

# 1 STANDARD OPERATING PROCEDURE (SOP)

## 1.1 SOP: Uterine Lavage Specimen Collection, Processing, Shipping and Storage

### 1.1.1 Uterine Lavage

#### SOP for obtaining and processing uterine lavage biospecimens

Medical University of Vienna, University of Washington and TwinStrand Biosciences V8.4 2-7-18

#### Part 1: Bedside collection

A bedside kit will be provided with the following parts:

- MEDICOPLAST MF 13005 catheter (Figure 1)
- Balloon syringe (2 mL)
- Pre-filled BD PosiFlush™ saline 10 mL syringe (x2)
- Pasteur transfer pipette
- Transport tube (15 mL empty falcon tube)
- Stabilizing medium (15 mL tube with 5 mL 3x solution)

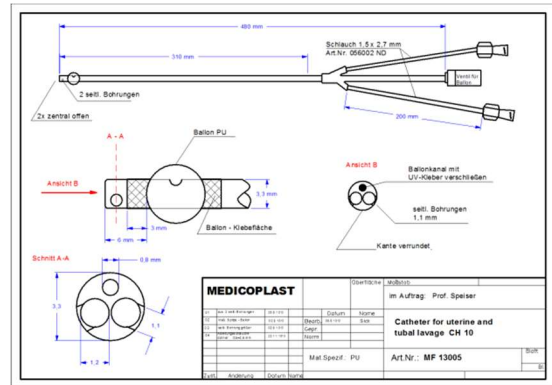


Figure 1. MEDICOPLAST MF 13005 catheter for uterine lavage

**CAUTION: Be careful to note which elements of the collection kit are sterile and which are not.**

The operator needs to provide the following material: speculum, betadine, swabs, forceps, Hegar's dilators, sterile gloves, method for pre-warming saline and appropriate draping material.

An online training video of the procedure can be found at the below web address and should be reviewed before the procedure. Please note that the collection tubes shown on the sterile drapes in the video are NOT sterile in the kits and should not be placed on the sterile field.

<https://vimeo.com/246795651/5eff1dc72d>

The protocol includes the following steps:

1. Under sterile conditions prepare the Speiser-catheter (Figure 2) by priming the two flush ports with normal saline pre-warmed to body temperature. First, use one of the pre-filled, pre-warmed syringes to fill the 2mL syringe with 1 mL of saline and connect it to the balloon port to later inflate the balloon at the tip of the catheter. Second, attach the remainder of this pre-filled syringe to one catheter port and flush until fluid comes out the tip and exactly 8 mL remains in the syringe. Third, attach the other pre-filled syringe to the other port and flush until exactly 2 mL remains in the syringe.
2. Clean of the cervix with antiseptic solution.
3. Infiltrate the cervix with lidocaine anesthetic using a 20G spinal needle. This is performed by initial submucosal injection, followed by a deeper infiltration at the 6 and 12 o'clock position (not needed when collecting intraoperatively). Cervical infiltration with local anesthetic is not required for patients having procedure performed under general anesthesia.
4. Grasp the cervix with a tenaculum at the 12:00 o'clock position and insert the prepared catheter through the cervical canal into the uterine cavity.

5. Inflate balloon with 1 mL of saline to seal the cervical canal and gently retract to engage.
6. If the cervix is too narrow to pass the 12G catheter, dilate it to 4.5 mm with Hegar's dilators.
7. Place the patient into the reverse Trendelenburg position, if not already done.
8. Push the plunger of the syringe containing 8 mLs of normal saline to slowly flush it into the uterine cavity and proximal tubes, while simultaneously gently pulling back on the plunger of the other syringe to aspirate the fluid, thereby slowly emptying one syringe while slowly filling the other. It is important to not flush in too quickly without concurrent aspiration on the other end, as fluid will be lost. The purpose of the 2 mLs of saline in the collection syringe is to allow brief back flushing if any plugging of the ports is encountered. Start first with flushing in 0.5 to 1mL of normal saline into the uterine cavity, before re-attempting to aspirate. After finishing the lavage, the balloon is deflated, and the catheter is removed.
9. Then, the lavage fluid is transferred from the syringe into the transport tube and the appropriate volume of 3x stabilization solution is added. If all 10mL of the lavage were recovered, use the Pasteur pipette to transfer the full 5mL of stabilizing medium to the lavage. If less is recovered, add only half the volume of the lavage worth of stabilizing medium.
10. Mix gently by inverting several times.
11. Stabilized sample is then carried to the on-site lab at room temperature for same day processing including on-site filtration and then frozen storage within a maximum 24 hours from collection.

