Standard Operating Procedure: Sample Collection and Preservation of Blood and Endometrial Tissue

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1. PURPOSE

This SOP describes the process of how to obtain and preserve blood and endometrial tissue to be used for primary cell culture, molecular assays, and tissue morphology, as developed in the NIH UCSF Human Endometrial Tissue and DNA Bank.

2. SCOPE

This procedure applies to blood and endometrial samples collected for research performed at USCF or by other NIH sponsored investigators.

3. RESPONSIBILITIES

3.1 It is the responsibility of the Principal Investigator and Director of the NIH UCSF Human Endometrial Tissue and DNA Bank to maintain this procedure.

3.2 It is the responsibility of each sample collection operator to understand the contents of this procedure and adhere to the procedure.

3.3 It is the responsibility of the manager or director to ensure that this procedure is followed and that all personnel adhere to the procedure.

4. REFERENCES AND DOCUMENTS

4.1 CHR Application for the NIH UCSF Human Endometrial Tissue and DNA Bank H52883-27873


4.3 Endometriosis: Natural History, Diagnosis, and Outcomes study, UCSF Field Procedures Manual, Sep 2007

4.4 Handling, Transport, and Storage of Specimens for Molecular Methods, CLSI Approved Guideline (MM13-A), Aug 2007

4.5 The UCSF Guide for the Research Use of Human Biological Specimens: Collecting, Banking and Sharing Specimens, May 2005
5. DEFINITIONS AND ABBREVIATIONS

5.1 Cervix: Neck of the uterus that extends down into the vagina.

5.2 DEPC Water: deionized water treated with diethylpyrocarbonate (DEPC). The DEPC treated water is sterilized by autoclaving. This process creates RNase and DNase free water which is recommended in any application where RNA is involved.

5.3 Endometrium: The mucous membrane lining the uterus (endo).

5.4 Morcellate: A surgical process of cutting tissue into small pieces for removal from the body.

5.5 Myometrium: The middle layer of the uterine wall consisting of smooth muscle cells and supporting stromal and vascular tissue (myo).

5.6 O.C.T gel: Embedding medium for freezing tissue specimens to ensure Optimal Cutting Temperature (O.C.T).

5.7 Perimetrium: The outer membrane (serosa) surrounding the uterus.

5.8 Pipelle® (Cooper Surgical, Trumbull, CT): A thin plastic tube that is inserted into the uterus that is used to collect endometrial samples. Samples are collected by aspirating the tissue into the plastic tube.

5.9 RNAlater®: Commercially available tissue medium from Qiagen (Valencia, CA) that is added to endometrial tissue to preserve the RNA and allow transport at room temperature.

5.10 TTM: Tissue Transport Medium consisting of Hanks balanced salt solution (HBSS) without phenol red, magnesium, and calcium plus the addition of 10% fetal bovine serum (FBS) and 1× penicillin and streptomycin solution.

6. MATERIALS AND REAGENTS

6.1 Materials needed in tote kit for a minimum of one sample collection

- Containers and Paperwork:
  - Styrofoam container for keeping samples cold or cooler tote
  - Liquid Nitrogen container filled at least 1/3 full of liquid nitrogen
  - Copy of consent form
  - CHR study approval letter or IRB approval
  - Sample Collection Record Form (one for each subject)
• Equipment:
  • Alcohol wipes
  • Biohazard plastic bags (>2)
  • Disposable blue absorbent pad or equivalent
  • Disposable gloves (latex free) and safety goggles
  • Numbering sheet for Sample ID numbers
  • Patient Labels
  • Pencil and Sharpie pen or equivalent
  • Pipelle® collection device
  • O.C.T. gel (manufacturer)
  • O.C.T. tissue mold (>2)
  • Test tube rack or storage box
• Instruments:
  • Sterile forceps (> 2)
  • Sterile scalpel
  • Sterile transfer pipettes (>2)
  • Sterile tweezers
  • Sterile empty tubes and dishes
  • Sterile Petri dish
  • Sterile 2 mL cryovials; white cap (>6)
  • Heparin green top Vacutainer™ Tubes, BD, Franklin Lakes, NJ
  • Red top Vacutainer™ 10 mL tubes
• Sterile pre-filled tubes
  • 15 mL sterile tube containing 7 mL TTM (>2)
  • 2 mL sterile vial filled with approximately 1.0 to 1.5 mL of 10% formalin (>2)
  • Sterile 2 mL prefilled capped tubes containing 0.5 -1mL RNA-Later® (>5)
  • 50 mL sterile tube containing 26 mL of RNase and DNase free water and 4 mL of 10 × PBS

