



# **Biorepository Protocols**

**March 2007**

## **About the Australian Biospecimen Network**

The Australasian Biospecimen Network (ABN) is a professional organisation established in 2002 to provide a forum to discuss technical, legal / ethical, and managerial issues relevant to the supply and use of human biospecimens in medical research. In particular, the ABN is comprised of groups and individuals with an interest in tissue banking. Tissue banking is the storage (with patient consent) of tissues removed during therapeutic procedures that have been identified by a pathologist as excess to diagnostic needs, and the supply of these tissues for use in ethics approved research projects.

ABN-Oncology, a subgroup of the ABN, is a network of Australian Tissue Banks that collect biospecimens for cancer research. In July 2004 ABN-Oncology was awarded an NHMRC Enabling Grant to support some of its activities. This funding will be used to:

- Support and enhance existing tissue collection at the 6 member banks
- Establish a new national Mesothelioma Tissue Bank
- Enable the development of a web-based specimen location search page to create a single point of contact for researcher enquiries.

Cancer specimens collected by ABN-Oncology Tissue Banks include melanoma, mesothelioma, ovarian, breast, colon, gastric, haematological, lung, paediatric, and prostate cancers.

The aims of ABN-Oncology are to increase the rate of tissue collection and provide standardised collection protocols and best practice guidelines, and streamline researcher access to tissue samples in order to facilitate important research into the causes and treatment of cancer. Furthermore, the web-based search page will have the scope to allow other tissue banks to join and expand the network over time.

The ABN executive committee is composed of:

Daniel Catchpoole – Children’s Hospital at Westmead

Anna deFazio – Westmead Institute for Cancer Research, Westmead Millennium Institute

Lisa Devereux - Peter MacCallum Cancer Centre

Michelle Fleming – ABN-Oncology Project Officer

Jenette Creaney – Sir Charles Gardiner Hospital

Christopher Schmidt - Queensland Institute for Medical Research

Heather Thorne - kConFab

Nikolas Zeps - Western Australian Research Tissue Network

**Abbreviations**

ACD	Acid Citrate Dextrose
DEPC	Diethylpyrocarbonate
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic Acid
EBV	Epstein Barr Virus
EDTA	Ethylenediaminetetraacetic acid
FCS	Fetal Calf Serum
H & E	Haematoxylin and Eosin
HSA	Human Serum Albumin
NBF	Neutral Buffered Formalin
NHMRC	National Health and Medical Research Council
OCT	Optimum Cutting Temperature
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PHA	Phytohaemagglutinin
RNA	Ribonucleic Acid
RPMI 1640	Roswell Park Memorial Institute (Tissue Culture Medium)
Tris-EDTA	Tris(hydromethyl)aminomethane-EDTA
Freezing Mix	10% DMSO, 20% FCS, RPMI 1640

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## Introduction

With the recent advances in genomic and proteomic research, and greater emphasis on the importance of translational research, the need for human tissue samples to be used in research has grown. Therefore, there is increased demand for tissue banking services and biorepositories that store tissue for research use. During this time, the ethical and regulatory environment for using human tissue in research has also become increasingly complex. Properly organised Tissue Banks are an appropriate response to this situation, ensuring that the interests of patients are protected and also that important research can proceed.

In particular, there has been an increase in need for large numbers of tissue specimens for use in new technologies such as tissue micro-arrays, as well as higher demand for a variety of good quality and well annotated specimens for genomics and proteomics research. With this higher demand for specimens and advances in the development of innovative technologies, both new and established tissue banks are now faced with several challenges (Holland et. al. 2003). These include:

- Obtaining a maximum amount of information from a limited number of specimens.
- Making provisions for the use of future technologies.
- Ensuring that specimens are stored in a way that allows application of current as well as emerging research techniques.
- Protecting the interests of specimen donors.

Although it is important for tissue banks to remain flexible in order to keep pace with new technology and approaches in biospecimen research, a certain level of standardisation will always be needed for this research to be replicable and valuable in the long term.

The literature on this subject indicates that the development of standard operating procedures for the collection, processing, and storage of specimens is best practice (Eiseman et. al. 2003, Somiari et. al. 2004, Mager et. al. 2004). As it is inevitable that several individuals (and possibly several laboratories) will handle the specimens, either at the collection or at the processing stages, using clear and explicit standardised protocols reduces experimental variability and ensures each specimen is of a similar high quality (Eiseman et. al. 2003).

Therefore, it is important that tissue banks have in place protocols for specimen processing and storage that are standardised and that address the above challenges.

Although biospecimen collection and banking is now an important facilitator of research, very little has been published on the selection and validation of the protocols used (Holland et. al. 2003). With this document, the Australasian Biospecimen Network hopes not only to provide information for new biorepositories or biorepositories developing new protocols, but also to add to the literature surrounding best practice principles in tissue banking.

## **Section 1:**

### **Ethical Issues when using Human Biospecimens**

#### **Basic Principles**

In Australia the collection, storage, and distribution of human biospecimens for scientific research purposes must be carried out in compliance with state and federal legislation as well as the guidelines contained within sections 15 and 16 of the NHMRC National Statement on Ethical Conduct in Research Involving Humans, 1999. An often important component of using human specimens is the requirement to use clinical information, and the s95A guidelines on the 1988 Privacy Act (NHMRC 2001) are specifically relevant to this. Briefly, these guidelines require that all research involving human specimens be reviewed and approved by a Human Research Ethics Committee (HREC). One of the fundamental principles outlined is the requirement for informed and voluntary consent by the person from whom the samples are taken. However, there are instances in which this requirement may be waived by an HREC. The following section details the guidelines laid out in the National Statement relevant to developing a process that ensures full compliance with these ethical and legal requirements. Each state or territory may have additional legal requirements and the ABN recommends that you obtain copies of the relevant legislation or take advice on your compliance with these. Some of this documentation may be available from the ABN by request.

#### **Informed Consent**

The National Statement states in section 1.7 that the ethical and legal requirements of consent have two aspects, the provision of information and the capacity to make a voluntary choice. So as to conform with ethical and legal requirements, obtaining consent should involve:

- (a) provision to participants of information, at their level of comprehension, about the purpose, methods, demands, risks, inconveniences, discomforts, and possible outcomes of the research (including the likelihood and form of publication of research results); and
- (b) emphasis of a subject's voluntary choice to participate.

Further in section 15.1 the National Statement states that the fundamental ethical principle to be observed in the use of human tissue samples for research is respect for the person and this is reflected in:



- a) the provision to the donor of full information about the purposes of the sampling, and/or the plan of the research proposal;
- b) consent by the donor to the use of the sample;
- c) the professional removal of samples to be used;
- d) provision for appropriate and secure storage of tissue samples;
- e) provision and maintenance of appropriate and secure systems to ensure confidentiality and privacy in the recording, storage and release of data; and
- f) accountability in the care and usage of such samples.

Prior to developing a program of collecting samples it is important to carefully examine whether or not you are able to satisfy all components of section 15.1 as these will be used by the HREC to guide them on determining whether or not your application will be determined to be ethical.

### **Obtaining Consent**

The most common method for demonstrating that a person has given informed consent is by use of a patient information sheet and consent form, the latter being a document that the person donating their samples and/or permitting access to their health information is required to sign. It is important to note that the consent form is not a legal document but instead has the status of 'evidence' that a person has been provided appropriate information to make a voluntary choice. By signing they are providing evidence that they understand and consent to comply with the specific requirements asked of them, usually detailed in bullet form above the signature.

Since this document is the only evidence that consent has been obtained it is important to keep this documentation safely filed and readily accessible. A copy of this form should be given to the donor, with the original remaining on file at the tissue bank.

The development of the Patient Information Sheet and Consent Form should be done with specific reference to fulfilling the relevant requirements laid out in the National Statement. The ABN uses a variety of these which differ due to the varying nature of the samples to be collected and the patient groups to be collected from. Examples of these are listed in Appendix 1. However, there are some basic principles that should be followed and these are detailed as follows.

**1) The purpose of collection must be described and it is often important to detail who is doing it. For example you would need to have a general statement such as:**

**WHAT AM I GIVING CONSENT FOR?**

We, << insert name >> are requesting your consent to allow us to use, for research purposes, tissue / blood etc samples. We would like to use these samples for medical research into <<disease>> in an attempt to further understand this disease in the human body; specifically to identify possible causes, improve diagnoses and possibly identify new treatments in the future. Much medical research is now focusing on genetics as an important factor in human disease.

**It may also be helpful to detail what will not be done, e.g.**

**WHAT WILL NOT BE DONE WITH YOUR TISSUE / BLOOD / MEDICAL RECORDS**

Your tissue and blood samples will NOT be used for research that involves reproductive cloning. No tissue or health information will be released to a third party unless it is to carry out research that has been approved by a Human Research Ethics Committee (each institution / hospital has one of these.)

**2) The methods used (e.g. biochemical and genetic studies) should be specified where known, but if banking samples for more open-ended use then a statement similar to the following will be suitable:**

**WHAT WILL BE DONE WITH MY SAMPLES?**

Researchers will investigate the cells in your <<blood and tissue>> samples, including looking at the genetic information contained in your genes. This will be carried out along with using information taken from your medical records to enable researchers to see how your genes relate to your diagnosis and your general health.

It is useful to describe whether the samples will be used only for a specific study or may be used for others (with appropriate approval by an HREC) in the future.

**3) Details of the demands on the participant specifying what is required from them are required. This should detail if any additional procedures or inconveniences will be asked or imposed upon them, e.g.**

**DOES THE DECISION TO PARTICIPATE OR NOT TO PARTICIPATE AFFECT MY CARE IN ANY WAY?**

The choice to participate is entirely up to you. Your care will not be affected in any way by your decision.

**CONSENT FOR AN ADDITIONAL BLOOD SAMPLE**

We are also requesting your consent to donate and allow us to use approximately 30 ml of blood for research purposes.

**4) Details of where the samples will used and any financial aspects should be included in this section, e.g.**

**INTERSTATE OR OVERSEAS RESEARCH**

Your tissue and blood samples may be sent interstate or overseas, the researchers become involved with a particular study that requires this. If this occurs, the researchers involved are required to demonstrate to a local HREC that the study meets the appropriate Australian ethical and privacy standards.

**COMMERCIAL ISSUES**

Our research is mostly directed to improving understanding of disease. Sometimes the research will lead to findings that result in the development of a commercial test or treatment that may be overseen by pharmaceutical companies. Australian law indicates that there is no financial reward or payment to you in such an event.

**5) Some tissue banks may find it useful to structure their consent forms so that potential participants can indicate which aspects of sample donation that they wish to be part of, e.g. by the use of tick boxes (for an example of this, the kConFab consent form can be viewed at [www.kconfab.org](http://www.kconfab.org)).**

*Care must be taken when designing such options to ensure that they do not lead to the creation of sub-collections that are of too few a number to be useful in research projects.*

**6) Potential risks, such as psychological distress, or possible discomforts, such as bruising after blood is taken, should be detailed. This should also detail how information arising from the study may or may not be fed back to donors.**

### **POTENTIAL RISKS**

If you allow us to use your blood or tissue, these samples are not intended for use in your diagnosis or treatment. It is possible, however, that future studies may result in new information being gained about diseases or potential diseases that you carry. Some of this information may have health implications for yourself and your family, including your descendants.

Where there is clear evidence of findings of medical importance to you or your family, contact will be made, via << insert name >>, through approved medical channels to ensure proper care is available to you. At that time you have the right to choose what information or investigations you would like to proceed with.

Some people experience bruising or may feel faint after giving blood. All procedures will be carried out by suitably qualified staff and your welfare is their priority.

**7) It is also useful to advise the donor to think more broadly about the possible issues arising from donating samples that may still be available for research after their death. Please note that Privacy Legislation means that a donor may not give consent on behalf of anyone else or any medical information about other persons without their express consent, e.g.**

If you decide to donate samples to the << insert name >>, it is advisable that you consider informing family members that you have donated tissue samples to the << insert name >>. You may also wish to discuss with family members the reason you decided to donate tissue for medical research.

**8) Both the option and mechanism by which the donor may withdraw their consent must be detailed, e.g.**

**WHAT IF I CHANGE MY MIND?**

The blood and/or tissue you donate will be stored until it is used up or until you contact the << insert name >> to request it be destroyed. If you wish to have your sample withdrawn, or if you wish to stop the << insert name >> accessing your medical records, you need to notify << insert name >> in writing. A letter confirming the destruction of your tissue samples and/or links to your medical records will then be sent to you.

**9) Details of how the donor's privacy and confidentiality will be protected should be detailed, e.g.****CONSENT FOR ACCESS TO YOUR MEDICAL RECORDS:**

In addition to asking your permission to utilise some of your blood and tissue for research, we also need to ask your permission for access to identified health information kept about you that is relevant to medical research. Such medical records may originate from hospitals, General Practice records, diagnoses by private specialists you have seen in the past, and information that is held on you by the Department of Health.

Your details will be held in strict confidence at all times. Samples will be identified by code only in all publications and communications. We will abide by all state and Federal Privacy legislation at all times.

It is important to note that there may be circumstances where your medical information kept by the << insert name >> could be obtained through legal processes (such as a court order).

**10) Responsibility of those doing the collection and those doing the research should be mentioned e.g.****RESPONSIBILITIES**

The <<insert name>> promises to store your sample carefully, but cannot guarantee against inadvertent loss that is beyond our control.

**Cultural / Ethnicity Considerations**

- Interpreters may need to be provided.

- Information should be provided to participants in a language they understand, whether written or via an interpreter.
- Consent forms and information need to be culturally appropriate.
- If samples / information are being collected only from a specific community, it is essential to gain consent from community leaders as well as from individuals.
- For more information, please refer to page C16 of “Research Involving Human Biological Materials: Ethical Issues and Policy Guidance Volume II” (NBAC 2000).

### **Tissue Collection for Open-Ended rather than Project Specific Research**

There is accumulating evidence that patients who consent to donate their tissue are keen to see that the maximum benefit will be obtained by its use. Consent should be broad and open (*Proposal 12-2* in the ALRC’s “Essentially Yours” discussion paper would provide guidelines for obtaining consent for unspecified research), delegating responsibility to HRECs to regulate appropriate usage. To consider a system of requiring re-consent would not only be impractical but could also be considered a re-invasion of privacy of an individual since the person would have to be contacted again.

In practice consent is given for a specific area of research, eg asthma or cancer, even where a specific project is in mind. Additional information regarding the specific nature of the project for which the samples are collected is often provided, with the caveat that the future studies will not be limited to this specific endeavour. In a recent American survey (Wendler & Emanuel 2002), the majority of donors gave their consent for research. Importantly, 91.9% of respondents would not impose greater safeguards on research using their sample for a different disease. The NSW Human Tissue and Anatomy Legislation Amendment Bill, 2003 suggests that patients may give broad or restricted consent at their own discretion.

### **Waiver of Consent**

In the NHMRC National Statement there are provisions within paragraphs 15.7, 15.8, 16.10j, 16.12 and 16.13 that allow for the storage of samples for future use and the waiver of re-consent. Importantly there is an enormous collection of tissue in Pathology archives, which has been retained for the purposes of clinical audit. It is apparent that this is a potentially invaluable resource for medical research. Whilst provisions under 16.13 of the National Statement allow waiver of consent, as do s95 and s95A guidelines on the Privacy Act

regarding health information, there is an urgent need to make clearer the circumstances under which these waivers are granted. Those wishing to use such material will have to be able to balance the need for consent against the logistical difficulties of obtaining it. This may include stating that a large proportion of those from whom tissue is being used have already died, or that the cost of recontacting the patients is too large. These arguments must include reference to any likely harms arising from the research and the likely positive outcomes from doing the work. There is no set formula for balancing this and different ethics committees may take differing views on where the balance lies. It is important that the researchers discuss these issues in detail with their local HREC before undertaking such work to determine how difficult this may or may not be. In practice, it is always best to seek consent wherever possible.

### **Providing Research Results to Donors**

The question of whether or not to provide research results to participants and tissue donors is often discussed within the research community, and support for providing results is increasing (Rigby & Fernandez 2005). However, as yet, there is little guidance from regulatory policies as to whether or not this should be done (Fernandez et al 2004). Providing results to research participants is an issue that particularly affects tissue banks, given that they are responsible for recruiting tissue donors, and are usually the gate-keepers of any identifiable information.