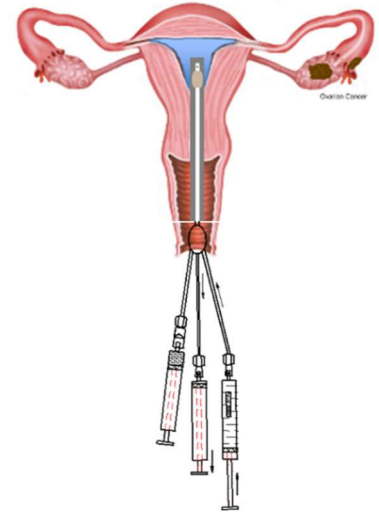


Figure 2. Uterine lavage concept

## **Part 2: Local lavage filtration**

This step takes place in a laboratory, near the site of collection. The goal is to filter the uterine lavage to remove clusters of endometrial cells. Then the uterine lavage is centrifuged to produce a cell pellet, which will be used for DNA extraction. Note that the filter used for filtration and the supernatant after centrifugation are not discarded but are stored frozen for potential future analyses. Processing occurs at room temperature.

A filtration kit with the following parts will be provided:

- Container #1 for cell pellet (50mL conical tube)
- Container #2 for filter (Screw top plastic jar containing 5 mL of Qiagen buffer ATL)
- Container #3 for lavage supernatant (15mL conical tube)
- Gravity flow 100 micron cell strainer

The facility needs to provide the following: test tube rack, swinging bucket centrifuge compatible with 50 mL conical tubes, -80°C freezer, dry-ice and packaging for frozen shipping.

The filtration protocol includes the following steps:

1. Place empty 50 mL conical tube (**container #1**) into a tube rack and place the filter on top of this tube. Pipette the ~15 mL of stabilized lavage fluid into the filter using disposable pipette. Very viscous samples may clog the filter. In this case re-aspirate, the fluid from the filter and re-apply. Repeat several times if necessary.
2. Place the filter itself into **container #2** with the mesh side down and gently swirl to cover debris in filter with the included 5 mL of buffer and close the container tightly. Store in 2x2 box.
3. Centrifuge container #1 at 3000 g with brake off for 10 minutes at room temperature.

4. Carefully aspirate the supernatant and transfer it to **container #3**. Be careful to not disturb cell pellet. Attempt to remove as much residual stabilizing medium as possible, using a smaller pipette as a final step.. Close tubes tightly. Store container #3 in 6x6 (5" tall) box. Store container #1 in 4x4 (5" tall) box.
5. Freeze all three containers at -80°C.
6. Ship the 3 containers (uterine lavage cell pellet, filter, uterine lavage supernatant) on dry ice. This shipment could also include paired blood, plasma and serum prepared from concurrently collected peripheral blood.

### **Part 3: Central lab DNA extraction**

This step takes place at the NCI Frederick lab. DNA is extracted from the uterine lavage cell pellet (container #1). The filter (container #2) and uterine lavage supernatant (container #3) are archived at -80°C for future use.

DNA extraction will be performed with standard Qiagen DNeasy Blood and Tissue kit (Cat #69506) using the spin-column protocol for purification of total DNA from animal tissues following manufacturer protocols and eluted into a standard buffer (10mM Tris HCl pH 7.5-8, 0.1mM EDTA). Ideally lysis and proteinase K digestion should be carried out at 37°C for 2 hours rather than 56°C for 10 minutes, but this is not critical. The optional RNase step should be followed. The DNA will be quantified with Nanodrop and Qbit; Nanodrop OD to inform on total nucleotide content and the 260/280nm ratio as a quality metric. The shipment manifests will list the sample volume, Nanodrop concentration, 260/280 ratio, and the Qbit concentration. The DNA will be divided, with **500ng each** sent to TwinStrands and McGill. If less than 2,000 ng is recovered, send 1/4 of what is available to each site.

Upon extraction, DNA will be kept at -80°C (-20°C non-frost free freezer is OK for short term storage). Repeated cycles of freeze-thaw will be minimized so aliquoted into 500ng each for shipping to TwinStrands and McGill, and the rest stored as long-term biobank fractions prior to initial freezing.

DNA from Buffy coat (Appendix 3) will be extracted with the same kit following the included protocol for peripheral blood. Ideally lysis and proteinase K digestion should be carried out at 37°C rather than 56°C, but this is not critical. The optional RNase step should be followed. **2uG** should be sent to TwinStrands and the remaining much larger amount of DNA banked.

All extractions and sample handing will be carried out under maximally clean conditions, well away from any facility that does PCR amplification for NGS. NCI Frederick will ensure careful handling to avoid cross contamination or contamination with traces of operator DNA. Duplex Sequencing is extremely sensitive and haplotypes of contaminating DNA and will be readily detected and will compromise mutational analysis.