PROCEDURE	Placenta Collection	
PREPARED BY	GAPPS Staff	
DATE ADOPTED		
REVIEWED BY	SIGNATURE	REVIEWED DATE

REVISED BY	SIGNATURE	REVISED DATE

SUMMARY OF CHANGES TO THIS SOP

Version: 4.2 –
1. Frozen tissues will be immediately snap frozen on dry ice
2. Frozen tissue samples will now be placed in 5 mL vials prior to freezing.
3. Placenta weight only taken if placenta will **NOT** be examined by a pathologist.
4. Placenta pictures will no longer be performed.

Version: 4.3 –
1. Control Punches for Placenta Punch RNAlater and Placenta Punch Flash Frzn added.

Version: 4.4.1 –
1. Added “Placenta to Pathology” check box to req. form
2. Added “Placenta Weight” field to req. form
3. Added “Placenta dimensions” field to req. form

Version: 4.4.2 –
1. Added “Membrane Roll” procedure for formalin membrane
2. Added 15ml Brown cap Sarstedt tube for membrane roll

PURPOSE

This Standard Operating Procedure (**SOP**) is intended for the collection, processing, and storage of human placental and umbilical cord tissues.

SCOPE

This procedure describes the isolation, processing, temporary and long-term storage of human placental tissues obtained after cord blood has been removed. It does not describe the collection of cord blood, or any assays or analysis of the placenta.

AUTHORITY AND RESPONSIBILITY

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.
3. The site's PI and Coordinator is responsible for the implementation of this procedure at their site and for ensuring that all appropriate personnel are trained and sign "Acknowledgement of Understanding" document for this SOP.
4. All health care providers and technicians who implement this SOP at study sites are responsible for reading and understanding this SOP prior to performing the procedures described.
5. All health care providers and technicians are expected to be trained and follow the procedures described in any of the GAPPS SOPs and have their signature on file at the collection site.

LIMITATIONS

Placenta processing can only occur on tissue that meets the following criteria to maintain consistancy and quality of tissue:

1. Cord blood collection for GAPPS or other entities should occur before tissue collection.
2. All tissues collected for freezing or for preservation in RNAlater **MUST** be collected and processed less than 30 minutes after participant delivers placenta. IF the placenta is not available until 30 minutes after delivery then exclude frozen and RNAlater tissue types and collect all other tissue types and store as indicated in this SOP.
3. Placenta should be fresh and have not been treated with any preservatives or reagents (eg. Formalin or Ethanol) before tissue collection.

SAFETY

1. Required Training for processing
   a) Blood borne pathogens
   b) Standard laboratory practices including centrifuge safety
   c) Chemical safety training
   d) Fire safety
2. Risks
   a) Sharps hazard
   b) Blood and biofluid exposure
   c) Chemical exposure, inhalation hazard (formaldehyde, 5%)
   d) Flammable liquids hazard (70% ethanol)
3. Required safety equipment
   a) Lab coats/scrubs
   b) Face shield/safety goggles

c) Closed toed shoes
d) Gloves
e) Flammable liquids cabinet
f) Formalin waste container

All health care providers and technicians are expected to be trained and follow universal precautions when handling biological or hazardous materials when performing the any procedures described in any of the GAPPS SOPs.

EQUIPMENT

Recommended vendors and equipment are listed. Unless otherwise specified, equipment of equal or better quality than those recommended can be used.

1. PREPARATION & PROCESSING

a. Clean scalpel handle with disposable blades
b. Clean scissors
c. Clean forceps
d. Extra-long histology forceps (optional; for dry ice bath freezing)
e. Large dissecting board
f. Dry ice bath or dry ice
g. 2-8°C refrigerator
h. -70/-80°C freezer for storage
i. Bleach bucket or equivalent sanitizing agent

REAGENTS

Recommended vendors are listed. Unless otherwise specified, reagents of equal or better quality than those recommended can be used.

