



MTN 005 Laboratory Training

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Objectives

- ◆ Overview of Lab testing
- ◆ HIV Testing Algorithm
- ◆ Collection and processing of IVR
- ◆ Specimen Management
- ◆ Supplies
- ◆ Q&A



SSP Section 12: Laboratory

- ◆ This will be your best resource for MTN 005 lab questions
- ◆ Make sure you have the most current version
 - ◆ Available at www.mtnstopshiv.org



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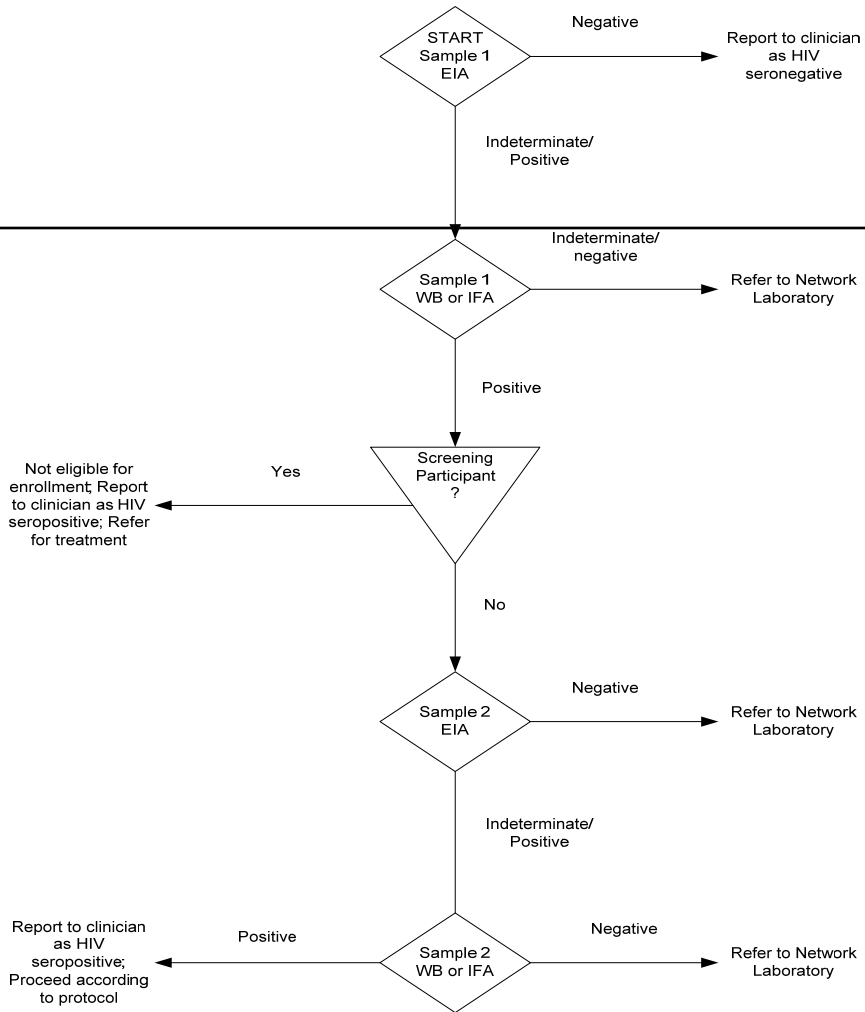
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Blood Specimens

- ◆ Red top tube or Green top tube
 - ◆ Syphilis Serology
- ◆ Whole blood: purple top tube
 - ◆ Plasma Archive (visit 2.0)
 - ◆ Draw enough for 3 cryovials with 1.5 mL each of plasma
 - ◆ Stored on site until requested by NL
 - ◆ HIV Test (visit 2.0 and 6.0)
 - ◆ EIA testing done at local lab
 - ◆ Follow algorithm located in SSP and protocol

HIV Testing Algorithm



Note:

US Sites: 1 rapid EIA

India Site: 1 non-rapid EIA

Note:

Indeterminate Sample 2 WB or IFA- refer to Network Lab for guidance



Urine Specimens

- ◆ hCG Pregnancy Test
 - ◆ Quidel Quick Vue
 - ◆ Testing done at local/clinic lab
- ◆ Urinalysis
 - ◆ Siemens Multistix® or Uristix®
 - ◆ Testing done at local/clinic lab



Cervical specimens

- ◆ NAAT for GC/CT
 - ◆ Gen-Probe Aptima swab. Place in transporter. RT
 - ◆ OR
 - ◆ ProbeTec, Becton Dickinson swab. Place in transporter. RT
 - ◆ Testing done at local laboratory
- ◆ Biomarkers
 - ◆ Dacron swab in cryovial with 400uL PBS
 - ◆ Store at $\leq -70^{\circ}\text{C}$ within 8 hour of collection
 - ◆ Ship to NL at the end of study
- ◆ Pap Smear
 - ◆ Only if indicated. Perform according to local SOP



