

STANDARD OPERATING PROCEDURES FOR THE COLLECTION OF PERINATAL SPECIMENS FOR RESEARCH

**Research Centre for Women's and Infants' Health
(RCWIH) BioBank**

**Mount Sinai Hospital
Samuel Lunenfeld Research Institute**

(abbreviated version)

December 16th 2010

MOUNT SINAI HOSPITAL
Joseph and Wolf Lebovic Health Complex

Samuel Lunenfeld Research Institute



TABLE OF CONTENTS

SECTION	PAGE
Introduction	1
Section 1 - Perinatal Specimen Collection	1
1.1. Protocols for obtaining perinatal specimens	1
1.1.1. Umbilical cord blood	1
1.1.1.1. Umbilical cord blood collected in standard collection tubes or vacutainers	1
1.1.1.2. Umbilical cord blood collected in closed-system collection bags	2
1.1.2. Placental tissue	4
1.1.2.1. Measurement of placental weight	4
1.1.2.2. Processing of placental tissue cores	4
1.1.2.3. Preparation of snap-frozen tissue	7
1.1.2.4. Preparation of paraformaldehyde-fixed tissue	8
1.1.2.5. Preparation of tissue frozen in OCT cryomolds	8
1.1.2.6. Preparation of fresh tissue for culture	10
1.1.3. Umbilical cord tissue	10
1.1.4. Fetal membranes (amnion and chorion)	10
1.1.5. Parental samples (buccal swabs, saliva samples, etc.)	12

LIST OF FIGURES

FIGURE	PAGE
Section 1 - Perinatal Specimen Collection	
1.1. Collection of umbilical cord blood using standard collection tubes or vacutainers	2
1.2. Collection of umbilical cord blood using closed-system collection bags	3
1.3. Preparation of the placenta for core sampling	5
1.4. Extraction and processing of placental tissue cores	6
1.5. Preparation of placental tissue OCT cryomolds	9
1.6. Processing of umbilical cord tissue	10
1.7. Processing of fetal membranes to produce a membrane roll for fixation	11

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

INTRODUCTION

All research 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 1.0 August 2010) that were developed and approved by the RCWIH BioBank Governance Committee. This document outlines only the methods followed by BioBank Clinical Research 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. PERINATAL SPECIMEN COLLECTION

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.

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.

1.1.1.1. Umbilical cord blood collected in standard collection tubes or vacutainers

1. Position the placenta with the fetal surface and umbilical cord facing up (Figure 1.1 (A)), and prepare a sterile 3cc syringe fitted with a sterile 18G blunt end needle.
2. Carefully insert the needle into the umbilical vein (above the clamp site) at a suitable angle that enables penetration of one vessel wall but not both (Figure 1.1 (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 that avoids placing the hands immediately behind the intended site of penetration (*i.e.* by pulling the cord slightly taught and supporting the cord just above the site of penetration, inserting the needle tip away from the supporting finger).



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

3. After inserting the needle tip within the vessel, gently withdraw the syringe plunger 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 collected, withdraw the needle from the umbilical cord and insert it into the top of an appropriate blood collection tube or vacutainer, and depress the plunger to transfer the contents of the syringe to the collection tube (Figure 1.1 (C)).
5. Withdraw the needle from the collection tube and dispose the needle and syringe in a biohazardous sharps container.
6. Promptly invert the collection tube several 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 applications it is sufficient to leave the mixed blood sample at room temperature for the duration of sampling).

1.1.1.2. Umbilical cord blood collected in closed-system collection bags

1. Position the placenta with the fetal surface and umbilical cord facing up (Figure 1.2 (A)), and prepare a sterile 16G safety needle attached to a 250ml blood collection bag containing CPD anticoagulant solution.
2. Carefully insert the needle into the umbilical vein (above the clamp site) at a suitable angle that enables penetration of one vessel wall but not both (Figure 1.2 (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 inserting the needle tip within the vessel, blood should 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 1.2 (B)). Do not let the bag hang unsupported as this may pull the needle out of the umbilical cord, resulting in blood loss and/or potential injury.