The receipt of results from research involving an individual's samples may have a direct impact on their quality of life (Fernandez et al 2004). For example, providing research results can have several benefits, as it demonstrates respect for persons, affirms the key role of the donor, and acknowledges that a person has made an important contribution (Fernandez et al 2004, Rigby & Fernandez 2005, Snowdon et al 1998). It can also act as a vital public relations tool, demonstrating research benefits and stimulating public enthusiasm for research. (Fernandez et al 2003). However, providing results can result in harms as well, such as emotional distress and survivor guilt, and may also have the potential to impact health insurability (Rigby & Fernandez 2005).

Research has shown that despite the possibility of harm, participants would still prefer to have access to research results (Snowdon et al 1998, Schultz et al 2003). Dawson (2003) found that a common view of members of the public was that it was 'only fair that donors received feedback'. Furthermore, in Schulz's study (2003) it was found that in most cases,

participants did not have increased levels of distress in response to the receipt of research results.

The fact that results will be offered in the future should be built into the consent process (Partridge 2002, Dawson 2003). For example, donors may be given the opportunity to ‘opt-out’ of receiving research results, and those who choose to opt-out should not be sent the results (Snowdon et al 1998, Fernandez et al 2004). The consent form should also make it clear to participants that should they choose to receive results, their identifying details will be kept on the tissue bank database.

The procedure for providing feedback to donors should be considered early in the development of protocols so that the logistics are in place and the process is fully understood by the patient and the researchers, and any expected costs need to be incorporated into the tissue bank’s budget. When developing a procedure, there are several issues to be considered, including the timing of the results release, whether generalised or individual results are released, and determination of the best method of informing donors. For example, when considering when to release results, it is best to ensure that results have been peer-reviewed, but not yet released to the media (Partridge 2002, Fernandez et al 2004).

When deciding whether generalised or individual results are released, the probability of the research providing clinically relevant and reliable information should be considered (Partridge 2002). Unless individual results are likely to have sufficient predictive value (for example, some genetic studies), it is best to only provide generalised results.

Finally, the best way to inform donors of their research results need to be determined. If generalised results are being provided, it is recommended that this be provided in the form of a regular newsletter (Eiseman et al 2003, Schultz 2003). The newsletter should provide contact numbers for donors if they seek further information, and be in lay language (Schultz 2003, ALRC 2003). Should individual results be supplied, it is best to have a health professional discuss the results in person with the donor (Dawson 2003, NBAC 2000).



## **Section 2:**

### **Guidelines on Collection of Biospecimens**

The methods used to collect biospecimens will vary depending on how the specimens will be processed and what is intended to be the end use. The following are general guidelines for collection:

#### **Labelling of Biospecimens**

- Each specimen should be labelled in such a manner that the labelling will survive all potential storage conditions, in particular dry ice and liquid nitrogen. Ink used on the label should be resistant to all common laboratory solvents.
- Labels should be printed with a linear barcode if possible, thus providing a direct link to database software. However, it is also important to include human-readable indications of contents.
- All specimens should ideally be labelled with at least 3 human-readable forms of identification on them, for example patient number, specimen number, patient date of birth.
- Suggested information for the label is the tissue bank's unique identifier number, sample type, and date of collection / banking, plus a barcode if available.

#### **Collection of Blood**

Summary tables of the general blood collection and processing protocols are included in Appendix 2. If collecting and processing serum or plasma for proteomics, please refer to Section 4.

- All blood should be treated as potentially infectious.
- Staff handling blood should check their Hepatitis B titre every 2 years.
- It is 'best practice' to take tissue bank blood samples concurrently with routine clinical blood samples, so as to minimise additional inconvenience and discomfort to patients.
- In the case of lymphoma, leukaemia, and myeloma donors, blood should be collected by fresh venipuncture rather than from an existing line.
- Blood may be collected into EDTA, ACD, Lithium Heparin, or into a clotted tube containing separating gel. Either EDTA and ACD tubes can be used if DNA is to

be extracted or lymphocyte cell lines to be made, however, ACD is more appropriate if there is to be an extended time lapse between blood collection and processing. Lithium Heparin is generally only used if cytology studies are being performed. If DNA is to be extracted from the blood or lymphocyte cells lines made, collecting into Lithium Heparin is not recommended as DNA yields are less and the recovered white blood cells are not as suitable for transformation, and any residual Lithium Heparin may interfere with PCR applications (Yokota et. al. 1999).

- Generally, EDTA tubes are used, however, ACD tubes can be used for blood from remote locations, as viable white blood cells can be recovered up to 10 days after collection (kConFab Protocols, 2005).
- Ensure tubes are clearly labelled as per the above recommendations.
- The amount of blood usually collected varies for different diseases. In most cases, 2 tubes (18-20 ml) blood is an ideal collection amount. This volume collected is guided by ethics clearance. However, sometimes there will be less than the expected amount, and in this case priorities need to be set as to the final blood products made. Reduced volume of blood in a tube containing additives should be noted so as to avoid confounding of results by variation in additive concentration
- Time of bleed and time of freezing should always be recorded, as well as any variations to the processing protocol.
- Blood should be transported at room temperature, although some proteomic applications require transport on dry ice.
- All blood should be processed within 48 hours of collection. Cell viability decreases rapidly after 48 hours, resulting in poor cell structure in slide preparations, or degradation of proteins and nucleic acids.
- Serum and plasma should be stored within 2 hours (House 2005, unpublished data).

### **Collection of Solid Tissues**

- Treat all tissue as potentially infectious.
- The collection process should be carried out in the most aseptic conditions possible.
- The intact operative specimen should be sent as soon as possible to pathology.

- It is considered to be ‘best practice’ to collect and process specimens within one hour of excision (Eiseman et. al. 2003). Transfer of specimens must be carried out as quickly as possible in order to minimize the effect of hypoxia upon genetic expression, and degradation of RNA and other tissue constituents.
- Ensure receptacle is clearly labelled. Specimens are useless if they are not correctly identified.
- For transport from surgery to pathology, or to the repository, specimens should remain fresh (not fixed) and be placed in a closed, sterile container on wet ice. Do not immerse them in liquid of any kind.
- The collection of samples for research should never compromise the diagnostic integrity of a specimen. Ensuring that only tissue exceeding diagnostic needs is banked means that patient care is never compromised, and helps retain public confidence in donating.
- It is important to have a pathologist supervise the procurement of the tissue (for quality assurance purposes).
- The pathologists will examine the sample, and, allowing adequate tissue for histological diagnosis and assessment of margins, will remove a portion of the tumour and adjacent normal tissue, if appropriate for the tissue bank.
- When selecting specimens, those areas with massive ischaemia and / or necrosis should be avoided.
- The anatomical site from which the tissue is taken must be recorded.
- Tissue bank staff will be present in pathology to freeze and fix the tissue as quickly as possible to maximise the RNA preservation.
- Samples requiring snap freezing can be frozen in a dewar of liquid nitrogen or on dry ice at the time of collection. Do not allow direct contact of the tissue with the liquid nitrogen.
- The approximate time that elapses before freezing and fixation should be noted.
- Tissue may be embargoed for an agreed period with the donating pathologist (commonly 2 weeks) before homogenising or cutting in case of diagnostic recall.
- If the tissue is being delivered from a remote site, ensure good communication between the sender and the receiver.
- Tissue should be placed in labelled histocassettes on biopsy pads if being sent from remote locations. They should be sent in liquid nitrogen or dry ice. For

remote sites where dry ice / liquid nitrogen isn't readily available, tissue collections into RNA<sub>later</sub> act as a good alternative (see page 63 for supplier).

### **Collection of Urine**

- When in transit, urine collections should be maintained on ice or refrigerated.
- Plastic or glass containers should be clean and dry, and have a 50-3000ml capacity, wide mouth, and leak-proof cap.
- Depending on the analyte to be measured, a preservative may be added.
- Urine should be processed and stored within 48 hours.

### **Collection of Buccal Cells**

The collection of buccal cells is logistically not difficult and does not require highly trained staff; thus, it may be more feasible than blood collection when staff are not on site, or the population chosen for a study is geographically diverse. Buccal cell collection should therefore be considered when non-invasive, self-administered, or mailed collection protocols are required (Steinburg et. al. 2002). Donors who do not give blood should also be asked to donate a buccal cell specimen; however, buccal cell collection will yield only limited amounts of DNA in comparison to blood.

A collection kit (containing mouthwash, 50 ml plastic tube, plastic biohazard bottle, and courier packaging) is mailed or given to the participant, along with an instruction sheet.

- The participant is to brush their teeth as usual, rinse their mouth well twice with water, and then wait 2 hours. They should not eat or drink anything other than water during this time.
- After 2 hours, 10 ml commercial mouthwash should be poured into the tube, and then 10 ml tap water added. This diluted mouthwash should be placed into the mouth (without swallowing) and swished around vigorously for 30 seconds.
- The mouthwash should then be spat back into the plastic tube and the tube should be sealed tightly.
- The sample should be sent back to the tissue bank immediately for processing, or stored at 4°C until sent (it should be sent within 24 hours).

### **Collection of Bone Marrow**

- Treat all bone marrow aspirates as potentially infectious.
- Staff should check their Hepatitis B titre every two years.

- Samples should be collected in an EDTA tube.
- For leukaemia and myeloma donors, a 2 ml aspirate should be collected in a 4 ml Lithium Heparin or EDTA tube.
- Transport bone marrow aspirates at room temperature.
- Process bone marrow aspirates within 24 hours of collection.

### **Collection of Associated Data**

The following base dataset of clinical and specimen data should be collected / recorded with each biospecimen (providing adequate consent has been obtained):

- Tumour Bank donor ID code
- Donor age at time of collection
- Gender of donor
- Consent status
- Involvement in clinical trial / study
- Primary Cancer Site
- Anatomical site of specimen collected (e.g. lymph node)
- Tumour morphology (histological sub-type e.g. adenocarcinoma)
- Tumour behaviour (benign, pre-malignant, malignant)
- Tumour grade
- Location of stored specimen
- Specimen ID code
- Preservation method
- Tissue condition (tumour/non-tumour/interface)
- Time (in mins) elapsed between tissue removal and fixation/freezing
- History of freezing/thawing
- Amount of tissue collected, and amount left over in storage

## **Section 3:**

### **Guidelines on Processing of Blood Specimens**

The following are protocol guidelines derived from the methods used by tissue banks in the ABN-Oncology group. However, it must be noted that these methods have been developed with a specific end use of specimens in mind, and so may not be suitable in all cases.

If serum and plasma are being collected, it is important to prioritise the separation of them so that these fractions can be frozen as soon as possible.

Note: summary tables of the blood collection and processing protocols are included in Appendix 2.

#### **Guthrie Cards**

Guthrie Cards are made from pure cotton and can be used for the extraction of DNA. Blood spot collection should be considered as alternative to whole blood when protocols call for easier collection and cheap room-temperature storage (Steinberg et. al. 2002). Always handle Guthrie Cards wearing gloves and only by the upper corner, marked out for labelling. Do not allow the card to come into contact with any unclean surface e.g. bench, base of hood.

(use EDTA / ACD tubes to make Guthrie Cards)

1. Mix blood thoroughly by inversion before starting.
2. Wipe top of vacutainers with ethanol before opening.
3. Use the fullest vacutainers to make 2 Guthrie cards by placing 40 µl of blood in the circle using a P200 pipette. Wipe the top of the vacutainer with 70% alcohol before removing the lid.
4. Air dry thoroughly in the back of the Class II Biological Safety Cabinet.
5. Store in a paper envelope (not plastic) at room temperature. Protect from moisture and rodents.

#### **Blood Pellets (White Cells)**

Blood Pellets can be used for the isolation of DNA.

(from EDTA / ACD tubes)

1. Transfer blood from the original tube to a labelled 50ml tube.
2. Fill tube with Tris-EDTA buffer (e.g. Fluka 86377) and mix vigorously. Place on ice for 5 to 10 minutes.

3. Spin as soon as possible at 1200g for 10 minutes.
4. Carefully pour off supernatant into a beaker containing chlorine bleach, e.g. Diversol (5g / 1lt beaker). This may need to be suctioned off as the pellet can be slippery. Briefly vortex the pellet and add 50 ml Tris-EDTA buffer. Shake vigorously.
5. If division of the sample is necessary, at this point pour 25 ml of sample into another falcon tube.
6. Spin both tubes at 1200g for 10 minutes.
7. Repeat washing if red cells persist.
8. Carefully pour off supernatant.
9. Using a swirling motion, remove the pellets (and a small volume of supernatant) with a P1000 pipette and transfer to 2 labelled cryovials.
10. Store in -80°C until DNA required.

### **Plasma**

Plasma can be used for bioassays, plasma DNA isolation, proteomic analysis, and biomarker discovery.

(from EDTA / Lithium Heparin / ACD tubes)

1. Spin one vacutainer (about 9 ml) at 815g for 10 minutes at 4°C to separate plasma.  
NOTE: Ensure rotor is balanced.
2. After wiping each tube with alcohol, remove about 3 ml plasma (but not the white cells in the buffy coat). Tube can be retained for white blood cell extraction.
3. Transfer to a clean, labelled 15 ml tube.
4. Centrifuge at 3200g for 10 minutes at 4°C.
5. Aliquot plasma into 1 ml labelled cryovials (3 to 4 aliquots).
6. Place in liquid nitrogen dewar to snap freeze.
7. Store at -80°C.

The purpose of double spinning the plasma is to remove all cellular contaminants so that the plasma is suitable for plasma DNA analysis. It is extremely important, therefore, not to disturb the buffy coat after the first spin, and any pellet after the second spin.

For more information on plasma processing for proteomics, please refer to Section 4.

### **Platelet Poor Plasma**

Platelet-poor plasma can be used for the isolation of plasma DNA.

(from EDTA tubes)

1. Spin blood at 3200g for 12 minutes at room temperature.
2. Pipette off plasma using a plastic pasteur pipette. Transfer into tube.
3. Spin plasma at 3200g for 10 minutes at 4°C.
4. Aliquot into 1 ml aliquots in labelled cryovials.
5. Store at -80°C.

For more information on plasma processing for proteomics, please refer to Section 4.

### **Serum**

1. Spin blood at 1200g for 10 minutes.
2. Aliquot 1 ml into labelled cryovials.
3. Place into liquid nitrogen dewar or dry ice to snap freeze.
4. Transfer to -80°C freezer.

For more information on serum processing for proteomics, please refer to Section 4.

### **White Blood Cells**

White blood cells can be used for DNA extraction and the creation of cell lines.

(from EDTA / ACD tubes)

1. Transfer the remaining blood from the plasma spin to a labelled 50 ml tube containing 10 ml RPMI 1640.
2. After alcohol swabbing the lid of this tube, aliquot 3 ml Ficoll into each of 2 clearly labelled 15 ml tubes.
3. Carefully layer 9 ml diluted blood onto each tube of Ficoll. Treat gently, do not mix, but spin as soon as possible.
4. Spin at 450g for 30 minutes. Note: when centrifuging, do not use brake.
5. Remove most of the top layer (RPMI 1640) using a 1 ml Eppendorf tip and discard (≈3-4 ml) into waste container containing chlorine bleach (concentration as per suppliers instructions).
6. Collect white blood cells with the same Eppendorf tip using a swirling motion to 'vacuum up' white blood cells. Do not take too much Ficoll (third layer), as it is toxic to the cells. Place the white blood cells in a labelled 15ml tube containing 10ml RPMI.
7. Spin at 450g for 10 minutes.
8. Pour off the supernatant into a waste container containing chlorine bleach. Add 3 ml of cold freezing mix (10% DMSO, 20% FCS, RPMI 1640) and resuspend.



9. Dispense the white blood cells into 3 x 1 ml labelled cryovials which have been sitting on ice.
10. Place on ice. Place vials in a rate-limiting freezer as per the protocol in Section 12: Guidelines on the Storage of Biospecimens (page 53). This should be done as soon as possible as DMSO is toxic at room temperature.
11. Transfer on a weekly basis to two liquid nitrogen tanks. Storing each tube in a different tank means that should one tank malfunction and the biospecimen be lost, the specimen in the other tank will still be unaffected.