6.2 List of reagents requiring preparation (See appendix 1 for how to prepare these reagents)

• 4% paraformaldehyde solution
• Phosphate Buffered Saline (PBS) pH 7.2 (1×)
• DEPC Water
• Tissue Transport Medium (TTM)
6.3 List of required reagents commercially available (use current vendor or equivalent)

- 16% paraformaldehyde (Electron Microscopy Science, Hatfield, PA)
- RNA later® (Qiagen)
- Liquid nitrogen
- 10× PBS (Fisher Scientific, Pittsburg, PA)
- 10% buffered formalin (Fisher Scientific, Pittsburg, PA)
- RNase and DNase free water (if DEPC water is not available)
- DEPC reagent (SigmaAldrich, St Louis, MO)
- Hanks Balanced Salt Solution without phenol red, calcium, and magnesium (HBSS) (Invitrogen, Carlsbad, CA)
- Fetal Bovine Serum; charcoal stripped (FBS) (Gemini Bioproducts West Sacramento, CA)
- 100 × Penicillin and Streptomycin solution (P/S) (Invitrogen)

7. Sample Collection Approvals

7.1 A biological use authorization number may be required to work with human derived samples in academic institutions. Contact the Biological Safety Committee or equivalent committee to obtain a number.

7.2 The Committee on Human Research (CHR), or other Institutional Review Board (IRB), will review all specimen collection protocols.

7.3 CHR or IRB approval is needed before the sample collection protocol can be implemented. Refer to Appendix 2 for an example of an approval letter.

7.4 Patient consent is necessary before obtaining endometrial tissue and blood samples. Refer to Appendix 2 for an example of a patient consent form for sample collection, Subject’s bill of rights, and HIPAA compliance (PHI) form.

7.4.1 The patient must be consented before the procedure is performed. Pre-operative visits are often a good time to approach the subject in participating in the research study.

7.4.2 The PI or his/her associates, e.g., the Research Associate (RA), will begin the consenting process by introducing her/his-self and explaining the purpose of the study and sample collection procedures to the subject.

7.4.3 Once satisfied that the participant thoroughly understands the informed consent documents, ask the participant to sign the appropriate forms.

7.4.4 The signature of a witness to the participant’s signature is also required.
7.4.5 A copy of the signed consent is provided to the patient.

7.4.6 A copy of the signed consent is also placed in the patient’s chart.

7.4.7 The original signed consent is kept by the PI in a study binder in a secure location.

8. **Blood Sample Collection.**

8.1 **Blood Sample Collection Procedure**

8.1.1 Blood collection is to be performed by a licensed phlebotomist, nurse or anesthesiologist. The collection can occur at the pre-op visit or in the operating room from the consented individual.

8.1.2 Collect two 10 mL red top tubes (with or without a serum separator) of blood and one green top Heparin tube for plasma. Mix the Heparin tube gently after collection to ensure the anticoagulant is thoroughly mixed with the blood to prevent the blood from clotting.

8.1.3 Label the tubes with the subjects sample ID number.

8.1.4 Place the tubes in a rack in an upright position and transport to the lab within two hours at room temperature. If there is a delay > 2 hours in getting to the laboratory, place the blood samples on ice until processing.

8.1.5 Record the time of collection on the associated SOP form.

8.2 **Serum and Plasma Processing Procedure**

8.2.1 Serum and plasma processing will occur in the laboratory. Follow blood borne pathogen standard for handling human blood and tissue.

8.2.2 Allow the red top tubes to clot for 30-60 minutes at room temperature in an upright position.

8.2.3 Centrifuge the green top and the red top tubes for 15 minutes at a speed of approximately 2500 rpm (1500 x g). Optimal time between blood draw and processing is less than 4 hours but should not exceed 24 hours.

8.2.4 Carefully remove the tube stopper while under a biological safety cabinet or place a blood block barrier material over the stopper before opening to eliminate aerosols.

8.2.5 Using a sterile plastic transfer pipette carefully remove the serum or plasma from the blood tube making sure not to disturb the cell layer.

8.2.6 Fill a 2 mL cryovial approximately ¾ full with serum or plasma.
8.2.7 Repeat step 8.2.6 until all the serum or plasma has been transferred.

Note: If cells are accidentally mixed with the serum or plasma, the aliquot vial can be re-centrifuged and the serum or plasma can be transferred to a new cryovial.

8.2.8 Label the cryovials with the subject’s sample ID number followed by a dash and the aliquot number. For example, UCSF-635-1, where the prefix is the site’s initials, the XXX number is the sample ID, and the -1 represents the aliquot number.

8.2.9 Place serum and plasma aliquots at \(-80^\circ\) C for long term storage.

8.2.10 Record sample processing and storage times on associated SOP form.

9. **Tissue Sample Collection Preparation**

9.1 Prepare a sample collection tote kit with materials listed in section 6.1.

9.2 Fill liquid nitrogen container with liquid nitrogen from the storage tank. Generally the container is filled about 1/3 full. Add more if there will be a delay in getting the samples to the laboratory.

9.3 Fill cryovials and tubes with appropriate medium. Refer to section 6.2.

9.4 Fill a biohazard bag with ice in advance if ice will not be available at the collection site.

9.5 Fill out SOP form with any patient information obtained during the patient consent visit and assign a study number to this patient case from the study log.

10. **Tissue Sample Collection Procedure**

10.1 Endometrial Biopsy Samples collected as an outpatient or in the operating room.

10.1.1 An *endometrial biopsy* sample is obtained by the physician using a disposable endometrial suction device (Pipelle® or similar device). The device is inserted through the cervix and passed to the fundus (top) of the uterus. The device’s plunger is withdrawn to create gentle suction and rotated within the uterine cavity to obtain an endometrial sample.

10.1.2 The physician hands the Pipelle® to the RA for distribution of the sample, or takes a more active role in the sample preparation by either expressing the aspirated material into a Petri dish or distributing the sample into appropriate tubes as described below.

10.1.3 A portion of the sample is used for the patient’s diagnostic evaluation, where appropriate, which is generally a formalin preserved sample for pathology review. The remaining biopsy material is used for the Tissue and DNA bank samples.
10.1.4 The remaining aspirated material is distributed into the appropriate tubes with the following priority for the Tissue and DNA bank:

Endometrial Biopsy Samples

- **1. RNA sample tube(s)** consist of endometrial tissue and is flash frozen in liquid nitrogen (Sample vial labeled #1). If liquid nitrogen is not available, dry ice can be used. Transfer sample(s) to a -80°C freezer for long term storage.
- **2. RNA later® sample** consists of endometrial tissue submerged in RNA later® medium. Store the sample at room temperature (20-25°C) or on ice for up to 24 hours and refrigerate for up to 7 days. Freeze at -80°C for long term storage.
- **3. 10% formalin sample** consists of endometrial tissue placed in 10% formalin. The sample is to be kept on wet ice or stored at 2-8°C up to 24 hours until processed.
- **4. Fresh cell sample** consists of endometrial tissue placed in Tissue Transport Medium (TTM). The sample is to be kept on wet ice or stored at 2-8°C up to 24 hours until processed.
10.1.9 Place any remaining sample in empty 2 mL cryovials for additional RNA sample vials (samples will be frozen as described in 10.1.5).

10.1.10 Label tubes with appropriate study number assigned from the study log.

10.1.11 Fill out SOP form with collection information and times when samples were processed.

10.2 Hysterectomy Sample Collection

10.2.1 General considerations

10.2.1.1 Follow operating room gowning and sterile procedures.

10.2.1.2 Confirm that the attending physician and surgical nurse know that the patient has consented to participate in the tissue bank collection procedure and review the samples that will be taken. This usually occurs in the “Time Out”.

