FEMALE GENITAL SECRETIONS COLLECTION, PROCESSING, AND STORAGE PROCEDURES

1. REAGENTS

1.1 Obtaining Sno-Strips™:

Sno-strips™ may be purchased through Akorn, 800-535-7155, product number 17478-0401-01. As of 01/01/2004 they are priced at $12.50 per box of 100. Akorn has a minimum order of $25.00, so two boxes will need to be purchased at one time.

1.2 Obtaining NASBA lysis buffer or 4 M guanidinium isothiocyanate with mecaptoethanol:

Aliquots of NASBA lysis buffer or 4M guanidinium may be provided from protocol to protocol. If neither is provided by the protocol, the NASBA lysis buffer may be purchased from Bio Merieux, 800-345-8151, product number 284046 for a box of 50 tubes containing 0.9 mLs of lysis buffer per tube.

1.3 Obtaining cytobrush collection kits for protocols requiring Digene collection kits (please note, the Digene collection kits are typically used for HPV measurements; cytobrushes for collecting samples for pathogens other than HPV, including HIV, can generally be found in the local clinic):

Digene collection kits for cytobrush specimens for some protocols may be obtained from the ACTG Central Repository through your site pharmacist. If they are not provided by the protocol, they may be purchased directly from Digene, 800-344-3631, product number 5126-1220. Please note, Digene changed the product name and number at the end of 2003, if you have or receive product 5122-1220 these are the same kits as 5126-1220.

2. SPECIMEN COLLECTION

The subject must refrain from any kind of sexual activity, douching, and inserting any intravaginal products for at least 48 hours prior to the collection of vaginal/cervical specimens. Samples will be collected by the following methods in the following order: 1) Endocervical wicks (Sno-Strips™), 2) vaginal swab, 3) cervicovaginal lavage (CVL), 4) cervical swab, 5) endocervical cytobrush, and 6) Pap smear.

2.1 Endocervical canal fluid collected by Sno-Strip™ wicking. The purpose of this collection procedure is to obtain endocervical canal fluid for viral RNA quantification.

2.1.1 If the Sno-Strips™ are to be stored in NASBA lysis buffer, the buffer must be crystal free before you begin. Most crystals will dissolve by placing the lysis buffer tube at room temperature for a few hours. If crystal remain, vortex the tube until they are gone.

2.1.2 Gently insert an unlubricated speculum into the vagina.

2.1.3 Sno-Strips™ will be used as wicks to collect primarily cell-free virions from the endocervical fluid. If excess mucus or menses clot has accumulated near the cervical os, a large cotton-tipped cotton swab may be used to gently remove this material before inserting the Sno-Strips™.

2.1.4 Using forceps (ring or sponge forceps work well), gently insert three Sno-Strips™ simultaneously into the vagina, place through the cervical os into the distal endocervical canal, and hold in place to adsorb sample. Each Sno-Strip™ adsorbs approximately 8 µL of specimen. Adsorption usually takes approximately one minute, but may take a little longer.

2.1.5 Hold the narrow end of the three strips over and slightly inside one labeled plastic transport tube (1.5-mL cryovial) containing 500 µL of NASBA lysis buffer or 4 M guanidinium isothiocyanate with mercaptoethanol. Cut the strips at the junction of the shoulder and neck of the Sno-Strip™ with scissors, allowing the narrow end to fall into the cryovial tube buffer.

2.1.6 Send the sealed vial to the local laboratory for processing.

2.2 Vaginal Swab

2.2.1 A Dacron swab is inserted and rotated 360 degrees in all four quadrants of the vaginal vault.

2.2.2 The swab is then placed into a sterile tube containing 2 mL of 1X PBS or non-bacteriostatic normal saline.

2.2.3 Rotate the swab 360 degrees against the inside of the tube to remove as much fluid as possible. Vortex the vial if this is possible; this procedure will aid in extracting the fluid from the swab.

2.2.4 Place the fluid in a cryovial and discard the Dacron swab.

2.2.5 Specimens should be transported to the laboratory with one hour. If this is not possible, place the specimen on ice or refrigerate at 4°C until transport, up to 4 hours.

2.3 Ectocervicovaginal lavage (CVL). The purpose of this collection procedure is to obtain a washing of virus and cells from the ectocervix and fluid from the posterior vaginal fornix for viral and immunologic studies.

2.3.1 Draw up 10 mL of either nonbacteriostatic normal saline (saline for irrigation) or 1X phosphate buffered saline in a 10-mL syringe. Cut a sterile plastic transfer pipette just below the bulb, throw the bulb away and place the pipette tip on the syringe. Alternatively, a 14-gauge angiocath can be inserted over the tip of a 10-mL syringe. It may be helpful to seal the junction with parafilm.

2.3.2 Introduce the syringe through the speculum at the opening of the cervical os, but do not insert in os.

2.3.3 Aim a continuous stream of saline directly at and into the os to bathe the cervix and the ectocervix.

2.3.4 Allow the fluid to pool into the posterior fornix and aspirate into the same syringe.

2.3.5 Repeat this procedure exactly 5 times with the same fluid; do not add any additional saline or PBS to the specimen.