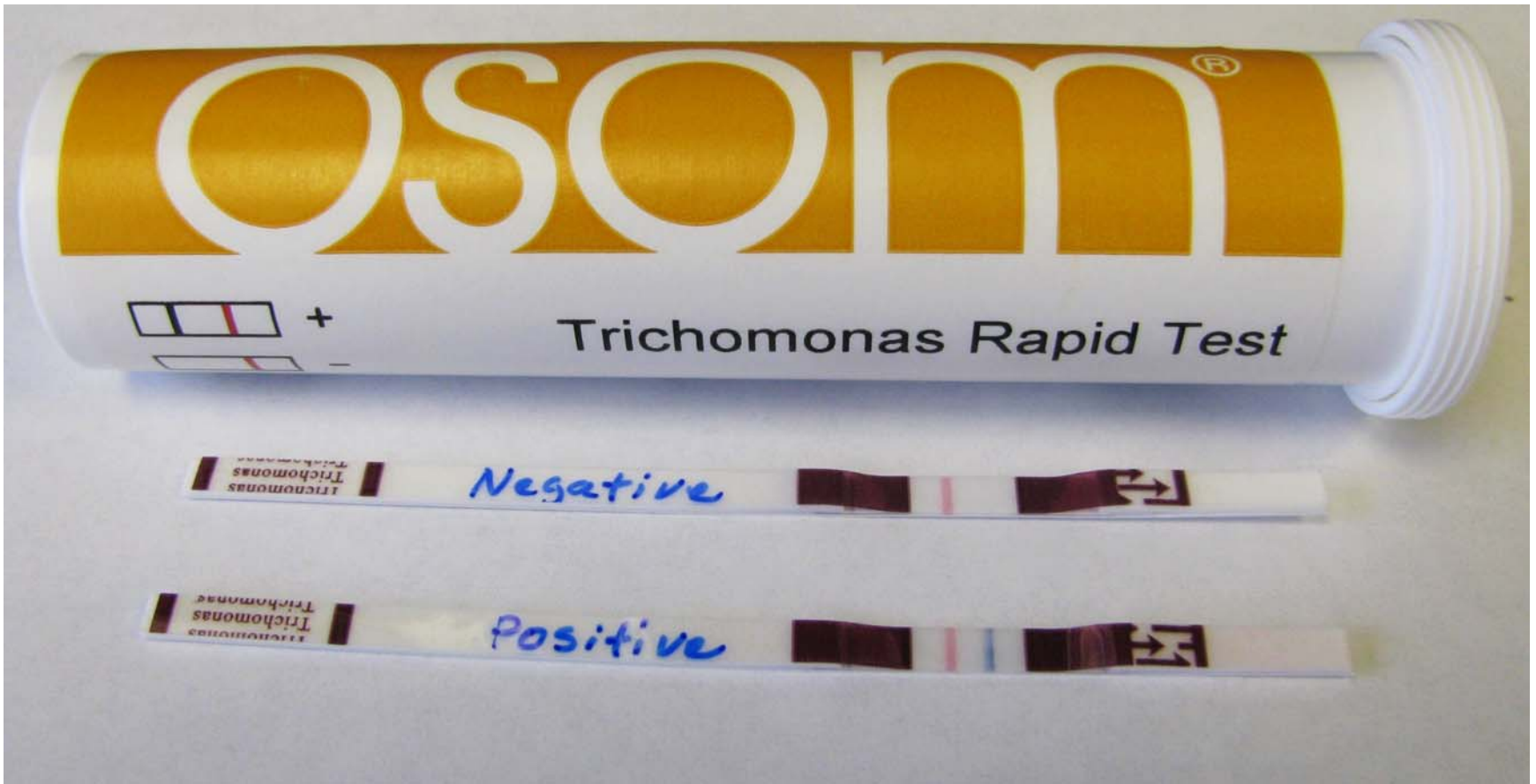
Vaginal specimens

- ◆ OSOM rapid *Trichomonas vaginalis* test
 - ◆ Dacron swab in dry tube for transport
 - ◆ Store sample at either room temp or 4°C
 - ◆ Test must be done with in 24 hours if sample is at RT
 - ◆ Test must be done with in 36 hours if sample is at 4°C
 - ◆ Testing done at local lab/clinic lab