10.2.1.3 Have a copy of the study approval letter from the CHR and written informed consent document available for review upon request.

10.2.1.4 Label all samples with the sample ID number assigned from the study log.

10.2.1.5 The procedures in sections 10.2.4 -10.2.12 are to be performed while in the operating room (OR). If a laboratory is located near the OR, steps 10.2.10-12 can be performed in the lab using tissue slices placed in the TTM tube. Tissue can then be removed from the TTM tube and processed as described for each step.

10.2.2 Samples to obtain from a hysterectomy specimen, when an intact uterus is provided, are depicted in Figure 2.
10.2.3 Refer to tables 1 and 2 for the samples to collect in order of priority depending on the quality of specimen:
Table 1: Priority of Sample Collection from a "Good Quality" Specimen

<table>
<thead>
<tr>
<th>Priority</th>
<th>Sample Type</th>
<th>Tissue</th>
<th>Container/Medium</th>
<th>Process Area</th>
<th>Transport Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RNA</td>
<td>endometrium</td>
<td>2 mL cyrovial(s)</td>
<td>OR</td>
<td>flash frozen/ Liq. Nit</td>
</tr>
<tr>
<td>2</td>
<td>RNA Later</td>
<td>endometrium</td>
<td>2 mL cyrovial/ RNA later</td>
<td>OR</td>
<td>RT/ ice</td>
</tr>
<tr>
<td>3</td>
<td>Formalin</td>
<td>endo/myo slice</td>
<td>2mL cryovial/10% formalin</td>
<td>OR</td>
<td>RT/ ice</td>
</tr>
<tr>
<td>4</td>
<td>4% paraformaldehyde*</td>
<td>endo/myo slice</td>
<td>freshly made 4% paraform.</td>
<td>OR</td>
<td>ice/2-8 C</td>
</tr>
<tr>
<td>5</td>
<td>O.C.T.*</td>
<td>endo/myo slice</td>
<td>OCT gel in mold</td>
<td>OR</td>
<td>flash frozen/ Liq. Nit</td>
</tr>
<tr>
<td>6</td>
<td>Fresh cells</td>
<td>endo/myo slice</td>
<td>15 mL tube/TTM</td>
<td>OR</td>
<td>ice/2-8 C</td>
</tr>
</tbody>
</table>

* If necessary, slices of tissue for these samples can be placed in the "Fresh Cell" tube for subsequent processing

Table 2: Priority of Sample Collection from a "Poor Quality" Specimen

<table>
<thead>
<tr>
<th>Priority</th>
<th>Sample Type</th>
<th>Tissue</th>
<th>Container/Medium</th>
<th>Process Area</th>
<th>Transport Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Formalin</td>
<td>endo/myo slice</td>
<td>2mL cryovial/10% formalin</td>
<td>OR</td>
<td>RT/ ice</td>
</tr>
<tr>
<td>2</td>
<td>4% paraformaldehyde</td>
<td>endo/myo slice</td>
<td>freshly made 4% paraform.</td>
<td>OR</td>
<td>ice/2-8 C</td>
</tr>
<tr>
<td>3</td>
<td>O.C.T.</td>
<td>endo/myo sawlice</td>
<td>OCT gel in mold</td>
<td>OR</td>
<td>flash frozen/ Liq. Nit</td>
</tr>
</tbody>
</table>

10.2.4 Aseptically cut the uterus open using a sterile scalpel.

10.2.4.1 Place the forceps into the cervical canal and slightly up into the uterus to help hold the uterus in place while cutting.

10.2.4.2 Cut the uterus lengthwise between the forceps in order to locate the uterine cavity.

10.2.5 Determine the quality of the tissue.

10.2.5.1 A good quality sample will have a pinkish red endometrial layer that will be clearly visible and will be suitable for collection of endometrial tissue for isolation RNA and/or fresh cells (see sections 10.2.8 and 10.2.12 below).

10.2.5.2 A poor quality sample will look like myometrial tissue only. Poor quality samples will not yield any visible endometrial tissue when scraped with the blade of a scalpel and are thus not suitable for isolation of RNA and/or fresh cells (see sections 10.2.8 and 10.2.12 below).

