

## Title: Gyn Cytology Specimen Submission and Handling

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**Principle:** The cervicovaginal (Papanicolaou) smear is a test for detection of premalignant cellular changes which are precursors to carcinoma of the cervix. The Pap smear, when used as part of a regular screening program, has been credited with reducing the incidence of cervical cancer worldwide.

Please note that the Pap smear is a screening test with an irreducible false negative rate. The ability to detect significant disease by cervical screening can be optimized by regular Pap smear examinations and HPV co-testing per current ACOG recommendations.

**Purpose:** The purpose of this procedure is to establish procedures for patient preparation, collection methods, labeling, specimen preservation and conditions for specimen transport.

**Scope:** This procedure applies to the Pre-Analytical Department personnel at Sierra Pathology Laboratory, (SPL).

**Note: All Cytology Fixative (spray or liquid) is flammable!**

Use with adequate ventilation and avoid breathing vapors. Prolonged exposure may cause mild irritation, drying, cracking, or contact dermatitis. Persons with pre-existing skin disorders, eye problems, impaired liver, kidney or respiratory function may be more susceptible to the effects of this substance.

### Specimen Collection:

- Patient Preparation:
  - Pap should be taken anytime in the cycle except during menses. Ideal time is midcycle.
  - Patient should not douche or use vaginal medications for 48 hours prior to collection of Pap smear.
- Preparation of Cervix:
  - Lubricate the speculum with warm water or minimal lubricant. Excess lubricant may make interpretation of smear difficult. It is recommended that lubrication be used sparingly or not at all.
  - Insert the speculum in a manner to make the entire cervix visible.

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- Clean the cervix with cotton ball or 8" rectal swab. Remove blood or mucus.
- Conventional Technique:
  - Label the specimen with the patient's name in hard lead PENCIL on the frosted end of the glass slide. If the slide is received unlabeled or misidentified, it will not be processed and the physician will be notified. It will be the physician's responsibility to correct errors in specimen identification.
    - A labeled Pap Pak is not ideal patient identification. The slide itself should be labeled.
    - Always allow the smear to dry after fixative has been applied and before closing the slide holder.
      - Wet smears adhere to cardboard folders.
      - Artifacts, cellular distortion, and loss of cytologic material may occur if cardboard fibers are dried into the smear.
    - Transport the smear in Pap Pak or cardboard slide folder, securely fastened with scotch tape to prevent slide loss.
      - If the smear is to be mailed, a special mailer with a plastic slide protector sleeve must be used inside the mailing envelope.
      - Flat cardboard slide folders offer inadequate protection from breakage in transit.
  - With a wooden or plastic spatula, firmly scrape the entire ectocervix and squamocolumnar junction. Assistant may smear the specimen on the upper two thirds of the smear while you collect the endocervical sample with the cytobrush. The cytobrush should be inserted so the upper end of the bristles are at the external os. Cytobrush should be rotated 180-360°. Immediately transfer the endocervical sample onto the lower one third of the slide by rotating the cytobrush and applying adequate pressure to flatten the bristles.
  - Immediately fix the smear with the cytology fixative to prevent air drying.
    - When coating or spray fixatives are used, the nozzle of the spraying apparatus should be held approximately 8-10 inches from the slide. Holding the spray fixative container too close to the slide can result in cellular artifacts while holding the spray fixative container too far from the slide may result in drying artifact or uneven fixative.
  - For Hormonal Cytology (Maturation Index), submit a lateral vaginal wall sample.
    - Obtain first before any of the above specimens to avoid contamination by cervical os endocervical samples.

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- Gently scrape the upper third of the lateral vaginal wall.
- Spread on slide and immediately spray fix.
- Clinical history is required for this examination.
  
- Liquid Base Techniques:
  - ThinPrep Collection Techniques
    - Using a pen, label a ThinPrep vial with patient's name and site of smear if other than cervix.
    - The specimen may be collected using a spatula and endocervical brush combination or by using a cervical broom.
      - If using the spatula/brush combo:
        - With a plastic spatula, firmly scrape the entire ectocervix and squamocolumnar junction. Immediately place spatula in vial and swirl vigorously. Discard spatula. Do not leave in vial.
        - Insert brush into the cervical os until only the bottom bristles show. Rotate brush ¼ to ½ of a turn in one direction. Immediately place brush in vial and rotate 10 times, pushing against the walls of the vial to dislodge as much specimen as possible. Discard brush. Do not leave in vial.
    - If using the cervical broom:
      - Insert the cervical broom into the cervical os until the smaller bristles make firm contact with the cervix. Rotate 360° 3-5 times in one direction.
      - Immediately place broom in the vial and swirl vigorously. Push the broom into the bottom of the vial several times to dislodge as much specimen as possible. Discard broom: do not leave in vial.
  - For Hormonal Cytology (Maturation Index), submit a lateral vaginal wall sample.
    - Obtain first before any of the above specimens to avoid contamination by cervical or endocervical samples.
    - Gently scrape the upper third of the lateral vaginal wall.
    - Swirl collection device(s) in vial as described above and cap vial.
    - Clinical history is required for this examination.
  
