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Cytopathology

1

Version 2.0
I. REQUISITION:

Peterson Laboratory Services is a Clinical Laboratory Improvement Amendment (CLIA) certified laboratory. Our certification is your assurance of the highest possible testing standards.

In compliance with CLIA regulation, guidelines have been established for the acceptance or rejection of cytology specimens. Appropriately labeled specimens must be accompanied by a cytology requisition. **Information appearing on the slide or ThinPrep vial and the requisition MUST match identically.**

Complete a cytology requisition (yellow, form 15) noting the following information:

1. **PATIENT NAME:** Patient name on the requisition must exactly match the patient name as it appears on the slide or ThinPrep vial label.

2. **DATE OF COLLECTION**

3. **PATIENT’S DATE OF BIRTH**

4. **PATIENT ADDRESS:** If the ordering physician/practitioner participates in the Pap Reminder system, patient address is needed to generate the patient reminder letter.

5. **PHYSICIAN/PRACTITIONER NAME**

6. **SOCIAL SECURITY NUMBER/PATIENT ID:** Please provide patient social security number or other patient identifier.

7. **PERTINENT CLINICAL INFORMATION:** The following information is required by regulation for accurate interpretation of the specimen:
   a) Date of last menstrual period
   b) Source of material submitted (cervix, endocervix or vagina)
   c) Hormonal status (pregnant, postpartum, post-menopausal, etc)
   d) Exogenous hormone therapy (birth control pills, estrogen therapy, etc.)
   e) History of abnormal cytology
   f) Date of last gynecological testing and the diagnosis
   g) Any other pertinent history

II. SPECIMEN LABELING:

ThinPrep Vial: Using a permanent marker, record patient name and ID number on the vial.

Conventional Slide: Using a Number 2 lead pencil, record the patient’s first and last name on the frosted end of the slide.

**IMPORTANT:**

If the name on specimen requisition does not exactly match the name on the slide or ThinPrep vial, the specimen will be rejected and returned to the originating facility. Please correct the discrepancy and re-submit the specimen.
III. Conventional Pap Smear Specimen Collection

A. Patient Preparation

1. It is advisable for the patient to avoid using vaginal suppositories, creams or douches for twenty-four (24) hours prior to collection.

2. Water may be used to lubricate and warm the speculum; however, lubricant jellies should not be used. Position the speculum so that the entire face of the cervix appears at the end of the speculum. A sample from this area is necessary for adequate specimen collection.

B. Sample Collection

1. For a sample to be considered adequate, it is necessary to obtain a specimen from both the ectocervix and the endocervix. Successful collection requires careful inspection of the cervix and localization of the transformation zone.

2. To sample the endocervix, an endocervical brush is gently inserted into the endocervix, turned 360 degrees and withdrawn. The cytobrush is NOT recommended for use on pregnant patients. Vigorous scrubbing of the endocervix should be avoided as bleeding and patient discomfort may result.

3. It is important to obtain a smear that is not obscured by blood, mucus or inflammatory exudate. If there is mucus or other discharge present, gently remove with a swab. Discard swab. Do not clean the cervix by washing with saline as it may result in an acellular smear.
4. To sample the ectocervix, use a scraper to gently, but firmly scrape the transformation zone (squamocolumnar junction). Be careful to avoid removing pieces of tissue with the scraper.

2. Fix the smear immediately using cytology spray fixative. Failure to fix smears immediately may result in smears that are unsatisfactory for evaluation.

**TWO SLIDE TECHNIQUE**

1. The endocervical brush and ectocervical scraping are each spread on individual slides as soon as they are obtained.

2. Fix the smears immediately using the cytology spray fixative. Failure to fix smears immediately may result in smears that are unsatisfactory for evaluation.

**NOTE:** Care must be taken to fix the smear as quickly as possible. Air drying begins within seconds of applying the smear to the slide. Air drying produces artifacts, cellular distortion and may lead to misinterpretation of smears.