10.2.6 Examine the uterine cavity and determine which half of the cavity will be used for sample collection.
10.2.6.1 Obtain the endometrium sample from the upper 2/3 of the uterus. The lower uterine segment and the area around the tubal ostia are to be avoided since they are not steroid hormone dependent portions of the endometrium.

10.2.6.2 Avoid obtaining endometrium that overlies a fibroid located close to the endometrial cavity.

10.2.6.3 Always ensure adequate tissue for examination by the pathologist on both parts of the endometrial cavity.

10.2.7 Sample collection for a morcellated uterus

10.2.7.1 Place the sample in a Petri dish and examine the pieces closely.

10.2.7.2 Note the curvature of the tissue pieces and the textural differences between the tissue types (endometrium, myometrium, and perimetrium) in order to collect the proper sample.

10.2.7.3 Collect the samples in order of priority listed in Tables 1 and 2.

10.2.8 Collection of RNA sample and RNA\textregistered later sample

10.2.8.1 Scrape the endometrium with the blade of the scalpel until a small visible amount of tissue is seen on the blade and place the tissue in the empty 2 mL cryovial for the RNA sample or place the tissue into the 2 mL cryovial containing the RNA\textregistered later medium.

10.2.8.2 Repeat this procedure until 4-6 sample tubes have been collected for the RNA sample and 1-2 RNA\textregistered later samples have been collected.

10.2.8.3 Flash freeze the RNA sample tissue tubes in liquid nitrogen.

10.2.8.4 Transport the RNA\textregistered later samples at room temperature or on ice.

10.2.8.5 Record processing times and information on the SOP form.

10.2.9 Collection of 10% formalin-fixed samples

10.2.9.1 Slice a piece of the uterus containing the endometrial and the myometrial tissue and place in the vial containing 10% formalin.

10.2.9.2 Label the sample with the sample ID number.
10.2.9.3 Transport the formalin tissue sample at room temperature or on ice and process within 2 hours of collection.

10.2.9.4 Record processing times and information on the SOP form.

10.2.10 Collection of the 4% paraformaldehyde-fixed samples (PFA)

10.2.10.1 Prepare 4% paraformaldehyde solution by breaking a paraformaldehyde vial and adding contents (10 mL) to a 50 mL tube containing 26 mL of DNase and RNase free water, and 4 mL of 10X PBS.

Note: Prepare solution just before use. Divide up the 40 mL contents into 15 mL tubes depending on the number of samples to process.

10.2.10.2 Add a slice of the uterus containing endometrial and myometrial tissue to 4% PFA solution using sterile forceps.

10.2.10.3 Label the 4% PFA tube with the sample ID number.

10.2.10.4 Transport the 4% PFA sample at 2-8°C and process within 2 hours of collection.

10.2.10.5 Record processing times and information on the SOP form.

10.2.11 Collection of O.C.T Sample

10.2.11.1 Add O.C.T gel to the plastic O.C.T. mold

10.2.11.2 Slice a piece of the uterus (1-2 cm square) containing the endometrial and the myometrial tissue and place into the O.C.T gel.

10.2.11.3 Position the tissue near the bottom of the mold with the endometrial side of the tissue away from the edges. Use forceps to gently push away any air bubbles from the sample.

10.2.11.4 Flash freeze the sample by placing the tissue mold in liquid nitrogen. Use long forceps to carefully place the mold face up into the canister.

10.2.11.5 Record processing times and information on the SOP form.

10.2.12 Collection of Fresh Cells

10.2.12.1 Slice a piece of the uterus containing the endometrial and the myometrial tissue and place it in the 15 mL tube containing 7 mL of TTM.

10.2.12.2 Place tissue tube on ice.
10.2.12.3 Record processing times and information on the SOP form.

11. Sample Processing and Storage in the Laboratory

11.1 Labeling of sample cryoboxes for sample storage and retrieval

11.1.1 Place samples in cryoboxes for freezer storage (RNA, RNA/tex®, and O.C.T. mold).

11.1.2 Fill out the freezer location information on the SOP form starting with the freezer number, freezer shelf, rack number, and box identification number.

