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RESEARCH CENTRE FOR WOMEN’S AND INFANTS’ HEALTH (RCWIH) BIOBANK
STANDARD OPERATING PROCEDURES FOR THE COLLECTION OF PERINATAL SPECIMENS
FOR RESEARCH

INTRODUCTION

All human tissue specimens obtained by the Research Centre for Women’s and Infants’ Health (RCWIH) BioBank program at Mount Sinai Hospital are collected, stored, and distributed in accordance with the policies of the Mount Sinai Hospital Research Ethics Board as outlined by the RCWIH BioBank ethics proposal (REB approval: REB # 10-0128-E).

The following document has been prepared based on Chapter 4 of the RCWIH BioBank Standard Operating Procedures (Version 4.0 January 15, 2015) that were developed and approved by the RCWIH BioBank Governance Committee. This document outlines only the methods followed by BioBank Clinical Research Assistants and Associates for the preparation and processing of specimens. For a complete copy of the RCWIH BioBank Standard Operating Procedure please contact BioBank Management (rcwih.biobank@lunenfeld.ca).
1.0 PREPARATION FOR TISSUE SAMPLING

1.0.1 Preparation of the sampling station

Prior to receiving the placenta the work station should be prepared by removing the computer keyboard and mouse, and covering the work surface with absorbent blue pads. To increase the amount of available work space, a large tray is used to cover the sink; the tray is covered with a blue absorbent pad for collections and may be temporarily removed for cleaning of instruments following a collection.

In addition to the labeled vials, tubes, cryomolds, and other collection materials, the following materials should be set up so that they are easily accessible during the collection (refer to Figure 4.3):

A. Disposable gloves (one box to use as needed), or surgical gloves
B. Sharps disposal container (to dispose of cord blood collection syringe and needle(s))
C. Container of disinfectant wipes (virox)
D. Appropriate rack to hold 50ml tubes
E. Appropriate rack to hold 2ml cryovials
F. Box of kimwipes
G. Disinfectant wipes
H. 1-2 X disposable kidney shaped bowl
I. 1 X 10cm Petri dish (for placental membrane roll)
J. Pins
K. Forceps
L. Scissors
M. Scale (set up with a blue absorbent pad lining the bowl and tared to zero)
N. 1-2 x ACD blood collection bag (if sampling umbilical cord blood)
O. 18G blunt end syringe (as many as needed, if required for sampling umbilical cord blood)
P. Blunt Filled needle (18G) (as many as needed, if sampling umbilical cord blood with syringe)
Q. Styrofoam cooler containing dry ice
R. Liquid nitrogen canister (recently filled and containing sufficient amount of liquid)
   - Pre-chilled 1X PBS without Ca\(^{2+}\) or Mg\(^{2+}\) (kept at 4°C)
   - Pre-chilled 4% paraformaldehyde (kept at 4°C)
   • Yellow biohazard garbage bag (to receive collection materials in contact with biohazardous materials)
1.0.2 Personal protection equipment to be worn during specimen collection

All RCWIH BioBank staff are expected to take all necessary steps to ensure personal safety during sampling, and to handle all human specimens in a manner that is suitable for potentially harmful infectious status*.

Appropriate personal protection equipment including:

- Powder-free, latex-free, medical examination quality disposable gloves (surgical gloves should be used in cases of known potentially harmful infectious status, see RCWIH BioBank SOP for Sample Collections Involving Blood Borne Pathogens)
* Eye glasses/goggles/shield
* Surgical mask (with shield in cases of potentially harmful infectious status)
* Hospital-issued scrub suit or laboratory coat
* Closed-toe, non-slip footwear

* all patients consented by the BioBank for placental sampling are to be pre-screened and verified as being HIV-, HepB- and HepC-negative, however it is the best practice to treat all human specimens as potentially infectious and exercise the necessary precautions to avoid infection. In addition, wearing appropriate personal protection equipment (specifically gloves and mask) eliminates the potential for contamination of the samples by collection staff. Investigator specific requests may require collection of HIV-, HepB- and/or HepC-positive (or other positive infection status) specimens. Refer to the RCWIH BioBank SOP for Sample Collections Involving Blood Borne Pathogens for safe tissue sampling protocols from potentially harmful infectious status cases.