1. Placental Processing Reagents

a. RNAlater: (Ambion, catalog # AM7021, 500 mL)

b. Formalin Solution: 10% normal buffered formalin (~50ml Formalin; 60mL cup) (Fisher, catalog # 23-005-500). Store at room temperature (15-30°C) until used. Discard if visible signs of contamination, such as color change or cloudy appearance, develop.

c. Ethanol Solution: 70% histology grade ethanol solution (Fisher, catalog # A405P-4, 4L)

d. Disinfectant: 10% Bleach bucket & spray bottle

TISSUE PROCESSING

There are two approved methods of snap-freezing collected samples: ethanol-dry ice bath and dry ice.

1) Ethanol-Dry Ice Bath Method: Using long histology forceps lower processed tissue for freezing into dry ice-chilled ethanol. Hold momentarily as vial freezes before proceeding to slowly submerge the entire specimen.

2) Dry Ice Method: Fill box with pelleted dry ice or broken up block dry ice.

NOTE: Collect RNAlater and frozen specimens only if it has been < 30 minutes after delivery of the placenta. If it has been > 30 minutes after delivery of the placenta, skip collection of tissue for RNAlater and for freezing but collect all other specimen types.

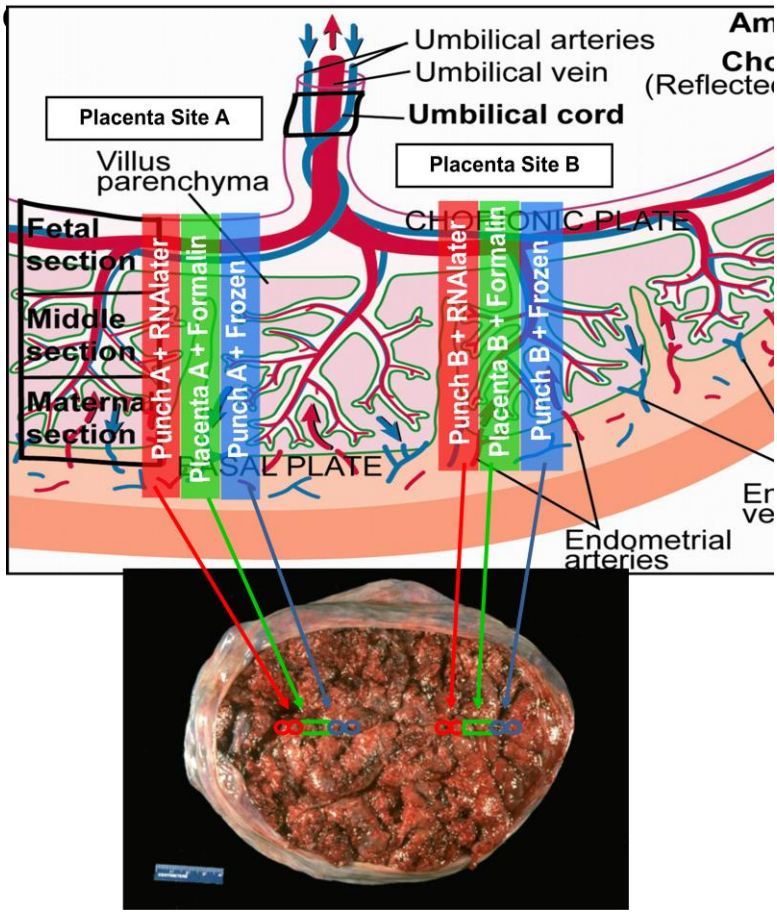
MULTIPLE BIRTHS

It is important to note that in the event of multiple births or the presence of multiple placentas and/or cords, each set should be treated as a separate specimen collection with its own requisition form (e.g. designated Twin A and Twin B). If there is one placenta with multiple cords each of the cords need to be designated as a separate specimen. All the procedures below reflect the processing of a single placenta.