osom
Trichomonas Rapid Test





Trichomonas Rapid Test

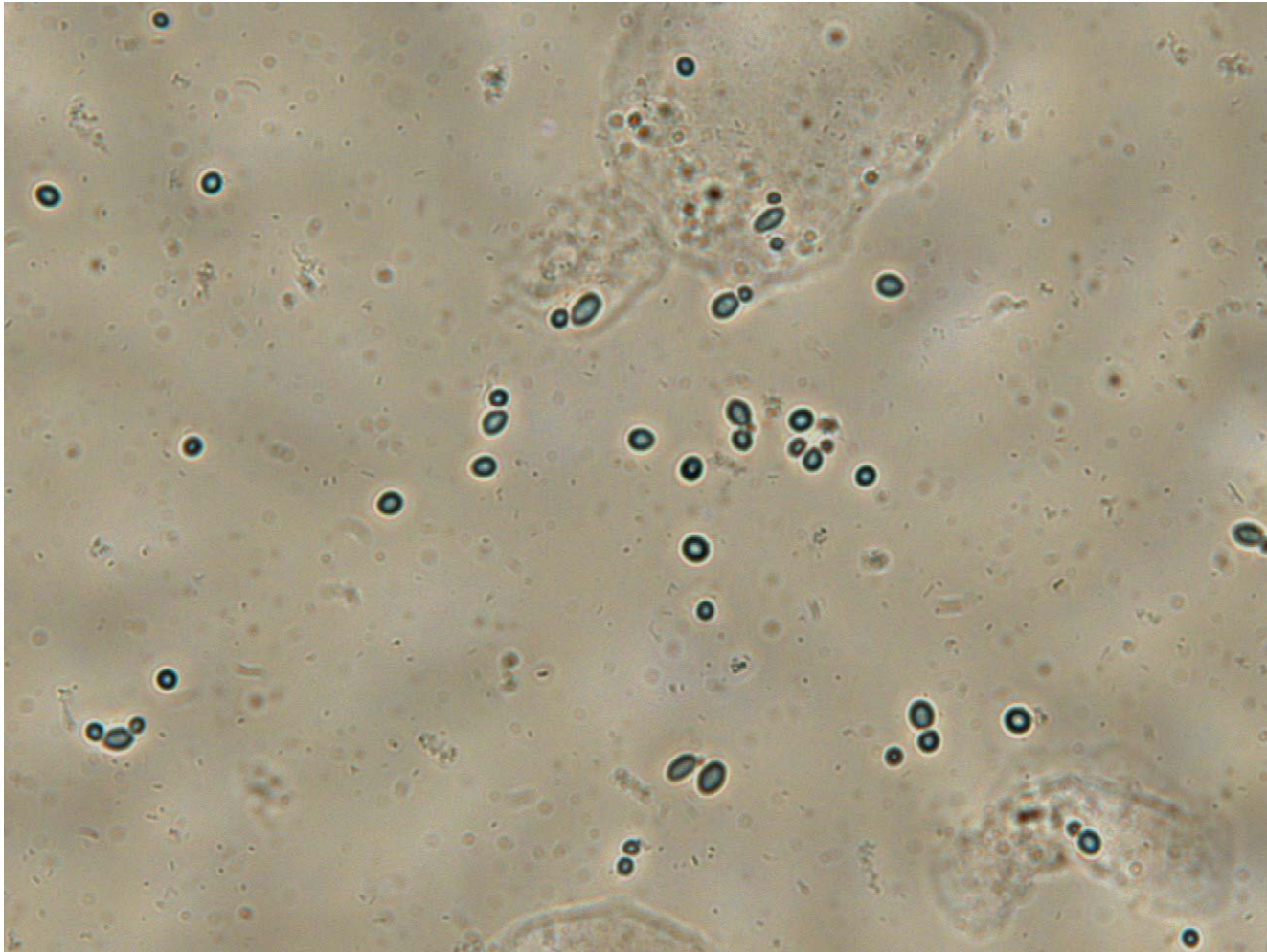
Trichomonas
Negative

Trichomonas
Positive

Vaginal specimens

- ◆ pH, only if indicated
 - ◆ pH strips with range 3.6 to 6.1
- ◆ Wet mount, only if indicated
 - ◆ Saline wet mount for clue cells.
 - Positive=20% clue cells per 400X
 - ◆ KOH for yeast, only if indicated
 - Positive=budding yeast cells or pseudohyphae