### **Non-Lymphocytes**

(for EDTA tubes)

1. After following the white blood cell protocol, remove the layer of white cells sitting on top of the red blood cells with a 1 ml Eppendorf tip.
2. Collect 1 ml of non-lymphocyte fraction from both the tubes of blood remaining after the white blood cells protocol. The cells sit on the surface of the red blood cells and are visible as a white haze. Use a swirling motion and place into a labelled 10 ml tube containing 5 ml RPMI.
3. Spin at 450g for 10 minutes. Brake can be used here.
4. Remove the supernatant into waste container containing chlorine bleach from the non-lymphocyte pellet.
5. Resuspend 1 ml in 600 µl of Freezing Mix (page ii).
6. Transfer non-lymphocyte pellet to a labelled cryovial.
7. Place on ice. Place vials in a rate-limiting freezer (see Section 12, page 53). This should be done as soon as possible as DMSO is toxic at room temperature. Leave vials in a rate-limiting freezer for a minimum of 4 hours and up to 4 days before transferring them into liquid nitrogen storage containers.

### **Buffy Coat**

The buffy coat is a thin, grayish-white layer of white blood cells (leukocytes) and platelets covering the top of the packed red blood cells after 450g centrifugation.

(from EDTA / Lithium Heparin / ACD tubes)

1. After having spun the blood, take buffy coat off with about 100 µl of plasma using a disposable sterile pasteur pipette. NOTE: be careful not to lift red cells (if possible).

2. Aliquot as appropriate into labelled cryovials.
3. Place in liquid nitrogen dewar to snap freeze.
4. Transfer to liquid nitrogen freezer.

**Whole Blood**

(from EDTA tubes)

1. Dispense 50 µl DMSO into two 1 ml sterile cryovials.
2. Invert EDTA tube twice then add 450 µl of blood to each cryovial.
3. Invert cryovial to mix the whole blood with the DMSO. NOTE: DMSO is cytotoxic at room temperature, therefore as soon as it is mixed with blood it should be placed in a rate limiting freezer filled up with 200 ml of isopropanol (refer to Section 12, page 53).
4. Transfer to -80°C after at least 4 hours.

## **Section 4:**

### **Collection and Processing of Serum and Plasma for Proteomics**

As proteomics is an emerging field, there is a lack of concrete evidence as to the best way to collect and process serum and plasma. There are also many different factors that can affect proteomic profiles, and so researchers are often reluctant to focus on just one factor (e.g. storage). However, the conclusions from several recent studies are presented here:

#### **Collection Tube Type**

- The choice of tube in which to collect blood depends on the expected downstream uses of the serum / plasma.
- Platelets are most stable in Sodium Citrate tubes, and therefore these tubes may be suitable if there will be a long elapse of time between collection and processing (Banks et al 2005) (i.e. greater than 2 hrs). However, as these tubes contain liquid, they dilute the blood sample, which can act to lower immunoassay measurements (Rai et al 2005).
- Samples in Lithium Heparin are also reasonably stable, but Heparin binds with a significant number of proteins. This can result in interference with some affinity processes, as Heparin can compete for or prevent binding of molecules to charged surfaces (Rai et al 2005, Banks et al 2005)
- Blood samples in EDTA tubes are slightly less stable than those in other tube types, and so must be processed quicker, however there is evidence that EDTA may act to inhibit the breakdown of proteins by proteases (Banks et al 2005). EDTA tubes are not recommended for assays where metal ions are necessary as it binds with them (Rai et al 2005).

#### **Processing Considerations**

- Serum that has been collected in clotting activator tubes should be processed 30-60 minutes after venipuncture to minimise the effect of coagulation events (Banks et al 2005)
- Plasma and serum in other tubes should be processed within two hours of the blood draw to allow analysis of the maximum profile of proteins in the blood sample (Rai et al 2005, House 2005 - unpublished data).

- To ensure that platelet contamination is minimised, be careful to not centrifuge blood at too low a speed, and also when removing platelets from the blood sample. Ensure platelets are removed before freezing (Banks et al 2005)
- Serum and plasma should be snap frozen and stored in liquid nitrogen (Morente et al 2006). As with any biospecimen, it is best to minimise the number of freeze-thaw cycles.
- Make sure all variables are tracked in your database, e.g. tube type, storage temperature, time to freezing (Rai et al 2005).

## Section 5:

### Guidelines on Processing of Solid Tissue Specimens

Careful and well-documented processing of tissue specimens is crucial to the overall usefulness of the repository as a resource for scientific research. These protocols are recommended methods for preserving solid tissue.

#### Snap Freezing

- Ideally, all tissue must be snap frozen between 20 minutes and 1 hour after removal from the patient (Morente et. al. 2006). If this is not possible, the specimen should be put on ice until dissection. The delay time should be recorded on the tissue bank database.
- Freezing by direct immersion in liquid nitrogen is a valid method, although it is more damaging to the tissue and hinders subsequent microscopic examination, especially when microdissection techniques are required.
- For liquid nitrogen snap freezing, the protocol is:
  1. Place the tissue sample into a clearly labelled plastic disposable histokinette cassette (note: glass or pop-top vials should not be used as they may break or pop open). If more than 1 piece of tissue is to be frozen, place each piece into the same specimen container, separated by a piece of parafilm.
  2. Optimal size for snap freezing or RNAlater permeability is 0.5 cm<sup>3</sup>, although smaller fragments should still be frozen. If there is sufficient material, freeze multiple samples.
  3. The cassette is wrapped in foil and a label is placed on the outside of the foil parcel. The foil will prevent desiccation of the tissue. This parcel is then put into the liquid nitrogen dewar.
  4. The cassettes are then transferred into the -80°C freezer for two weeks (in case of diagnostic recall), and then to the liquid nitrogen tanks for long-term storage.
- The ideal medium for rapid freezing is isopentane that has been cooled to its freezing point (-160°C). Isopentane is ideal because it is an efficient cryoconductor, and, when compared to liquid nitrogen, results in less damage to tissues (Morente et al 2006).

- **To cool isopentane to its freezing point**, the vessel containing the isopentane must be introduced in another container of liquid nitrogen. The freezing point approximately corresponds to the moment when opaque drops begin to appear in the isopentane.
- Slow freezing by placing tissue in a refrigerator, chest freezer, or cryostat must be completely avoided, as it results in the formation of ice crystals.

## OCT Embedding

The OCT protocol is used for freezing tissue samples when good preservation of histologic detail is required. OCT samples can also be used for DNA and RNA extraction.

1. Fill dewar with liquid nitrogen.
2. Pour approximately 200-300 ml isopropanol into a plastic beaker.
3. Place the beaker into liquid nitrogen. Note: do not put beaker into liquid nitrogen before pouring in the isopropanol or the beaker will crack.
4. While isopropanol is chilling, prepare tissue.
5. Form a foil 'boat' approximately 12 mm in diameter. Place a label on the side of this 'boat'. Commercial plastic moulds can also be used.
6. If tissue is too big for the 'boat', divide as appropriate in a biohazard hood using a scalpel blade and a petri dish.
7. Put 2 drops of OCT into the 'boat' and place tissue on top in correct orientation for cutting.
8. Carefully pour OCT on top of tissue so that it is well covered. Do not allow any air bubbles to form.
9. Label a 20 ml tube and also place a label on the inside of the tube.
10. When isopropanol is very cold (you can judge this by the viscosity - should be like honey), remove the beaker from the liquid nitrogen and hold the foil 'boat' in the isopropanol using forceps. Do not submerge.
11. The tissue block should freeze slowly from the outside in, obvious by the whitening of the OCT.
12. When completely white, place the OCT block inside the labelled tube and drop into the liquid nitrogen canister. Note: rather than storing the OCT block inside a tube, a snap-locked bag can be used instead as long as it is tightly sealed to prevent desiccation.
13. Store OCT blocks in a -80°C freezer.
14. Allow the isopropanol to warm up before pouring back into the recycling bottle.

## Formalin Fixation

- Tissue specimens should not be bigger than 1.5 x 1 x 0.5 cm.
  - Specimens will be fixed in 10% neutral buffered formalin (NBF) (e.g. Confix Blue) for a minimum of 4 hours and a maximum of 48 hours, after which time they will be embedded in paraffin following conventional techniques.
  - It is best practice for tissue to be processed within 24 hours.
1. Using a pencil, label a cassette and a ticket for inside. Put the ticket inside the cassette with the tissue and place it into a 10% NBF container.
  2. Tissue fixation time is dependent on tissue size. Small tissue pieces (10 x 10 x 3mm) fixed in 10% NBF for 6 to 24 hours will generally show good cytological preservation and immunolocalisation, with a minimum of antigen masking.
  3. Process the blocks on the Tissue Tek VIP processor using program 9 (see Table 1).

TABLE 1: PROGRAM 9 – FORMALIN FIXATION

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

4. Choose the mould size appropriate to the tissue.
5. Sit the mould underneath the wax dispensing nozzle and leave for a few seconds to warm up.
6. Dispense enough wax to fill the bottom of the mould.
7. Take out cassette from the processor and drain off wax for a few seconds. Place onto embedding platform where it is warm, break off the lid of the cassette (throw away) with forceps and take out tissue.
8. Place tissue into mould with warmed forceps, orientate it, hold it down and slide mould over to small cold plate for quick setting (note: it is important to be careful

here as if the wax cools too much, the next layer of wax will snap off when the block is being sectioned).

9. Flatten tissue with forceps to make a level surface.
10. Place the cassette, with the ticket inside, on top of the mould, fill with wax.
11. Place onto -5°C cold plate to set.
12. Once set, scrape off excess wax from sides.
13. A section is cut from each block and stained with Haematoxylin & Eosin (H & E).  
Sections should undergo a pathology review by a pathologist.
14. File block away.

### **Alcohol Fixation**

1. Tissue is placed into 70% alcohol made with DEPC water for a minimum of 4 hours.
2. The tissue is transferred to a labelled cassette.
3. The blocks are processed on the Tissue Tek VIP processor using program 9 (see table 1).
4. The blocks are then embedded in paraffin wax as per 'Formalin Fixation' steps 4 to 14.

### **Cutting and Staining of Frozen Tissue**

- The cryostat should only be used by a trained technical assistant.
- Depending on specific needs, the standard thickness of sections cut is 4 µm.
- The protocol for Haematoxylin and Eosin (H & E) staining for frozen tissue is outlined in Table 2.
- When staining unfixed frozen sections, the following should be noted:
  - The section may have a tendency to lift from the slide more easily during staining. To overcome this tendency, adhesive slides are used.
  - Eosin staining is depressed.
  - Nuclear staining is enhanced.



TABLE 2: H &amp; E STAINING FOR FROZEN TISSUE

10% Formalin	1-2 minutes
Water	10-20 seconds
Harris Haematoxylin	1 minute
Water	10-20 seconds
Acid Alcohol (1%)	1-2 seconds
Water	10-20 seconds
Scotts Tap Water	10-20 seconds
Water	10-20 seconds
Eosin (1%)	30 seconds
Wash briefly, dehydrate, clear and mount	

### Cutting and Staining of Formalin Fixed Tissue

- The microtome can only be used by a trained technical assistant.
- Depending on specific needs, the standard thickness cut is 4 µm.
- The protocol for H & E staining for formalin fixed tissue is outlined in Table 3.
- Note: de-waxing and dehydration should be performed in the fume hood.

TABLE 3: H &amp; E STAINING FOR FORMALIN FIXED TISSUE

<b>Dewaxing</b>	
Xylene	3 minutes with occasional agitation
Xylene	3 minutes with occasional agitation
Alcohol (100%)	10 dips
Alcohol (100%)	10 dips
Alcohol (95%)	10 dips
Alcohol (70%)	10 dips
Water	10 dips
<b>Staining</b>	
Harris Haematoxylin	3 minutes
Running water	1 minute with gentle agitation
Acid Alcohol (1%)	5 dips or 10 seconds
Running water	1 minute with gentle agitation
Scotts Tap Water	1 minute
Running Water	2 minutes with gentle agitation
Alcohol (70%)	10 dips
Alcohol (95%)	10 dips
Eosin (1%)	30 seconds
<b>Dehydration</b>	
Alcohol (100%)	10 dips
Alcohol (100%)	10 dips
Alcohol (100%)	10 dips
Alcohol (100%)	10 dips
Xylene	10 dips
Xylene	10 dips and leave
Slides are now ready to coverslip with DPX®	

Note: Histolene can be used as a less toxic alternative to Xylene

## Homogenising Tissue

1. Place a sheet of bench coat on an open area of the lab floor.
2. Fill a one litre dewar and a two litre esky with liquid nitrogen.
3. Remove two steel homogenisers from the -80°C freezer and place in the esky (An esky can be used instead of a dewar as steel homogenisers can easily crack the dewar if dropped). Wait until the liquid nitrogen ceases bubbling.
4. Place tissue, numbered tubes, and spatulas into the 1 litre dewar.
5. Place metal tube block onto dry ice in an esky.
6. Weigh the tissue to obtain an estimate of the number of 250 mg aliquots.
7. Remove the homogeniser from the esky and place it in the class II safety cabinet.
8. Place tissue sample into the base to the homogeniser and put pestle on top.
9. Remove from the class II safety cabinet and place on the bench coat on the floor.
10. Place the wood block on top of the pestle as a shock absorber. Using a hammer, hit the wood 3 to 5 times.
11. Return the unopened homogeniser to the class II safety cabinet.
12. Remove the pestle. Break and mix powder with a frozen spatula. If repeat crushing is necessary, replace the pestle and repeat steps 8 and 9.
13. Loosen the lid of a screw top tube but do not remove.
14. Transfer powder to each of the labelled frozen tubes using a spatula and place in the metal block with their lids closed and transfer from the Class II Safety Cabinet to the balance for weighing. Each aliquot should be 250 mg.
15. Store tubes in liquid nitrogen.
16. Allow the used homogenisers to defrost at room temperature for approximately 2 hours in the safety cabinet.
17. In the safety cabinet, soak the homogenisers in a container of 2% Decon for 20 minutes.

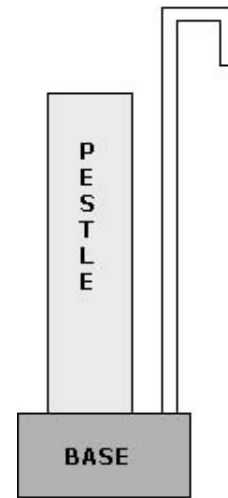


Figure 1: Steel Homogeniser

18. Wash and scrub the homogenisers in 2% Decon. Rinse well with tap water, followed by distilled water, and finally in 70% alcohol.
19. Leave the homogenisers to air dry at room temperature.
20. Wrap the homogenisers in foil and return them to the -80°C freezer for at least 3 hours or overnight before usage. It is important that homogenisers are completely dry before being frozen, otherwise base and pestle cannot be separated.

### **Preservation of RNA using RNAlater**

- Follow the recommended protocol for use of RNAlater.
- Tissue should be immersed for 12 hours before snap freezing in liquid nitrogen.
- RNAlater tissue should be stored at -80°C.

### **Preservation of RNA in Frozen Tissue Using RNAlater**

- This protocol is aimed to isolate intact RNA from archived frozen tissue for Laser Capture Microdissection (LCM) purposes.
- The tissue is assumed to be preserved at -80°C before RNAlater treatment.
- The protocol for preserving RNA in frozen tissue is as follows (Guo & Catchpoole 2003):
  1. Tissue should be placed in the cryomoulds and immersed in the OCT medium before frozen-sectioning with a cryotome.
  2. Tissue sections should be approximately 10µm in size and placed on glass slides.
  3. Immediately cover the frozen sections with 1.5ml RNAlater. Soak the sections for 1 hour at room temperature.
  4. Carefully decant and discard the solution.
  5. To remove any residue RNAlater and OCT medium, cover the sections in 1ml DEPC treated water. Carefully decant and discard the solution.
  6. Proceed **immediately** to LCM and RNA isolation.
  7. RNeasy RNA isolation kit (Qiagen) is recommended.

## **Section 6:**

### **Guidelines on Processing of Other Specimens**

These protocol guidelines are based on those presently used by ABN-Oncology tissue banks. However, depending on the planned end use of the sample, other processing methods may be more suitable.

#### **Urine Processing**

- Vapour-phase liquid nitrogen storage is recommended. Proteins, hormones and metabolites will all be stable if stored at -80°C or vapour-phase liquid nitrogen.
- Record total urine volume.
- Mix well to ensure homogeneity.
- Ensure cryovials are labelled.
- Aliquot according to the volume necessary for the proposed analysis and / or storage.

#### **Processing of Pleural Effusions**

1. Record the total volume fluid processed.
2. Working in the hood, place fluid into 50 ml tubes for centrifugation.
3. Spin at 4°C, 450g for 15 minutes.
4. Decant fluid into waste.
5. Gently resuspend cells in 50 ml cold PBS. Spin at 4°C, 450g for 10 minutes.
6. Decant supernatant. Combine all pellets into one tube. Resuspend with 50 ml cold PBS. Spin at 4°C, 450g for 10 minutes.
7. Take approximately 100 µl of the cell pellet and make a smear on a microscopic slide. Stain using the H & E procedure. This is to give an estimation of the tumour cell content.
8. Transfer the pellet into freezing vials that have been clearly labelled. Divide according to the size of the pellet. Absolute figures are difficult to provide but try to freeze at least 2 and no more than 6 vials.

Notes:

- Fluid may also need to be frozen for proteomic analysis
- Cells can also be frozen in Trizol.

- Whole effusion could also be frozen. This all depends on what you envisage the end application to be.

### **Buccal Cell Processing**

- On arrival at the laboratory, transfer mouthwash to 15 ml conical test tubes.
- Add 35 ml Tris-EDTA to the mouthwash sample and spin at 450g for 5 minutes.
- Decant supernatant and discard.
- Wash cells twice, each time with 45 ml Tris-EDTA.
- Resuspend cell pellet in 500 µl Tris-EDTA and transfer to 2 ml labelled cryovials.
- Store sample at -80°C.

### **Processing of Bone Marrow Aspirates**

#### **Bone Marrow Aspirate Smears –**

- Ensure slides are clearly labelled.
- Store in a slide box at room temperature.

#### **Bone Marrow Aspirate –**

1. Aliquot bone marrow into 500 µl aliquots in a labelled cryovial.
2. Add equal volume of freezing solution to bone marrow.
3. Place on ice. Place vials in a rate limiting freezer. Transfer to -80°C as soon as possible as DMSO is toxic at room temperature. Store at -80°C.

#### **Mononuclear Cells –**

1. Using a 1 ml Eppendorf pipette, transfer the bone marrow to a labelled 50 ml tube containing 10 ml RPMI 1640. Record the amount of bone marrow received.
2. Aliquot 3 ml Ficoll, after alcohol swabbing lid, into each of 2 labelled 10 ml tubes.
3. Carefully layer the diluted bone marrow onto each tube of Ficoll. Treat gently, do not mix, but spin as soon as possible.
4. Spin at 450g for 30 minutes.
5. Remove most of the top layer (RPMI 1640) using a 1 ml Eppendorf tip and discard (≈ 3 to 4 ml) into a waste container containing chlorine bleach.
6. Collect 1 ml white blood cells with the same Eppendorf tip using a swirling motion to vacuum up the white blood cells. Do not take too much Ficoll (third layer) as it is toxic to the cells. Place the white blood cells into a labelled 15 ml tube containing 10 ml RPMI.
7. Spin at 450g for 10 minutes.

8. Pour off the supernatant into a waste container containing hypochlorite powder or chlorine bleach (concentration to supplier's instructions). Ensure volume remaining is approximately 1000  $\mu$ l.
9. Count 120  $\mu$ l and record the number of cells (a cell counter such as a Sysmex can be used here). Cell count and total volume (1000  $\mu$ l) should be inserted in database when entering aliquots. Increase the total volume to 2 ml in the case of very large cell pellets. Adjust final count accordingly.
10. Make up to 3 ml with the freezing mix and resuspend (where the cell pellet is extremely large, divide into 2 vials).
11. Dispense the white blood cells into 1 ml labelled cryovials.
12. Place on ice. Place vials in a rate limiting freezer ('Mr Frosty') as soon as possible as DMSO is toxic at room temperature. Leave vials in the rate limiting freezer for up to 4 days (refer Section 12, page 53) before transferring them into the storage containers.
13. Transfer on a weekly basis to the liquid nitrogen tanks.

## **Section 7:**

### **Guidelines on Establishment of Cell Lines**

The following protocols are examples of the processes used in ABN-Oncology tissue banks to establish cell lines.

#### **General Guidelines**

- Collect blood in ACD / EDTA tubes.
- Cryopreserve white blood cells for future transformation (see above) or transformed lymphoblastoid cells in a medium containing 10% DMSO as the cryoprotectant.
- Freeze cells using a controlled rate of freezing – approximately 1°C per minute to -80°C (refer Section 12, page 53).
- Store in a liquid nitrogen tank within 72 hours of freezing (immersed in liquid nitrogen is best, but vapour storage is acceptable if properly managed).

#### **Establishing Permanent Lymphoblastoid Cell Lines (LCL's)**

**CAUTION:** All lymphocyte transformations must be performed in PC2 facilities. The technician should wear double gloves whilst working with viable viral supernatants and remove the top pair before leaving the biological cabinet. Plugged tips should only be used during the transformation procedure. This is to prevent aerosol contamination with the viral supernatant. Gloves and all disposable products (including plates and tubes) should be placed in a plastic bag and the bag securely sealed before it is removed from the biological cabinet. All waste must then be discarded in a biological biohazard bag for incineration.

Day 0 -

- Thaw an aliquot of the EBV viral supernatant on ice (refer Beck et. al. 2001).
- Thaw cells as described in “Successful transformation of cryopreserved lymphocytes: a resource for epidemiological studies” (Beck et. al. 2001).
- Spin cells down at 1200 rpm for 5 minutes. Discard supernatant.
- Resuspend the cell pellet in 200 µl of viral supernatant.
- Incubate at 37°C for 90 minutes.
- Add 900 µl of warm RPMI 1640 + 15% FCS + 2% PHA to the cell suspension. PHA stimulates mitosis in lymphocytes thereby inducing protein, DNA and RNA synthesis.

- Add 150 µl of the cell suspension to each well in a 96 well plate.
- Incubate for 8 days in a well humidified incubator at 37°C, 5% CO<sub>2</sub>.

Day 8 -

- Pool 2 of the 8 wells into 1 well in a 24 well plate. Repeat this step for the remaining 6 wells.
- Add 350 µl of warm RPMI 1640 + 15% FCS + 2% PHA to each well. Freeze 1 vial of cells at this stage.
- Incubate for 4 days at 37°C, 5% CO<sub>2</sub>.

Day 12 -

- Add 750 µl of warm RPMI 1640 + 15% FCS + 2% PHA to each well.
- Incubate for 4 days at 37°C, 5% CO<sub>2</sub>.

Day 16 -

- Examine the plates under an inverted microscope. A successful transformation is evident by the presence of growing viable cells. Typically the cells grow as tight non-adherent clusters of cells, resembling a ball of cells. However, in some instances cells may have not as yet clumped together, instead growing as discrete cells.
- Pool 2 of the 4 wells into a T25 flask. Add 2.5 ml of RPMI 1640 + 10% FCS. Repeat this step for the remaining two wells (this will act as the duplicate for each cell line).
- Incubate the cell line (keeping the flask upright as the cells grow in clumps on the surface of the flask), at 37°C, 5% CO<sub>2</sub>.

Day 20 -

- Remove flasks from the incubator.
- To determine whether the cell line needs re-feeding, look at the acidity of the medium and the density of the cell clumps. The colour of the medium acts as an indicator of the metabolic output of the cells. If the medium is yellow (acidic) and the cell clumps are numerous, the cell line is ready for feeding. Add 5 ml of warm RPMI 1640 + 10% FCS to only one of the two flasks. Only one flask of a pair is fed at a time, this is to prevent possible contamination of both flasks at one time.
- Incubate the cultures at 37°C, 5% CO<sub>2</sub>, keeping the flasks upright.



## Day 21 -

- Remove flasks from the incubator.
- Add 5 ml warm RPMI 1640 +10% FCS to the other flask.
- Incubate the cultures at 37°C, 5% CO<sub>2</sub>, keeping the flasks upright.

## Day 24 -

- Remove flasks from the incubator.
- Determine whether they require re-feeding. If the cell clumps are numerous and the medium is yellow, then add 30mls of warm RPMI 1640 + 10% FCS to one of the two flasks.
- Incubate the cultures at 37°C, 5% CO<sub>2</sub>, keeping the flasks upright.

## Day 25 -

- Remove flasks from the incubator.
- Add 5 ml warm RPMI 1640 +10% FCS to the other flask.
- Incubate the cultures at 37°C, 5% CO<sub>2</sub>, keeping the flasks upright.

Incubate the cell lines until they become confluent, i.e. the cell clusters become numerous and the medium acidifies. Disperse the cell clumps by pipetting the cell suspension several times with a 10 ml pipette. Take a 0.5 ml aliquot of cells from the flask and perform a cell count. There should be  $\approx 4 \times 10^5$  viable cells / ml.

Take 5 ml of the cell suspension from each flask and place it in separate 15 ml centrifuge tubes. Spin the cells down at 1200 rpm for 5 minutes. Discard the supernatant and resuspend the pellet in each tube with 1 ml of freezing solution. Add the cell suspension to a pre-labelled cryotube. Freeze the cells down as described in Beck et. al. (2001). Ensure the cryotubes are labelled. The two tubes represent the first freeze of each cell line and act as a back up source in case of future contamination. The two tubes are stored at -80°C and their location is recorded in the database.

## Potential Problems and Observations :

- Some cultures take longer than 24 days to reach confluency (the medium is pink and the clumps few). In this situation, remove 5 ml of the spent medium and replace with

5ml of fresh RPMI 1640 + 10% FCS. Incubate until the culture reaches confluency. Replace the spent medium every two weeks, if cell growth is slow.

- If cultures have not reached a stage where they can be subcultured after 3 months, they are discarded and the transformation is regarded as a failure.

### **Establishing a Cell Line from Melanoma Metastases**

1. Melanoma tissue (open biopsy, bronchoscopy, thoroscopy) is dissected in a sterile field to remove gross fat. A sliver selected by the surgeon must be supplied to pathology for diagnostic confirmation. The remaining tissue may be dissected into pieces suitable for specific applications. No specific minimum size suitable for establishing a cell line can be nominated, since large proportions may be necrotic.
2. Tissue is placed in cold sterile transport medium, typically saline or RPMI-1640 with gentamicin (40 micrograms / ml). Place tumour samples from different excision sites in different tubes. Ensure that tube is labeled. Place tubes on ice immediately. Record the number of tubes, patient ID number, and time of surgery. In addition record the excision site, approximate size, and any comment on the morphology of each tumour sample.
3. Tissue is transported at 2°C - 8°C, and should be processed within 48 hours of surgery.
4. Tissue fragments are mechanically disaggregated using crossed, single-use scalpels, in a 10 cm deep skirt petri dish, with a depth of 1 - 10 mm of sterile RPMI-1640. Mechanical processing is inefficient, but reduces fibroblast contamination.
5. Strain suspension through a sterile 100 micron mesh (BD).
6. Ficoll cells if a high level of debris is evident following disaggregation (270g / 30 minutes, one wash).
7. Resuspend in 10% FCS / RPMI-1640 supplemented with gentamicin and culture at serial dilutions in a flat-bottom, tissue culture grade plastic 96-well plate, avoiding outside wells. Typically, begin two-fold serial dilutions at 20,000 cells / well (require a total of  $0.4 \times 10^5$  cells). If excess cells are available, store in 10% DMSO / 4% human serum albumin in a rate limiting freezer at -1°C / minute, according to standard techniques, at  $1 - 5 \times 10^6$  cells / ml / vial.
8. Culture in 36°C humidified CO<sub>2</sub> incubator (5% - 8%) and change 50% of medium weekly.

9. Distinguish rapidly growing fibroblasts and lymphoblastoid cells from melanoma cells. Select wells that have selective growth of melanoma cells or in which melanoma cells ultimately outgrow fibroblasts (typically at 2- 4 months).

About 45% of tumours will establish in this way. A further 15% require extended culture, up to 8 months. A small proportion (5%) require very extended culture, up to 18 months. Care must be taken during this time to change the medium weekly, and maintain sterility.

10. Store in 10% DMSO / 4% human serum albumin in a rate limiting freezer at  $-1^{\circ}\text{C}$  / minute, according to standard techniques, at  $0.5 - 2 \times 10^6$  cells / ml / vial. Storage in HSA (compared to FCS) may facilitate shipping to certain countries.
11. Following storage, re-culture an aliquot in antibiotic-free medium. Harvest cells and supernatant at 7 days, and store for mycoplasma and sterility testing (Tryptone soy broth and thioglycollate). Mycoplasma testing should be done by two independent methods (e.g., PCR, Hoechst staining, Gen-Probe Kit, bioMerieux *Mycoplasma*IST2).
12. Perform expression profiling of melanoma cells to confirm melanoma origin.
13. Confirm patient origin by DNA fingerprinting (ABI profiler plus), usually compared to EBV-transformed matched LCL.

## Section 8:

### Isolation of RNA

#### Isolation of Total RNA from Frozen Bone Marrow using Trizol LS

Notes: 1. Reference: Barbaric D, Dalla-Pozza L, Byrne JA: A reliable method for total RNA extraction from frozen human bone marrow samples taken at diagnosis of acute leukaemia. *J Clinical Pathology* 2002, 55:865-867

2. This method can also be applied for frozen peripheral blood samples

2. Key Reagent: Invitrogen:10296-028, Trizol LS reagent

3. All reagents and tips must be RNase-free

1. Add appropriate volume of Trizol LS into a 50-ml Falcon tube (see table)

Add appropriate volume of 5 M Acetic acid into the same tube (see table)

BM VOLUME (ml)	VOLUME ADDED (ml)		
	Trizol LS (0.75 ml Trizol LS /0.25 ml BM)	5 M Acetic acid (30 ul/0.75 ml Trizol LS)	Chloroform (0.2 ml/0.75 ml Trizol LS)
≤ 0.5	1.5	0.06	0.4
0.75	2.3	0.09	0.6
1.0	3.0	0.12	0.8
1.25	3.8	0.15	1.0
1.5	4.5	0.18	1.2
2.0	6.0	0.24	1.6

2. Remove the bone marrow sample from -80°C (bone marrow sample is supposed to be kept in 2 ml cryovial). Immobilise it using the desktop vice. Cut the cryovial open with a Stanley knife using autoclaved blade.

3. Remove the bone marrow pellet from the vial rapidly in frozen state and place immediately into a Nagler tube containing Trizol LS and 5 M Acetic acid.

4. Homogenise completely using high-speed agitation Polytron Blender.

5. Incubate at room temperature for 5 min.

6. Add appropriate volume of chloroform (see table). Shake vigorously for 15 sec to emulsify.
7. Incubate for 3 min at room temperature.
8. Centrifuge at 12000 g for 15 min at 4°C.
9. Transfer top aqueous phase to a fresh tube.
10. Optional: (if contamination is suspected in the aqueous phase)  
Centrifuge at 12000 g for 10 min at 4°C.  
Transfer top aqueous phase again to another fresh tube.
11. Aliquot up to 750 ul of the aqueous phase and distribute to 1.5 ml microcentrifuge tubes.
12. Add 1:1 v/v of isopropanol to each tube. Mix well by inverting the tubes.
13. Incubate at room temperature for 10 min.
14. Centrifuge at 12000 g for 15 min at 4°C.
15. Decant supernatant carefully. Wash RNA pellet once with 1.3 ml of 75% ethanol. Mix well.
16. Centrifuge at 7500 g for 5 min at 4°C.
17. Decant ethanol carefully. Air-dry the RNA pellet for 10 minutes. Resuspend each RNA pellet in 10-30 ul RNase-free H<sub>2</sub>O in each tube.
18. Proceed to quantitation and quality control (refer to “RNA Quantitation and Quality Control” below).

### **Clean-up of Total RNA using RNeasy Kit**

- Notes: 1. Key Reagents: QIAGEN: 74104, RNeasy Mini Kit (Buffer RLT, Buffer RPE are provided in the kit by manufacturer)
2. All reagents and tips must be RNase-free
  3. A maximum of 100 ug total RNA can be used in this protocol.