1.1 Protocols for obtaining perinatal specimens

The following sampling protocols are to be performed for samples collected for research purposes only and/or for the purpose of tissue banking.

**NOTE:** during handling and placental sampling by RCWIH BioBank staff care must be taken to avoid unnecessarily altering the state in which the placenta is received as this could impact the subsequent evaluation by MSH Pathology. In cases where twin placentas are sampled all umbilical cord clamps used to designate the respective twin should be left in the manner received and should be in agreement with the description provided on the Pathology requisition form completed by hospital staff. Any cord knots (true or false) or entanglements should be left in the manner received and not untangled (particularly for monochorionic-monoamniotic gestations).

1.1.1 Umbilical cord blood

The umbilical cord should be clamped immediately after delivery to prevent blood loss, and the collection of umbilical cord blood should take place as soon as possible in order to avoid formation of clots in the vessels.

**NOTE 1:** For twin pregnancies (or other multiple births) the clinical staff will distinguish the cords belonging to each infant using cord clamps. This designation must remain when the placenta is sent to Pathology. When the cords are identified by surgical clamps, it is necessary to attach new plastic cord clamps to distinguish the twins and this information should be clearly conveyed on the Pathology requisition form that accompanies the placenta.

**NOTE 2:** When collecting samples from a monochorionic monoamniotic twin placenta the umbilical cords may be tangled or knotted. It is very important that the cords are left in the original state, without attempting to untangle the cords or knots as this is an important aspect of evaluating these placentas by clinical pathologists. Under those conditions, attempts should be made to trace the individual cords to their insertion sites to allow clear identification of corresponding placental regions.
1.1.1.1 Umbilical cord blood in standard collection tubes or vacutainers

**Figure 1.1.** Collection of umbilical cord blood using standard collection tubes or vacutainers (see text for description).

1. With the placenta positioned with the fetal surface and umbilical cord facing up (Figure 4.4 (A)), umbilical cord blood is drawn from the umbilical vein using a sterile 10cc syringe fitted with a sterile 18G blunt end needle.

2. The needle is carefully inserted into the umbilical vein (above the clamp site) at a suitable angle that enables penetration of one vessel wall but not both (Figure 4.4 (B)).
   - **CAUTION NOTE:** extreme care should be taken at this stage as the umbilical cord may be difficult to hold securely and penetrate, and there is a potential for personal injury as a result of needle puncture. To eliminate the risk of injury the cord should be held in such a way to avoid placing the hands immediately behind the intended site of penetration (i.e. by pulling the cord slightly taught and supporting the cord slightly above the site of penetration, inserting the needle tip away from the supporting finger).

3. After insertion of the needle tip within the vessel, the syringe plunger is gently withdrawn to draw blood into the syringe. It may be necessary to gently move the needle tip within the vessel (without removing it completely) to maintain collection of blood as the plunger is withdrawn.

4. After the required amount of cord blood has been withdrawn, the cord is clamped above the site of venipuncture. The needle is then withdrawn from the umbilical cord and inserted into the top of an appropriate* blood collection tube or vacutainer, the plunger is then depressed gently to avoid production of bubbles and the contents of the syringe are transferred to the collection tube (Figure 4.4 (C)).

5. Withdraw the needle from the collection tube and carefully dispose the needle + syringe in a biohazardous sharps container.

6. Promptly invert the collection tube a few times to mix the blood sample with any anticoagulants or other agents within the tube, and if necessary transfer to the appropriate storage condition (for most cases it is sufficient to leave the mixed blood sample at room temperature for the duration of the sampling).

7. Return any surgical instruments that were received with the placenta at the time of collection and proceed with the rest of the sample processing.
1.1.1.2 Umbilical cord blood collected in closed-system collection bags

Figure 1.2. Collection of umbilical cord blood using closed-system collection bags (see text for description).

1. With the placenta positioned with the fetal surface and umbilical cord facing up (Figure 4.5 (A)), umbilical cord blood is drawn from the umbilical vein using a sterile 16G safety needle attached to a 250ml blood collection bag containing CPD anticoagulant solution.