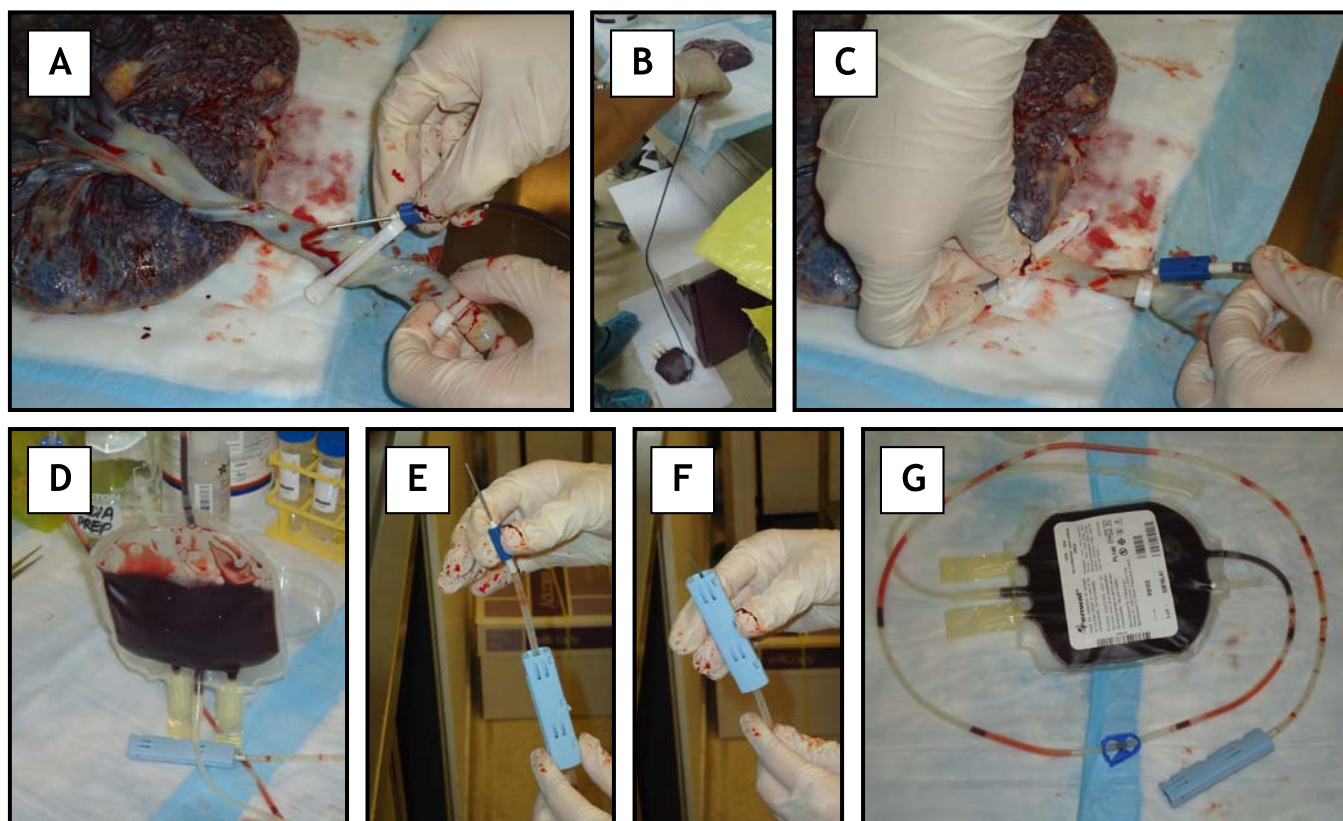


Figure 1.2. Collection of umbilical cord blood using closed-system collection bags (see text for description).

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 1.2 (C)). To avoid personal injury be extremely careful not to move the needle tip while the secondary clamp is applied. 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.
 - **RECOMMENDATION:** prior to sampling open 1-2 cord clamps so that they are within reach during the blood collection to facilitate re-clamping if necessary.
5. When sufficient blood has been collected (see the example in Figure 1.2 (D)), carefully remove the needle tip from the umbilical cord and move the safety clip up the tubing towards the needle until the tip is completely enclosed (Figure 1.2 (E-F)).
6. Promptly mix the blood bag gently to ensure adequate mixing of the blood and anticoagulant solution.
7. Return the bag to the original packaging and store at 4°C.

1.1.2. Placental tissue

1.1.2.1. Measurement of placental weight

The fresh, untrimmed placental weight should be determined prior to sampling.

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).
2. Using a standard scale (with the bowl pre-lined with an 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. Care should be taken to avoid transfer of loose blood clots and any other fluids that may be trapped within membranes or contained in the bowl containing the placenta.
3. Record the wet, untrimmed placental weight.
4. Remove the placenta from the scale and proceed with the rest of the sample processing.

1.1.2.2. Processing of placental tissue cores

All specimens of placental tissue collected for the purpose of tissue banking by the RCWIH BioBank are processed in the following manner to obtain pooled samples from each of the 4 placental quadrants.