The fixative provided is 95% alcohol with carbowax added to prevent drying of the sample. The nozzle of the spraying apparatus should be held approximately twelve (12) inches from the slide. Adequate amounts of fixative should be applied to completely wet the surface.
COLLECTION PREPARATION

Thinprep Collection Techniques

The detection of cervical cancer and its precursors, as well as other gynecologic abnormalities, is the primary purpose of obtaining a cervical cell sample. In general, the guidelines state that it is important to obtain a specimen that is not obscured by blood, mucus, inflammatory exudates or lubricant.

Patient Information

- The patient should be tested 2 weeks after the first day of her last menstrual period, and definitely not when she is menstruating.

  Even though ThinPrep reduces obscuring blood, clinical studies have demonstrated that excessive amounts of blood may still compromise the test and possibly lead to an unsatisfactory result.

- The patient should not use vaginal medication, vaginal contraceptives or douches during the 48 hours before the exam.

Specimen Collection Preparation

- Lubricant jellies should not be used to lubricate the speculum

  Even though lubricant jellies are water soluble, excessive amounts of jelly may compromise the test and possibly lead to an unsatisfactory result.

- Remove excess mucus or other discharge present before taking the sample. This should be gently removed with ring forceps holding a folded gauze pad.

  The excess cervical mucus is essentially devoid of meaningful cellular material and when present in the sample vial may yield a slide with little or no diagnostic material present.

- Remove inflammatory exudate from the cervical canal before taking the sample. Remove by placing a dry 2 x 2 inch piece of gauze over the cervix and peeling it away after it absorbs the exudates or by using a dry protoswab or scopette.

  The excess inflammatory exudate is essentially devoid of diagnostic cellular material and when present in the sample vial may yield a slide with little or no diagnostic material present.

- The cervix should not be cleaned by washing with saline or it may result in a relatively acellular sample.
Broom-Like Device Protocol

**Obtain...**

...an adequate sampling from the cervix using a broom-like device. If desired, use lukewarm water to warm and lubricate the speculum. Water-soluble gel lubricant sparingly applied to the posterior blade of the speculum can be used if necessary. 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 five times.

**Rinse...**

...the broom as quickly as possible 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, swirl the broom vigorously to further release material. Discard the collection device.

**Tighten...**

...the cap so that the torque line on the cap passes the torque line on the vial.

**Record...**

...the patient’s name and ID number on the vial, and the patient information and medical history on the cytology requisition form.

**Place...**

...the vial and requisition in a specimen bag for transport to the laboratory.
**Brush/Spatula Protocol**

**Obtain…**
...an adequate sampling from the ectocervix using a plastic spatula. If desired, use lukewarm water to warm and lubricate the speculum. Water-soluble gel lubricant sparingly applied to the posterior blade of the speculum can be used if necessary. 1 Select contoured end of plastic spatula and rotate it 360 degrees around the entire exocervix while maintaining tight contact with exocervical surface.

**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.

**Obtain…**
...an adequate sampling from the endocervix using an endocervical brush device. Insert the brush into the cervix until only the bottommost fibers are exposed. Slowly rotate 1/4 or 1/2 turn in one direction. DO NOT OVER-ROTATE.

**Rinse…**
...the brush as quickly as possible in the PreservCyt solution by rotating the device in the solution 10 times while pushing against the PreservCyt vial wall. Swirl the brush vigorously to further release material. Discard the brush.

**Tighten…**
...the cap so that the torque line on the cap passes the torque line on the vial.
IV. INTERPRETATION OF Pap TESTING REPORTS

The following information specifically describes epithelial cell abnormalities.

