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ROUTINE HISTOLOGY SPECIMEN COLLECTION

MATERIALS NEEDED

1. Instruments for collection
2. Requisition form with the following information:
   Patients name
   Date of birth
   Sex
   Physician's name and health facility submitting specimen
   Tests requested for each specimen submitted
   Billing information
   Date of collection
   Source of specimen, be as descriptive as possible
   Clinical history, when appropriate
   Requisitions not containing the proper information will be rejected.
3. Container of 10% neutral buffered formalin. Container must bear OSHA approved Formalin labeling
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. Label the specimen container with two patient specific identifiers. . **All containers must have two patient identifiers or will be rejected.** Acceptable identifiers include: Patient name, first and last (initials are not acceptable), date of birth, hospital number, social security number. Identifiers should not be placed on the lid of a specimen container as lids can be switched,
3. The specimen container should also be labeled with the specimen source, either directly or using a reference to the requisition.
4. Ensure the lid is correctly screwed on tightly.
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 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.

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

Criteria for rejection

1. **Specimens received without an accompanying requisition will be returned to the point of origin for correction.**
2. **Specimens with containers that do not contain two patient identifiers will be returned to the point of origin for correction.**
3. **Specimens with multiple containers which are identically labeled, such that the sources cannot be determined will be returned to the point of origin for correction.**
4. **Specimens that are improperly packaged, or damaged to the point of compromising either specimen integrity or safety of handling, will be returned to the point of origin for correction.**
5. **For specimens that have requisitions missing one or more elements listed above, the submitting facility will be contacted by the receiving party at Pensacola Pathologists. If the missing information can be resolved remotely, then the case can be accessioned normally. If not, the specimen will be returned to the point of origin for correction.**
6. **See GEN 1-22 SPECIMEN REJECTION POLICY for more information.**

Transporting Specimens

1. **Specimens should be properly packaged and checked for leaks.**
2. **A specimen tracking log should be filled out containing all of the specimens that are being transported. Medspeed tracking barcode stickers should be applied to all specimens.**
3. **Specimens submitted concurrently can be placed together in a tertiary container along with the tracking sheet and placed in the designated Medspeed pickup area. For specimens which originate on the Sacred Heart Pensacola campus the specimens can be delivered to the Pathology Department directly.**

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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% buffered 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 and date of birth or medical record number. **All slides and containers need two patient identifiers**.
2. Place the aspirate in a container of 10% buffered formalin and label it "A"
3. Place the bone marrow core biopsy (if applicable) in a container of 10% buffered formalin and label it "B".
4. Completely label the specimen container with patient's name, date of birth, 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.

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GYNECOLOGIC CYTOLOGY SPECIMENS

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. *Note: Excessive amounts of blood may compromise the test and lead to an unsatisfactory result.*
2. Not use birth control foams, jellies or other vaginal creams or vaginal medications or douches for 48 hours prior to the test. *Note: These are interfering substances and may lead to an unsatisfactory result.*

Test Requisition

Under the supervision and guidance of the physician (authorized individual), a laboratory requisition must be legibly and accurately filled out. Printed orders from an EMR system are acceptable as long as they have the required information. The requisition form must have the following information:

1. Patient's name (any name change in the past 5 years should be noted)
2. Date of birth
3. Menstrual status (LMP, hysterectomy, pregnant, postpartum, hormone therapy)
4. Previous Pap history to include:
   - Abnormal cervical cytology results
   - Previous treatment, biopsy or surgical procedure and results.
   - Indicate if patient is at high risk for cervical cancer.
5. Source of specimen, e.g. cervical, endocervix, vaginal

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6. Appropriate clinical history should include:
   - Hormone/contraceptive use
   - Relevant clinical findings (abnormal bleeding, grossly visible lesion, History of Cancer, HPV, radiation or chemotherapy)
7. Patient's billing and insurance information.
8. Date of specimen collection
9. Physicians name and clinic address
10. Requests for additional testing from liquid Pap, i.e., HPV, HPV Genotype 15/18/45, CT/GC/TV.

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.
4. A pap collected in any fixative other than the ThinPrep Preservcyt solution (e.g., formalin vial) is not acceptable for the ThinPrep instrument. Any Pap or Aptima Specimen Transport Tube submitted in expired media will be rejected.
5. The physician/office will be notified of rejected specimens and instructed to re-collect.