2. The needle is carefully inserted into the umbilical vein (above the clamp site) at a suitable angle that enables penetration of one vessel wall but not both (Figure 4.4 (A)).
   - **CAUTION NOTE:** extreme care should be taken at this stage as the umbilical cord may be difficult to hold securely and penetrate, and there is a potential for personal injury as a result of needle puncture. To eliminate the risk of injury the cord should be held in such a way to avoid placing the hands immediately behind the intended site of penetration (i.e. by pulling the cord slightly taught and supporting the cord slightly above the site of penetration, inserting the needle tip away from the supporting finger).

3. After insertion of the needle tip within the vessel, blood will start to flow automatically through the collection tube towards the bag. To assist with the flow of blood, position the collection bag securely below the placenta on a clean surface or disposable pad (Figure 4.5 (B)). Do not let the bag hang unsupported as this may pull the needle out of the umbilical cord, resulting in blood loss.

4. If the flow of blood diminishes it may indicate the presence of a clot or collapsed area of the vessel. To clear the needle tip or reposition the needle, carefully apply a secondary clamp to the cord above the needle insertion site (Figure 4.5 (C)). To avoid personal injury, be extremely careful not to move the needle tip while the secondary clamp is applied. (NOTE:
prior to sampling open 3-4 cord clamps so that they are within reach during the blood collection to facilitate re-clamping if necessary). Remove the needle, clear any clotted blood from the tip and re-insert the needle above the secondary clamp site to re-establish the flow of blood.

5. When sufficient blood has been collected (see the example in Figure 4.5 (D)), the needle tip is carefully removed from the umbilical cord and the safety clip is moved up the tubing towards the needle until the tip is completely enclosed (Figure 4.5 (E-F)).

6. The blood bag is promptly shaken gently to ensure adequate mixing of the blood and anticoagulant solution.

7. The bag is then returned to the original packaging, labeled with the corresponding sample ID number, and stored at 4°C until distribution to the specific investigator or further processing.

1.2 Placental tissue

1.2.1 Measurement of placental weight

All placentas sampled by the BioBank are to be weighed prior to sampling to determine the fresh, untrimmed weight.

1. For singleton placentas, remove any remaining cord clamps using clamp scissors (insert the clamp hinge in the open jaws of the clamp scissors and compress the scissor handles to cut the hinge).

**NOTE:** for twin pregnancies (or other multiple births) the clinical staff will distinguish the cords belonging to each infant using cord clamps; this designation must remain when the placenta is sent to Pathology and therefore the clamps should not be removed for weighing.

2. Using a standard scale (with the bowl pre-lined with a blue absorbent pad and tared to zero), carefully transfer the whole placenta (including the membranes and umbilical cord) to the scale and allow scale to come to rest (Figure 4.6).

3. Record the placental weight on the Sample Log and BioBank Data Collection Form.

4. Remove the placenta from the scale and proceed with the rest of the sample processing.
1.2.2 Processing of placental tissue cores

All specimens of placental tissue collected for the purpose of tissue banking are to be processed in the following manner using a non-systematic, quadrant sampling method. For studies which use an alternative sampling protocol that has been approved by the BioBank Manager refer to Appendix A.

1. Carefully position the placenta on a blue absorbent pad with the maternal surface facing up, paying close attention to position the umbilical cord as needed to ensure that the placental lies as flat as possible on the table surface (Figure 4.7 (A)).
2. Observe any fetal membranes covering the maternal surface of the placenta (if the membranes are predominantly on the fetal side of the placenta proceed to step 4). Attempt to locate the site or rupture or opening in the membranes (Figure 4.7 (B)). If it is necessary to disrupt the membranes to expose the placental surface, gently grasp the membranes (using forceps) in the center of the placental disc and elevate the membranes slightly to allow a small incision to be made using scissors.
3. Carefully increase the opening in the membranes so that the membranes can be transferred to the fetal side of the placental progressively exposing the full maternal surface of the placenta (Figure 4.7 (C-D)). Membranes should not be trimmed off, but can be folded underneath the placenta at this stage.
4. Observe the maternal surface of the placenta. Carefully remove any large blood clots on the surface using a closed pair of forceps (NOTE: to avoid damaging the tissue forceps should be held roughly parallel to the placental surface and passed gently over the surface to draw blood clots away. Alternatively, blood clots can be removed carefully by hand if preferred to avoid tissue damage).
5. Select a quadrant of the placenta to be sampled. Using forceps gently grasp an area of tissue that is at least 1.5 cm away from the closest edge of the placental disc AND at least 1.5 cm away from the center of the placental disc (typically containing the umbilical cord insertion site) (Figure 4.8 (B)).