Before start: 1).  $\beta$ -Mercaptoethanol ( $\beta$ -ME) must be added to Buffer RLT before use. Add 10 ul  $\beta$ -ME per 1 ml Buffer RLT. Buffer RLT is stable for 1 month after addition of  $\beta$ -ME. Mix thoroughly.

2). Buffer RPE: Add 4 volumes of 100% ethanol before using for the first time.

1. Adjust RNA sample to 100 ul with RNase-free water. Add 350 ul of Buffer RLT including  $\beta$ -ME. Mix thoroughly.
2. Add 250 ul of ethanol (96-100%) to RNA. Mix well by pipetting. Do not centrifuge.

3. Apply 700 ul of the sample to RNeasy mini column. Centrifuge for 15 sec at >8000 g. Discard the flow-through and collection tube.
4. Transfer the RNeasy column into a new 2 ml collection column. Pipet 500 ul Buffer RPE onto the RNeasy column. Centrifuge for 15 sec at >8000 g. Discard the flow-through.
5. Add another 500 ul Buffer RPE to the RNeasy column. Centrifuge for 2 min at >8000 g to dry the membrane. Discard the flow-through and collection tube.
6. Optional: Place the RNeasy column in a new 2 ml collection tube. Centrifuge at full speed for 1 min.
7. To elute, transfer the RNeasy column to a new 1.5 ml collection tube. Pipet 30-50 ul RNase-free water directly on the RNeasy silica-gel membrane. Centrifuge for 1 minute at >8000 g.
8. If the expected RNA yield is >30 ug, repeat step 7 again. The first eluate may be used to improve the final concentration, but the yield will be 15-30% less than the yield obtained using a second volume of RNase-free water
9. Proceed to quantitation and quality control (refer to “RNA Quantitation and Quality Control” below)

## Section 9:

## RNA Quantitation and Quality Control

## Quantitation of RNA with Spectrophotometry

1. Dilute sample RNA in 1×TE buffer, adjusting the A260 reading on the spectrophotometer between 0.1~1.0

Dilution times	1:10	1:50	1:100
RNA (ul)	1	1	1
1×TE buffer (ul)	9	49	99

2. Determine  $A_{260}$  and  $A_{280}$  with spectrophotometry
3. Measured RNA (ug/ul) =  $A_{260} \times 0.04 \times \text{dilution times}$  (1  $A_{260}$  = 40 ug/ml, 1:100 dilution)

The RNA purity ( $A_{260}/A_{280}$ ) should be between 1.7~2.1.

**Reagent Recipe:**

1 × TE buffer pH 7.4 (1L):	1M Tris·Cl, pH 7.4	10 ml
	0.5M EDTA, pH 8.0	2 ml

## Quantitation of RNA using RiboGreen

Notes: 1. RiboGreen is a fluorescent nucleic acid stain method which is more sensitive than spectrophotometry. A linear dynamic range of 10 ng/mL to 1 µg/mL can be achieved by the use of a high concentration of the dye. By the use of a lower concentration of the dye, a lower range of 2.5 ng/mL to 50 ng/mL can be measured.

2. Key Reagents: Molecular Probes: R-11490, Quant-iT™ RiboGreen® RNA Assay Kit
3. All reagents and tips must be RNase-free

### Reagents Preparation:

1. 1×TE: (100 ml): (20×TE is provided in the kit)  
20×TE 5 ml + DEPC H<sub>2</sub>O 95 ml (See “Reagents Recipe” of Quality Control of total using Denaturing Agarose Gel Electrophoresis)
2. RiboGreen Reagent: (On the day of experiment)
  - (1). For high-range: (200-fold)  
(100 ul/ sample)  
(eg. 6 samples + 5 samples for Standard Curve = 11 samples  
 $11 \times 100 = 1100$  ul  
5.5 ul of RiboGreen RNA quantitation reagent + 1094.5 ul of 1×TE)

(2). For low-range: (2000-fold)

(100/sample)

(eg. 6 samples + 5 samples for Standard Curve = 11 samples

$11 \times 100 = 1100 \text{ ul}$

0.55 ul of RiboGreen RNA quantitation reagent + 1100 ul of  $1 \times \text{TE}$ )

3. Standard RNA:

(1). For high-range: (2 ug/ml: 50-fold dilution of RNA Stock solution)

1800 ul is sufficient for 10 times of both high and low-range Standard Curve

36 ul of RNA Stock + 1764 ul of  $1 \times \text{TE}$

For 5 times of both high and low-range Standard Curve

18 ul of RNA Stock + 882 ul of  $1 \times \text{TE}$

(2). For low-range: (100 ng: 20-fold dilution of 2 ug/ml RNA solution)

1700 ul is sufficient for 10 times of low-range Standard Curve

85 ul of 2 ug/ml RNA solution + 1615 ul of  $1 \times \text{TE}$

For 5 times of both low-range Standard Curve

42.5 ul of RNA Stock + 807.5 ul of  $1 \times \text{TE}$

### Reagents Preparation for Standard Curve

1. For high-range:

Volume (ul) of $1 \times \text{TE}$	Volume (ul) of 2 ug/ml RNA Stock	Volume (ul) of 200-fold Diluted RiboGreen Reagent	Final RNA Concentration in RiboGreen Assay
0	100	100	1 ug/ml
50	50	100	500 ng/ml
90	10	100	100 ng/ml
98	2	100	20 ng/ml
100	0	100	blank

2.

3. For low-range:

Volume(ul) of $1 \times \text{TE}$	Volume(ul) of 100ng/ml RNA Stock	Volume(ul) of 2000-fold Diluted RiboGreen Reagent	Final RNA Concentration in RiboGreen Assay
0	100	100	50 ng/ml
50	50	100	25 ng/ml
90	10	100	5 ng/ml



98	2	100	1 ng/ml
100	0	100	blank

**Sample Preparation:**

1. Dilute the experimental RNA solution in 1×TE to a final volume of 100 ul in 96-well microtiter (eg. 1 ul of experimental RNA + 99 ul of 1×TE).
2. Add 100 ul of the aqueous working solution of the RiboGreen Reagent to each sample.

**Incubation:**

For all samples including samples of experiment and standard curve, mix well and incubate for 2 to 5 minutes at room temperature, protected from light.

Read on Fluorometer.

**Quality Control of Total RNA using Denaturing Agarose Gel Electrophoresis****1. Gel Preparation:**

- a. Make 1% agarose gel solution by diluting agarose into DEPC water, heating in microwave until completely dissolved, then cool in the air to ~60°C.

Solution Volume (ml)	50	100	200
Agarose (g)	0.5	1	2
DEPC H <sub>2</sub> O (ml)	36	72	144
10× MOPS buffer (ml)	5	10	20
37% formaldehyde(12.3M) (ml)	9	18	36

- b. Add appropriate volume of 10× MOPS buffer and 37% formaldehyde (12.3M).
- c. Pour the gel into the mould in a fume hood and wait ~1 hour to solidify the gel.

**2. Prepare the sample.**

- a. Using 1-5 ug RNA, adjust the volume to ~3 ul.

Note: Speedi-vac may be used to reduce the volume if the concentration of RNA is not high enough.

- b. Add 5-10 ul of Gel Loading Buffer to the sample. Ethidium Bromide can be added to the sample at final concentration of 10ug/ml.

For 100 ul of gel loading buffer:

48 ul	formamide
17.3 ul	37% formaldehyde (12.3M)
34.7 ul	loading dye (see reagent recipe)

- c. Heat denature sample at 55°C for 15 min. Cool on ice for 1min.
3. Electrophoresis: 60V for 2 hour in 1× MOPS buffer. (5V/cm until the blue dye front has migrated from a half to 2/3 of the way down the gel).
4. Result: Visualize the gel on a UV transilluminator.

#### Reagents Recipe:

DEPC H<sub>2</sub>O      Add 1 ml of DEPC in 2 Litre d.d.H<sub>2</sub>O

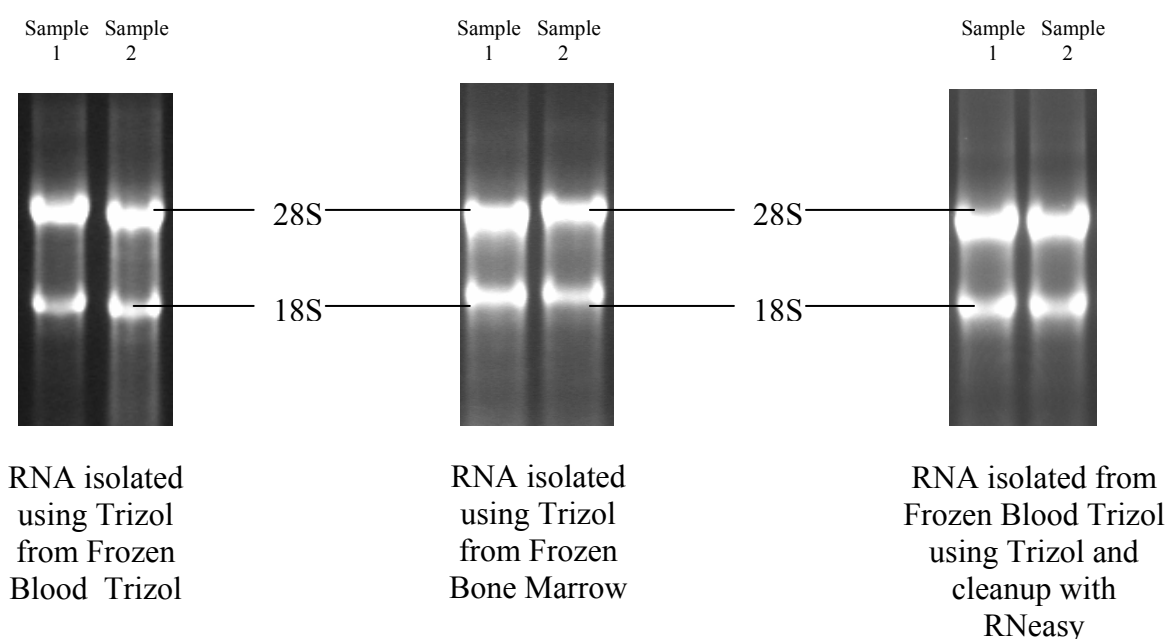
Incubate at 37°C overnight

Autoclave twice to burn off the DEPC.

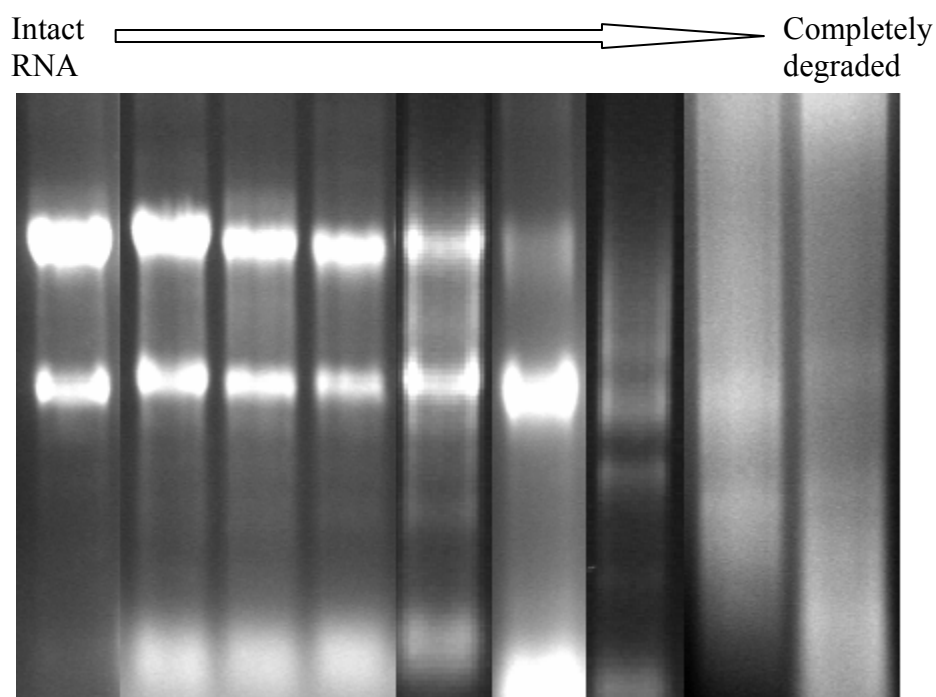
10× MOPS buffer (PH 7.0):    0.4M      MOPS  
    0.1M      sodium acetate  
    0.01M     EDTA  
    adjust PH to 7.0 with NaOH, keep at RT

Loading Dye:    80 ul      10× MOPS buffer  
                          50 ul      DEPC water  
                          50 ul      ethidium bromide (10 mg/ml)  
                          40 ul      sterile glycerol  
                          40 ul      saturated bromphenol blue in DEPC water  
                          keep at 4°C avoiding light.

#### RNA Quality of Different Samples and Methods



### Range of RNA Quality

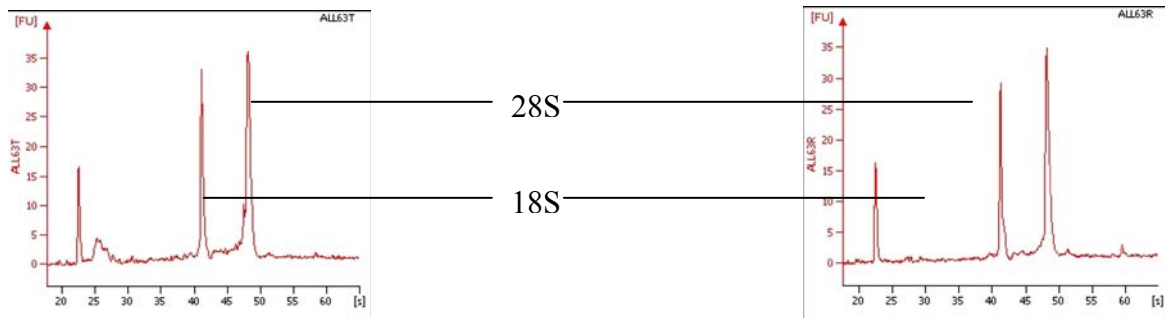


### Quality Control of Total RNA using Bioanalyser

Notes: 1. A RNA 6000 Nano chip can analyse 12 RNA samples in the 25-500 ng/μl range

2. RNA Integrity Number (RIN), which can be generated by Agilent 2100 expert Bioanalyser software, is a more accurate and sensitive indicator to assess the RNA integrity than 28S/18S. Compared with the standard RNA degradation pattern RIN is given to RNA sample, ranging from 10 (intact RNA) to 0 (completely degraded). RIN of 5 might not work for microarray experiments, but might work well for an appropriate RT-PCR experiment.

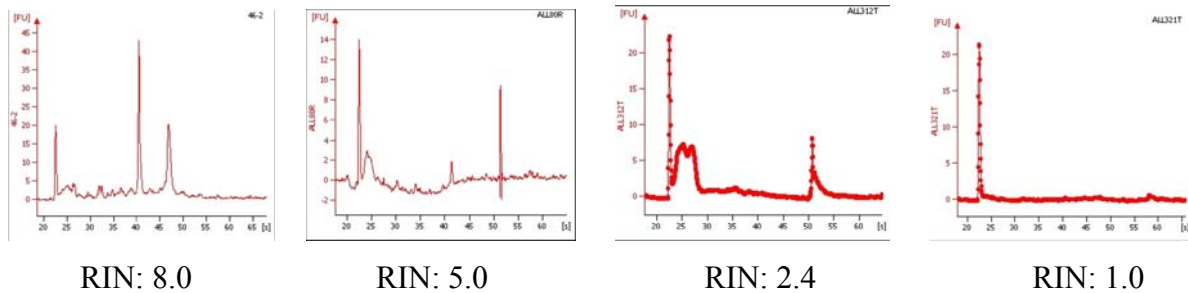
1. Adjust concentration of sample RNA between 25-500 ng/ul. Prepare 2 ul of each sample.
2. Follow the manufacturer instruction to mix the sample and RNA ladder with marker
3. Load the sample to Bioanalyser and run.