Tissue Processing Priority List:

1. Placental Disc Punches and Membrane RNA specimens
2. Frozen specimens
3. Placental membrane swab
4. Formalin preserved specimens
5. Placenta weight (if applicable)

**NOTE: Remember to clean all instruments after placenta collection



Note: placenta sizes, shapes and cord insertion points are highly variable. The images and descriptions are given based on average specimens

1. COLLECTING PLACENTAL DISC TISSUE PUNCHES for RNA and Frozen

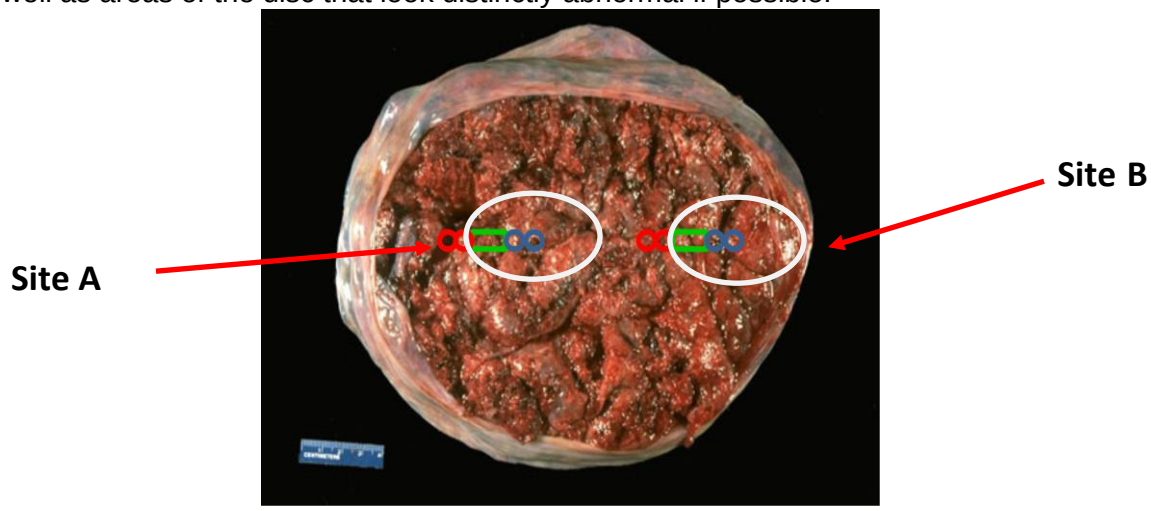
NOTE: These specimen types should **not be collected > 30 minutes** after delivery of the placenta

Required equipment:

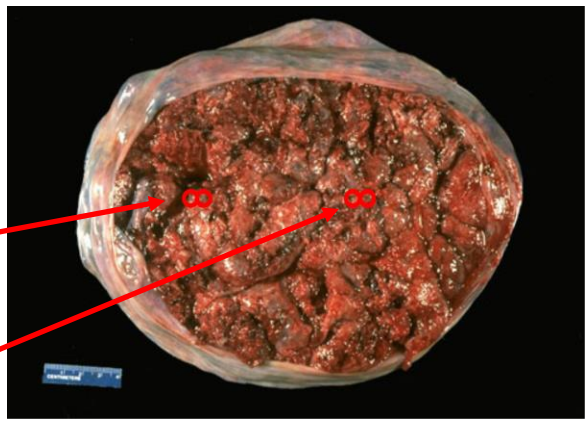
On Site:
1. Clean scissors
2. Clean dissecting and extra-long histology forceps (optional for dry ice bath freezing)
3. Container with dry ice bath or dry-ice

Supplied in Kit:
1. One 8mm biopsy punch
2. One 10mm biopsy punch
3. Two 5 ml tubes with RNAlater
4. Four 5ml tubes for frozen tissues
5. One 5ml tube for RNAlater and one 5ml tube for Flash FRZN Controls (in select kits only)

1.1. Select two sites separated by several centimeters on the placental disc (**Site A** and **Site B**), avoiding the site of umbilical cord insertion and disc edge (see diagram below). These collection sites will be used for the duration of this protocol. Avoid any visible lesions as well as areas of the disc that look distinctly abnormal if possible.