1. Position the placenta on an absorbent pad with the maternal surface facing up, paying close attention to position the umbilical cord as needed to ensure that the placenta lies as flat as possible on the table surface (Figure 1.3 (A)).
2. If the fetal membranes have been inverted during delivery and now cover the maternal surface attempt to locate the site of rupture or opening in the membranes (Figure 1.3 (B)) (if the membranes are predominantly on the fetal side of the placenta proceed to step 4). 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 1.3 (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
 - **RECOMMENDATION:** 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.

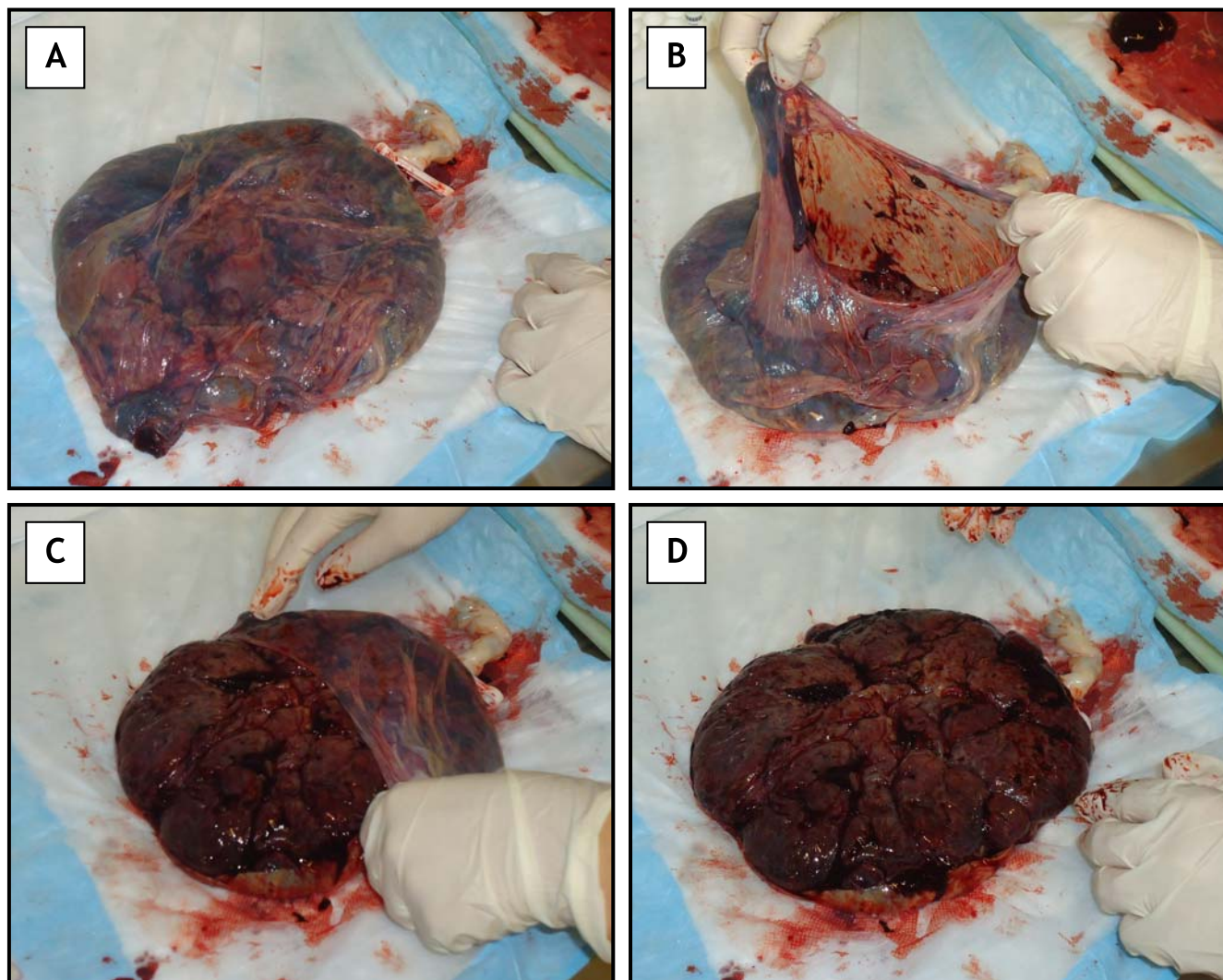


Figure 1.3. Preparation of the placenta for core sampling (see text for description).