Transporting Pap Specimens

1. Document the patient's name and specimen type and number of containers/specimens on the specimen tracking sheet. Ensure all containers are labeled with 2 patient identifiers.
2. The specimens are transported to the cytology laboratory by courier. If the specimens are to be mailed, contact the cytology laboratory for instructions. Specimens must be shipped in accordance with applicable local and national transportation regulations.

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3. ThinPrep specimens should be stored and transported from 15°C (59°F) to 30°C (86°F) and should be tested within 6 weeks of collection.

Method I – Conventional Pap

Materials

Speculum (without lubricant)

Spatula and Endocervical brush or plastic cervix broom

Frosted end glass slide and pencil

Pap smear requisition & Pre-printed patient label

Cytology spray fixative (contains alcohol, flammable, poison)

Biohazard specimen transport bag

Labeling the Sample

1. Write the patient's name and DOB 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. Inserted speculum into the vagina without lubrication. Warm water may be used to facilitate insertion of the speculum.
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.

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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.

Method I – Conventional Pap

Techniques for Sample Collection

Spatula and Endocervical Brush Technique

1. Obtain an adequate sampling from the ectocervix using a plastic or wooden spatula.
   - Rotate the notched end of the spatula that corresponds to the contour of the cervix 360° around the circumference of the cervical os.
   - Spread the sample on the spatula evenly and thinly lengthwise down one half of the labeled slide surface, using a single uniform motion. Immediately spray with cytology fixative. Generally, spray fixatives should be 6-10 inches from the glass slide when applied.
   - Discard the spatula.
2. Obtain an adequate sampling from the endocervix using an endocervical brush device.
   - Insert brush into the cervix until only the bottom-most fibers are exposed.
   - Slowly rotate the brush one full turn 360°.
3. Roll the brush as quickly as possible 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. Spray fixative 6-10 inches from the glass slide.
   - Discard the brush.
4. Tighten the vial cap so that the black torque line on the cap passes the black line on the vial.
5. Label slides with patient's name and DOB (2 identifiers).
6. Place slide(s), when dry, in slide holder and put in the specimen transport bag and seal. Place requisition in the pouch outside the sealed bag.

Broom-Like Device Technique

1. Obtain and adequate sampling from the cervix using a broom-like device.
   - Insert the central bristles of the broom into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix.
   - Push gently, and rotate the broom in a clockwise direction 5 times.
2. Transfer the sample with a single paint stroke motion 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. Spray fixative 6-10 inches from the glass slide.
   - Discard the broom.
3. Label slides with patient's name and DOB (2 identifiers).
4. Place slide(s), when dry, in cardboard slide holder and put in specimen transport bag and seal. Place requisition in the pouch outside the sealed bag.

ThinPrep Pap Test

Method II – ThinPrep Pap (preferred)

Synonyms – Liquid-based Pap test

Specimen Collection

Materials

Speculum (without lubricant)

Spatula and Endocervical Brush or plastic Cervix Broom

ThinPrep PreservCyt Solution Vial

Pap smear requisition

Pre-printed patient label

Biohazard specimen transport bag

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Warning: PreservCyt solution contains Methanol which is poisonous. May be fatal if swallowed, harmful vapors, flammable. Store at 15°C - 30°C away from heat or sparks.

Labeling the Sample

Write the patient's name, collect date and date of birth on the PreservCyt Solution vial (all specimen containers must have 2 patient identifiers). Pre-printed patient labels are preferred.

ThinPrep Pap

Specimen Collection Preparation

1. Lubricant jellies should not be used to lubricate the speculum. *Note: If lubricants are used do not use any with ingredients that contain "carbomers" or carbopol polymers". These products will interfere with processing and may result in an unsatisfactory result.*
2. Gently remove with gauze excess mucus or other discharge present before taking the sample.
3. The sample should be obtained before application of acetic acid.

Techniques for Sample Collection

Spatula and Endocervical Brush Technique

1. Obtain an adequate sampling from the ectocervix using a plastic spatula.
   - Rotate the notched end of the spatula that corresponds to the contour of the cervix 360° around the circumference of the cervical os.
   - Rinse the spatula as quickly as possible into the PreservCyt Solution vial by swirling the spatula vigorously in the vial 10 times.
   - Discard the spatula.
2. Obtain an adequate sampling from the endocervix using an endocervical brush device. • Insert brush into the cervix until only the bottom-most fibers are exposed.