RNA isolated using Trizol  
from Frozen Blood Trizol  
28S/18S: 1.8  
RIN: 10

RNA isolated from Frozen Blood  
Trizol using Trizol and cleanup  
with RNeasy  
28S/18S: 1.8  
RIN: 10

#### RIN with different RNA quality



## Section 10:

### Extraction of Genomic DNA from Blood

Notes: 1. This method is suitable for 5 ml of whole blood. Approximately 100-200 ug DNA can be isolated.

2. This method can be applied for both fresh and frozen blood.

#### Reagents

##### TLB (Tris Lysis Buffer), 500 ml

11% Sucrose (W/V)	55 g	Sucrose
5 mM MgCl <sub>2</sub>	2.5 ml	1M MgCl <sub>2</sub>
10 mM Tris-HCl pH 8.0	5 ml	1M Tris-HCl pH8.0
1% Triton X 100 (W/V)	5 g	Triton X 100

Aliquot to 40 ml each and store at -20°C

##### NLS (Nuclei Lysis Buffer), 500 ml

0.1M Tris-HCl pH 8.0	50 ml	1M Tris-HCl pH 8.0
20mM EDTA pH 8.0	20 ml	0.5M EDTA pH 8.0
0.15 M NaCl	15 ml	5M NaCl

Autoclave and store at RT

#### Sample Processing

Frozen blood should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.

1. Add 5 ml blood to 40 ml TLB buffer, mix well to lyse the red blood cells. Spin at 4°C at 4800 rpm for 15 minutes.
2. Discard the supernatant carefully and leave the white blood cells untouched.
3. Add 5.5 ml of NLS buffer and resuspend the white blood cells gently. Add 0.25 ml 20% SDS and mix well to dissolve the pellet.
4. Incubate at RT for 5 min. Add 200 ul of fresh made proteinase K (10 mg/ml). Mix well and incubate at 50°C for 3 hours.

**Plasma Protein C Inhibitor (PCI) Purification**

1. Add 0.6 ml of PCI
2. Shake gently for 10 min.
3. Spin 5 min at 1500 rpm
4. Transfer the upper aqueous phase into a new 1.5 ml tube.

**Isopropanol Precipitation**

1. Add 4 ml of 7.5 M  $\text{NH}_4\text{AC}$  to 8 ml of sample to obtain final concentration of 2.5 M  $\text{NH}_4\text{AC}$ .
2. Add 12 ml of 100% isopropanol
3. Mix gently by inversion.
4. The precipitation is achieved when the precipitate floats.
5. Recover the DNA in a new 1.5 ml tube.
6. Rinse 3 times with 1.5 ml of 70% Ethanol.
7. Air dry 10 min.

**Extraction of Genomic DNA from Blood using FlexiGene DNA Kit**

- Notes: 1. Key Reagents: QIAGEN: 51204, FlexiGene DNA Kit (Buffer FG1, FG2, FG3 included)
2. This protocol is suitable for 1-3 ml of whole blood.

- Before start:
1. Resuspend the lyophilized QIAGEN protease in Buffer FG3 (hydration buffer): 0.3 ml when using the 50 ml FlexiGene DNA Kit
  2. Frozen blood should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.
  3. Calculate the total volume of blood to be processed. For every 1 ml of blood, mix together 500 µl Buffer FG2 (denaturation buffer) and 5 µl reconstituted QIAGEN Protease (see following table). The Buffer FG2/QIAGEN Protease mixture should be prepared not more than 1 hour before use.
  4. Heat a heating block or water bath to 65°C.

## Volumes of Buffer FG2 and QIAGEN Protease required for different batch volumes

Total volume of blood in batch (ml)	1	3	6	12	18	36
Volume of Buffer FG2 (ml)	0.5	1.5	3	6	9	18
Volume of QIAGEN Protease (µl)	5	15	30	60	90	180

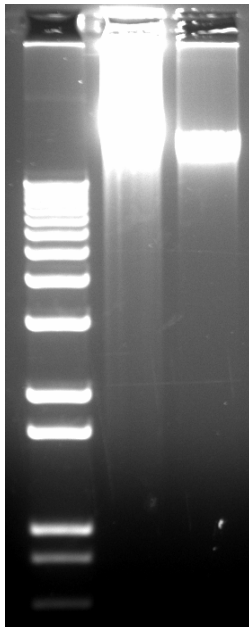
## Procedure

1. Pipet 5.0 ml Buffer FG1 into a 10 ml centrifuge tube. Add 2 ml whole blood and mix by inverting the tube 5 times.
2. Centrifuge for 5 minutes at 2000g in a swing-out rotor.
3. Discard the supernatant and leave the tube inverted on a clean piece of absorbent paper for 2 minutes, taking care that the pellet remains in the tube. Note: In rare cases the pellet may be loose, so pour slowly. Inverting the tube onto absorbent paper minimizes backflow of supernatant from the rim and sides of the tube onto the pellet.
4. Add 1.0 ml Buffer FG2/QIAGEN Protease (see “Before start”), close the tube, and vortex immediately until the pellet is completely homogenized. Inspect the tube to see that homogenization is complete. Note: when processing multiple samples, vortex each tube immediately after addition of Buffer FG2/QIAGEN Protease. Do not wait until buffer has been added to all samples before vortexing. Usually 3–4 pulses of high-speed vortexing for 5 seconds each are sufficient to homogenize the pellet. However, traces of pellet with a jelly-like consistency (often barely visible) may remain. If these traces are seen, add a further 300 µl Buffer FG2 and vortex again.
5. Invert the tube 3 times, place it in a heating block or water bath, and incubate at 65°C for 10 min. Note: The sample changes colour from red to olive green, indicating protein digestion.
6. Add 1.5 ml isopropanol (100%) and mix thoroughly by inversion until the DNA precipitate becomes visible as threads or a clump. Note: complete mixing with isopropanol is vital to precipitate the DNA and must be checked by inspection. For samples with very low white blood cell counts, in which the DNA may not be visible, invert the tube at least 20 times.
7. Centrifuge for 3 min at 2000 g. Note: if the resulting pellets are loose, centrifugation can be prolonged or a higher g-force can be used.
8. Discard the supernatant and briefly invert the tube onto a clean piece of absorbent paper, taking care that the pellet remains in the tube. Note: In rare cases the pellet may be loose,

so pour slowly. If the white blood cell count of the sample was sufficiently high, the DNA should be visible as a small white pellet.

9. Add 1.5 ml 70% ethanol and vortex for 5 seconds.
10. Centrifuge for 3 min at 2000 *g*. Note: If the resulting pellets are loose, centrifugation can be prolonged or a higher *g*-force can be used.
11. Discard the supernatant and leave the tube inverted on a clean piece of absorbent paper for at least 5 min, taking care that the pellet remains in the tube. Note: In rare cases the pellet may be loose, so pour slowly. Inverting the tube onto absorbent paper minimizes backflow of ethanol from the rim and sides of the tube onto the pellet.
12. Air-dry the DNA pellet until all the liquid has evaporated (at least 5 min). Note: Avoid over-drying the DNA pellet, since over-dried DNA is very difficult to dissolve.
13. Add 300  $\mu$ l Buffer FG3, vortex for 5 seconds at low speed, and dissolve the DNA by incubating for 1 hour at 65°C in a heating block or water bath. Note: If the DNA is not completely dissolved, incubate the solution overnight at room temperature. If a reduced volume of Buffer FG3 is used, the incubation time may need to be prolonged.

Marker   Sample 1   Sample 2



Genomic DNA isolated using  
FlexiGene DNA kit from  
Frozen Blood



## **Section 11:**

### **Guidelines on Database Requirements**

Following are some general guidelines on basic biorepository database requirements:

- Qualman et al (2004) state that ideally, a biorepository database should ensure that data is:
  - Maintained in a standard format,
  - Available over a long time,
  - Able to be distributed to others as needed,
  - Collected from satellite sites and combined.
  
- A biorepository database must be able to:
  - Provide all samples with a unique identifier generated by a standard coding mechanism. This identifier should be assigned at the time of collection, specific clinical and epidemiological data should be identified with the same number, and this number should be used to track a biospecimen from collection through processing, storage, and distribution (NCI 2005).
  - Keep track of consent (and withdrawal of consent).
  - Track original and current quantities of a biospecimens, as well as record when samples are exhausted (Qualman et al 2004).
  - Record sample location and biospecimen movements within or out of the biorepository (NCI 2005).
  - Provide reports and audit information.
  - Monitor quality control (Qualman et al 2004).
  - Store data securely and authenticate users to protect against unauthorised access to the data.
  - Have tiered levels of access so that users can only perform those operations for which they have permission (NCI 2005).

## Section 12:

### Guidelines on Storage of Biospecimens

The cooling rate and method of storing specimens can have serious effects on the viability of cells. Storage can affect the quality of the sample and determines whether future use is possible. The following must be taken into account when freezing specimens:

- Storage vessels – vessels used for storing samples which are to be frozen need to be able to withstand the sudden exposure to low temperatures that occurs with snap freezing, to be leak-proof even at low temperatures, and to be stable when stored for long periods of time at low temperatures (Morente et al 2006).
- Rate of cooling - the rate of cooling controls the size of ice crystals and how fast they are formed, which may affect cell recovery. A uniform cooling rate of -1°C per minute from ambient temperature is effective for a wide variety of cells.
- Storage - the temperature at which frozen specimens are stored affects the length of time after which cells can be recovered in a viable state. The lower the storage temperature, the longer the viable storage period.
- The critical temperature for sensitive organisms and cells is generally considered to be -140°C, the glass transition temperature, at which all metabolic activities cease.

Any freezers should have appropriate back-up protocols in case of equipment failure.

Below is a list of suitable long-term biospecimen storage temperatures. Note that the temperature list is the **maximum** acceptable. For example, while storage of some biospecimens at -80°C is acceptable, at this temperature metabolic activity has not completely ceased (ISBER 2005), but has only slowed down, and therefore cells cannot be stored for as long as those which are stored at lower temperatures.

#### Long-term Blood Product Storage

- Guthrie cards should be stored at room temperature.
- Whole blood can be stored at -80°C.
- Serum can be stored at -80°C.
- Blood pellets can be stored at -80°C.
- Non-lymphocytes can be stored at -80°C
- Plasma can be stored at -80°C.

- Buffy coat should be stored in liquid nitrogen.
- White blood cells should be stored in liquid nitrogen.

### **Long-term Storage of Cell Lines**

- Cell lines should be stored in liquid nitrogen.

### **Long-term Solid Tissue Storage**

- Snap frozen tissue should be stored in liquid nitrogen.
- Homogenised tissue should be stored in liquid nitrogen.
- Tissue preserved in *RNAlater* can be stored at -80°C.
- Formalin-fixed tissue should be stored in paraffin wax at room temperature.
- Alcohol-fixed tissue should be stored in paraffin wax at room temperature.
- H & E slides should be stored at room temperature.

### **Long-term Storage of Other Tissues**

- Buccal cells can be stored at -80°C.
- Urine can be stored at -80°C or in vapour phase liquid nitrogen.
- Bone marrow aspirate can be stored at -80°C.
- Bone marrow aspirate smears (on slides) should be stored at room temperature.
- Pleural effusions should be stored in vapour phase liquid nitrogen.

Ensure the storage locations are clearly recorded.

Note: Vapour phase liquid nitrogen storage is preferable over liquid phase storage. Use of vapour phase liquid nitrogen avoids the safety hazards inherent in liquid phase storage. Although the temperature of liquid phase liquid nitrogen is lower (-196°C), the temperature of vapour phase liquid nitrogen (-150°C) is still below the crucial glass transitional temperature.

### **Controlled Rate Freezing**

Before being placed in long-term storage, vials need to be cooled to -80°C at a rate of 1°C / minute. This can be achieved using a Nalgene “Mr Frosty” Cryo 1 Degree Celsius Freezing Container filled with 200 ml of isopropanol. Place vials in ‘Mr. Frosty’ and leave for a

minimum of 4 hrs and maximum of 4 days before transferring them into liquid nitrogen storage containers (Note: store 'Mr Frosty' at room temperature when not in use).

**Retrieval Considerations**

- Although slow cooling is generally best to ensure cell viability, the opposite is required when thawing from the frozen state. Agitation of the vial / ampoule in a 37°C water bath is preferable, but may be detrimental to certain cell types if the process is too lengthy.
- The rate and method of thawing specimens can have serious effects on the viability of cells.
- Repeated freeze / thaw cycles are to be avoided.
- As required according to the specimen type, maintain proper temperature of specimens during the retrieval process. For frozen specimens, keep vials on dry ice or in liquid nitrogen during the process.
- If specimens are to be shipped to an outside location, contact recipient at least 24 hours prior to shipment.
- In addition to temperature of storage, handling during removal from storage will affect viability of cells. Every time an ampoule / vial is exposed to a warmer environment even briefly, it experiences a change in temperature.

## Section 13:

### Guidelines on Transporting Biospecimens

When preparing to transport biospecimens, it is important to consider shipping time, distance, climate, season, method of transport, and regulations as well as the type and number of biospecimens to be sent and their intended use. Below are some general guidelines:

#### Packaging

- Use appropriate insulation, e.g. for 8°C to -20°C use gel packs, for -80°C use dry ice, and if samples need to be kept at -150°C, transport them in a dry shipper containing liquid nitrogen (NCI 2005).
- Ensure enough refrigerant is included to allow for a 24 hour delay in shipping (ISBER 2005). If dry ice is being used, ensure the packaging is vented i.e. packaging should not be taped shut to the point where any build-up of carbon dioxide gas cannot be released. If wet ice is being used, the packaging needs to be waterproof.
- IATA regulations require that when transporting biological specimens, 3 layers of packaging should be used:
  1. Watertight primary (inner) packaging, for example a zip-lock bag or cryotubes.
  2. Watertight secondary packaging, for example a Styrofoam container. Absorbent material should be placed between this and the outer packaging in case of leaks.
  3. An outer packaging, such as cardboard, of adequate strength for its capacity, weight, and intended use.
- Formalin-fixed paraffin embedded samples should be shipped at room temperature in insulated packaging to protect from extreme fluctuations in temperature (NCI 2005).
- Guthrie cards should be transported in water-tight plastic bags, and can be shipped at room temperature.
- Slides need to be cushioned to avoid breakage. Shipping temperature depends on processing eg fixed, stained and mounted slides can be shipped at room temperature whereas frozen sections for further processing should be shipped on dry ice.
- Desiccants can also be used for samples sensitive to humidity (ISBER 2005), e.g. Guthrie Cards.

**Paperwork**

- Before sending biospecimens, the recipient needs to be notified and a material transfer agreement (or similar) received (NCI 2005).
- Both the sender and the recipient need a log so that they can track packages (ISBER 2005).

**Regulations**

- Follow all relevant regulations, in particular the IATA “Infectious Substances and Diagnostic Specimens Shipping Guidelines 2005” ([www.iata.org](http://www.iata.org)), and label all packages appropriately (NCI 2005).
- When shipping biospecimens overseas, be aware of the receiver country’s requirements prior to the initiation of the shipment, and ensure that the consignment adheres to these regulations (ISBER 2005).

## Section 14:

### Guidelines on Quality Control for Biorepositories

Quality control is the system of technical activities measuring the attributes and performance of a process, or item, against defined standards, to verify that the stated requirements are met (ISBER 2005).

Each biorepository should have a quality management plan, which should describe procedures for conducting audits of:

- Equipment maintenance and repair
- Staff training records
- Data management
- Record keeping
- Protocols
- Quality of biospecimens.

The following are quality control protocols which can be used to ensure that quality of specimens is maintained.

#### **Solid Tissue**

**Histopathology:** H&E stained cryosections should be examined by a pathologist to determine tissue content (tumour cells, benign tissue, necrotic areas, etc.) and to verify tissue and disease type.

**RNA and DNA integrity:** mechanisms should be implemented to monitor RNA and DNA integrity and correlate with other features including time to freezing of original specimen, storage conditions, length of storage etc.

**Immunohistochemistry:** Staining of fixed tissues should be assessed and monitored (Morente 2006).

**DNA fingerprinting:** Random samples should be DNA fingerprinted and compared with matched blood samples to verify tissue identity.