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 (Figure 1.4 (B)).
 - **RECOMMENDATION:** the regions selected for sampling should be representative of the whole placenta, and should not contain the umbilical cord insertion site, areas of obvious thrombosis, infarcts, or other abnormalities that do not reflect the majority of the tissue.
6. Using scissors make 4 equal sized 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 1.4 (B-C)). Cuts should 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 1.4 (D-F)).

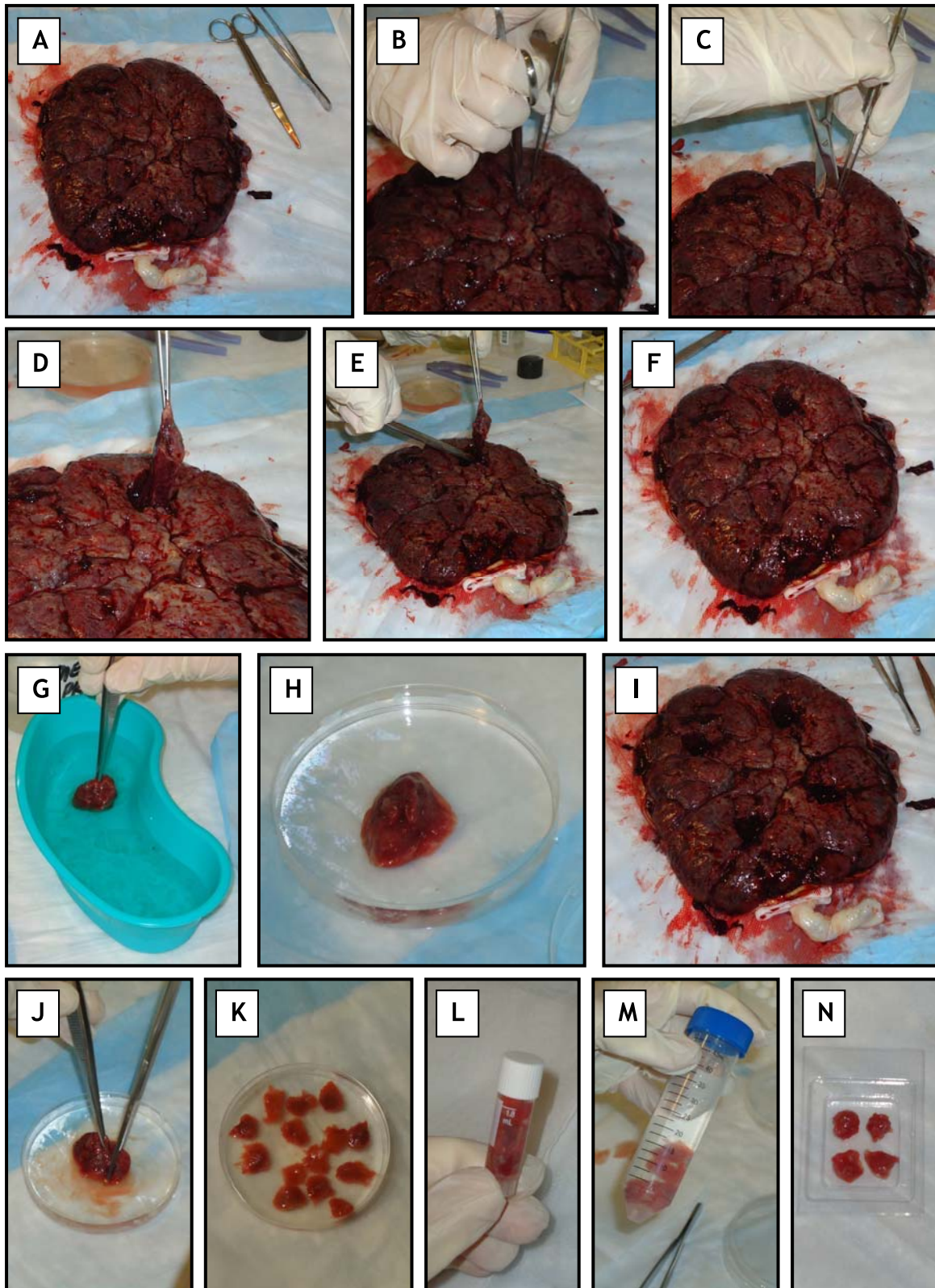


Figure 1.4. Extraction and processing of placental tissue cores (see text for description).