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- Slowly rotate the brush ¼ or ½ turn in one direction. Do not over-rotate.

3. Rinse the brush as quickly as possible into the PreservCyt Solution vial by rotating the device in the solution 10 times while pushing against the PreservCyt vial wall. Swirl vigorously to further release material.
   - Discard the brush.
4. Tighten the vial cap so that the black torque line on the cap passes the black line on the vial.
5. Label vial with patient's name and DOB (2 identifiers).
6. Place vial in the specimen transport bag and seal. Place requisition in the pouch outside the sealed bag.

ThinPrep Pap

Broom-Like Device Technique

1. Obtain and adequate sampling from the cervix using a broom-like device.
   - Insert the central bristles of the broom into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix.
   - Push gently, and rotate the broom in a clockwise direction 5 times.
2. Rinse the broom as quickly as possible into the PreservCyt Solution vial.
   - Push the broom into the bottom of the vial 10 times, forcing the bristles apart.
   - Swirl the broom vigorously to further release material.
   - Discard the broom.
3. Tighten the cap so that the black torque line on the cap passes the black line on the vial.
4. Label vial with patient's name and DOB (2 identifiers).
5. Place vial in the specimen transport bag and seal. Place requisition in the pouch outside the sealed bag.

Aptima Assays for ThinPrep Pap

Synonyms-

Molecular testing, PCR, DNA/RNA testing, Genotyping, HPV reflex test, CT/GC, Trichomonas

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ANCILLARY TESTING FROM LIQUID PAP VIALS

1. Collect cervical specimens in ThinPrep Pap test vials containing PreservCyt solution with broom-type or cytobrush/spatula collection device or Aptima Unisex Swab Specimen collection kit for Endocervical specimens, according to the manufacturer's instructions.
2. Indicate on the requisition if HPV DNA testing (High Risk), HPV DNA 16/18/45 Genotyping testing, Chlamydia, Gonorrhea or Trichomonas testing is desired.
3. If a standing order is desired for HPV reflex testing to be run on all ASC-US Paps for that physician, then submit a signed request on office stationary to the cytology lab.
4. Transport the ThinPrep liquid cytology specimens at 2°C to 30°C. Prior to transfer the ThinPrep cytology specimens should be stored at 2°C to 30°C, with no more than 30 days at temperatures above 8°C.

APTIMA MULTITEST SWAB SPECIMEN COLLECTION KIT

1. Partially peel open the swab package. Remove the swab. Do not touch the soft tip or lay the swab down. If the soft tip is touched, the swab is laid down, or the swab is dropped, use a new Aptima Vaginal Swab Specimen Collection Kit.
2. Hold the swab, placing your thumb and forefinger in the middle of the swab shaft covering the score line. Do not hold the swab shaft below the score line.
3. Carefully insert the swab into the vagina about 2 inches (5 cm) past the introitus and gently rotate the swab for 10 to 30 seconds. Make sure the swab touches the walls of the vagina so that moisture is absorbed by the swab and then withdraw the swab without touching the skin.
4. While holding the swab in the same hand, unscrew the cap from the tube. Do not spill the contents of the tube. If the contents of the tube are spilled, use a new Aptima Vaginal Swab Specimen Collection Kit.
5. Immediately place the swab into the transport tube so that the score line is at the top of the tube.

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6. Carefully break the swab shaft at the score line against the side of the tube.
7. Immediately discard the top portion of the swab shaft.
8. Tightly screw the cap onto the tube.
9. Label tube with date, patient's name and date of birth. Place in bag and send to lab with request. **ALL CONTAINERS MUST HAVE TWO PATIENT IDENTIFIERS.**

Specimen Transport and Storage

Vaginal swab specimens must be transported to the laboratory in the provided swab specimen transport medium and tube. Vaginal swab specimens must be transported to the laboratory at 2ºC to 30ºC and tested within 60 days of collection. If longer storage is needed, refer to the appropriate Aptima assay package insert. Note: Specimens must be shipped in accordance with applicable national and international transportation regulations.