**Researcher Feedback:** mechanisms should be implemented to record researcher feedback on sample quality and results.

**Whole Blood**

- DNA is made from the Guthrie card spot and the blood pellet from the same participant using Qiagen whole blood DNA kits.
- A PCR using 6 different microsatellite markers is performed and then run on a sequencing gel.
- The DNA profile of the Guthrie card versus the blood pellet is assessed for each participant.
- If either the Guthrie card or blood pellet DNA doesn't amplify or match for any one sample, the DNA extraction is repeated, PCR amplified and re-analysed.
- All errors between the Guthrie card and the blood pellet DNA should immediately be reported. In addition, DNA will be re-extracted and re-analysed.
- Should discordance between the Guthrie card and the blood pellet still occur, it should be reported to the Tissue Bank manager who will act on it accordingly.

**EBV Cell Lines**

- EBV cell lines are made from selected participants using one of the white blood cell freezes.
- DNA is made from the Guthrie card spot and the EBV cell pellet from the same participant using Qiagen whole blood DNA kits.
- PCR and gel analysis is performed and assessed as per the 'Whole Blood' quality control protocol (above).
- Discordance between the Guthrie card and the EBV cell pellet DNA will be reported and acted upon accordingly.

**Tumour Cell Lines**

- Tumour cell lines are made from selected participants using malignant tissue fragments or cells
- DNA is made from the Guthrie card spot and the tumour cell pellet from the same participant using Qiagen whole blood DNA kits.
- PCR and gel analysis is performed and assessed as per the 'Whole Blood' quality control protocol (above).
- Discordance between the Guthrie card and the tumour cell pellet DNA will be reported and acted upon accordingly.



- *Mycoplasma* testing should be performed when the cell line is established using an accepted method (e.g. PCR, Hoechst staining, Gen-Probe Kit, bioMerieux *Mycoplasma*IST2).

### **Biospecimen Storage**

- Audits should be conducted to check that biospecimen storage locations concur with database records (Morente et al 2006).
- Storage vessels (tubes, cassettes etc) should be checked to ensure they have remained intact (Morente et al 2006).
- Storage conditions should be monitored by a central alarm system and / or local alarms.
- Documented procedures should be put in place to monitor liquid nitrogen deliveries.
- Back-up systems and enough empty freezer space should be allowed in case quick transfer of specimens from malfunctioning freezers is required.
- Freezers and liquid nitrogen stores should have limited or controlled access.
- Having multiple storage sites (on or off-site) ensures that all specimens will not be destroyed in the case of freezer malfunction or other emergency situations.
- Periodic inventory audits should be conducted to certify location and identity of specimens.

## **Section 15:**

### **Suggestions for ‘Conditions of Use’ and ‘Access to Biospecimens’ Forms**

It is important to gain written agreement from researchers that they will adhere to the conditions of biospecimen use. Suggested clauses that could be included in a ‘Conditions of Use’ form are listed below:

- Specimens should be used in the manner described on the application form and only for the project approved.
- Specimens will not be given or sold to other investigators, nor used to produce commercial products.
- No attempts should be made by the investigator to identify the patient or to determine patient information other than what has already been supplied.
- The tissue bank does not accept any responsibility for the inadvertent provision of incorrect tissue.
- The tissue bank does not guarantee that mRNA will be obtained from samples.
- The tissue bank will do its utmost to ensure that the tissues are harvested and stored as well as possible, but cannot guarantee the condition of the material.
- All samples should be handled with the utmost care to prevent infection with pathogens. No responsibility will be taken by the tissue banks for injury or illness that may occur to staff handling the samples.
- The costs of packaging and transport of the specimens will be borne by the individual / institution requesting the samples and not by the tissue bank.
- The investigator/s is required to provide a report at the completion of the project indicating the percentage of samples which have provided useful information.

- At study completion or after publication, all viable remaining samples / data can be returned to the tissue bank for use by others.
- The investigator of the project will be required to acknowledge the tissue bank in any publications or presentations resulting from work on tissue bank samples.
- A copy of any abstracts / publications resulting from the use of tissue bank samples should be forwarded to the tissue bank.
- If research findings are made with supplied tissues that have implications for the patient or their family, the investigator/s must notify the tissue bank. This should be clearly defined with the relevant HREC governing the activity of the tissue bank.
- Violation of these conditions may render the individual and / or institution ineligible for future tissue bank applications.

For more information, please refer to the NHMRC 'Access to Facilities Policy' in Appendix 3.

## **Section 16:**

### **Suggested ‘Application for Use’ Form Requirements**

The NHMRC recommends that all biorepositories develop a ‘Request for Biospecimen Access’ form that is available electronically (NHMRC 2005) (Appendix 3). Possible suggestions that could be incorporated into these forms include the following requirements:

- A scientific protocol for the research project, including a rationale for the number and amount of specimens required.
- A list of the specific material required.
- Evidence that the project has obtained ethical clearance.
- For projects that have not already been peer reviewed, a list of 3 people to review the project.
- A list of relevant publications authored by the Chief Investigator within the last 5 years.
- A suggested timeline for the supply of tissue specimens.
- Evidence of a grant application approval or other funding source.
- Information on resources available for the project.
- An outline of consulting agreements, collaborations, and research projects between investigators named on the application and commercial organisations.

## Section 17:

### Laboratory Suppliers

Below is list of companies that supply some of the items required for the operation of a biorepository. This section is not necessarily an endorsement of the products; rather, it is intended as a reference list for newly established biorepositories. Products mentioned in the above sections that are not included in this list are generically available from most laboratory suppliers.

ITEM	SUPPLIER	CATALOGUE NO.
Fetal Calf Serum	Trace	Cat # 15010 –0500V
Bio bottles	Marair Tel 03 9335 2699	Cat # B/ 2.5
Bio bottle outer cartons	Marair Tel 03 9335 2699	Cat # BC/2.5
Small bio bottle Shipper infectious substance	Labtek Tel 07 3881 1388	Cat # LBSLABPAK11
Ficoll Paque research grade	Pharmacia-Biotech Tel 02 9850 3333	Cat # 17-1440-02
Cryocolour colour coded tops	Medos Tel 03 9562 6888	Mauve Cat # 3-75922 Pink Cat # 3-75884 Grey Cat # 3-75906 *White inserts are free with freezing vials
Tris-EDTA X 100 conc	Sigma Tel 03 9839 2930	
DMSO	BDH	Cat # 10323.4L
Vacurette 9 ml EDTA tubes	Greiner	
ACD tubes	Interpath Tel 03 9457 6277	Cat # 455055
RNAlater	Ambion	Cat # 7021
Gen-probe <i>Mycoplasma</i> detection kits	bioMerieux Australia Tel 1800 333 421	
Guthrie Cards	Whatman Tel 03 9787 7830	
RNAlater (500ml)	Ambion	Cat # 7021
‘Mr Frosty’ Freezer	Integrated Science Tel 03 9819 7399	
Steel Homogeniser	Refer Figure 1	

OCT medium	Tissue Tek	
Lithium Heparin	Greiner	

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Also:

Australian Ovarian Cancer Study Laboratory Manual, accessible from Nadia Traficante, Peter MacCallum Cancer Centre, [n.traficante@petermac.org](mailto:n.traficante@petermac.org)

Children's Hospital at Westmead Tumour Bank Standard Operating Procedures, accessible from Dan Catchpoole, Children's Hospital at Westmead, [DanielC@chw.edu.au](mailto:DanielC@chw.edu.au)

kConFab Laboratory Manual, accessible from Heather Thorne, Peter MacCallum Cancer Centre, [h.thorne@petermac.org](mailto:h.thorne@petermac.org)



Queensland Institute of Medical Research Cell Line Establishment Protocol, accessible from Chris Schmidt, Queensland Institute of Medical Research, [ChrisS@qimr.edu.au](mailto:ChrisS@qimr.edu.au)

Peter MacCallum Tissue Bank Laboratory Manual, accessible from Lisa Devereux, Peter MacCallum Cancer Centre, [Lisa.Devereux@petermac.org](mailto:Lisa.Devereux@petermac.org)

Australian Mesothelioma Tissue Bank Consent and Information Forms, accessible from Jenette Creaney, Sir Charles Gardiner Hospital, [creaneyj@cyllene.uwa.edu.au](mailto:creaneyj@cyllene.uwa.edu.au)

Western Australia Research Tissue Network Laboratory Manual, accessible from Lydia Glavinas, Western Australia Research Tissue Network, [Lydia.Glavinas@health.wa.gov.au](mailto:Lydia.Glavinas@health.wa.gov.au)

## Appendix 1

### WA RESEARCH TISSUE NETWORK - CONSENT FORM

#### Consent for tissue/blood taking and tissue/blood banking for clinical research

I, \_\_\_\_\_ of \_\_\_\_\_  
have read the Information Brochure entitled "WA RESEARCH TISSUE NETWORK".  
(Doctor or health professional) ..... has explained to me and I  
understand the consequences involved in my voluntary donation of tissue and/or blood for the  
WA Research Tissue Network. **I have had an opportunity to ask questions and am  
satisfied with the answers given**

*In making my donation, I understand and agree that:*

the **tissue/blood** (which in this consent form, includes its constituents and any cell lines  
derived from the **tissue/blood**) will be used in relation to **(WRITE IN PROJECT NAME)**  
samples of any **tissue/blood** or derived cell line/s held in a bank will be discarded upon my  
written request to the WA Research Tissue Network Project Manager,  
I will be approached by a medical professional in regard to potentially important health  
information that arises from this research that may impact upon me or my family. At that time  
I may choose what further information or investigations I would like to proceed with,  
I can request to know more specific details of any studies that used my samples at any time by  
contacting the WARTN,  
Research results, and the fact that I have made this donation, will not be revealed to any 3<sup>rd</sup>  
party not directly part of medical research without my written consent, except under subpoena

The WA Research Tissue Network will not be liable for any loss of or damage to, the  
**tissue/blood** used in accordance with this form,

I will not benefit financially if this research leads to development of a new treatment or  
medical test,

Storage of and access to my **tissue/blood** will be managed by an Advisory Committee and  
only released where the research proposal has been approved by a Human Research Ethics  
Committee,

I understand that international research collaboration using my tissue/blood will only take  
place where researchers abide by equal or more stringent regulations of privacy and ethics as  
those in Australia, as assessed by a Human Research Ethics Committee

I give permission to access health information about me related to the research area defined in  
point 1 above, such as is kept in a medical record or by the WA Department of Health, to  
assist medical research only where the research proposal has been approved by a Human  
Research Ethics Committee.

I **Do** ☐ **Do Not** ☐  
consent to the storage and use of my tissue and/or blood (delete as appropriate) for  
biochemical and genetic based medical research.

.....  
Name of Patient

.....  
Signature of Patient

.....  
Date

.....  
Name of Doctor.....  
Signature of Doctor.....  
Date

At present there are no international or national laws governing the storage of human cell lines/tissues; however, this form is consistent with NH&MRC National Guidelines for medical research involving humans that were used by the **King Edward Memorial Hospital** Human Research Ethics Committee (HREC) to approve its use. If you have any ethical concerns you can contact the Secretary of the HREC on 08 9340 8221. All study participants will be provided with a copy of the Information Sheet and Consent Form and a copy will be put in their Medical Record.

## **AOCS INFORMATION BROCHURE**

We are conducting a national medical research study to look at the causes of ovarian cancer. **For this study we need to talk to women with ovarian cancer, women who are having ovarian surgery and women who do not have ovarian cancer.** You have been invited to take part in this study and this brochure will tell you what the study is all about. Before you decide to take part in the study we want you to have all the facts. Please read this brochure carefully and discuss any questions you have with a member of the research team. If you agree to take part in the study we will ask you to fill out some questionnaires. You will also be asked if you will donate a small sample of blood and urine and, if you are about to undergo surgery, some of your tissue that is removed at surgery.

### **WHO IS CONDUCTING THE STUDY?**

The study is being conducted by scientists at the Queensland Institute of Medical Research (QIMR) and Peter MacCallum Cancer Institute (PMCI) together with doctors at many of the big hospitals in Australia. The study has been approved by the Research Ethics Committees of these institutions in accordance with the guidelines of the National Health and Medical Research Council of Australia.

### **WHY DO YOU WANT ME TO TAKE PART?**

We are doing this national study to try to find out what causes ovarian cancer so we can try to prevent it from occurring in future generations of Australian women. We want to enrol more than 2000 women in the study to look at genes and environmental factors that might influence whether or not a woman gets ovarian cancer. If you take part your donation may help discover new tests for cancer or new treatments to prevent or cure disease.

### **WHAT WILL BEING IN THE STUDY MEAN FOR ME?**

Being in the study will involve the following steps:

- (1) completing two questionnaires: one about your general health, lifestyle and well being and another about your diet (this should take between 30 and 60 minutes)
- (2) a short interview (20-30 minutes) conducted somewhere convenient to you or by telephone
- (3) giving permission for the doctors conducting the study to access your relevant medical and pathology records (including tumour material) and clinical cancer genetic test results (if applicable)
- (4) *if you are agreeable*, we would like to collect a small blood sample and a urine sample
- (5) *if you are about to have surgery*, we would also like permission to keep some of the tissue that is removed at surgery.

There will be no direct benefit to you from taking part in the study however the results of the study are likely to help people who have cancer and other diseases in the future.

There will be no cost to you and if you do not want to take part this will not affect your future medical care in any way. You will also be free to withdraw from the study at any stage if you no longer wish to continue.

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**IF I GIVE A BLOOD SAMPLE, WHAT WILL IT BE USED FOR?**

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We will ask your consent to collect a small amount of blood from a vein in your arm (30ml or about 6 teaspoons). If you agree to this and are having surgery the blood sample will be collected at that time. If you are not having surgery, a trained research nurse will take the sample when she collects your questionnaires. Alternatively you could have the sample taken by your GP or local pathology lab.

We will use your blood sample to do biochemical and genetic tests (see section on Genetic Testing on page 4) to look for factors that make someone more or less likely to get cancer or that affect how a cancer responds to treatment. We may also use your blood to make long-lived cell lines and will keep some of your sample indefinitely for future research studies (see the section on 'Future Research' below). For example, a number of blood tests are currently done to detect certain types of cancer and it is important to have blood samples from people with and without cancer to evaluate these tests.

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**IF I GIVE A URINE SAMPLE, WHAT WILL IT BE USED FOR?**

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We will ask your consent to collect a small urine sample. This will be stored indefinitely in a freezer for future biochemical and genetic research studies into ovarian cancer (see the section on 'Future Research' below). For example, it may be possible to develop urine tests to detect certain types of cancer and it is important to have samples from people with and without cancer to evaluate these tests.

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**WHAT WILL BE DONE WITH MY TISSUE?**

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If you are having surgery your doctor will remove some body tissue to do some routine tests. The results of these tests will be given to you by your doctor and will be used to plan your care. If there is any remaining tissue we would like to save this for cancer research. We will not take any extra tissue for this study.

Your tissue will be stored in a freezer and will be used only by qualified Medical Researchers for biochemical and genetic studies of cancer. These studies aim to understand more about what causes ovarian cancer and why treatment seems to work for some women but not for others. We may also use your tissue to make long-lived cell lines. Your tissue will be valuable for this research whether or not you have cancer. These studies have to be approved by the Scientific and Human Research Ethics Committees at the QIMR and PMCI and at the institution carrying out the research. These bodies abide by the ethical and scientific principles set out by the National Health and Medical Research Council of Australia.

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**HOW IS MY PRIVACY PROTECTED?**

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When you fill out a questionnaire or donate a sample of blood or urine or tissue to this study we will make every effort to protect your privacy.

- All your questionnaires and samples will be stored securely in such a way that they cannot immediately be identified as having come from you. They will be labelled with a unique barcode number so that they do not get confused with questionnaires or samples from someone else.
- Any identifying information (your full name, address etc) will be stored separately from the samples and information you provide. Access to this identifying information is restricted to a small number of senior members of the study team.
- No information that could be used to identify you or your family will be included in any report on the results of the study.

- This is an independent research study that is funded by the “Ovarian Cancer Program” of the U.S. Army Medical Research and Material Command. Representatives of the U.S. AMRMC are eligible to review research records as a part of their responsibility to protect human subjects in research.