11.1.3 Assign a cryobox number based on consecutive numbering. For example, if the last box that was used was #22, the next new box will be labeled box #23.

11.2 RNA Samples

11.2.1 Remove frozen samples from liquid nitrogen using long forceps.

11.2.2 Ensure the sample is labeled properly with the sample number and date.

11.2.3 Place samples in labeled cryobox and place in -80°C freezer.

11.2.4 Record storage time and freezer location on the SOP form.

11.3 RNA/tex® Samples

11.3.1 Remove sample from refrigerator and follow steps 11.2.2 – 11.2.4 above.

11.4 Fresh Samples

11.4.1 Alert research personnel that fresh sample tissue is available for processing in the refrigerator.

11.4.2 Trained laboratory personnel are to perform the protocol for fresh cell preparation within 24 hours of tissue collection.

11.4.3 Contact laboratory manager if cells have been stored in TTM longer than 24 hours to determine if the processing should continue or cells are to be discarded.

11.4.4 Record if the cells were processed. If yes, record the time the sample was placed in digestion media. Record all information on the SOP form.

11.4.5 If the sample was processed outside of the 24 hour processing window, the sample will be flagged as a protocol deviation in the database.
11.5 O.C.T Block

11.5.1 Remove the frozen mold from liquid nitrogen, cover with foil, label with sample ID, place in labeled cryobox and place in the -80°C freezer.

11.5.2 Record sample preparation time and date along with freezer location information on the SOP form.

11.6 4% PFA-fixed sample for ethanol fixation

11.6.1 Carefully decant the 4% PFA into a chemical waste container.

11.6.2 Rinse the tissue by adding approximately 7 mL or equal volume of 1× PBS back to the tube containing the tissue.

11.6.3 Decant the 1× PBS down the sink and add an equal volume of 50% ethanol.

11.6.4 Allow the tissue to sit in the 50% ethanol for a minimum of 5-10 minutes.

11.6.5 Decant the 50% ethanol into the chemical waste and add an equal amount of 70% ethanol. The tissue can stay in the 70% ethanol until it is paraffin embedded.

11.6.6 Record when the 4% PFA was decanted off the sample to start of the ethanol fixation process on the SOP form.

11.6.7 If the sample was processed outside of the 2 hour processing window, the sample will be flagged as a protocol deviation in the database.

11.7 10% Formalin Sample for Ethanol fixation

11.7.1 Follow ethanol fixation steps 11.6.1–11.6.5 for the formalin sample.

11.7.2 Record when the 10% formalin was decanted off the sample to start of the ethanol fixation process on the SOP form.

11.7.3 If the sample was processed outside of the 2 hour processing window, the sample will be flagged as a protocol deviation in the database.

12. Sample Usage

12.1 RNA frozen tissue or tissue preserved in RNAlater® is used for molecular studies such as RNA gene expression.

12.2 10% Formalin samples are used for histologic and pathologic evaluation and dating of the endometrium.

12.3 Fresh tissue samples are used to isolate cells for cell culture experiments.

12.4 4% PFA samples are used for immunohistochemistry studies.
12.5 O.C. T samples are used for tissue frozen sections.
### DOCUMENT HISTORY

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<th>Effective Date</th>
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### AUTHORIZATION SIGNATURES

Print name and date under appropriate title below:

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Appendix One:

REAGENT PREPARATION

Buffers: 1× PBS from 10× PBS

**Phosphate Buffered Saline (PBS) pH 7.2:** prepare as described below and store in an appropriately labeled bottle at 2-8º C for up to six months:
Make a 1/10 dilution of the 10× PBS using DEPC or DNase and RNase free water as diluent
For example; (1 liter bottle)
- Add 100 mL of 10× PBS to a 1 litter bottle
- Add 900 mL of DEPC water to the 1 litter bottle

**4% Paraformaldehyde Solution (PFA)**
To a 50 mL sterile tube add the following reagents:
- 26 mL of DCPE water
- 4 mL of 1× PBS
- Add 10 mL of 16% paraformaldehyde by breaking the ampule and adding it to the tube
- Mix gently and add to sample.