APTIMA ASSAY SPECIMENS

Limitations and Interfering Substances:

1. After collection, transport and store swab specimen transport tube at 2°C to 30°C. Assay within 60 days of collection. Store collection kits at room temperature (15°C -30°C) until the expiration date printed on pouch label.
2. Aptima swab for use only with Hologic/Gen-Probe Aptima Assays CT/GC, and Trichomonas tests. Cannot be used for HPV testing or culture.
3. Dispose of transport medium (contains sodium azide) in accordance with federal, state and local regulations.
4. Specimens were evaluated in the Aptima Combo 2 CT/GC and Trichomonas assay for interference by blood, gynecological lubricants, and spermicides. The data indicated no assay interference by these substances. The effects of tampon use, douching and specimen collection variables have not been assessed for their impact on the detection of CT or GC or Trichomonas.

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5. ThinPrep specimens were evaluated in the Aptima HPV assay for interference by blood, glacial acetic acid, gynecological lubricants, spermicides and anti-fungal medications. The data indicated no assay interference by most of these substances with the exception of two lubricants that contained Polyquaternium 15 and one anti-fungal medication that contained tioconazole.
6. Aptima assays are not intended for the evaluation of suspected sexual abuse or for other medico-legal indications.
7. Aptima results should be interpreted in conjunction with other lab and clinical data available to the clinician.

APTIMA URINE SPECIMEN COLLECTION KIT SPECIMEN COLLECTION AND HANDLING OF FEMALE URINE FOR:

Chlamydia trachomatis (CT)

Neisseria gonorrhoeae (GC)

Trichomonas vaginalis (TV)

*** Patient should not have urinated for at least 1 hour prior to specimen collection.***

1. Direct patient to provide first-catch urine (approximately 20 to 30 mL of initial urine stream) into urine collection cup free of any preservatives. Collection of larger volumes of urine may result in specimen dilution that may reduce test sensitivity. Female patients should not cleanse labial area prior to providing specimen.
2. Remove cap from urine specimen transport tube and transfer 2 mL of urine into urine specimen transport tube using the disposable pipette provided. The correct volume of urine has been added when the fluid level is between the black fill lines on urine specimen transport tube label.
3. Re-cap urine specimen transport tube tightly. This is now known as the “processed urine specimen.”

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4. Label tube with date, patient's name and date of birth. Place in bag and send to lab with request. **All containers must have two patient identifiers.**

Specimen transport and storage

1. After collection, transport and store processed urine specimens in the Aptima urine specimen transport tube between 2˚C to 30˚C until tested.
2. Processed urine specimens should be assayed with the Aptima assay for CT, GC and/or TV within 30 days of collection.
3. If longer storage is needed, freeze between -20˚C to -70˚C for up to 12 months after collection in the Aptima assay for CT and/or GC. For the Aptima assay for TV, freeze at < -20˚C for up to 12 months.
4. Urine samples still in primary collection container must be transported to the lab between 2˚C to 30˚C.
5. Transfer urine sample into Aptima urine specimen transport tube within 24 hours of collection.
6. Transfer urine sample into Aptima urine specimen transport tube within 24 hours of collection.

Note: The Aptima assays are FDA approved tests.

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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. The Pap report will include the HPV and CT/NG/TV test results.

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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
  - -intra-utérine 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

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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)

NONGYNECOLOGIC CYTOLOGY SPECIMENS

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MATERIALS NEEDED

1. Instruments for Collection

2. Non-Gyn Cytology Form (requisition or EMR printed orders) - Complete the form with the following information; patient's name, age, sex, physician's name, address and health facility submitting the specimen, billing information, date of collection, exact source of specimen and clinical history.

4. Container with Cytology Fixative/Preservative – CytoLyt (clear), PreservCyt (clear) for fluid specimens and Cytology Spray Fixative or jar of 95% Alcohol for smear preparation specimens. All fluid cytology specimens should be sent fresh (preferable) to the laboratory or collected in a cytology fixative. Fresh specimens should be refrigerated (ice pack in cooler for car transport) until they are taken to the lab.