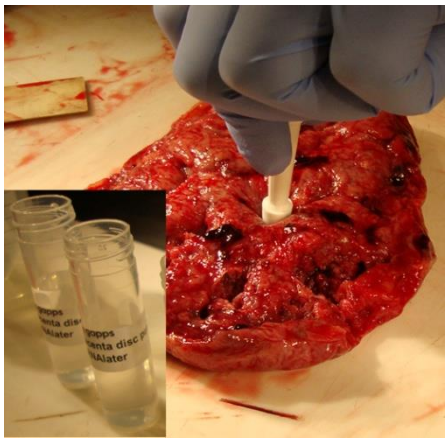


1.2. From the maternal side take **four 8 mm full-thickness vertical tissue punches** from the placental disc for **RNAlater**. A recommended technique is to twist punch during sampling and to thread the punch completely through the disc; this ensures complete sampling and inclusion of both fetal and maternal membranes.



1.2.1. <u>Two, 8 mm tissue punches from Site A:</u>
into Placenta Punch 1 + RNAlater

1.2.2. <u>Two, 8 mm tissue punches from Site B:</u>
into Placenta Punch 2 + RNAlater

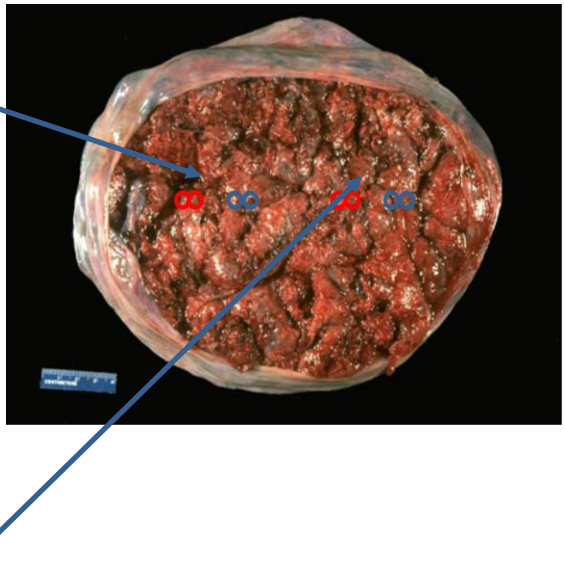


1.3. **Two plugs of tissue from each site** are placed in their respective GAPPS pre-labelled 5 mL tubes **(Site A and Site B),** with ~3 ml of **RNAlater**. If the kit contains a **Placenta Punch RNAlater Control** tube, take an additional punch adjacent to Site A and place it in the GAPPS pre-labeled 5 ml **RNAlater Control** tube. Store all samples at 4ºC before shipping to GAPPS.

(Note: In certain cases the punch is not effective at getting a full-thickness sample, particularly if the placenta is very soft, as seen in premature gestations (<28 weeks). In this situation a 0.5 cm full-thickness slice retrieved by scalpel or scissors can be taken and placed in a larger container with RNAlater (require 10 times the volume of RNAlater to tissue volume). If submitting a slice of placenta in RNAlater, rinse in sterile PBS first to wash away excessive maternal blood.)

1.4. For frozen placenta specimens, take one 10mm punch biopsy starting from the maternal side of the placenta from **Site A** in an area adjacent to the RNAlater sampling site (see diagram below). Use forceps to place 10mm plug into 5ml vial labeled ***Placenta Punch – 1 + Flash Frzn***. Repeat the 10mm biopsy punch in an area immediately adjacent to first sampling in Site A. Use forceps to place 10mm plug into the second 5ml vial labeled ***Placenta Punch – 1 + Flash Frzn***.

1.4.1. Two, 10 mm Tissue Punch, Site A: *labeled as Placenta Punch – 1 + Flash Frzn*



1.5. Perform the steps outlined above for the second collection site, **Site B**, in an area adjacent to the RNAlater sampling site (see diagram). Use forceps to place the first 10mm plug into 5 ml vial labeled ***Placenta Punch – 2 + Flash Frzn*** and the second 10mm plug into the second 5ml vial labeled ***Placenta Punch – 2 + Flash Frzn***. If the kit contains a **Placenta Punch Flash Frzn Control** tube, take an additional punch adjacent to Site A and place it in the GAPPS pre-labelled 5 ml **Flash Frzn** Control tube.