2.3.6 Transfer the fluid to a sterile 15 mL conical test tube.

2.3.7 Transport the laboratory within 1 hour of collection. If this is not possible, place specimens on ice or refrigerate at 4°C until transport, up to 4 hours.

2.4 Endocervical Swab

2.4.1 A Dacron swab is gently inserted 1 cm into the cervical os and rotated 360 degrees.

2.4.2 The swab is then placed into a sterile tube containing 2 mL of 1X PBS or non-bacteriostatic normal saline.

2.4.3 Rotate the swab 360 degrees against the inside of the tube to remove as much fluid as possible. Vortex the vial if this possible; this procedure will aid in extracting the fluid from the swab.

2.4.4 Place the fluid in a cryovial and discard the Dacron swab.

2.4.5 Specimens should be transported to the laboratory with one hour. If this is not possible, place the specimen on ice or refrigerate at 4°C until transport, up to 4 hours.

2.5 Endocervical canal cytobrush. The purpose of this collection procedure is to obtain primarily cells for viral DNA quantification.

2.5.1 Gently insert a cytobrush with a plastic shaft 1 cm into the cervical os and rotate exactly 360 degrees. Bleeding usually occurs with the cytobrush and this should be noted.

2.5.2 The cytobrush must be placed in the appropriate Digene collection vial or vial containing 0.5mL of NASBA lysis buffer or 4M guanidinium isothiocyanate with mecaptoethanol before drying occurs. The end of the cytobrush is snapped off with scissors so that the brush portion can fit into the transport tube. The cytobrush should snap easily, particularly if they are scored with a pair of scissors approximately 2 cm from the brush end of the handle.

2.5.3 Place the brush in the vial, being certain that the brush is immersed in the transport medium, and so that the scored area is approximately even with the lip of the vial, hold the bottom of the handle and tube with one hand while snapping off the top of the handle with the other. Firmly tighten the lid of the cryovial.

2.5.4 Cytobrushes in this fluid can remain at 4°C for up to 72 hours. Clinicians should send the sealed vial to the local laboratory for processing.

3. Laboratory Specimen Processing

3.1 Sno-Strip™ specimens-LDMS spec code CER/NON/SNO/LYB: At the processing laboratory, vortex each vial for 5 seconds, label and freeze upright at -70°C. Do not remove the Sno-Strips from the vial.

3.2 Cytobrush specimens LDMS spec code CER/NON/CTB/GIT or LYB: At the processing laboratory, vortex each vial for 5 seconds, label and freeze upright at -70°C. Do not remove the cytobrush from the vial.

3.3 Cervicovaginal lavage: In the laboratory note volume, color and presence of gross blood or mucous in the specimens.

3.3.1 If whole CVL is desired, briefly vortex to ensure equal concentrations in each aliquot. Pipette off desired amount of whole CVL. Label and store as 1.0 mL aliquots. Freeze as soon as possible at -70°C. LDMS spec code:CVL/NON/CVL

3.3.2 Centrifuge remaining CVL at 600-800 x *g* for 10 minutes.

3.3.3 Aspirate the supernatant (including any mucous) and store as 1.0 mL aliquots. Label and store at -70°C. LDMS spec code: CVL/NON/FLD

3.3.4 Resuspend the cell pellet in 10 mL of PBS.

3.3.5 Centrifuge at 600 x *g* for 10 minutes.

3.3.6 Repeat steps 4 and 5 once and resuspend cells in 1 mL of PBS.

3.3.7 Divide one-half of the cell suspension (0.5 mL) between two labeled microfuge tubes; i.e., 0.25 mL/microfuge tube.

3.3.8 Centrifuge these cell suspensions at the highest speed in a microfuge for 3 minutes.

3.3.9 Aspirate the supernatants and store at -70°C as dry cell pellets. LDMS spec code: CVL/NON/PEN

3.3.10 Take the remaining 0.5 mL cell suspensions and cryopreserve using the ACTG Consensus Freezing Protocol that can be found at http://aactg.s-3.com/pub/download/vir/freezingprotocol.doc. Store these specimens in vapor phase liquid nitrogen. LDMS spec code: CVL/NON/CLN/DMS

3.4 Endocervical Swab-LDMS specimen code CER/NON/SWB/NSL:
Please note that this procedure may vary if swab is being collected for pathogens other than HIV such as Candida, BV, HPV, and other STDs.

3.4.1 Vortex well.

3.4.2 Divide into 0.5 mL aliquots.

3.4.3 Label and store at –70°C as soon as possible.

3.5 Vaginal Swab-LDMS specimen code VAG/NON/SWB/NSL

3.5.1 Vortex well.

3.5.2 Divide into 0.5 mL aliquots.

3.5.3 Label and store at –70°C as soon as possible.

Procedure: ACTG Female Genital Secretions, Collections, Processing and Storage Procedures

Prepared by: ACTG Laboratory Technologist Committee

Preparation Date: 01 June 2004

Date Implemented into the Laboratory: _____

Updated on:

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Reviewed by: Date:

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Supersedes Archived Protocol: DAIDS Virology Manual for HIV Laboratories, Version January 1997