5. Container labels. Pre-printed patient labels for containers are preferred or write the patient's name, date of birth, medical record number, source of specimen and doctor's name on the specimen container label. **All containers must have two patient identifiers.**

5. Microscopic Glass Slides with Frosted End and Slide Holder for prepared slide specimens - Write the patient's name and a second identifier (DOB or Hosp.#) on the frosted end of the slide with an ordinary lead pencil or permanent felt tip marker. Do not use an ink pen. It washes off in the staining procedure. **All slides must have two patient identifiers.**

6. Specimen Transport Bag and Vivid Pathology tracking sheet.

CRITERIA FOR REJECTION

1. Specimens should be sent to the lab as soon as possible and unfixed specimens should be refrigerated until they are processed. Improper fixation or undue delay in transport may alter the cellular content. Any microscopic distortion of material due to improper fixation or degeneration of cells will be noted on the final report. Cytology specimens received in formalin will be rejected.
2. Specimens not labeled, improperly labeled, or discrepancy between container label and requisition are not acceptable and the physician/office will be notified and asked to correct the problem. The specimen may be returned to the office if improperly labeled.
3. Syringes received with an attached needle are not acceptable.

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4. If the cellular content is scanty, or obscured by blood or pus at the time of screening, a statement will be added to the report. The specimen may be reported as Unsatisfactory for Evaluation and will need to be recollected.
5. Specimens ordered by unauthorized person.
6. Empty specimen containers.
7. Leaking containers or contaminated requisitions.

STORAGE AND STABILITY REQUIREMENTS FOR NONGYN SPECIMENS

1. Slides and preservative fluid store at room temperature (15°C - 30°C). Preserved specimens stable 3 weeks.
2. Fresh specimens keep refrigerated. Transport in cooler with ice packs. Receipt by lab within 24 hours is preferred, however, most nongyn specimens stable up to 3 days refrigerated.
3. Cytolyt & PreservCyt preservatives contain Methanol. Spray fixatives and alcohol slide containers contain ethanol, methanol and isopropyl alcohols. Poisonous and flammable. Store away from heat or sparks. See Safety Data Sheets for safe handling, storage and disposal.

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NONGYN SPECIMEN COLLECTION AND PRESERVATION

Sputum

When a pulmonary lesion is suspected, a complete sputum series should be examined. This usually consists of a fresh morning specimen each day for three days. A post bronchoscopy specimen may be included in the series.

Method I: Early Morning Spontaneous Deep Cough Technique

1. Patient is given a labeled specimen collection cup containing CytoLyt Solution. One cup should be provided each morning for three consecutive days.
2. Caution the patient that only sputum is to be collected, not material from sinus drainage or saliva.
3. Patient should rinse mouth with water.
4. Instruct the patient to cough deeply several times the first hour after awakening and expectorate into the collection cup.
5. Place lid tightly on specimen cup and shake for a few seconds.

Method II: Sputum Induction Technique

If the patient is non-productive of satisfactory specimens, the induction technique should be administered. Various aerosol instruments are available and instructions for use accompany each. The object of the aerosolization is to introduce a significant amount of water into the lungs. Irritants or mucolytic agents can be added.

1. Explain the procedure to the patient.
2. Before beginning, ask the patient to clear his throat and wash his mouth out with water.
3. Administer the aerosol.
4. Sputum should be expectorated into a collection cup.

Sometimes, if an adequate sample cannot be produced using an aerosol, the patient will have a productive cough within the next 24 hours. The patient should be given a collection cup and instructions for collecting a sputum sample during this period of time. Note: The specimen will be reported as Unsatisfactory if it is not a deep cough and does not contain alveolar macrophages.

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Bronchial Washings and Bronchoalveolar Lavage

1. After the specimen is collected, put the entire specimen into a collection container with a tight sealing lid. A separate container should be used for microbiology tests. If multiple tests are ordered on the same sample, the slip should indicate that the specimen is to be shared with another department or lab.
2. Label each container with two patient identifiers information and exact source (site) of specimen.
3. Indicate on slip requests for special stains (e.g., lipid or hemosiderin laden macrophages, fungal, pneumocystis).

Note: In order to localize the tumor to a lung or specific lobe, separate bronchoscopes should be used for each area of investigation; otherwise contamination of specimens may occur.

Bronchial Brushing

1. Direct smears may be made by quickly rotating the brush gently on a glass slide labeled with the patient's name and DOB or Hospital #. Fix immediately by placing slides into the container of 95% alcohol.
2. Immediately after the brush is withdrawn from the bronchoscope, cut the wire a short distance from the brush and insert into a tube of normal saline or a cytology preservative (obtained from the cytology department).
3. Label each container and slide with two patient identifiers and exact source (site) of specimen.