**DEPC Water**
To a one liter container add the following reagents:
- Add 1 mL of DEPC reagent to 1 liter of deionized water
- Allow the DEPC reagent to dissolve over night at room temperature
- Mix and autoclave per standard autoclaving procedure for liquids

**Tissue Transport Medium (TTM)**
**HBSS containing 10% FBS, 1× P/S:** To a 1L container, add the following reagents:
- 890 mL HBSS (without phenol red, Ca, or Mg)
- 100 mL FBS
- 10 mL 100× P/S
## Attachment: Sample Collection Record Form

<table>
<thead>
<tr>
<th>Site ______ / Sample ID ______</th>
<th>Doctor ______________________</th>
<th>Consent Yes No</th>
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</thead>
</table>

### Patient Information

<table>
<thead>
<tr>
<th>Sample Location</th>
<th>Type of Sample</th>
<th>LMP __________</th>
<th>Date of Last Pap ________</th>
<th>Pap Result Normal Abnormal__________</th>
<th>Gravidity/Parity______</th>
<th>Smoker Yes No</th>
<th>Regular cycles Yes No</th>
<th>Weight __________</th>
<th>OCP Yes No</th>
<th>Endometriosis Yes No Not sure</th>
<th>Height__________</th>
<th>Ethnicity __________</th>
<th>Age__________</th>
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### Blood Collection

<table>
<thead>
<tr>
<th>Blood Collection</th>
<th>Yes No</th>
<th>Time sample centrifuged____ Time sample frozen____</th>
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</thead>
</table>

### Tissue Collection (check all that apply)

<table>
<thead>
<tr>
<th>Tissue Collection</th>
<th>Date/Time tissue removed from patient _<em><strong><strong><strong>/</strong></strong></strong></em></th>
<th>Date/Time sample processing complete _<em><strong><strong><strong>/</strong></strong></strong></em></th>
<th>Was the sample morcellated? Yes No</th>
<th>Comments:</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA sample # vials ____</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNALater® sample # vials ____</td>
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<td>Fresh cells</td>
<td></td>
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<tr>
<td>O.C.T. # molds ____</td>
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<tr>
<td>Formalin sample # vials ____</td>
<td></td>
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<tr>
<td>4% PFA # tubes ____</td>
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### Laboratory Processing

<table>
<thead>
<tr>
<th>Sample</th>
<th>Activity</th>
<th>Time</th>
<th>Date</th>
<th>Freezer #</th>
<th>Shelf #</th>
<th>Rack #</th>
<th>Box #</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA</td>
<td>Placed in - 80 C Freezer</td>
<td></td>
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<tr>
<td>RNA-Later</td>
<td>Placed in - 80 C Freezer</td>
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<tr>
<td>OCT block</td>
<td>Placed in - 80 C Freezer</td>
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<tr>
<td>Fresh cells</td>
<td>Processed ☐ yes ☐ no</td>
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</tr>
<tr>
<td>4% PFA</td>
<td>4% PFA removed</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>10% Formalin</td>
<td>10% Formalin removed</td>
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### Administrative Tracking

<table>
<thead>
<tr>
<th>Will an operation report be received?</th>
<th>Yes No</th>
</tr>
</thead>
<tbody>
<tr>
<td>If yes, was an endometriosis diagnosis confirmed?</td>
<td>Yes No Needs MD review</td>
</tr>
<tr>
<td>If endo, what stage:</td>
<td>minimal mild Moderate Severe</td>
</tr>
<tr>
<td>If review is needed, operation report referred to</td>
<td>Date: ________</td>
</tr>
<tr>
<td>Reviewer Interpretation: Endo Stage: minimal mild Moderate Severe Previous history/ no endo found at time of surgery</td>
<td></td>
</tr>
</tbody>
</table>

### Date Form completed ______________________ | Initials ____________________
Training Form

By signing the table below the signee has acknowledged they have read and understood this version of the SOP.

<table>
<thead>
<tr>
<th>Date</th>
<th>Print name</th>
<th>Title</th>
<th>Signature</th>
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