6. Using scissors make 4 equal vertical cuts (each ~1.5 cm in length), straight down and at right angles to each other to form a square-shaped tissue core (Figure 4.8 (B-C)). Cuts are to be made to a depth that allows excision of a nearly full-thickness core that excludes the chorionic plate at the fetal surface. Make a final cut horizontally just above the chorionic plate to completely free the tissue core (Figure 4.8 (D-F)).

7. Wash the tissue core in a clean kidney-shaped bowl or beaker containing cold 1X PBS (this is a preliminary step to remove excess blood) (Figure 4.8 (G)). Transfer the tissue core to a clean area on a blue absorbent pad. Carefully roll or blot the tissue on the absorbent pad to remove excess blood and PBS (Figure 4.8 (H)).
Figure 1.5. Extraction and processing of placental tissue cores (see text for description).
8. Repeat steps 5 through 7 for the remaining 3 quadrants of the placenta. (Figure 4.8 (I)).

9. After excising, washing and blotting the 4 tissue cores, using the scissors and forceps cut each respective core into several smaller pieces that are roughly 0.5cm³ each (Figure 4.8 (J-K)). Tissue cores are to be processed in such a way that minimizes mechanical stress (i.e. compression, stretching, tearing, etc.) on the tissue as much as possible.

10. Transfer the tissue samples to the appropriate cryovials, tubes, or cyromolds as described in section 4.3.2.3. through 4.3.2.6. (Figure 4.8 (L-N)). **NOTE:** the sequence in which the respective samples are prepared should be consistent across all sample collections, and should be performed in the following order:

- Snap-frozen tissue (cryovials)
- Fixed tissue (50 ml tubes)
- Fresh tissue for culture (50 ml tubes)
- Other processed samples

### 1.2.3 Preparation of snap-frozen tissue

Each vial of snap-frozen tissue collected for the purpose of tissue banking is to contain a representative, pooled sampling of the placental tissue, prepared in the following manner:

1. Using forceps carefully collect one 0.5cm³ piece of tissue from one of the 4 processed tissue cores on the absorbent blue pad (avoid causing mechanical stress such as compression as much as possible by grasping the tissue at one edge only, or by using the forceps to scoop the tissue up from the dish).
2. Transfer the sample to a pre-labeled 2 ml cryovial.
3. Repeat steps 1 through 3 for the remaining 3 cores so that each cryovial contains a total of 4 tissue pieces, one from each of the 4 respective dishes (Figure 4.8 (L)).
4. Repeat steps 1 through 4 for all of the required cryovials.
5. Transfer the cryovials to a liquid nitrogen canister to snap-freeze the tissue.

- **CAUTION NOTE:** use appropriate precautions and care when transferring samples to the liquid nitrogen canister (refer to Appendix C for information regarding the safe handling of liquid nitrogen).
6. At a convenient time (i.e. after all other tissue samples have been processed) transfer the frozen cryovials to a designated long-term cryostorage system (refer to section 4.6 for details of sample transfer, and section 6.1 for information about long-term storage of tissue cryovials).
7. Record the primary location of the cryovials in the Sample Log.

### 1.2.4 Preparation of paraformaldehyde-fixed tissue

Each 50 ml tube of fixed tissue collected for the purpose of tissue banking is to contain a representative, pooled sampling of the placental tissue, prepared in the following manner:

1. Using forceps carefully collect one 1cm² piece of tissue from one of the 4 cores of processed tissue on the blue absorbent pad (avoid causing mechanical stress such as compression as much as possible by grasping the tissue at one edge only, or by using the forceps to scoop the tissue up from the dish).
2. Transfer the sample to a pre-labeled 50 ml tube containing 15 ml of cold 4% paraformaldehyde in PBS*.
• **CAUTION NOTE:** care should be taken to avoid inhalation of paraformaldehyde vapors by keeping the tube closed as much as possible and removing the lid only when necessary to add the tissue sample (refer to the product MSDS found in Appendix C for additional information regarding safe work practices when working with paraformaldehyde).