1.5.1. Two, 10 mm Tissue Punch, Site B: *labeled as Placenta Punch – 2 + Flash Frzn*

1.6. Immediately following collection freeze vials on dry ice. Surround specimens completely to facilitate rapid freezing.

1.7. Once frozen, store samples at -80ºC before shipping on dry ice to GAPPS.

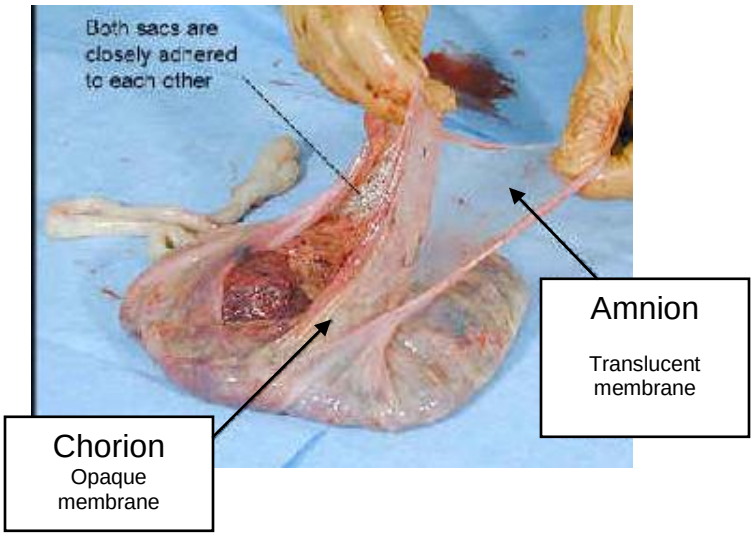
2. MEMBRANE SWAB

Required equipment:

On Site:
1. Forceps

Supplied in Kit:
1. Sterile swab
2. 1.8mL cryo-vial

2.1. Using clean forceps, gently pull apart Amnion and Chorion membranes along the edge previously cut to make the membrane strip. Probe between the membranes and collect cells with a sterile swab. See below picture showing separation of amnion and chorion:



2.2. Place the swab end into the 1.8ml cryo-vial labelled ***Membrane Swab + Frozen***, breaking off the head of the swab. Save the wooden stick for the formalin membrane roll below.

2.3. Cap the vial and freeze on dry ice.

2.4. Store at -80°C and ship on dry ice to GAPPS.

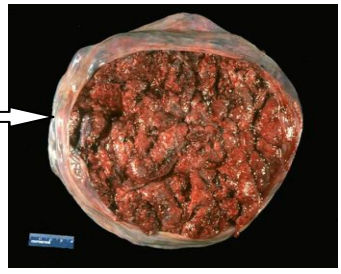
3. COLLECTING PLACENTAL MEMBRANE TISSUE

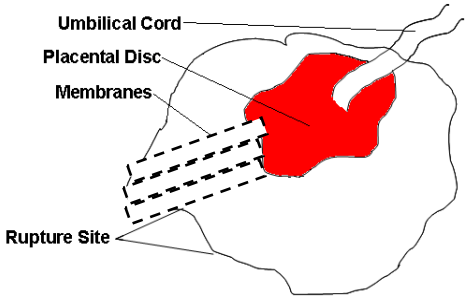
Required equipment:

On Site:
1. Clean scissors
2. Container with dry ice bath or dry ice
3. Formalin
4. Clean dissecting forceps and extra-long histology forceps (optional for dry ice bath freezing)

Supplied in Kit:
1. Two 5 ml tubes
2. One 5 ml sterile tube with RNAlater
3. One 15ml conical tube for formalin

Membrane Rupture Site

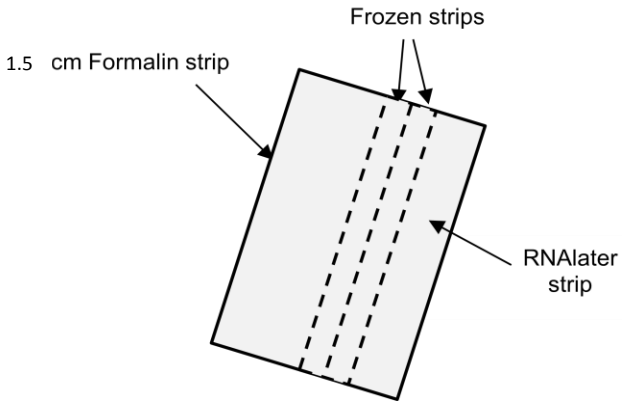




3.1. Locate the placental membranes. Orient the placenta so that the disc lays flat and the membranes are as spread out as much as possible without damaging them. The point of membrane rupture is easier to see from the maternal surface (see picture above left with arrow).