Breast Nipple Secretions

Method I
Nipple secretions should be collected by applying the slide directly to the nipple. Gently express discharge and smear droplet across the slide. Immediately fix the smear with cytology spray fixative. Follow the directions on the spray bottle. Allow to dry (5 to 10 minutes) and place in slide container for transportation to the cytology laboratory.

Method II (Preferred)
Immobilize breast and hold container under breast. Gently express secretions directly into a cytology preservative such as Cytolyt or directly into a ThinPrep Pap smear vial.

Label each container and slide with two patient identifiers and site (right or left breast).

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Gastric and Esophageal Brushing

Method I (Preferred)

Direct smears may be made by quickly rotating the brush gently on a glass slide labeled with the patient's name and DOB or Hosp.#. Fix immediately with cytology spray fixative. Follow the directions on the spray bottle. Allow to dry (5 to 10 minutes) and place in slide container for transportation to the cytology laboratory.

Method II

Immediately after the brush is withdrawn from the instrument, cut the wire a short distance from the brush and insert into normal saline or a cytology preservative solution (obtained from the cytology department).

Label each container and slide with two patient identifiers.

Gastric and Esophageal Washings

After the specimen is collected, put the entire specimen into a collection container. Refrigerate until transported to cytology.

Body Cavity Fluids, and Other Fluids

1. The fluid is collected in plain **unheparinized** tubes, or other suitable containers. Glass bottles are not recommended for transport. (Minimum required amount 8cc, maximum accepted amount 500cc). *Note: Do not send multiple large containers of body fluid to the lab, 50cc-100cc is the desired amount for cytology processing.*
2. Place container in sealed biohazard bag.
3. Keep the specimen refrigerated until transport to the lab.

Cerebrospinal Fluid

1. Perform spinal lumbar tap. The first few drops may be discarded if very bloody. Place in clean conical plastic tube with screw top lid.
2. Draw as much fluid as clinical judgement allows. (Minimum required amount 2 cc).
3. CSF should be refrigerated immediately and sent to lab ASAP.

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Urine

Specimen types may include voided, catheterized, intraoperative washings of bladder, ureters, renal pelvis.

Patient preparation:

1. Hydrate the patient by administering one glass of water every 30 minutes for a 3 hour period. IV fluid or diuretics can be given to increase urinary output.
2. Female patients should be catheterized to avoid vaginal contamination.
3. After the 3 hour period, have the patient void or catheterize. Discard specimen. One hour after discarded specimen, have the patient void and save the specimen.

Method I (Preferred)

Place urine in a leak proof container and send the labeled specimen to the lab immediately or refrigerate until it can be brought to the lab.

Method II

1. If refrigeration is not available and transport is not available the same day, the urine may be added to a cytology fixative.
2. After the specimen is collected, add the specimen to an approximately equal volume of CytoLyt or PreservCyt Solution. Add the specimen to the 30 ml of fixative in the CytoLyt Solution collection cup. If there is more specimen than the collection cup will hold, discard the remaining specimen.
3. Contact the cytology department for CytoLyt or PreservCyt Solution.

Tzanck Prep for Viral Inclusions

1. Scrape lesion with scalpel and spread onto slide. Label slide with two patient identifiers.
2. Slide may be air dried or sprayed with a cytology fixative.
3. Fill out requisition with location of lesion and indicate if slide is dry or fixed.
4. Place in cardboard holder for transport. Place holder & request slip in transport bag.

Fine Needle Aspiration

Sampling of lesion:

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1. Position the patient to ensure best access to the lesion/mass, as well as the maximal safety and comfort of the patient.
2. Palpate the mass carefully. Differentiate between the normal structures and the lesion/mass.
3. Swab the skin area with an alcohol swab. Most superficial FNAs are performed without local anesthesia.
4. Stabilize the mass against deeper tissues with the index and middle fingers, or thumb and forefinger.
5. Start with .5ml to 1ml of air in syringe. Insert needle to edge of the lesion and apply negative pressure.
6. Move the needle back and forth in the lesion with short (<1cm) strokes and minimal redirecting of the needle.
7. Release negative pressure if blood or specimen appears in needle hub (or 15-20 strokes if hub is empty).

Sample Preparation:
Method I

1. Have nearby a collection container with cytology preservative (CytoLyt Solution or PreservCyt). *Note: Alcohol is not acceptable*.
2. After the aspiration biopsy has been completed and the needle withdrawn, disconnect the needle from the syringe, fill the syringe with air, reconnect syringe and needle and expel the specimen into the Cytology Solution.
3. Then draw the fixative into the syringe to wash out remaining specimen. Expel into collection container.