3. Repeat steps 1 through 3 for the remaining 3 tissue cores so that each tube contains a total of 4 tissue pieces, one from each of the 4 respective cores (Figure 4.8 (M)).

4. Repeat steps 1 through 4 for all of the required fixed tissue samples.

5. Transfer** the tube containing 4% paraformaldehyde and tissue to a shaker or rocker at room temperature, and shake/rock for 24 h. Alternatively, if tissues are friable or subsequent transfer after 24 hours is not possible due to work scheduling conflict the tissue may be fixed in a cold room or 4°C fridge for 72 hours. To facilitate accurate timing of the 24 hour period the sample IDs, time and date of collection and time and date of transfer (after 24 hours or 72 hours) should be recorded on the RCWIH BioBank PFA Sample Log (Appendix D).

6. After 24 hours on the rocker/shaker or 72 hours in the cold room, working in a fume hood or appropriately ventilated area, remove all of the paraformaldehyde liquid waste and collect in a designated container.

7. Wash the sample briefly one time in 70% ethanol. Discard wash in the designated container.

8. Add 10-15 ml of 70% ethanol to the tube containing the fixed tissue samples.

9. Store fixed tissues samples in 70% ethanol at 4°C until ready to be further processed for embedding in paraffin wax blocks (refer to Appendix B for further details regarding processing of fixed tissues).

* 4% paraformaldehyde should be prepared fresh from a 16% stock and kept at 4°C for no longer than 2-3 weeks (refer to Appendix B for protocols detailing the preparation of the 16% stock and 4% paraformaldehyde solutions).

** once samples have been transferred to the tube containing 4% paraformaldehyde, tubes can be left at room temperature (temporarily without shaking if no shaker is available) while the rest of the tissue processing and cleanup are completed. Transfer the sample to a rocker for 24 hours or a cold room/fridge at 4°C for 72 hours, as appropriate, at the earliest convenient time, but only after all other samples have been processed.

### 1.2.5 Preparation of fresh tissue for culture

Upon request, fresh tissue is provided in 50 ml tubes containing either cold PBS or an appropriate culture medium (to be specified and provided by the individual requesting the fresh tissue sample, along with any additional instructions regarding the storage and transfer of the sample). Typically only one representative sample of placental tissue is required, unless indicated otherwise.

1. Using forceps carefully collect one 1cm³ piece of tissue from one of the 4 processed tissue cores (avoid causing mechanical stress such as compression as much as possible by grasping the tissue at one edge only, or by using the forceps to scoop the tissue up from the dish). **Note:** investigator-specific requests may determine alternate sampling details. Consult BioBank Manager for all information regarding specific collection requests.

2. Transfer the tissue sample to a pre-labeled 50 ml tube containing 15 ml of PBS or media as required.

3. Store the sample at room temperature or 4°C as specified.
4. Promptly notify the individual requesting the sample that the tissue is available for pickup and make any necessary arrangements for the sample transfer.

**1.3 Umbilical cord tissue**

Following placental sampling, samples of umbilical cord tissue are collected (in the case of multiple gestations, each cord is to be sampled).

1. Using forceps, securely hold the distal end of the umbilical cord (Figure 1.6A)
2. Using scissors cut the required segments of cord, rinse in cold 1X PBS to remove excess blood and/or clots, blot briefly and process as follows (Figure 1.6 (B-C)):
   a. 1 segment (2 cm in length) is transferred to a 50ml tube containing 10ml 4% PFA, and fixed on a shaker for 24h at room temperature or in a cold room/fridge at 4°C for 72 hours (further processing is as described for PFA-fixed placental tissue in section 4.3.2.4)
   b. 2 segments (1cm in length) are cut to fit into 2ml cryovials (one segment per cryovial for a total of 2 biobanking specimens), and snap-frozen in liquid nitrogen (refer to section 4.6 for details of sample transfer, and section 6.1 for information about long-term storage of tissue cryovials).
   c. Additional samples are collected as required

**Figure 1.6.** Processing of umbilical cord tissue (see text for description).