3.2. First, with scissors cut a 0.5 cm wide strip of membranes from the placental rupture site to the edge of the placental disc (margin). Separate from the placental disc and transfer to the 5 ml tube labelled, ***Membrane + RNAlater***. Make sure that the specimen is in contact with the RNAlater following collection. Store at 4ºC before shipping to GAPPS.

3.3. Cut an additional slice 0.5 cm wide from point of rupture to the edge of the placental disc. Cut free at placental margin, bunch up with forceps, and place into a 5 ml tube labelled, ***Membrane + Flash Frzn***. Take another 0.5 cm wide membrane strip and place in the second tube labelled ***Membrane + Flash Frzn***. Immediately freeze in dry ice bath or on dry ice



3.4. Cut a final 1.0 ± 0.25 cm membrane strip from point of rupture to placental disc. Roll up the strip on the wooden stick from the membrane swab starting at the point of rupture and place in the 15ml brown capped tube labeled ***Membrane + Formalin***. If necessary to get a full length roll, cut the membrane at the halfway point and roll the remainder on a second stick. Cut the membrane from the placenta leaving a sliver of the placental disk at the terminus of the roll to identify orientation and transfer to the 15ml tube. Apply the associated PTID label to the formalin container and fill with a minimum of 10ml 10% formalin. Store at room-temperature. Transfer from formalin to 70% ethanol 48-72 hours after obtaining the specimen. Store at 4ºC before shipping to GAPPS.

4. COLLECTING UMBILICAL CORD TISSUE

The umbilical cord should be sampled from the fetal end of the cord remaining on the placenta after delivery. Avoid sections of the umbilical cord that were previously clamped or sustained needle punctures during the cord blood collection.

Required equipment

On Site:

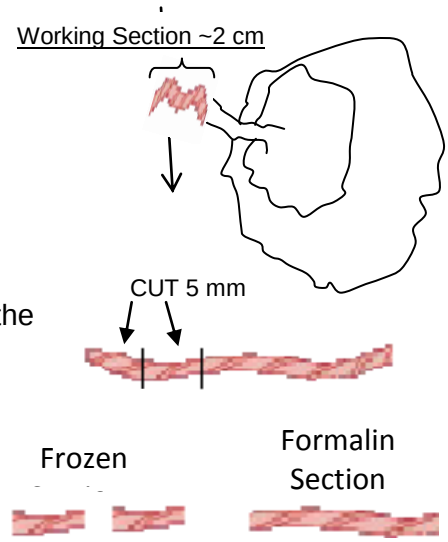
1. Clean scalpel/scissors
2. Container with dry ice bath or dry ice
3. Clean forceps and extra-long histology forceps (optional for dry ice bath freezing)

Supplied in Kit:

1. Two 5 ml vials
2. One specimen cup with 50-60 ml formalin

4.1. Remove section of umbilical cord adjacent to the clamp site. Cut two consecutive ~0.5 cm cross-sections for frozen tissue from the umbilical cord. Place each into the two 5 ml vials labelled: ***Cord + Flash Frzn***. Immediately freeze on dry ice. Store at -80 ºC and ship on dry ice to GAPPS.