KOH: budding yeast



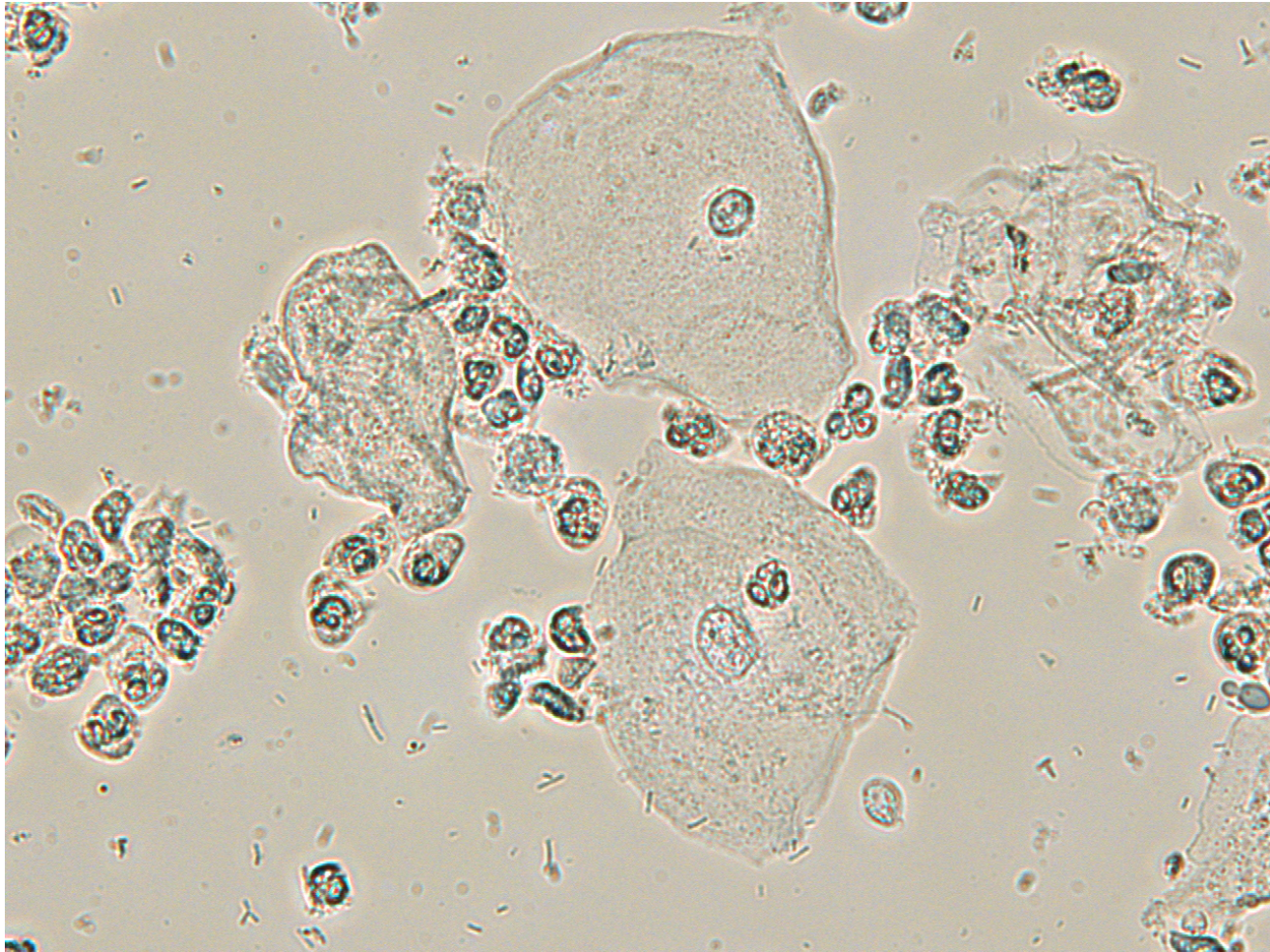
KOH: Amorphous debris



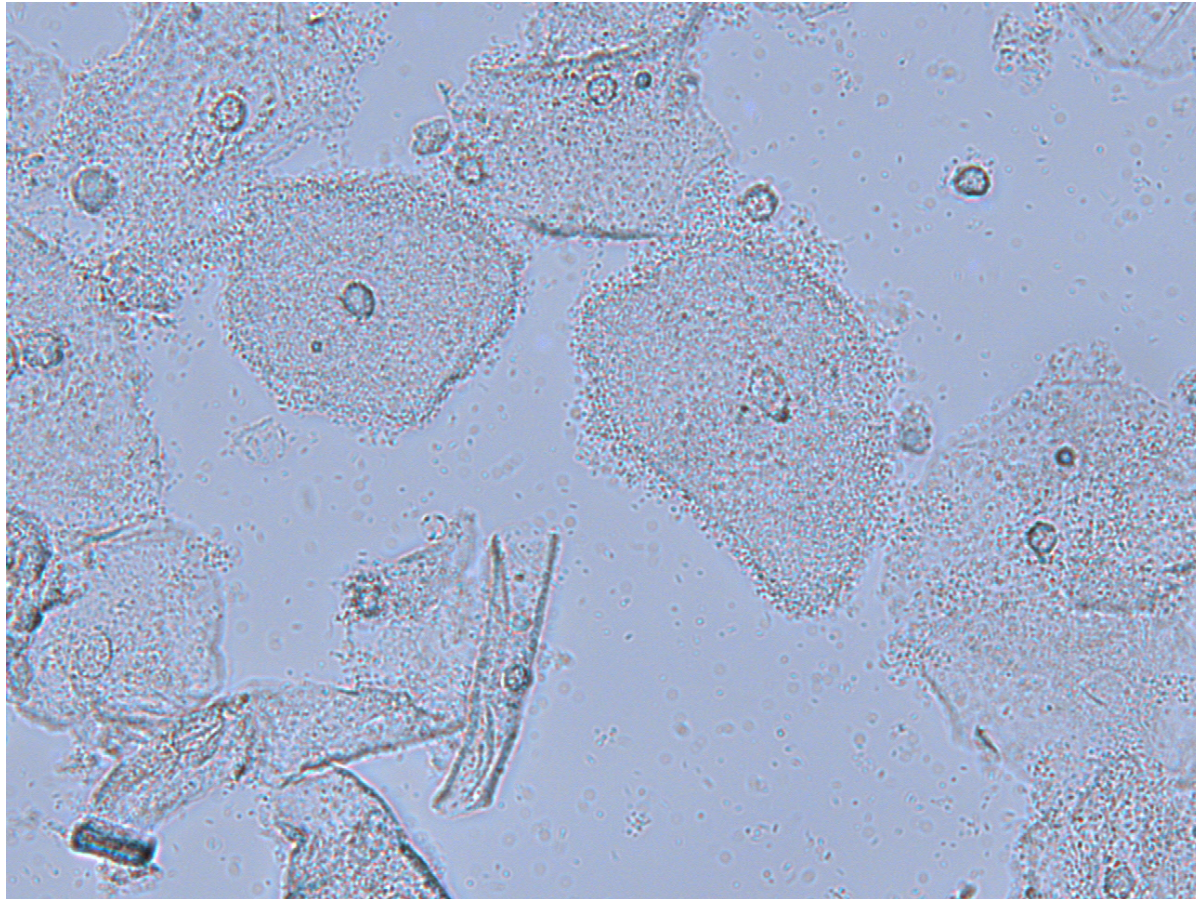
