



PROCEDURE	Processing Formalin-Fixed Placental Tissues	
PREPARED BY	GAPPS staff	
DATE ADOPTED		
REVIEWED BY	SIGNATURE	REVIEWED DATE

REVISED BY	SIGNATURE	REVISED DATE

SUMMARY OF CHANGES TO THIS SOP
Version 1.0

PURPOSE

This Standard Operating Procedure (**SOP**) describes a procedure for processing formalin-fixed placental tissues.

SCOPE

This procedure covers the processing of formalin-fixed placental tissues and placement into standard cassettes.

Authority and Responsibility for SOP's

1. The GAPPS Medical Director (or his/her designee) and Laboratory Manager have the authority to establish this procedure.
2. The GAPPS Laboratory and the QA monitors are responsible for the implementation of SOP documentation at participating sites.

Supplies

1. Cassettes-labeled with reagent-proof pen or pencil
2. Plastic tub with lid for ethanol
3. Metal cassette rack for plastic tub
4. 1.4-1.5 Liters of 70% ethanol (95% histology grade ethanol)
5. Plastic container to use as a liquid waste container
6. Small shipping biohazard bag
7. Plastic container lined for solid waste
8. Forceps
9. Scalpel and extra blades
10. Cutting board
11. 70% etOH and Bleach for cleanup

Supply Preparation

Add 1050 mL histological grade ethanol and 450 mL distilled water to get 70% ethanol.

Safety

All technicians are expected to be trained and follow universal precautions when handling biological or hazardous materials prior to completing this protocol.

Limitations of the Procedure

Procedure should be carried out in a biological safety cabinet. Take care when using scalpels and handling blades. Technician should wear a lab coat and double-glove to prevent transmission of blood-borne pathogens from placental tissue.

Cutting the Tissues and Placing in Cassettes for Processing Preparation

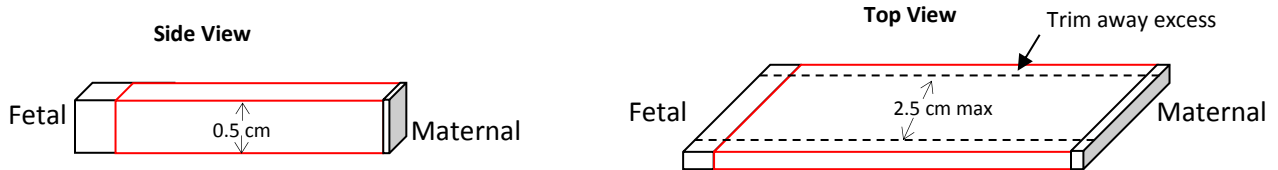
1. Label cassettes with the ID and tissue type.
2. Fill plastic tub with 1.4-1.5 Liters of 70% ethanol. Use the 95% histology grade ethanol.
3. Put metal rack in tub with ethanol to hold the samples in their cassettes.
4. Gather a plastic container to use as a liquid waste container.
5. Gather a small shipping biohazard bag and line another plastic container for solid waste.
6. Gather forceps and scalpel and extra blades.
7. Place everything in the hood.



8. Begin cutting sections of the tissues and place them in the proper cassette (see notes below). If one of the aspects is missing, report it on the specimen form. Enter this information digitally at the end.
9. Store the tissue filled cassettes in the 70% ethanol filled plastic tub.

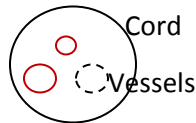
Placenta

You want a cross section of both maternal and fetal aspects. The fetal side has an opaque, tough membrane on it. The maternal side has a thin semi-clear membrane. Trim away the outer parts if you can, leaving a rectangular, flat piece. If it is large enough, cut several pieces and fit them in the cassette.



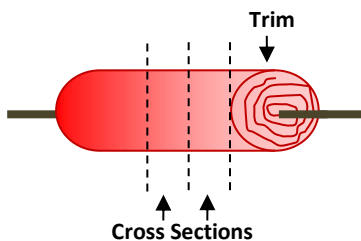
Cord

You want 2-4 cross sections of cord all facing the same direction in the cassette. The cord should have three vessels—two are easy to see, one is more difficult. If needed, trim a little of the cord so you get a flat surface.

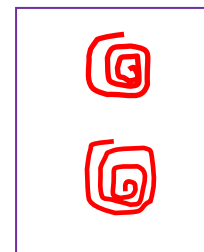


Membrane

The membrane consists of the amnion and the chorion. It should come wrapped in a roll around a wooden stick. If the roll has come undone slightly, reposition the membrane on the stick to form a tight roll. Trim away an outer piece of the membrane roll until it has a complete cross section. Cut a cross section of the membrane roll about 0.5 cm wide and place it in the cassette on its side. Cut one or two more cross sections and place in the cassette. Don't let them come unraveled!



Placement in Cassette



Clean Up

1. The plastic specimen cups go in the big biohazard bin.
2. Liquid waste goes into labeled ethanol waste container.
3. Solid waste goes in the big biohazard bin.
4. Used blades go in the sharps container.
5. Spray the cutting area with 70% ethanol and 10% bleach and wipe the area as best you can.
6. Turn off the hood light and fan.



7. Wash forceps and scalpel holder with bleach in sink.

Placing in Tissue Processor

1. Take the plastic tub with the cassettes to the Histology room.
2. Make sure the processor has liquid (ethanol) in it and is at the "Ambient" condition. Place all cassettes in the rack of the tissue processor and place the lid on top (cassettes will float during the cycle otherwise). Close the processor (lid).
3. Run the tissue processor. After the tissues are processed, embed them in paraffin.

Embedding the Tissues in Paraffin

1. Put the cassettes in the holding area in the tissue processor. Turn on the cryo by pressing the cryo button and let it cool until the cryo reaches below 0 degrees C.
2. Use the big molds because the tissues won't fit in the small ones.
3. Press tissue into the bottom of the mold. Hold it down with forceps and release some paraffin.
4. While holding it down, move the mold to the freezing area for a few seconds while the bottom part freezes. Put on the cassette.
5. Move it back to the heat and add more paraffin.
6. Move the mold to the big freezing area and let sit until it solidifies. Cassette will easily separate from the mold tray once all of the paraffin has solidified.
7. Carefully take the cassette out of the tray and use a knife to trim away excess paraffin.
8. You want to avoid air bubbles underneath the biopsy. In step 4, press it down a lot while it's freezing. If there are bubbles at the end, you can try to melt that side a little on the heating area so the bubbles fill themselves in. Make sure the tissue is entirely covered in paraffin.
9. File the cassettes in a cabinet, noting the location information digitally.