7. Briefly 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 1.4 (G)). Transfer the tissue core to a new 10cm tissue dish containing cold PBS (Figure 1.4 (H)).
8. Repeat steps 5 through 7 for the remaining 3 quadrants of the placenta, transferring each tissue core to a separate 10cm tissue dish containing cold PBS (Figure 1.4 (I)).
9. After excising the 4 tissue cores, use a pair of scissors and forceps to cut each core into several smaller pieces that are roughly 1cm³ each (Figure 1.4 (J-K)). Tissue cores should 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 cryovials, tubes, or cyromolds as described in section 1.1.2.3. through 1.1.2.6. (Figure 1.4 (L-N)).
 - **RECOMMENDATION:** the sequence in which the respective samples are prepared should be consistent across all sample collections, and in a sequence that maximizes the quality of the biomaterials to be studied. This sequence is typically the reverse order of the stability of the desired biomaterials (*i.e.* samples for which RNA will be isolated should be processed first to minimize RNA degradation, while samples used to prepare protein lysates or DNA can be processed afterwards, followed by samples that will undergo fixation, *etc.*).

1.1.2.3. Preparation of snap-frozen tissue

Each vial of snap-frozen tissue collected for the purpose of tissue banking by the RCWIH BioBank contains a representative, pooled sampling of the placental tissue, prepared in the following manner:

1. Using forceps carefully collect one 1cm³ piece of tissue from one of the 4 tissue dishes containing 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).
2. Briefly blot the tissue sample on a clean area of an absorbent pad to remove excess blood and PBS.
3. Transfer the sample to a pre-labeled 2 ml cryovial.
4. Repeat steps 1 through 3 for the remaining 3 Tissue dishes so that each cryovial contains a total of 4 tissue pieces, one from each of the 4 respective dishes (Figure 1.4 (L)).
5. Repeat steps 1 through 4 for all of the required cryovials.
6. Transfer the cryovials to a liquid nitrogen canister to snap-freeze the tissue.
 - **CAUTION NOTE:** use appropriate safety precautions and personal protective equipment when transferring samples to the liquid nitrogen canister.
7. 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, and record the primary location of the cryovials. Storage in the vapour phase of a liquid nitrogen storage system is used by the RCWIH BioBank and is recommended.

1.1.2.4. Preparation of paraformaldehyde-fixed tissue

Each sample of fixed tissue collected for the purpose of tissue banking by the RCWIH BioBank contains a representative, pooled sampling of the placental tissue, prepared in the following manner:

1. Using forceps carefully collect one 1cm³ piece of tissue from one of the 4 tissue dishes containing 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).
2. Briefly blot the tissue sample on a clean area of an absorbent pad to remove excess blood and PBS.
3. Transfer the sample to a pre-labeled 50 ml tube containing 25 ml of cold 4% paraformaldehyde in PBS.
 - **CAUTION NOTE:** care should be taken to avoid inhalation of paraformaldehyde vapors by working under properly ventilated conditions, keeping the tube closed throughout sampling and removing the lid only when necessary to add the tissue sample.
 - **RECOMMENDATION:** 4% paraformaldehyde should be prepared fresh from a 20% stock and kept at 4°C for no longer than 2-3 weeks.
4. Repeat steps 1 through 3 for the remaining 3 tissue dishes so that each tube contains a total of 4 tissue pieces, one from each of the 4 respective dishes (Figure 1.4 (M)).
5. Repeat steps 1 through 4 for all of the required fixed tissue samples.
6. Transfer the tube containing 4% paraformaldehyde and tissue to a shaker or rocker at room temperature, and shake/rock for 24 h. To facilitate accurate timing of the 24 h period the time of collection should be written directly on the sample tube.
 - **RECOMMENDATION:** 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 at the earliest convenient time, but only after all other samples have been processed.
7. While working in a fume hood or appropriately ventilated area, remove all of the paraformaldehyde liquid waste and collect in a designated container.
8. Wash the sample briefly once in 70% ethanol, and discard the waste in the designated container.
9. Add 15-20 ml of 70% ethanol to the tube containing the fixed tissue samples.
10. Temporarily store fixed tissues samples in 70% ethanol at 4°C until ready to be dehydrated and further processed for embedding in paraffin wax blocks.
 - **RECOMMENDATION:** the duration of temporary storage in 70% ethanol should not exceed two weeks

1.1.2.5. Preparation of tissue frozen in cryomolds

Each cryomold of frozen tissue collected for the purpose of tissue banking by the RCWIH BioBank contains a representative, pooled sampling of the placental tissue, prepared in the following manner:

1. Using forceps carefully collect one 1cm³ piece of tissue from one of the 4 tissue dishes containing 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).