KOH: pseudohyphae, budding yeast, and artifact



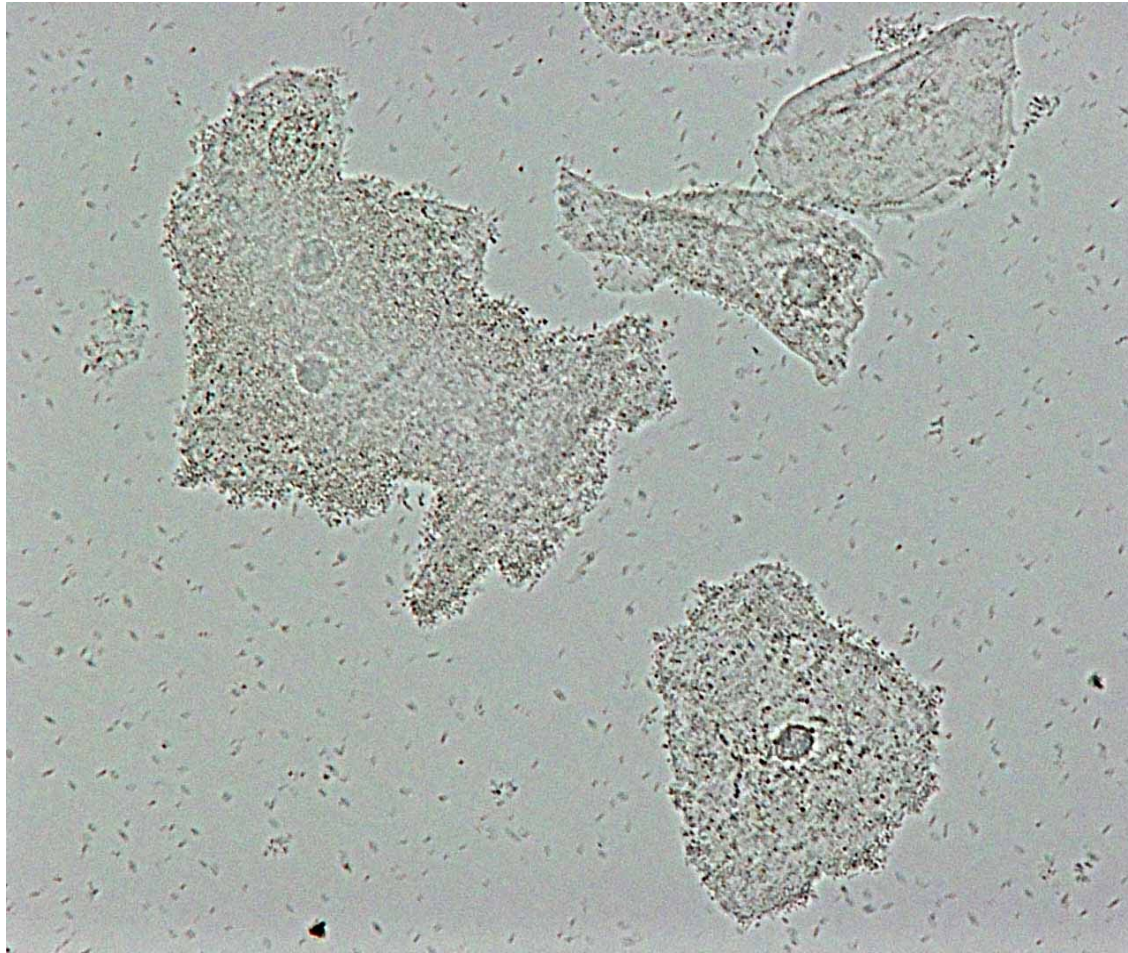
Saline wet mount: normal epithelial cells and WBC