A. ADEQUACY

Adequacy and diagnosis should be used in conjunction with clinical history to determine appropriate patient follow-up. The condition of the specimen received and the presence or absence of endocervical and/or squamous metaplastic cells is noted. The presence of excessive red blood cells, inflammation, scanty cellularity, or poor fixation of cells is also noted. ADEQUACY IS NOT A DIAGNOSIS

B. PATHOLOGIST REVIEW POLICY

Pap Smears with an atypical diagnosis will be reviewed by a pathologist. A separate charge will be made for the pathologist over read.

C. HORMONAL EVALUATION

Hormonal evaluation is a qualitative cytohormonal evaluation of the patient. HORMONAL EVALUATION IS NOT A DIAGNOSIS! In compliance with Bethesda 2001 guidelines for reporting Pap testing, hormone evaluation is reported out only if:

a) Estrogen effect is elevated for the patient age and/or history

b) Estrogen effect is decreased for the patient age and/or history

If no hormone evaluation is reported, you may assume that the estrogen effect is compatible with the patient’s age and history. The results should be used in conjunction with the patient’s history.

D. INFECTIOUS DISEASE SCREENING

Specimens are screened for cytologic evidence of infectious diseases, such as trichomonas, herpes, candida and human papillomavirus (HPV).

HPV testing can be performed from the ThinPrep specimen vial. HPV testing will determine the presence of LOW-RISK and/or HIGH-RISK HPV factors.

LOW-RISK HPV types are generally associated with warts or low-grade dysplasia. Testing for low-risk HPV is not necessary nor recommended.

HIGH RISK HPV types are associated with warts, low-grade lesions and high-grade lesions (including cancer).

Guidelines developed by the American Society for Colposcopy and Cervical Pathology (ASCCP) recommend HPV high risk testing only ordered on patients with atypical squamous cells of undetermined significance (ASCUS). The test must be ordered within 3 weeks of ThinPrep vial specimen collection.

Chlamydia trachomatis and Neisseria Gonorrhoeae testing by can also be performed from ThinPrep specimen collection vials. The Cytology Department must have a written request to run either HPV or CT/GC testing. If testing is desired, fax a request (form available on our website) to 785-537-3592, attention CYTOLOGY.
V. Pap Reminder System

Peterson Laboratory Services holds quality patient care to the highest standard. A patient reminder system is offered at no additional cost.

Pap Patient Letter

Letters are prepared by Peterson Laboratory Services and mailed directly from our facility to those patients with negative pap test results. The letter explains to the patient that their pap test has been interpreted as “normal.” The patient is instructed to telephone the attending physician’s office if they have questions or concerns.

If there is a question if a letter was sent to a patient, please refer to the patient report. A single asterisk (*) under the pathologist’s name on the left side indicates that a letter was sent. If no asterisk appears, it indicates that a letter was not sent.

Pap Reminder Letters

Letters reminding patients that they are due for Pap testing are available to our clients as arranged. The letters, stating that they, as a patient are due for a repeat Pap test, are prepared and sent to the physician’s office for mailing.

Non-Genital Cytology
I. COLLECTION / HANDLING PROCEDURES

The most accurate testing is achieved with highest quality specimens. Please submit at next courier pickup.

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<th>SOURCE</th>
<th>SPECIMEN REQUIREMENTS</th>
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<td>BODY FLUIDS (pleural, ascitic, etc.)</td>
<td>Fresh (no fixative required). Refrigerate. Submit the entire specimen.</td>
</tr>
<tr>
<td>BREAST CYST ASPIRATE</td>
<td>Fresh (no fixative required). Refrigerate.</td>
</tr>
<tr>
<td>BRONCHIAL WASHINGS</td>
<td>Fresh (no fixative required) or submitted in equal amounts of Cytolyt (available from our lab)</td>
</tr>
<tr>
<td>BRUSHINGS</td>
<td>Place brush(s) in small container with normal saline. Refrigerate.</td>
</tr>
<tr>
<td>CEREBROSPINAL FLUID</td>
<td>Fresh (no fixative required). Refrigerate.</td>
</tr>
<tr>
<td>FINE NEEDLE ASPIRATE</td>
<td>Refer to following special collection procedure</td>
</tr>
<tr>
<td>SPUTUM</td>
<td>First morning deep-cough specimen is recommended. Generally three (3) separate specimens are collected.</td>
</tr>
<tr>
<td>URINE</td>
<td>Fresh (no fixative required). Refrigerate. DO NOT COLLECT FIRST MORNING SAMPLE. Indicate on requisition if sample is voided or catheterized, as appropriate.</td>
</tr>
<tr>
<td>BLADDER WASHINGS</td>
<td>Fresh (no fixative required). Refrigerate.</td>
</tr>
<tr>
<td>SMEARS (including Tzanck)</td>
<td>Spray fix immediately. If collected from the head/neck/lymph area, please submit 2 air-dried slides also.</td>
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A. LABELING