2. Briefly blot the tissue sample on a clean area of an absorbent pad to remove excess blood and PBS.
3. Transfer the sample to a pre-labeled cryomold.
4. Repeat steps 1 through 3 for the remaining 3 tissue dishes so that each cryomold contains a total of 4 tissue pieces, one from each of the 4 respective dishes. Tissue pieces should be arranged in a 2x2 array, and carefully positioned so that a minimal amount of space remains between each tissue, while maintaining a clear margin around the outside of the cryomold (Figure 1.5 (A)).
5. Repeat steps 1 through 4 for all of the required cryomolds.
6. For each cryomold, apply enough Optimal Cutting Temperature (OCT) liquid medium around the outer margin of the cryomold and in the space between the tissue samples to just cover the 4 tissue pieces (Figure 1.5 (B)).
7. Transfer the cryomolds to a bath of dry ice (contained in a Styrofoam container or cooler) and carefully position the cryomolds so that they are stable and remain horizontal/level (this is to prevent the liquid medium from flowing out of the mold before freezing completely).

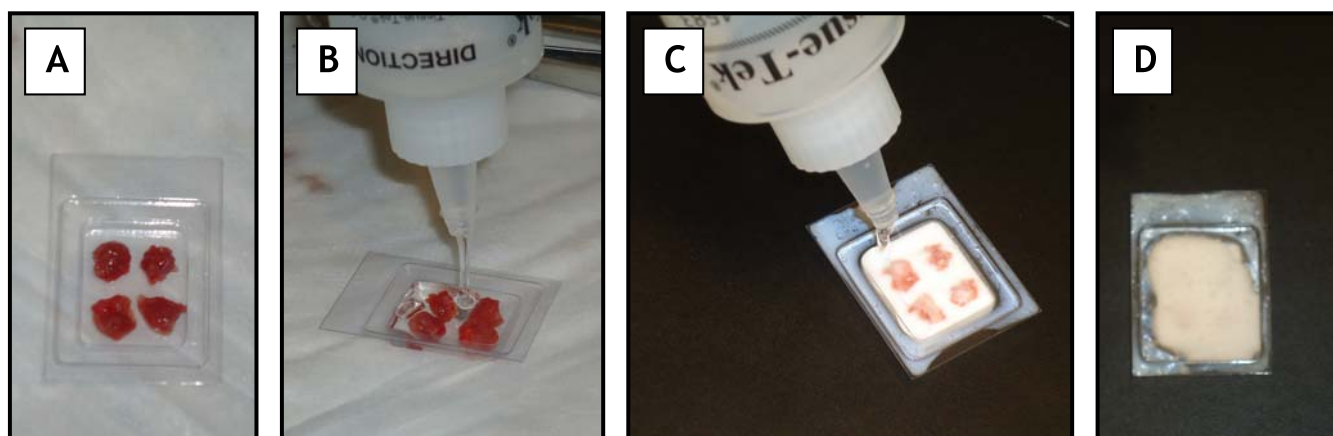


Figure 1.5. Preparation of placental tissue OCT cryomolds (see text for description).

8. Leave the cryomolds on the dry ice bath, carefully replace the lid of the Styrofoam container, and continue with the remainder of the tissue processing.
9. At a convenient time (*i.e.* after all other tissue samples have been processed), check to see whether the OCT medium is completely frozen (indicated by a change in opacity from clear in the liquid state to white and opaque when frozen). If there are any areas of exposed tissue, apply a thin, additional layer of liquid OCT medium to the top surface of the cryomolds to ensure full coverage of the tissue, and return to the dry ice bath (Figure 1.5 (C)).
10. After verifying that samples are completely frozen and properly covered by OCT medium (Figure 1.5 (D)), wrap the cryomold in a small piece of plastic or foil, transfer to an appropriate location in a designated -80°C freezer and record the primary location of the cryomolds.

1.1.2.6. Preparation of fresh tissue for culture

Fresh tissue can be prepared in either cold PBS or an appropriate culture medium and typically only one or two representative samples of placental tissue are required.

1. Using forceps carefully collect 1-2 1cm^3 pieces of tissue from the dishes containing 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).
2. Transfer the tissue sample(s) to a pre-labeled 50 ml tube containing 25 ml of PBS or media as required.
3. Store the sample at room temperature, 4°C or as required prior to experimentation.

1.1.3. Umbilical cord tissue

Following placental sampling, samples of umbilical cord tissue should be collected.

1. Using forceps, securely hold the distal end of the umbilical cord (Figure 1.6 (A))
2. Using scissors cut the required segments of cord, rinse in 1X PBS to remove excess blood and/or clots, blot briefly and process as follows (Figure 1.6 (B-C)):
 - a. Transfer 1 segment (2 cm in length) to a 50ml tube containing 4% PFA, and fix on a shaker for 24h at room temperature (further processing is as described for PFA-fixed placental tissue in section 1.1.2.4).
 - b. Cut 1 segment (2-3 cm in length) into smaller pieces as necessary to fit into 2ml cryovials, and snap-freeze in liquid nitrogen.
 - c. Collect additional samples as required.