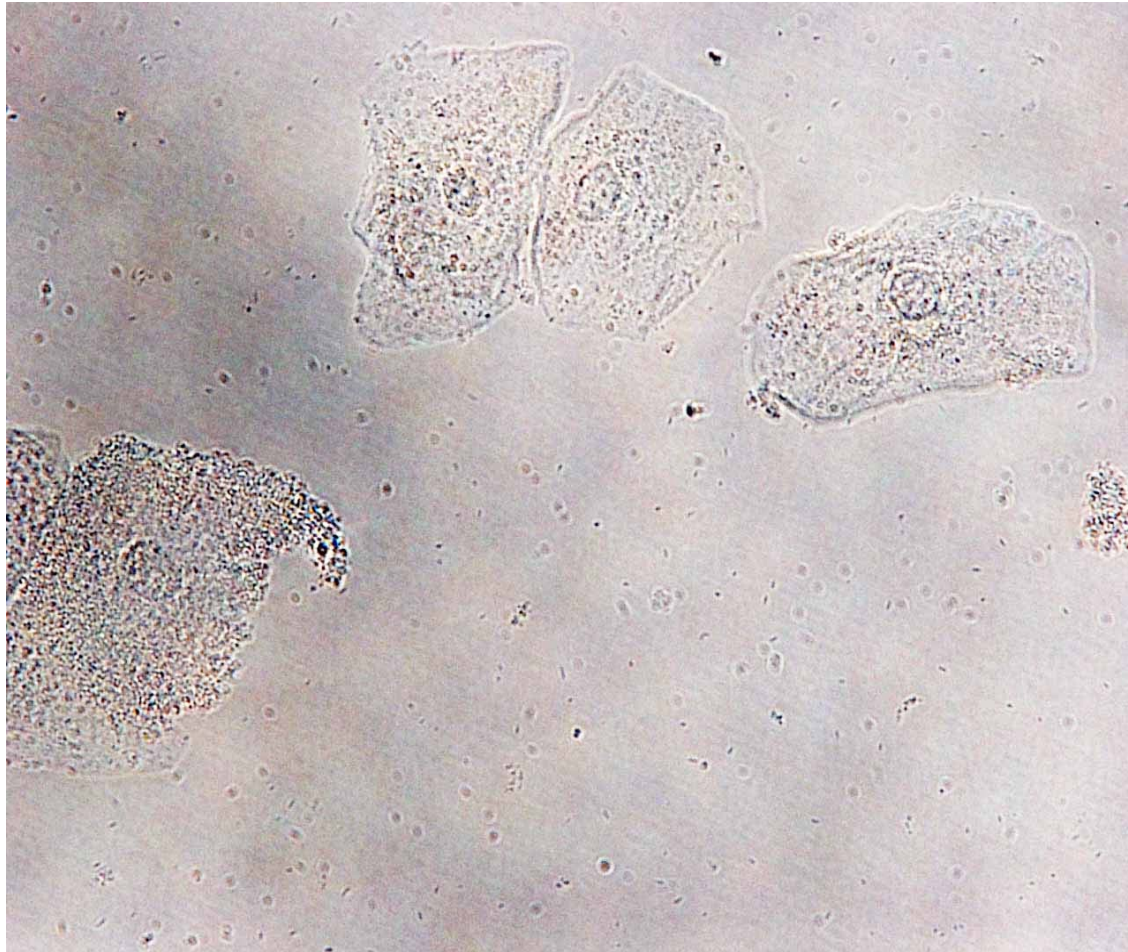
Saline wet mount: Clue cells



Clue cells



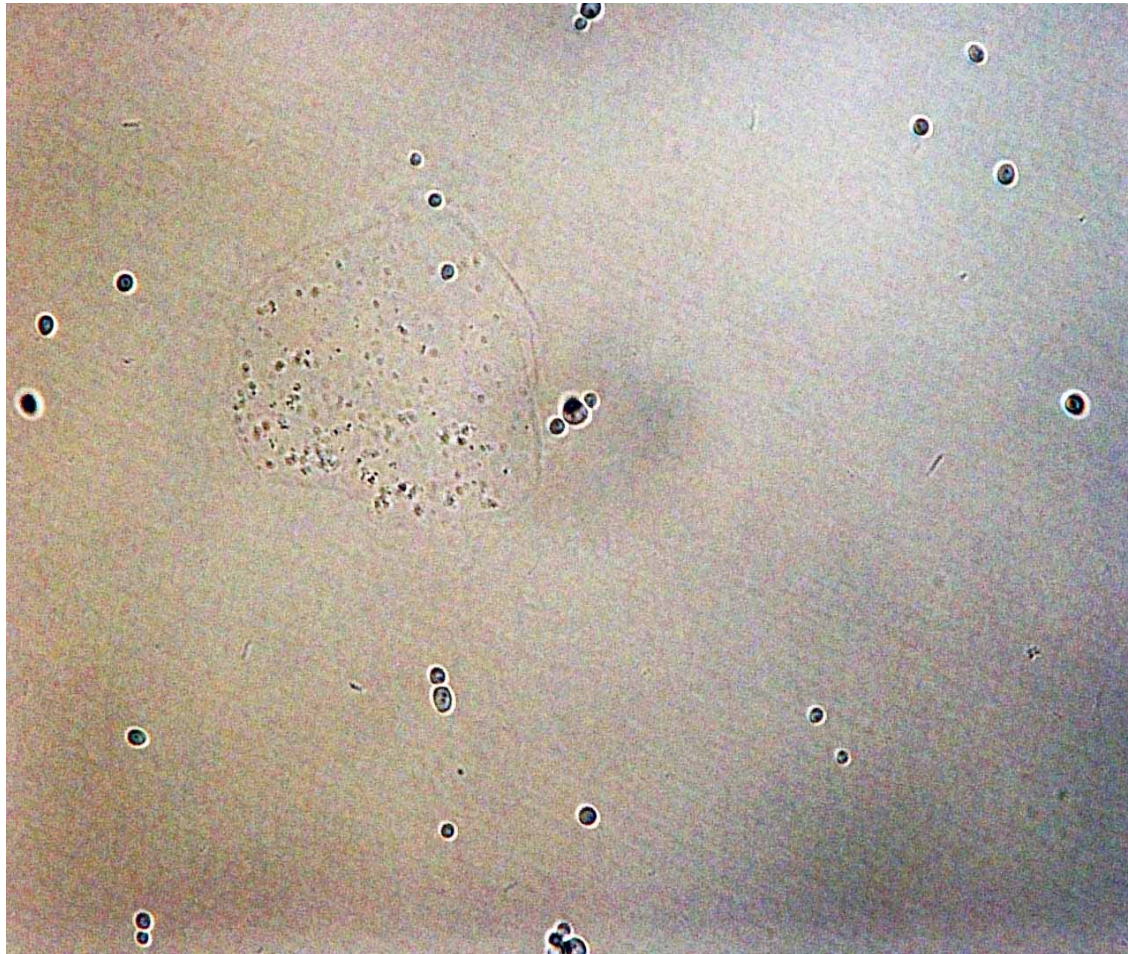
25% clue cells



Negative for clue cells



Budding yeast



Vaginal Specimens

◆ Microflora

- ◆ 2 Dacron swabs inserted into one Port-A-Cul transport tubes
- ◆ Specimen may be kept at RT for up to 4 hours. It must be refrigerated within 4 hours and shipped to NL with ice packs on the day of collection