1. Label glass slides with the following information:
   a. Patient first and last name
   b. Date of collection
2. Label the container with the following information:
   a. Patient first and last name
   b. Anatomic location of specimen
   c. Date of collection
3. Indicate on the label that the specimen bottle contains NORMAL SALINE

B. REQUISITION:

1. Complete a green PLS cytology requisition (GP3) with the following demographic information:
   a. Patient First and Last Name
   b. Physician
   c. Date of Birth
   d. SEX of patient
2. Additional required information in the NON-GENITAL CYTOLOGY area of the request form:
   a. Anatomic Location of Specimen
   b. Pertinent medical history
   c. Diagnosis

C. HANDLING:

1. Place slides in slide folders
2. Place slide folders and specimen container in PLS specimen transport bag
3. Fold requisition and place in side pocket of PLS specimen transport bag
4. Transport to PLS

NOTE: Slide containers and spray fixative are furnished upon request. Please contact the laboratory if you require supplies or additional information.
II. FINE NEEDLE ASPIRATE

COLLECTION/HANDLING PROCEDURE

Fine needle aspirate specimens may be submitted fresh or submitted as slides.

**FRESH:** Dependent upon volume of specimen obtained, specimens may be submitted in the syringe needle (draw up small amount of saline or alcohol to prevent drying) or the syringe itself. Larger volume specimens (ex: breast cyst aspirates) may be expressed into a container for submission.

**A. SLIDES:**
   1. 22-25 gauge needle
   2. 20 mL syringe
   3. Clean glass slides with frosted ends
   4. Spray fixative or 95% alcohol
   5. Pre-filled Cytolyt preservative tube
   6. Slide holder(s)

**B. COLLECTION:**
   1. The aspiration is usually performed under aseptic technique without local anesthesia. If local anesthesia is used, insert the needle adjacent to, but not into the lesion. Anesthetizing solution could dilute or distort the specimen.
   2. Attach a 22-25 gauge needle to the 20 mL syringe.
   3. Place the plunger of the syringe at the 5 mL mark. Secure the mass with one hand. Use the other hand to insert the needle into the mass.
   4. Draw negative pressure to the 20 mL mark, move needle in short stabbing motions, changing the angle of direction within the lesion.
   5. Return the plunger to the 5 mL mark to release the suction before withdrawing the needle. NEVER INJECT AIR!
   6. After withdrawing the needle, remove the needle from the syringe. Fill the syringe with air.
   7. Reattach the needle and carefully express one drop of specimen onto a glass slide. Use another glass slide or the needle tip to spread the specimen.
   8. IMMEDIATELY (within a few seconds) spray the slides with the fixative. Any delay will cause air drying and cellular distortion, thus rendering the specimen unsatisfactory for evaluation.
   9. If a lipoid lesion or lymph node is being examined, two (2) additional slides may be prepared and allowed to air dry without fixative.
   10. The contents of the needle and syringe should be expressed/rinsed into a Cytolyt tube.
   11. Submit the glass slides and saline container for cytology studies.