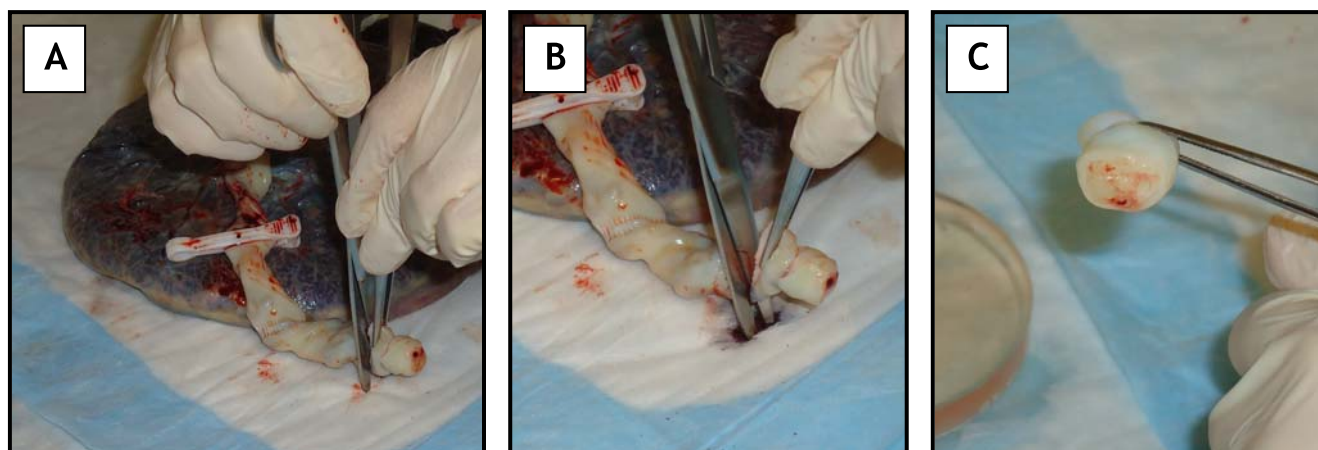


Figure 1.6. Processing of umbilical cord tissue (see text for description).

1.1.4. Fetal membranes (amnion and chorion)

Following the collection of umbilical cord tissue a sample of fetal membranes should be obtained. Although samples of the fetal membranes can be processed in any manner (*i.e.* fresh, fixed or

frozen), the following describes the preparation of a fetal membrane roll for tissue fixation and embedding in paraffin wax.

1. Identify a free margin of the fetal membranes (this will be the site of membrane rupture unless the membranes were previously cut during other steps of sample processing) (Figure 1.7 (A)).

