materials for the purposes contemplated by this Agreement will not infringe any third party intellectual property or other right which will interfere with the Recipient's ability to undertake the research. WSLHD makes no representation or warranty that the Materials are fit for the particular purpose for which it is required by the Recipient. The Recipient agrees to indemnify WSLHD and its servants and agents against any and all damages, expenses (including reasonable legal expenses), claims, demands, suits or other liability arising from the Recipient's use of the Materials and any progeny or modifications of the Materials.

14. WSLHD rights are transferable under this agreement.

15. The recipient must obtain and maintain adequate insurance in respect of the possession, handling, storage, use, transport and disposal of the Materials and must, on request, provide evidence of such insurance.

16. Neither party may commence any Court or arbitration proceedings (except proceedings seeking urgent interlocutory relief) in connection with this Agreement before complying with this clause [clause 15]. If a dispute arises under or in connection with this Agreement, a party must give the other party a notice specifying the dispute. Within five (5) Business Days after the notice is given, the parties (each represented by its chief executive officer or other person authorised by the party to bind it in connection with the dispute) must confer to resolve the dispute or to decide the method of resolving the dispute. Unless the parties otherwise agree, the dispute must be referred to mediation if not resolved within fifteen (15) Business Days after the notice is given. The parties must appoint a mediator within twenty (20) Business Days after the notice is given. If they fail to agree, the mediator must be nominated by the then current President of the Law Society New South Wales (NSW) Australia, or that President's nominee, who will also determine the mediator's remuneration. The mediation must be conducted in NSW, Australia in accordance with the Mediation

Rules of the Law Society of NSW Australia. The mediator assists in negotiating a resolution of the dispute. The mediator may not make a decision binding on the disputants, unless the disputants have so agreed in writing. The mediation ends if the dispute is not resolved within twenty (20) Business Days after the mediator's appointment

17. This Agreement is governed by the law in force in the state of New South Wales, Australia and the parties hereto submit to the non-exclusive jurisdiction of the courts of the state of New South Wales, Australia.

18. This Agreement can only be amended, supplemented, replaced or novated by another document signed by the parties. Neither party can assign or dispose of its rights under this Agreement without the written consent of the other.

19. Each party must do anything (including execute any document), and must ensure that its employees and agents do anything (including execute any document), that the other party may reasonably require to give full effect to this Agreement.

20. This Agreement contains the entire agreement between the parties about its subject matter. Any previous understanding, agreement, representation or warranty relating to that subject matter is replaced by this Agreement and has no further effect.

21. Any right that a person may have under this Agreement is in addition to, and does not replace or limit, any other right that the person may have.

22. Any provision of this Agreement which is unenforceable or partly unenforceable is, where possible, to be severed to the extent necessary to make this Agreement enforceable, unless this would materially change the intended effect of this Agreement.

23. This Agreement shall commence on the date it is signed by the last of the parties to sign it, and shall continue for the Term. A party may terminate this Agreement immediately by notice in writing if: (a) the other party is in breach of a material term of this Agreement which is not remedied within 30 days of receiving a notice, in writing to their address in this agreement, to do so; (b) the other party is in liquidation or provisional liquidation or under administration, has a controller or analogous person appointed to it or any of its property, is taken to have failed to comply with a statutory demand, is unable to pay its debts or is otherwise insolvent, takes any step that could result in becoming an insolvent under administration, enters into a compromise or arrangement with, or assignment for the benefit of, any of its members or creditors, or is involved in any analogous event.

24. Upon termination or expiry of this Agreement, the rights and obligations of the parties are at an end as to their future operation except for (a) the enforcement of any right or claim which arises on or has arisen before termination; and the Definitions and clauses 1 to 22 inclusive, which survive termination.

This Agreement shall be effective when signed by all parties, and its effective date is the latest of the dates set out below.

SIGNED on behalf of the

WESTERN SYDNEY LOCAL HEATH DISTRICT (WSLHD):

_____ **Date:**_____
WSLHD Representative

XXXX (The Recipient):

_____ **Date:**_____
Authorised Company Representative's Name
Company Position

WESTERN SYDNEY LOCAL HEALTH DISTRICT

MATERIALS TRANSFER AGREEMENT

SCHEDULE

1. WSLHD Scientist — Anna deFazio
   "Insert Recipient Institution" Scientist — Insert name of scientist

2. Materials/data from WSLHD

Insert description of material and/or data

3. Project

Project Title:

Project Duration:

Project Description: