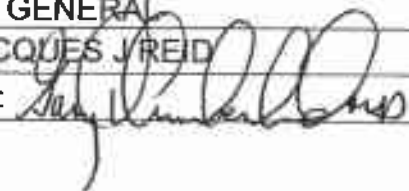


PENSACOLA PATHOLOGISTS, P.A.	
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AUTHOR: S JACQUES J REID	EFFECTIVE: MAR-04
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ROUTINE HISTOLOGY SPECIMEN COLLECTION

MATERIALS NEEDED

1. Instruments for collection
2. Requisition form with the following information:
 - Patients name
 - Age
 - Sex
 - Physician's name and health facility submitting specimen
 - Billing information
 - Date of collection
 - Source of specimen
 - Clinical history
3. Container of 10% neutral buffered formalin . Container must bear OSHA approved Formalin labelling.
4. Specimen transport bag

PROCEDURE

1. After collecting the specimen, place it in a container of 10% neutral buffered formalin
Large enough to have approximately 1:15 ratio of specimen to formalin.
2. Completely label the specimen container with patient's name, date of collection and Specimen source or type.
3. Ensure the lid is correctly screwed on tightly.
4. Place specimen in biohazard labelled specimen transport bag and seal.
5. Place completed requisition in the outer pocket of the transport bag.

RESULTS

Pathology reports on tissue specimens submitted for routine pathology diagnosis will be rendered within 24 to 48 hours, excluding weekends and holidays, with the exception of those cases requiring special procedures such as special stains or immunohistochemical stains. These may typically add an extra day for final diagnosis.

NOTE

Always use a specimen container large enough to accommodate the specimen.
If necessary use a larger container.

BONE MARROW SPECIMEN COLLECTION

MATERIALS NEEDED

1. Instruments for collection
2. Requisition form with the following information:
 - Patient's name
 - Age
 - Sex
 - Physician's name and health facility submitting specimen
 - Billing information
 - Date of collection
 - Source of specimen
 - Clinical history
3. 2 containers of 10% zinc formalin (if sending both clot and core biopsy)
Containers must bear OSHA approved formalin labelling.
4. Glass microscope slides for smears. (if applicable)
5. Specimen transport bag

PROCEDURE

1. Label the blood smears with the patient name. Preferably at least 4.
2. Place the aspirate in a container of zinc formalin and label it "A"
3. Place the bone marrow core biopsy (if applicable) in a container of zinc Formalin and label it "B".
4. Completely label the specimen container with patient's name, date of collection and Specimen source. Ensure that the lids are securely screwed on.
5. Place specimen in biohazard labelled specimen transport bag and seal.
6. Place completed requisition in the outer pocket of the transport bag.

RESULTS

Pathology reports on bone marrow specimens will be rendered within 24 to 48 hours, excluding weekends and holidays, with the exception of those cases requiring special procedures such as immunohistochemical stains. These may typically add an extra day for final diagnosis.

NOTE

In the event you do not have the zinc formalin, you may substitute 10% neutral buffered formalin.

GYNECOLOGIC CYTOLOGY SPECIMENS

Conventional Pap Smear

Specimen Collection, Adequacy, Requisition & Transportation

Materials

Speculum (without lubricant)
Spatula and Endocervical brush or plastic cervix broom
Frosted end glass slide and pencil
Cytology spray fixative
Pap Smear requisition
Slide holder and specimen transport bag

Patient Preparation

To optimize collection conditions, a woman should:

1. Schedule an appointment approximately two weeks (10-18 days) after the first day of her last menstrual period.
2. Not douche 48 hours prior to the test.
3. Not use tampons, birth control foams, jellies or other vaginal creams or vaginal medications for 48 hours prior to the test.
4. Refrain from intercourse 48 hours prior to the test.

Test Requisition

Under the supervision and guidance of the physician, a laboratory requisition must be legibly and accurately filled out before obtaining the cellular sample. The laboratory requisition is the main communication link between the physician and the laboratory. The requisition form should have the following information as required by CLIA '88.

1. Patient's name (any name change in the past 5 years should be noted)
2. Age and/or date of birth

3. Menstrual status (LMP, hysterectomy, pregnant, postpartum, hormone therapy)
4. Previous Pap history, abnormal cervical cytology results, previous treatment, biopsy surgical procedure and results.
5. Source of specimen, e.g. cervical, vaginal
6. Indicate if patient is at high risk for cervical cancer

Appropriate clinical history provided by the physician on the requisition should include:

1. Hormone/contraceptive use
2. Relevant clinical findings (abnormal bleeding, grossly visible lesion, etc.)

Patient's SSN, physician and health facility names, insurance information and medicare information if applicable.

Labeling the Sample

1. Write the patient's name on the frosted end of the slide.
2. Use an ordinary lead pencil. Do not use an ink pen. It washes off in the staining process.

Visualization of the Cervix for Collection of an Adequate Sample

1. Collection of a cervical cytology specimen is usually performed with the patient in the dorsolithotomy position.
2. A sterile, or single-use bivalve speculum of appropriate size is inserted into the vagina without lubrication. Warm water or saline may be used to facilitate insertion of the speculum. The position of the speculum should allow for complete visualization of the os and ectocervix.
3. The transformation zone is the site of origin for most cervical neoplasia and should be the focus of cytology specimen collection. The transformation zone may be easily visualized or may be high in the endocervical canal. Location varies not only from patient to patient, but in an individual over time. Factors producing variation include changes in vaginal pH, hormonal changes including pregnancy, childbirth, menopausal status, and hormonal therapy.

4. In postmenopausal patients or women who have received radiation therapy, cervical stenosis may prevent visualization of the transformation zone. It remains important to sample the endocervix in these patients. This may require more extensive clinical procedures.
5. If a patient has had a hysterectomy, a vaginal sample is sufficient, with particular attention to sampling the vaginal cuff.

Techniques for Sample Collection

Spatula and Endocervical Brush Technique

1. The ectocervix should be sampled before the endocervix/transformation zone. First, a sample of the ectocervix is taken using a plastic (or wooden) spatula. The notched end of the spatula that corresponds to the contour of the cervix is rotated 360° around the circumference of the cervical os.
2. The sample on the spatula is spread evenly and thinly lengthwise down one half of the labeled slide surface, using a single uniform motion. Immediately spray with fixative. **Follow the manufacturer's instructions on the container and package insert.** Generally, spray fixatives should be 6-10 inches from the glass slide when applied.
3. Sampling of the endocervix requires insertion of the endocervical brush into the endocervical canal until the bristles closest to the hand are visible. The brush is rotated 45-90° and removed.
4. The endocervical brush is then rolled along the remaining half of the labeled slide surface by turning the brush handle and slightly bending the bristles with gentle pressure. Immediately spray with fixative.

Broom-Like Device Technique

1. The ectocervix and endocervix are collected simultaneously. The central bristles of the broom are inserted into the endocervical canal until the lateral bristles bend fully against the ectocervix. The sampling device is rotated 360° in the same direction five (5) times while maintaining gentle pressure.
2. The broom is removed and with a single paint stroke motion the cellular sample is transferred down the long axis of the labeled surface of the slide. The broom is turned

over and the paint stroke motion is repeated over the same area. Immediately spray with fixative. Follow the manufacturer's instructions on the container and package insert. Generally, spray fixatives should be 6-10 inches from the glass slide when applied.

Transporting Specimen

1. Place slide(s), when dry, in slide holder.
2. Place slide holder with slide and completed requisition form in a specimen transport bag.
3. If the specimen is not transported by courier, but by mail, request a special mailer from the cytology laboratory.

Criteria for rejection

1. Slides that are broken beyond repair are unacceptable. The physician/office will be notified of the rejected specimen.
2. Improper collection technique or inadequate fixation may result in an unsatisfactory specimen. If the cellular content is scanty, air dried or obscured by blood or pus at the time of screening, a statement will be added to the report that the specimen is Unsatisfactory for Evaluation.
3. Specimens not labeled or improperly labeled are not acceptable and the physician/office will be notified and asked to correct the problem.

ThinPrep Pap Test

Specimen Collection, Adequacy, Requisition & Transportation

Patient Preparation

To optimize collection conditions, a woman should:

1. Schedule an appointment approximately two weeks (10-18 days) after the first day of her last menstrual period.
2. Not douche 48 hours prior to the test.
3. Not use tampons, birth control foams, jellies or other vaginal creams or vaginal medications for 48 hours prior to the test.
4. Refrain from intercourse 48 hours prior to the test.

Test Requisition

Under the supervision and guidance of the physician, a laboratory requisition must be legibly and accurately filled out before obtaining the cellular sample. The laboratory requisition is the main communication link between the physician and the laboratory. The requisition form should have the following information as required by CLIA '88.

1. Patient's name (any name change in the past 5 years should be noted)
2. Age and/or date of birth
3. Menstrual status (LMP, hysterectomy, pregnant, postpartum, hormone therapy)
4. Previous Pap history, abnormal cervical cytology results, previous treatment, biopsy or surgical procedure and results
5. Source of specimen, e.g. cervical, vaginal
6. Indicate if patient is at high risk for cervical cancer
7. Date of specimen collection
7. Submitting physician's name

Appropriate clinical history provided by the physician on the requisition should include:

1. Hormone/contraceptive use

2. Relevant clinical findings (abnormal bleeding, grossly visible lesion, etc.)

Patient's SSN, physician and health facility names, insurance information and medicare information if applicable.

Labeling the Sample

Write the patient's name and collect date on the PreservCyt Solution vial.

Visualization of the Cervix for Collection of an Adequate Sample

1. Collection of a cervical cytology specimen is usually performed with the patient in the dorsolithotomy position.
2. A sterile, or single-use bivalve speculum of appropriate size is inserted into the vagina without lubrication. Warm water may be used to facilitate insertion of the speculum. The position of the speculum should allow for complete visualization of the os and ectocervix.
3. The transformation zone is the site of origin for most cervical neoplasia and should be the focus of cytology specimen collection. The transformation zone may be easily visualized or may be high in the endocervical canal. Location varies not only from patient to patient, but in a individual over time. Factors producing variation include changes in vaginal pH, hormonal changes including pregnancy, childbirth, menopausal status, and hormonal therapy.
4. In postmenopausal patients or women who have received radiation therapy, cervical stenosis may prevent visualization of the transformation zone. It remains important to sample the endocervix in these patients. This may require more extensive clinical procedures.
5. If a patient has had a hysterectomy, a vaginal sample is sufficient, with particular attention to sampling the vaginal cuff.

Techniques for Sample Collection

Spatula and Endocervical Brush Technique

1. The ectocervix should be sampled before the endocervix/transformation zone. First, a sample of the ectocervix is taken using a plastic spatula. The notched end of the spatula that corresponds to the contour of the cervix is rotated 360° around the circumference of the cervical os.
2. The spatula is rinsed in the PreservCyt Solution vial by swirling the spatula vigorously in the vial 10 times.
3. Sampling of the endocervix requires insertion of the endocervical brush into the endocervical canal until the bristles closest to the hand are visible. The brush is rotated 45-90° in one direction and removed. Do not over-rotate.
4. The brush is rinsed in the PreservCyt Solution vial by rotating the device in the solution 10 times while pushing against the PreservCyt vial wall. If material still remains on the brush, take the spatula and scrape the material from the brush while holding it in the PreservCyt Solution vial. Discard the brush and spatula.
5. The vial cap is tightened so that the torque line on the cap passes the torque line on the vial.

Broom-Like Device Technique

1. The ectocervix and endocervix are collected simultaneously. The central bristles of the broom are inserted into the endocervical canal until the lateral bristles bend fully against the ectocervix. The sampling device is rotated 360° in the same direction five (5) times while maintaining gentle pressure.
2. The broom is removed and rinsed into the PreservCyt Solution vial by pushing the broom into the bottom of the vial 10 times, forcing the bristles apart. As a final step, the broom is vigorously swirled to further release material. Discard the device.
3. The vial cap is tightened so that the torque line on the cap passes the torque line on the vial.

Transporting Specimen

1. The vial is placed in the specimen transport bag and sealed. The requisition is placed in the pouch outside the sealed bag.
2. The specimens are transported to the cytology laboratory by courier. If the specimens are to be mailed, contact the cytology laboratory for instructions.

Criteria for rejection

4. A pap collected in any fixative other than the ThinPrep Preservcyt solution (e.g., formalin vial) is not acceptable for the ThinPrep instrument. The physician/office will be notified of the rejected specimen.
5. Improper collection technique may result in an unsatisfactory specimen. If the cellular content is scanty or acellular at the time of screening, a statement will be added to the report that the specimen is Unsatisfactory for Evaluation.
6. Specimens not labeled or improperly labeled are not acceptable and the physician/office will be notified and asked to correct the problem.

SurePath Pap Test

Specimen Collection, Adequacy, Requisition & Transportation

Patient Preparation

To optimize collection conditions, a woman should:

1. Schedule an appointment approximately two weeks (10-18 days) after the first day of her last menstrual period is preferred, however, a menstrual pap is acceptable.
2. Not douche 48 hours prior to the test.
3. Not use tampons, birth control foams, jellies or other vaginal creams or vaginal medications for 48 hours prior to the test.
4. Refrain from intercourse 48 hours prior to the test.

Test Requisition

Under the supervision and guidance of the physician, a laboratory requisition must be legibly and accurately filled out before obtaining the cellular sample. The laboratory requisition is the main communication link between the physician and the laboratory. The requisition form should have the following information as required by CLIA '88.

1. Patient's name (any name change in the past 5 years should be noted)
2. Age and/or date of birth
3. Menstrual status (LMP, hysterectomy, pregnant, postpartum, hormone therapy)
4. Previous Pap history, abnormal cervical cytology results, previous treatment, biopsy or surgical procedure and results
5. Source of specimen, e.g. cervical, vaginal
6. Indicate if patient is at high risk for cervical cancer
7. Date of specimen collection
8. Submitting physician's name
9. Indicate on slip if HPV DNA testing or other special testing from vial is requested

Appropriate clinical history provided by the physician on the requisition should include:

1. Hormone/contraceptive use
2. Relevant clinical findings (abnormal bleeding, grossly visible lesion, etc.)

Patient's SSN, physician and health facility names, insurance information and medicare information if applicable.

Labeling the Sample

Write the patient's name and collect date on the SurePath preservative solution vial.

Visualization of the Cervix for Collection of an Adequate Sample

6. Collection of a cervical cytology specimen is usually performed with the patient in the dorsolithotomy position.
7. A sterile, or single-use bivalve speculum of appropriate size is inserted into the vagina without lubrication. Warm water or saline may be used to facilitate insertion of the speculum. The position of the speculum should allow for complete visualization of the os and ectocervix.
8. The transformation zone is the site of origin for most cervical neoplasia and should be the focus of cytology specimen collection. The transformation zone may be easily visualized or may be high in the endocervical canal. Location varies not only from patient to patient, but in an individual over time. Factors producing variation include changes in vaginal pH, hormonal changes including pregnancy, childbirth, menopausal status, and hormonal therapy.
9. In postmenopausal patients or women who have received radiation therapy, cervical stenosis may prevent visualization of the transformation zone. It remains important to sample the endocervix in these patients. This may require more extensive clinical procedures.
10. If a patient has had a hysterectomy, a vaginal sample is sufficient, with particular attention to sampling the vaginal cuff.

Techniques for Sample Collection

Broom-Like Device Technique

1. The ectocervix and endocervix are collected simultaneously. The central bristles of the broom (Carvex-brush) are inserted into the endocervical canal until the lateral bristles bend fully against the ectocervix. The sampling device is rotated 360° in the same direction five (5) times while maintaining gentle pressure.
2. The entire head of the broom is removed from the handle and placed into the SurePath Solution vial.
3. Reseal the vial with the broom inside.

Transporting Specimen

1. The vial is placed in the specimen transport bag and sealed. The requisition is placed in the pouch outside the sealed bag.
2. The specimens are transported to the cytology laboratory by courier. If the specimens are to be mailed, contact the cytology laboratory for instructions.

Criteria for rejection

1. A pap collected in any fixative other than the SurePath preservative solution (e.g., formalin vial) is not acceptable for the SurePath instrument. The physician/office will be notified of the rejected specimen.
2. Improper collection technique may result in an unsatisfactory specimen. If the cellular content is scanty or acellular at the time of screening, a statement will be added to the report that the specimen is Unsatisfactory for Evaluation.
3. Specimens not labeled or improperly labeled are not acceptable and the physician/office will be notified and asked to correct the problem.

THE BETHESDA SYSTEM 2001

GYNECOLOGIC CYTOLOGY CLASSIFICATION

SPECIMEN ADEQUACY

SATISFACTORY

Satisfactory for evaluation but may include any quality indicators, e.g., absence of endocervical/transformation zone component, partially obscuring blood, inflammation, etc.

UNSATISFACTORY

Unsatisfactory for evaluation of epithelial abnormality because of reason specified and should be repeated.

Specimen rejected/not processed – report is **not** generated but physician is notified as to reason for rejection.

GENERAL CATEGORIZATION

- Negative for Intraepithelial Lesion or Malignancy
- Epithelial Cell Abnormality: See Interpretation/Result (specify squamous or glandular)
- Other: See Interpretation/Result (endometrial cells in a woman > 40 years of age).

AUTOMATED REVIEW

If case examined by automated device, specify device and result. Each Pap report will state if it has been reviewed by an automated screening instrument.

ANCILLARY TESTING

Provide a brief description of test methods and report the results. A comment will be added to each Pap report stating that HPV testing is pending as requested by each physician. A separate report will then be issued for the HPV and CT/NG testing results.

DESCRIPTIVE INTERPRETATION

NEGATIVE FOR INTRAEPITHELIAL LESION OR MALIGNANCY

Negative for squamous cell abnormalities or glandular cell abnormalities. Organisms and other non-neoplastic findings are included under this category.

ORGANISMS:

- *Trichomonas vaginalis*
- Fungal organisms morphologically consistent with *Candida* species
- Bacteria morphologically consistent with *Actinomyces* species
- Cellular changes consistent with Herpes simplex virus
- Shift in flora suggestive of bacterial vaginosis

OTHER NON-NEOPLASTIC FINDINGS

- Reactive changes associated with
 - inflammation (includes typical repair)
 - Radiation changes
 - intrauterine contraceptive device (IUD)
- Parakeratosis and/or hyperkeratosis
- Atrophy
- Glandular cells status post hysterectomy

OTHER

- Endometrial cells in a woman > 40 years of age
 - specify if negative for squamous intraepithelial lesion

EPITHELIAL CELL ABNORMALITIES

SQUAMOUS CELL

- Atypical squamous cells
 - of undetermined significance (ASC-US)
 - cannot exclude HSIL (ASC-H)
- Low grade squamous intraepithelial lesion (LSIL)

encompassing: HPV/mild dysplasia/CIN 1

- High grade squamous intraepithelial lesion (HSIL)
encompassing: moderate and severe dysplasia, CIS/CIN 2 and CIN 3
 - with features suspicious for invasion (if invasion is suspected)
- Squamous cell carcinoma

GLANDULAR CELL

- Atypical glandular cells (AGC)
 - specify endocervical, endometrial, or NOS
- Endocervical adenocarcinoma *in situ* (AIS)
- Adenocarcinoma
 - endocervical
 - endometrial
 - extrauterine
 - not otherwise specified (NOS)

OTHER MALIGNANT NEOPLASMS (SPECIFY)

MATURATION INDEX

The maturation of the vaginal squamous epithelium is hormone dependent. A maturation index is a rough indicator of hormone levels and is derived from the percentage of superficial, intermediate, and parabasal squamous cells present in the vaginal smear. Estrogens produce a high percentage of mature superficial squamous cells, and progesterone yields a high percentage of intermediate cells. Decreased or absent hormone concentrations result in parabasal or atrophic cellular patterns.

Smears for the maturation index must be prepared with cells from the lower third of the lateral vaginal wall. Cellular material from the endocervix is unsatisfactory as are smears contaminated with cervical cells. Infectious agents and inflammation can affect the MI.

The maturation index is reported as follows: (%P/%I/%S)

Marked estrogen effect	more than 75% superficial cells
Moderate estrogen effect	26% to 75% superficial cells
Mild estrogen effect	10% to 25% superficial cells
No significant estrogen effect	less than 10% superficial cells
Atrophic smear	more than 75% parabasal cells

MATURATION INDEX MUST BE TAKEN FROM THE LATERAL VAGINAL WALL

MATERIALS NEEDED

1. Microscopic Slide with Frosted End - Write the patient's name on the label end of the slide (frosted side) with an ordinary lead pencil. Also, indicate on the label (V) for vaginal smear if a cervical/endocervical smear is being collected also.
Liquid Pap Vial – Write the patient's name on the label. If a cervical/endocervical sample is being collected as well, place samples in two separate vials. Write the specimen source on each vial.
2. Cytology Spray Fixative – Use this fixative for conventional slide method.
3. Speculum – Use water, not lubricant, on speculum and shake off excess.
4. Collection Device – Wooden or Plastic Spatula
5. Gyn Cytology Form – Complete the form with patient information and mark Maturation Index.
6. Slide Holder for Glass Slide (Conventional Method)
7. Specimen Transport Bag – Place specimen in bag and seal. Place the completed requisition form in the pouch outside the sealed bag.

SPECIMEN COLLECTION AND PRESERVATION

Vaginal Scrape

1. Scrape the lateral wall of middle third of vagina.
2. Conventional Method - Spread evenly on slide and immediately spray with fixative. Allow to dry (5-10 minutes) and place in a slide holder. Mark slide as V (vaginal) if a cervical/endocervical smear is submitted also.
Liquid Pap Method – Place specimen in pap vial. Write specimen source (Vaginal) on the label if a cervical/endocervical specimen is submitted also.

ANCILLARY TESTING FROM LIQUID PAP VIALS

1. Collect pap as usual in a ThinPrep, SurePath or Digene cervical sampler specimen vial (DNA collection device).
2. Indicate on the requisition if HPV DNA testing (High Risk), Chlamydia or Gonorrhea testing is desired.
3. If a standing order is desired for HPV testing to be run on all ASC-US/ASC-H paps for that physician, then submit a signed request on office stationary to the cytology lab. The standing order request will remain active for one year.

DIGENE DNA CERVICAL SAMPLER SPECIMEN COLLECTION AND HANDLING

1. Collect Pap smear specimen before obtaining specimen for DNA testing. Collect DNA specimen prior to application of acetic acid or iodine if a colposcopy will be performed.
2. Remove excess mucus from the cervical os and surrounding ectocervix using a cotton or Dacron swab. Discard the swab.
3. Insert the brush 1- 1.5 cm into the os of the cervix until the largest outer bristles of the brush touch the ectocervix. Rotate it 3 full turns in a counter-clockwise direction. **DO NOT INSERT BRUSH COMPLETELY INTO THE CERVICAL CANAL.**
4. Remove the brush from the canal. Avoid touching the bristles to the outside of the tube or to any other object.
5. Insert brush to bottom of the Transport Tube. Snap off shaft at score line and cap tube securely.
6. Label tube with date and patient's name. Place in bag and send to lab with request.

ANCILLARY TESTING FROM LIQUID PAP VIALS

Notes: Do not use cervical brush with pregnant women.

Cervical specimens may be held for up to 2 weeks at room temperature and stored for an additional week at the lab at 2-8°C.

Use only with Hybrid Capture2 HPV and CT/GC DNA tests. Cannot be used for culture.

Store kits at 15-30°C until the expiration date printed on pouch label.

Dispose of transport medium (contains sodium azide) in accordance with federal, state and local regulations.