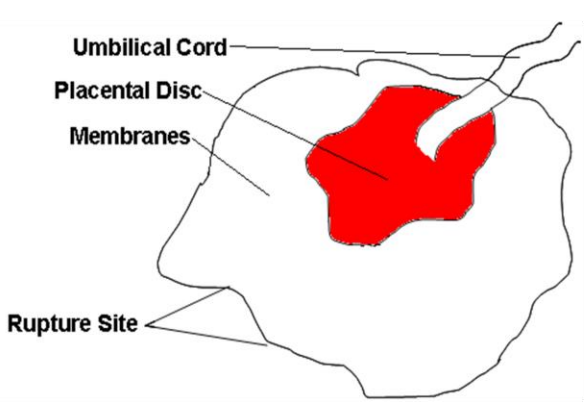
4.2. Cut an additional piece ~1 cm long and place into the designated formalin container, ***Cord + Formalin***. Ensure PTID label has been applied to the outside of the designated formalin container. Transfer from formalin to 70% ethanol 48-72 hours after obtaining the specimen. Store at 4ºC before shipping to GAPPS at ambient temperature.

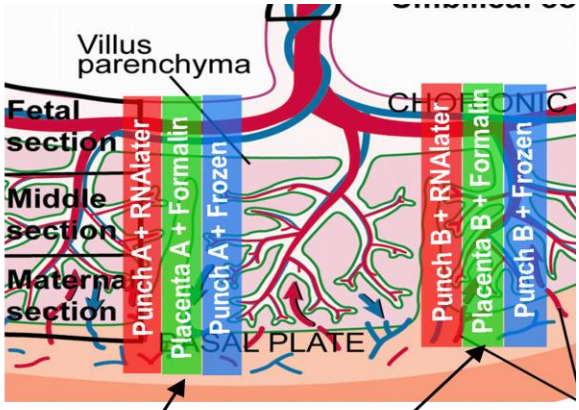


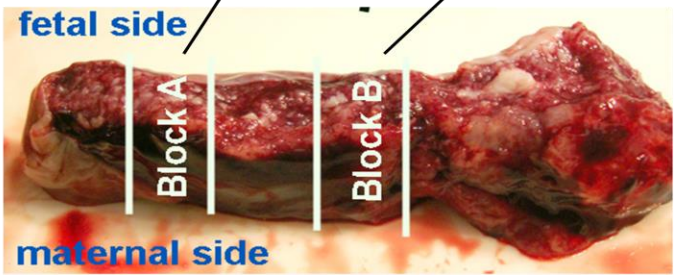
Required equipment:

On Site:
1. Scissors
2. Scalpel
3. Dissecting forceps
4. Formalin

Supplied in Kit:
1. Two specimen containers (1 sterile cup and one 50 ml conical tube) for formalin







5.1. Locate area between tissue punches sampled for RNAlater and frozen (see above)

5.2. Take a **full thickness sample (encompassing maternal and fetal aspect) located between biopsy punches** and place respectively in designated formalin container (***Placenta – A + Formalin*** and ***Placenta – B + Formalin***). Ensure PTID label has been applied to the outside of the designated formalin container

5.3. Transfer from formalin to 70% ethanol 48-72 hours after obtaining the specimen. Store at 4ºC before shipping to GAPPS.

6. Placental Weight:

Note: Placental weight is only required on placentas NOT examined by a pathologist

Required equipment:

On Site:	**Supplied in Kit:**
1. Sterile gauze 2. iBalance 2500 laboratory scale	n/a

6.1. The placenta should be laid out and excess blood should be wiped away with sterile gauze. Placenta should be taken to the processing area (this may vary at each site) and be trimmed. Use a metric scale to record the weight of the tissue. Make sure to "tare" the scale or subtract the weight of the empty container holding the tissue when weighing all tissue.

6.1.1. Trimming the Placenta: Membranes should be removed (trimmed) from the placental disc and cord cut ~5cm for point of insertion prior to weighing the disc. **Record the weight of the placenta on Laboratory Requisition Form.**

7. SPECIMEN STORAGE

7.1. Once frozen, store samples at -80ºC before shipping on dry ice to GAPPS.

7.2. Place all remaining placental tissues (e.g. cord, membranes, and disc) into a sealed storage container and dispose of according to respective institution's policies.

7.3. If tissue in formalin cannot be shipped to GAPPS within 2 days post collection, transfer formalin-fixed tissues to 70% ethanol after 48-72 hours of fixation.