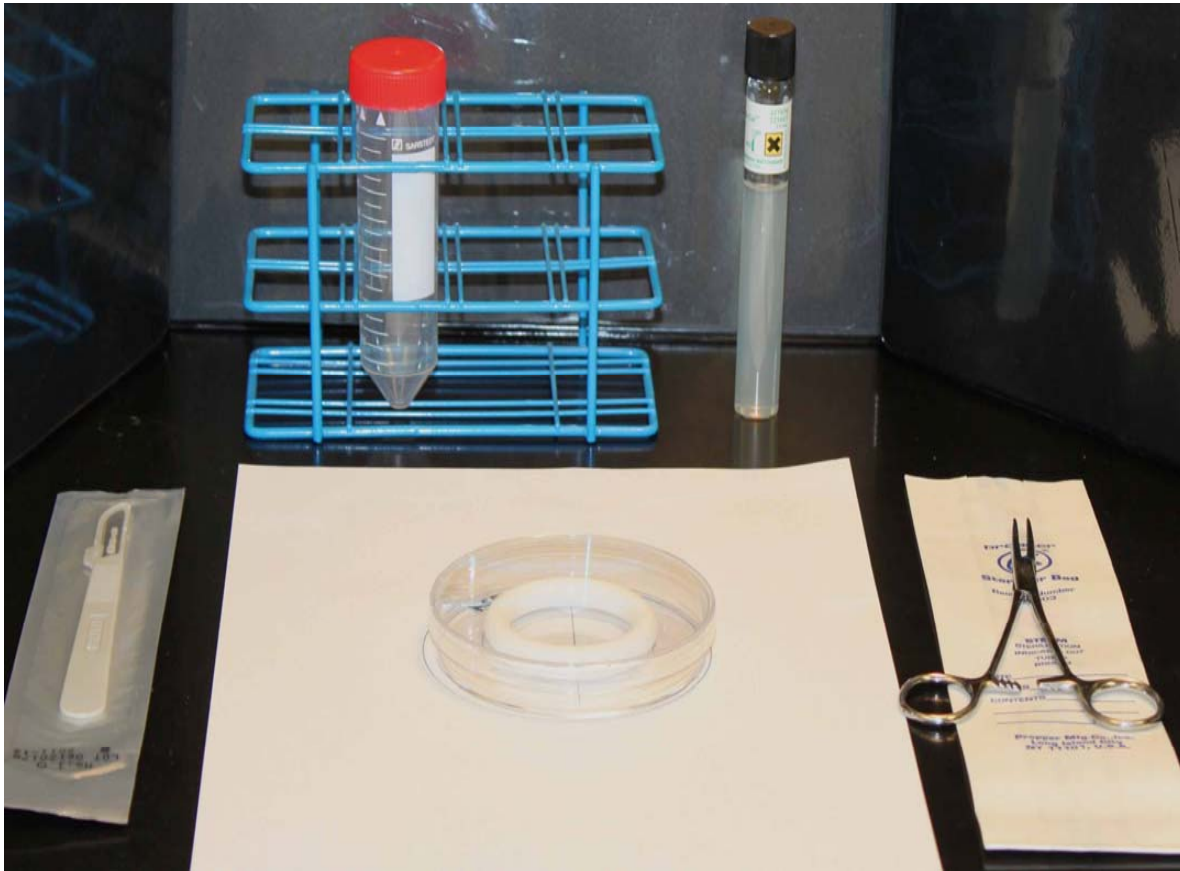
◆ Vaginal smear for Gram stain

- ◆ Roll swab onto 2 slides. Air dry. Ship one slide to NL

◆ HSV culture, only if indicated

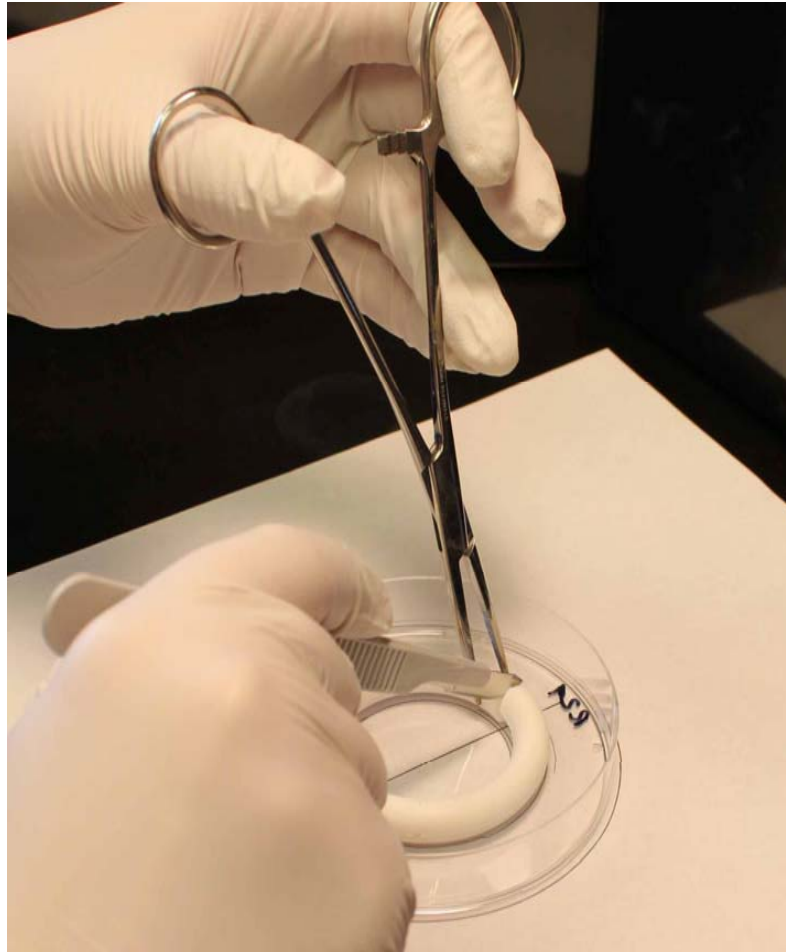
- ◆ Use specific transport media for herpes culture. Check with local lab for supplies.
- ◆ Testing done at local lab.

Processing IVR

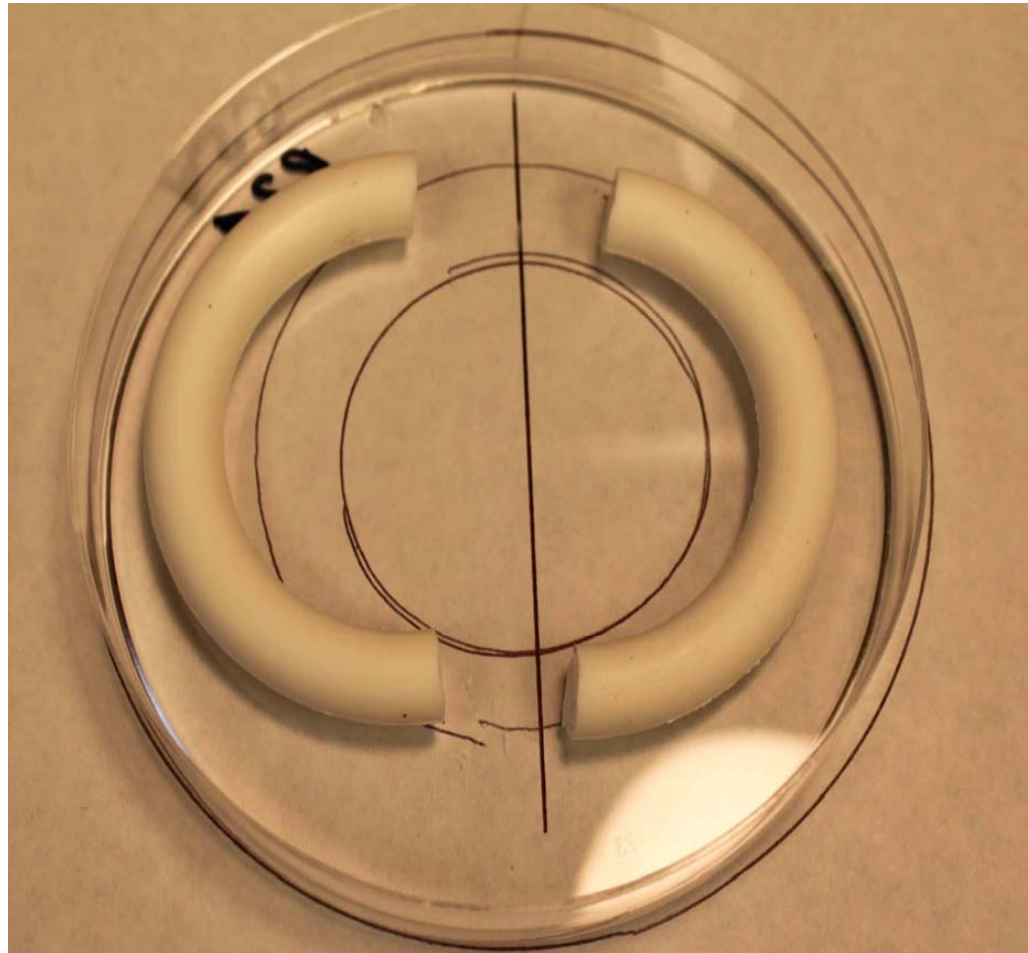


Use sterile scalpel, forceps, and petri dish for processing the ring.

Gently, hold the ring with the forceps and cut the ring in half



$\frac{1}{2}$ for culture and $\frac{1}{2}$ for biofilms staining



Gently insert ½ section into tube with paraformaldehyde
and leave for 1 hour to fix the cells



After 1 hour transfer the section into a tube with enough PBS to cover the ring for transport.