Fine Needle Aspiration

Method II (Preferred Method)

1. After the aspiration biopsy has been completed, the needle is removed from the syringe and air is drawn into the syringe barrel.
2. The needle is reconnected to the syringe. The material in the needle is carefully expelled in a single drop toward the label end of a glass slide, preferably a totally frosted slide. The open edge of the needle bevel is directed down toward the slide during expression of material. *Note: A second slide may be used as a shield to prevent the aspirated material from spraying.*

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3. Another slide is placed face to face with slide containing specimen. The specimen is allowed to spread without applying pressure. If tissue fragments are present, they may be flattened with very slight pressure. Gently pull slides apart horizontally with an easy sliding motion once the specimen pool stops spreading between the slides.

   ∆**CAUTION:** When disconnecting and reconnecting the needle, use a needle recapping device.

4. Fix immediately by dropping one slide into 95% Alcohol and allow the second slide to Air Dry.

5. Then draw the cytology preservative (CytoLyt, PreservCyt) into the syringe to wash out remaining specimen. Expel into collection container.

6. Repeat collection as necessary, trying to sample a different area and follow above steps.

7. If lymphoma is suspected, squirt some specimen into RPMI solution for flow cytometry.

8. Label all slides and containers with two patient identifiers and exact specimen site. Indicate all specimen sites on pathology requisition form.

Note: It is prudent to be prepared for the possibility of ancillary studies, such as: core tissue (placed in formalin), flow cytometry (RPMI solution), or microbiology/virology cultures.

Anal Brushing

Synonyms – Rectal brush, Anal Pap

Test Information:

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The incidence of invasive anal squamous cell carcinoma, a HPV related cancer is on the rise. Cytologic screening can aid in the detection of anal-rectal cancer and its precursor lesions. Diagnostic terminology as defined by the Bethesda System for Reporting Cervical Cytology (TBS 2001) is used. The Sensitivity and specificity of a single anal-rectal cytology specimen is comparable with that of a cervical cytology specimen.

Collection:

1. Either a fixed conventional slide or liquid-based anal-rectal cytology specimen is acceptable, but ThinPrep specimens are preferred.
2. Insert the cytobrush or water moistened Dacron swab 5-6 cm into the anal canal and rotate the brush/swab by sampling the anal canal transition zone.
    - Rinse the brush/swab by swishing vigorously in the PreservCyt solution (ThinPrep vial).
    - Seal cap on tightly on ThinPrep vial.
    - If slides are used the smears must be fixed immediately with a spray fixative or dropped into 95% alcohol.
3. Label the vial (or slides) with two patient identifiers (name/DOB), fill out lab requisition and place in biohazard bag for transport to the laboratory.

Specimen Limitations:
Specimens with scant cellularity or those that consist mainly of anucleated squames are unsatisfactory for evaluation. The presence of squamous metaplastic and rectal columnar cells is reported as an indication that the anal transition zone has been sampled.

TRANSPORTATION OF NONGYN SPECIMENS:

1. Place specimen containers in a specimen transport bag and seal. Place spray-fixed slides in a cardboard or plastic slide holder and tape closed, place in bag. Place the completed requisition in the pouch outside the sealed bag. Ensure all containers and slides are labeled with two patient identifiers and exact site of specimen.

2. Transport specimens to the cytology laboratory by courier. If specimens are to be mailed, contact the laboratory for instructions.

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CASE ASSINMENT

Cases will be distributed to pathologist's base on their subspecialties.

1. IT will run a daily log of all cases to be assigned. Those cases will be assigned to each pathologist based on their specialty.
2. Points are given to the complexity of each type of case. Vivid Pathology will try to distribute cases evenly when applicable.

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3. All cases that are in Ligolab will be assigned from the unassigned case list.
4. For Baptist only, Vivid will assign cases that have been accessioned in their Co-path. Vivid will run the unassigned case report and assign based on specialty. Vivid will make sure there are no other cases to be assigned once we have completed assignment.
5. All cases that have been assigned will go to their credenza or make a courier run to whatever location that are working for that day.

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Revised: Jan 2008, Jan 2014, Sep2015, Nov2015, July 2019