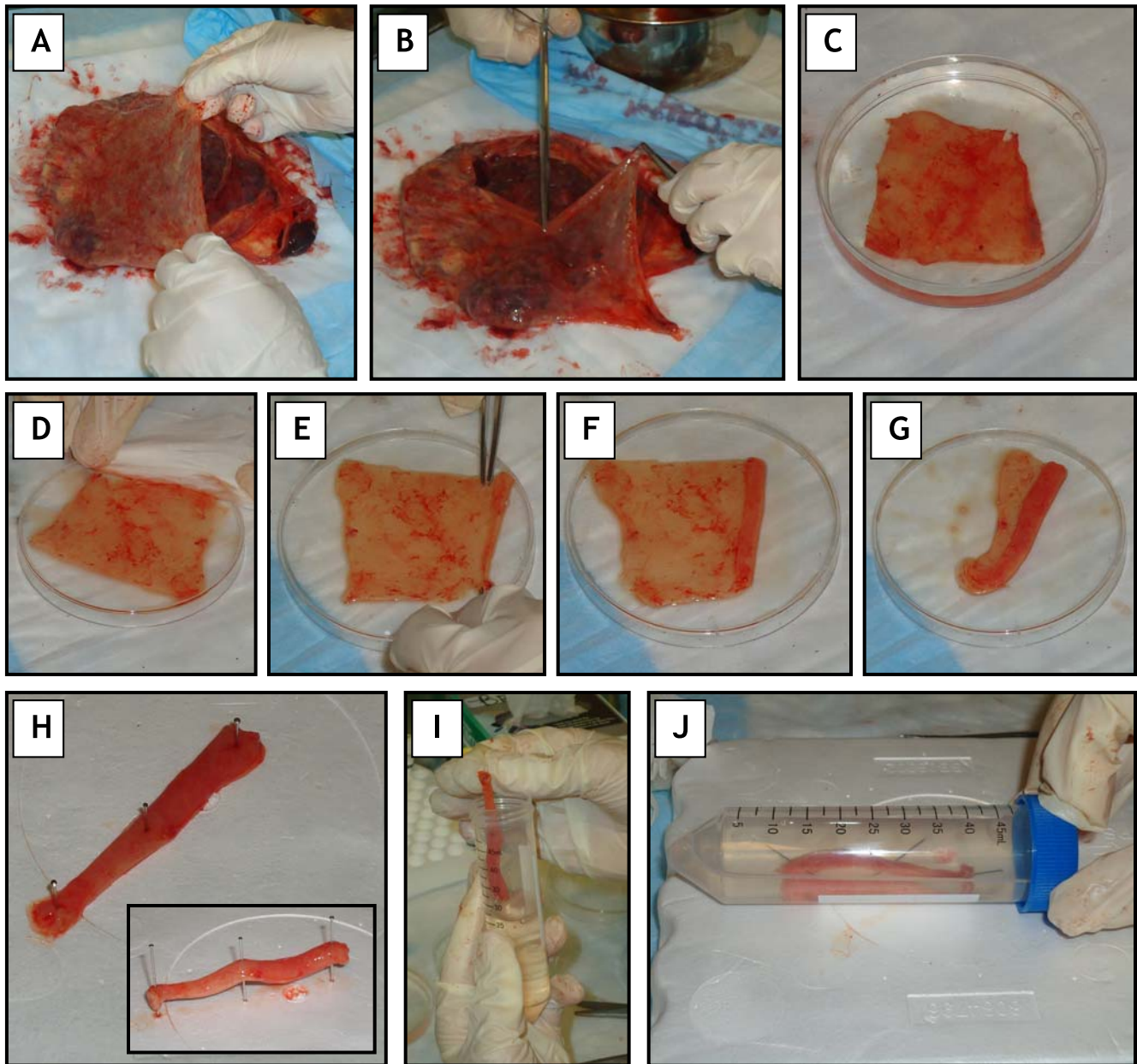


Figure 1.7. Processing of fetal membranes to produce a membrane roll for fixation (see text for description).

2. Without separating the amnion from the chorion, use forceps to grasp both membranous layers and proceed to cut a rectangular piece of membrane (approximate dimensions: 5-6 cm x 6-7 cm) (Figure 1.7 (B)).
3. Transfer the piece of membrane to a tissue dish containing cold 1X PBS (Figure 1.7 (C)). At this stage it is important to identify the orientation of the membrane with respect to the amnion and chorion; position the membranes in the dish with the amnion facing up so that upon rolling the membrane the amnion is internal and the chorion is external.
4. Transfer the membrane to the top lid of a tissue dish so that it lies flat (with the amnion facing up) and can be easily rolled (Figure 1.7 (D)). Blot any excess PBS using an absorbent pad.
5. Using forceps grasp both ends of the membranes along one of the shorter sides and flip or roll a small section of membrane so that it overlaps the adjacent area (Figure 1.7 (E)). Repeat several times, keeping the rolled area as tight as possible (Figure 1.7 (F-G)).
6. When the roll is complete and tight, transfer the roll to the smooth side of a Styrofoam platform (a cooler lid or vial rack are recommended). Insert pins through all of the layers of the membranes, at both ends and once in the middle to secure the roll (Figure 1.7 (H)). Gently slide the roll up the pins in preparation for removal from the Styrofoam platform (see inset in Figure 1.7 (H)).
7. Carefully transfer the pinned membrane roll to a 50ml tube containing 4% paraformaldehyde (Figure 1.7 (I)).
8. Store the sample at room temperature in a horizontal position for 15-20 minutes (Figure 1.7 (J)); this is to allow the tissue to fix partially and become somewhat rigid while the membrane roll is straight (storing the tube vertically before the tissue becomes rigid will result in a compressed, or curved membrane roll).
9. Continue to fix the membranes for 24h at room temperature on a shaker (further processing is as described for PFA-fixed placental tissue in section 1.1.2.4)

1.1.5. Parental samples (buccal swabs, saliva samples, etc.)

At an appropriate time (either prior to, or following delivery, as determined to be convenient for the donating individuals) parental samples (such as buccal swabs, or saliva samples) should be obtained. Samples should be collected using sterile, single-use collection kits according to the manufacturer's and/or investigator's instructions and stored accordingly.