Discard the paraformaldehyde in chemical discard

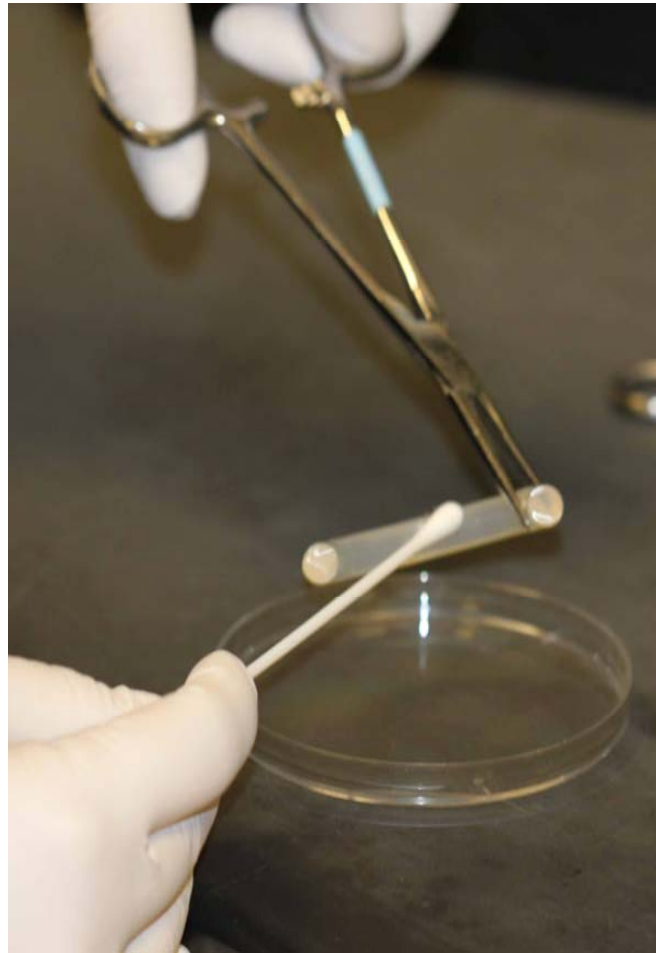


Insert a sponge cork into the tube and push it down to the ring to prevent the ring from moving.
Cap tightly.



Second half of ring:

Swab the entire $\frac{1}{2}$ section of ring with sterile swab.



Insert the swab into a Port-a-Cul transport tube.
Push the swab about half way into the tube, break
off the shaft, and cap tightly.



- ◆ Place the 2 Port-a-cul tubes in a biohazard bag with a paper towels wrapped around the tubes to protect from breakage and to absorb fluid in case of leakage. Add the slide holder to the bag with the port-a-cul tubes.
- ◆ Place the tube containing the ring section in a separate biohazard bag with a paper towel.
- ◆ Add ice packs to the package.
- ◆ Ship to the NL the day of collection.
- ◆ See shipping instructions listed in section 12.8.5 of SSP manual





Specimen Management

- ◆ Label specimen containers with SCHARP label
- ◆ All specimens must be tracked according to site Chain of Custody.
- ◆ CRFs are required for specimens reported to SCHARP.
- ◆ Specimens to be shipped to the Network Lab must be accompanied by an LDMS tracking sheet and entered into LDMS.

LDMS Specimen Tracking Sheet

For login of MTN-005 stored specimens into LDMS

| Participant ID <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> - <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> - <input style="width: 20px; height: 20px;" type="text"/> | | | Visit Code <input style="width: 20px; height: 20px;" type="text"/> . <input style="width: 20px; height: 20px;" type="text"/> | | Specimen Collection Date <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> / <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> / <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> | | |
|--|---|-----------------------------|--|---------------------|--|--|--|
| Site Number Participant Number Chk | | | <i>dd</i> <i>MMM</i> <i>yy</i> | | | | |
| # of TUBES or SPECIMENS | PRIMARY SPECIMEN | PRIMARY ADDITIVE | ALIQUOT DERIVATIVE | ALIQUOT SUB ADD/DER | INSTRUCTIONS FOR PROCESSING LAB | | |
| <input type="checkbox"/> | Blood (BLD) | EDT (purple top) | PL1/2 | N/A | At Enrollment (Visit Code 2.0): lab to make at least three (3) 0.5mL aliquots. | | |
| <input type="checkbox"/> | Vaginal Smear for Gram Stain (VAG) | NON (no additive) | SLD | GRS | Re-label with LDMS label. Store duplicate slides (one for on-site storage and one for shipping and reading at the NL). | | |
| <input type="checkbox"/> | India Only: Vaginal Swab for PCR (VAG) | NON (no additive) | SWB | N/A | Store swab at $\leq -70^{\circ}\text{C}$ locally. | | |
| <input type="checkbox"/> | US Only: Vaginal Swab for Culture (VAG) | PAC | SWB | N/A | Ship on ice packs to NL on the day of collection. | | |
| <input type="checkbox"/> | US Only: Cervical Swab for innate factors (CXS) | PBS | SWB | N/A | Store swab at $\leq -70^{\circ}\text{C}$ locally. | | |
| <input type="checkbox"/> | US Only: Swab of used IVR for Culture (IVR) | IVR | SWB | N/A | Ship on ice packs to NL on the day of collection. | | |
| <input type="checkbox"/> | US Only: Used IVR for FISH and SEM (IVR) | NON (no additive) | IVR | PFM | Ship on ice packs to NL on the day of collection. | | |

Shipping instructions

- ◆ Specimens to be shipped the day of collection:
 - ◆ Vaginal microflora (Port-a-Cul)
 - ◆ Slide for Gram stain
 - ◆ IVR microflora (Port-a-Cul)
 - ◆ IVR for staining (50 mL tube with PBS)

- ◆ Ship in Styrofoam box with ice packs
- ◆ Ship FedEx, overnight standard delivery
 - ◆ UN3373, Biological specimen sticker
- ◆ Notify Lorna Rabe by e-mail of the shipment
 - ◆ Attach the LDMS to e-mail



Shipping instructions

- ◆ Specimens to be batched and shipped at end of study:
 - ◆ Cervical swabs for biomarkers
 - ◆ plasma archive
- ◆ The NL will notify your site when to ship the above specimens with detailed instructions.



Supplies provided by NL

- ◆ Port-a-cul tubes (microflora)
- ◆ Dacron swabs
- ◆ Shipping containers (for specimens to NL)
- ◆ Cryovials with PBS (cervical biomarkers)
- ◆ 50 mL conical tubes (biofilm processing)
- ◆ Foam corks to stabilize IVR in tube during transport
- ◆ Petri dishes (biofilm processing)
- ◆ 2.5% paraformaldehyde (keep frozen until day of use)



Supplies not provided by NL

- ◆ Sterile forceps
- ◆ Sterile scalpel
- ◆ PBS
- ◆ Ice packs for shipping



Michele

Rometa

Lorna

Hilary

Melinda

Wayne

Lynda

Kevin

Lisa

Bridgete

May

Ted



Any Questions?