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### **CAN I WITHDRAW FROM THE STUDY?**

You may withdraw from the study at any time and this will in no way affect your medical treatment in the future. If you decide now that your tissue and blood can be kept for research, you can still change your mind later. Just contact us and let us know that you do not want us to use your samples. Any blood or tissue (or any products derived from them) that is left in the bank will then be destroyed.

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### **WILL I FIND OUT THE RESULTS OF THE RESEARCH USING MY TISSUE OR BLOOD?**

The results of research done with your tissue or blood are not likely to be available in the immediate future. This is because research can take a long time and must use tissue samples from many people before the results are known. We will not be able to give you the individual results from your samples (except in exceptional circumstances, see Genetic Testing on page 4) but everyone who takes part in the Australian Ovarian Cancer Study will be sent an information update to let you know in general, how the study is going. You can also contact the Study Team at any time if you have any questions about the study.

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### **FUTURE RESEARCH USING YOUR INFORMATION AND SAMPLES**

After we have finished this particular study we will keep the information and remaining samples that you give us indefinitely. In the future we may match your personal data against other health registers and we will use your samples for future biochemical and genetic studies of ovarian cancer. We may also contact you again to ask you to take part in a follow-up study but you will be under no obligation to do so. Any extra studies that use your samples will have to be approved by the Scientific and Ethics Committees at QIMR, PMCI and the institution carrying out the study before your material can be used. Any information or material given to researchers will be identified by a code only so it will not be possible for them to identify you in any way. You will not receive any notice of future uses of your information or samples.

There is a chance that information derived from the samples that you are donating under this study may, in the future, have some commercial value, for example if they lead to the development of a commercial product. You will not be compensated for your participation in the study or for any future value that your samples may be found to have. However, it is our intention that if money is generated as a result of research using your samples then some will be put into a special fund to be used for future research into ovarian cancer.

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### **WHAT IF I HAVE MORE QUESTIONS OR A COMPLAINT ABOUT THE STUDY?**

If you have any questions about the study, please call our *free AOCs Helpline* on **1800-222-600**

or:

Suzanne Moore (Project Manager) (07) 3845 3547

Or:

Dr Penny Webb (a study investigator) (07) 3362 0281

If you have a complaint or would like to speak to someone who is not involved in the study, you can contact:

- the Chairman of the **QIMR Human Research Ethics Committee** by phoning the Secretary on (07) 3362 0259
- the Ethics Coordinator, Human Research, at the **Peter MacCallum Cancer Institute** on (03) 9656 3771
- A representative from your hospital (see list on page 5)

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### **GENETIC TESTING AND THE AUSTRALIAN OVARIAN CANCER STUDY**

People respond differently to things they come into contact with such as smoke, alcohol, foods and medications. We think that the reason people respond differently is partly due to their genes. Some genes may make people more or less likely to become ill when they come into contact with particular factors. We are trying to find out which genes these are, in the hope that this will lead to new treatments that act in the same way as the ‘good’ genes. We would greatly appreciate your participation in the genetic part of the Australian Ovarian Cancer Study. However this decision is entirely your own and you do not have to give us any explanation if you do not want to take part.

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### **HOW DO WE GET A SAMPLE OF YOUR GENES?**

DNA is the genetic molecule of life and it carries the hereditary information that underlies the physical and behavioural characteristics of all living things. DNA makes up genes, and genes are arranged into larger structures called chromosomes.

There are two common ways of obtaining a sample of DNA – either from a blood sample, or by taking a swab of cheek cells from the inner lining of the mouth. When your sample arrives at our laboratory, we extract DNA from it. Scientists use centrifuges and chemicals to purify the cells and to extract purified DNA from these cells. The purified DNA will then be screened to look for genes which are thought to be related to ovarian cancer. If you have in the future, or have already had any other clinical genetic tests for cancer we seek your permission to access the results of these tests.

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### **WHAT IF WE FIND IMPORTANT GENETIC INFORMATION ABOUT YOU?**

It is possible that future research using your sample may result in new genetic information about your risk of getting cancer. If research findings are made that may have significant implications for you or your family, the researcher is obliged to submit a full report to a Scientific Committee who will then consider whether you or your family might benefit from the chance to learn more about this information.

It is your choice whether or not you wish to know of any important results and we will ask you if you want us to tell you if we do find any information that has significant implications for you or your family. We will also ask you if you want information to be given to a member of your family if we cannot contact you. **We will not give any information about you to members of your family without your permission.**

If we find some important information and you have declared that you do wish to know of any significant results, we will do our best to contact you to ask if you want to find out more about these findings. You would be given genetic counselling about the implications of receiving genetic information and, if you then decided that you wished to obtain your individual results, you would be asked to give a second blood sample so that the research results could be confirmed in an accredited testing laboratory.

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**ARE THERE ANY RISKS IF I PARTICIPATE?**

When a blood sample is taken there is a small chance that you might experience slight bleeding, minor bruising or pain. All blood samples will be taken by trained personnel who will be qualified to manage any such problems.

Currently in Australia, genetic testing does not affect your ability to obtain private health insurance (although there may be a waiting period for pre-existing conditions). It is, however, possible that in the future the fact that you have taken part in a study involving genetics may affect you or your family if you want to take out a new health, disability or life insurance policy. We will not pass on this information about you to anyone, including your family members, without your written permission unless lawfully obliged to.

**INDEX OF HOSPITAL ETHICS COMMITTEE CONTACTS****NEW SOUTH WALES:****Hunter Hospital**

The Professional Officer  
Hunter Area Research Ethics Committee  
C/- HAHS  
Locked Bag 1  
NEW LAMBTON NSW 2305  
Tel: (02) 4921 4950 Fax: (02) 4921 4818

**Royal Hospital for Women**

Ms. K Breheny  
Research Ethics Secretariat,  
Prince of Wales Hospital,  
Cnr. High and Avoca Streets  
Randwick NSW 2031  
Tel: (02) 9382 3583  
Email: brehenyk@sesahs.nsw.gov.au

**Royal North Shore Hospital**

Ms Tegan Cox  
C/- Research Office  
Level 4, Vindin House  
Royal North Shore Hospital  
St Leonards NSW 2065  
Tel: (02) 9926 8106 Fax: (02) 9926 6179

**Royal Prince Alfred Hospital**

Secretary, Ethics Review Committee  
C/- Research Development Office  
Room 608A, Level 6 Building 82  
Royal Prince Alfred Hospital  
Missenden Rd  
Camperdown NSW 2050  
Tel: (02) 9515 6766

**Westmead Hospital**

Ms Jillian Gwynne Lewis  
Patient Representative  
Westmead Hospital  
Westmead, NSW 2145  
Tel: (02) 9845 7014

**North Shore Private Hospital**

Westbourne Street  
St Leonards  
NSW 2065

**QUEENSLAND:****Wesley Hospital**

Wesley Hospital Multidisciplinary Ethics  
Committee  
PO Box 499  
Toowong Q 4066

**The Townsville Hospital**

Townsville Health Service District  
Institutional  
Ethics Committee  
PO Box 670  
Townsville Q 4810

**Mater Misericordiae Hospitals**

Ms Odette Petersen  
Mater Research Secretariat

**Royal Women's Hospital**

Ms Jenny Lonton  
Medical Services

Level 2, Aubigny Place  
Raymond Terrace  
South Brisbane, QLD 4101  
Tel: (07) 3840 1585 Fax: (07) 3840 8546

Level 4, Ned Hanlon Building  
Royal Women's Hospital  
Butterfield St, Hertson, Qld 4029  
Tel: (07) 3636 5490

**SOUTH AUSTRALIA:**

**Flinders Medical Centre**

Ms Carol Hakof  
Administrative Officer – Research  
Level 7, Flinders Medical Centre  
Bedford Park, SA 5042  
Tel: (08) 8204 4507 Fax: (08) 8204 4006

**Royal Adelaide Hospital**

The Chairman, Research Ethics  
Committee  
Royal Adelaide Hospital  
North Terrace, Adelaide 5000  
Tel: (08) 8222 4139

**VICTORIA:**

**Freemasons Hospital**

The Secretary, Research Ethics  
Committee  
Freemasons Hospital  
166 Clarendon Street  
East Melbourne 3002  
Tel: (03) 9483 3553

**Royal Women's Hospital**

The Secretary, Research Ethics  
Committee  
The Royal Women's Hospital  
Royal Women's Hospital  
132 Gratton Street,  
Carlton VIC 3053  
Tel: (03) 03 9344 2759



## **AUSTRALIAN OVARIAN CANCER STUDY**

### **CONSENT FOR RESEARCH PARTICIPANT COPY**

I have read this Information Brochure .....YES  
 The consequences involved in participation in this research study have been explained to me and I understand these .....YES  
 I have had an opportunity to ask questions and am satisfied with the answers I have been given ..... YES

**I** ..... *(please print name)* **hereby voluntarily consent to:**

(1) Participate in the AOCS study as described in the Information Brochure...YES NO

(2) The following samples being collected and used for biochemical and genetic analysis as described in the Information Brochure:

a. A small (30mls) blood sample ..... YES NO

b. A urine sample ..... NOT APPLICABLE..YES NO

c. A tissue sample ..... NOT APPLICABLE...YES NO

(3) Allow the AOCS researchers access to my:

- medical, oncology and pathology records (including tissue blocks / slides)from any treatment centres for the duration of the study .....YES NO

- *clinical cancer genetic test results* .....*NOT APPLICABLE YES NO*

I wish to be contacted if findings are made that have implications for me or my family ..... YES NO

I give permission for these findings to be revealed to members of my family if they request this information ..... YES NO

In making my donations I understand that:

- The tissue/blood (including its constituents and anything derived from it) will be stored indefinitely at PMCI/QIMR and will be used for this and future biochemical and genetic studies of cancer.
- The samples and questionnaires will be stored in a coded system to maintain confidentiality.
- There will be no cost, nor any financial benefit to me for participating in the study. If my samples lead to the development of a commercial product in the future I will not receive payment for this.
- If at any time I decide that I no longer wish to participate in the study, my samples will be discarded upon my written request to the Study Investigators. This will not affect my future medical treatment.
- The samples will remain in the custody of the PMCI and QIMR. They will be stored in good faith, but their suitability for future use cannot be guaranteed. Samples will not be used for purposes other than those agreed to in this consent form.
- All studies using my samples will have to conform with the ethical and scientific principles set out by the National Health and Medical Research Council of Australia, the *Privacy Act 1988* and the Guidelines approved under section 95A of the *Privacy Act (2001)*. I will not be notified about future use of my samples.
- I may be approached again to participate in future studies but I am under no obligation to do so.

**SIGNATURE:** ..... **Date:** .....

**Address:** .....

**WITNESS: Name:** ..... **Signature:** .....  
**Address:** ..... **Date:** .....

## Appendix 2

### QUICK REFERENCE TABLES – BLOOD COLLECTION & PROCESSING

Tube	Fraction	Comments
EDTA (purple top)	All	Good multi-purpose tube For proteomics – blood in EDTA is slightly less stable and must be processed quicker, but EDTA may inhibit proteases. Not appropriate for assays where metal ions are necessary. May be problematic for cytogenetic analyses (Vaught 2006)
ACD	Particularly good for white blood cells. Not for plasma.	Good if time lapse between collection and processing – viable white blood cells can be extracted up to 10 days after collection. As there is a large amount of ACD added to the tubes, they are not suitable for plasma as it will cause dilution.
Lithium Heparin	Plasma, Buffy Coat.	Generally only used for cytology studies, may interfere with PCR (Yokota et. al. 1999). Unsuitable for DNA extraction.
Serum Gel Separator Tube	Serum	May not be optimal for proteomics
Sodium Citrate (light blue)	Plasma	For proteomics - platelets are most stable in citrate, although it dilutes the blood sample, which can lower immunoassay measurements.
Clotted Tube (red top – plain)	Serum	

Blood Product	Use
Guthrie Cards	DNA extraction. Cheaper and easier alternative to whole blood.
Blood Pellets (white cells)	Isolation of DNA.
Plasma	Bioassays, proteomic analysis, and biomarker discovery.
Double Spun Plasma	Isolation of plasma DNA
White Blood Cells	DNA extraction and the creation of cell lines.
Non-lymphocytes	DNA extraction
Serum	Bioassays, proteomic analysis
Buffy Coat (in DMSO)	EBV cells transformation (note: excess red cell contamination may have detrimental effects on efficiency)
Whole Blood	DNA & RNA extraction

## **Appendix 3**



**Australian Government**

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**National Health and Medical Research Council**

# **ENABLING GRANTS ACCESS TO FACILITIES POLICY**

The purpose of this policy is to develop a generic set of guidelines to assist in the development of access policies for the facilities currently funded under the Enabling Grants Scheme. It should be noted that some issues will clearly vary from facility to facility and therefore each facility will need to develop specific guidelines in conjunction with the more general ones.

There is a need to address the problem of adequate access and “too much” access. The NHMRC feels it is necessary that fair and equitable access be available to all Australian researchers. Conversely, access clearly cannot be carte blanche and certain caveats will invariably need to pertain. The NHMRC is to conduct a 12 month administrative review to clarify, amongst other issues, what mechanisms have been put in place by each of the facilities including an **Access Policy**.

In formulating an access policy the following general principles should be taken into consideration:

There should be a clear and transparent access policy which enables equitable access for all Australian researchers; acknowledging that different internal and external rates may apply.

Access may, to some extent, be determined by limitations around the resource (e.g. a tissue bank may have limited amounts or numbers of a given tissue).

The process should be overseen by an independent body of eminent persons.

An appeals process for dispute resolution should be established. Broader issues of governance should also be addressed as they may influence the mechanism by which disputes are managed.

It is not the NHMRC's intention to influence the issue of international or commercial access. Such decisions are the prerogative of the facility so long as international or commercial access does not in any way compromise the access of Australian researchers or the quality or availability of the resource.

**Acknowledgement.** It is obviously mandatory that investigators acknowledge the facility in any published work that results from accessing the resource. It is suggested that each facility specify this requirement including the wording of the acknowledgement.

### **Specific suggestions:**

A specific request for access form be developed by the facility which is available electronically.

In most but not all instances, ethical and/or scientific approval of the research will be needed. It is suggested that this be an essential component early in the application process. In the case of tissue and tumour banks, it is suggested that approval for the project comes from the institutional scientific committee from whence the application is being made.

Applications so submitted should go to a properly constituted management committee of the facility for sign-off. It is essential that the management committee have a clear degree of independence (ie should not consist exclusively of investigators who access the facility) and have clear policies in place for the management of conflict of interest.

The independent appeals process may be institutionally-based, eg a university committee, but again it is essential that it be seen to be independent of any influence by that institution with clear independent membership external to the institute.

The issue of collaboration and authorship has been raised in discussions. This is clearly a sensitive and difficult issue. By their nature, such facilities are funded for the public good and ostensibly run, also for the public good, which in this case means the facilitation of first quality science. They are not seen as the province of one particular investigator or group of investigators and thus it is difficult to see a case for automatic authorship/collaboration status for the CI's on the original Enabling Grant application. On the other hand, in some cases, significant input may derive from members of the facility that goes beyond that defined in the simple acknowledgement. If it is envisaged that such situations will occur, then it is again suggested that a transparent, clearly articulated policy is developed up front to avoid conflict and to allow external bodies, ie the NHMRC, to arbitrate on the appropriateness of the policy. If such a policy is adopted, then again a clear, transparent and independent resolution process should be developed to avoid conflict of interest.

In certain circumstances it would be appropriate for the facility to consider issues of liability/risk management in the context of their policy development.

Grant applications dependent on access to the facility. Concern has been raised that in certain contexts, investigators have successfully gained grants from NHMRC, NIH, etc and then “demanded” access to the facility. This is clearly unacceptable. If a clear and transparent procedure is established up front and if individual investigators chose not to follow that procedure, then they, **not** the facility, put their grant at risk. In future rounds of all grant applications going through the NHMRC schemes, in addition to seeking sign-off on animal ethics, human ethics and biosafety, etc, a similar set of questions and boxes are included in all applications around investigators seeking access to facilities supported under the Enabling Grants Scheme.

It is strongly advocated that all processes and procedures around the facility are clearly documented so they are available for audit/scrutiny, and in addition to provide a basis for addressing criticisms if concerns are raised at a later date.