- SurePath Collection Techniques

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- Using a pen, label a SurePath vial with patient's name and site of smear if other than cervix.
- There are two recommended sampling devices for SurePath specimens. One is a broom type device and the other is the brush/plastic spatula combo. Versions of these products are available with snap off heads.
  - If using the spatula/brush combo:
    - With a plastic spatula, firmly scrape the entire ectocervix and squamocolumnar junction. Immediately place spatula in the vial and snap off the head. This can be done using several methods.
    - Insert brush into the cervical os until only the bottom bristles show. Rotate brush  $\frac{1}{4}$  to  $\frac{1}{2}$  of a turn in one direction. Immediately place brush in vial and snap off the head. Cap the vial firmly.
  - If using the cervical broom:
    - Insert the cervical broom into the cervical os until the smaller bristles make firm contact with the cervix. Rotate  $360^\circ$  in one direction, 2 times.
    - Immediately place broom in the vial and snap off the head. This can be done using several methods. Cap the vial firmly.
- For Hormonal Cytology (Maturation Index), submit a lateral vaginal wall sample.
  - Obtain first before any of the above specimens to avoid contamination by cervical or endocervical samples.
  - Gently scrape the upper third of the lateral vaginal wall.
  - Swirl collection device(s) in vial as described above and cap vial.
  - Clinical history is required for this examination.

### Procedure for Specimen Submission

- 1.0 **All specimen containers must be labeled with two patient identifiers.**

Label the specimen with the patient's name in pen directly on the specimen container, and at least one other identifier (e.g. DOB, SSN, MR number, etc).

  - 1.1 Slides may be labeled with only one identifier (patient name) in pencil on the frosted end of the slide.
  - 1.2 If the specimen is received unlabeled or misidentified, it will not be processed and the physician will be notified. It will be the physician's responsibility to correct errors in specimen identification.
- 2.0 For Conventional smears, be sure fixative has dried before closing the folder. Wet cardboard may adhere to the slide making smears suboptimal.

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- 3.0 Be sure the lid to the ThinPrep Vial or Surepath vial is securely fastened so that no fluid leaks out during transportation. This could lead to loss of diagnostic material.
- 4.0 Submit Pap smear with the Cytology Requisition form filled out in its entirety. The clinical history information requested is necessary for proper evaluation of the specimen and must minimally include:
  - 4.1 Patient's name
  - 4.2 Source of specimen
  - 4.3 Test to be performed (please indicate if maturation index is requested).
    - 4.3.1 For liquid based testing, be sure to indicate if reflex testing is requested.
    - 4.3.2 Check the "HPV" if HPV testing is requested.
    - 4.3.3 Check the "GC/CHLAMYDIA" box if testing for Gonorrhea or Chlamydia is requested.
  - 4.4 Number of vials submitted
  - 4.5 Requesting clinician's name (**Specimens are only accepted from physicians or other persons authorized under law**).
  - 4.6 Patient's date of birth
  - 4.7 Date of specimen collection
  - 4.8 Pertinent clinical history, such as:
    - 4.8.1 Date of the last menstrual period
    - 4.8.2 Menopausal status
    - 4.8.3 Current or recent past pregnancy
    - 4.8.4 Oral contraceptive or IUD use
    - 4.8.5 Estrogen replacement therapy
    - 4.8.6 Previous therapeutic procedures
    - 4.8.7 Prior abnormal history
  - 4.9 *Patients at increased risk for cervical carcinoma should be identified as such on the requisition form.*
    - 4.9.1 Check the "HIGH RISK" box on the requisition form for all patients at high risk;
    - 4.9.2 Check the "SCREENING" box for all patients with routine annual smears with no known previous abnormalities or current visible lesions.

### Results:

See [Gyn Cytology Reporting Criteria](#) for applicable adequacy and reporting criteria.

### Quality Control:

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- Limitations of the Procedure:
  - Air drying is the absence of fixation. Air drying produces artifacts and cellular distortion and may lead to misinterpretation of smears. Air drying of any Pap smear regardless of technique is not recommended. Delayed fixation can also result in air drying artifact.
  - In the case of ThinPrep, the use of lubrication when obtaining the Pap smear can severely limit the quality of the Pap smear. Presence of lubrication in the vial hinders the ability of the T2000 to deposit diagnostically significant cells onto the slide. It is recommended that lubrication be used sparingly or not at all.
  - Severe inflammation and blood can limit the collection of diagnostically significant cells. If blood or mucus is present on the cervix at the time of collection, it is recommended that the cervix first be swabbed to remove this material before collecting the Pap smear.
- Any slide that is received broken must be documented on the cytology requisition. If portions of the slide can be salvaged, they are to be attached to another glass slide with a spring clip and hand stained. The broken portion is then mounted on a full glass slide with mounting medium and coverslipped according to the [Coverslipping Slides](#) procedure.
- See [Specimen Acceptability and Rejection Criteria](#) for applicable specimen criteria and procedures for rejection of unsatisfactory specimens.

### Related Documents:

- [Coverslipping Slides](#)
- [Gyn Cytology Reporting Criteria](#)
- [Specimen Acceptability and Rejection Criteria](#)

### References:

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- Bibbo, M (1991) Comprehensive Cytopathology. W.B. Saunders Company.
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- Koss, L.G. (2006) Diagnostic Cytology and its Histopathologic Bases, 5<sup>th</sup> Ed. Lippincott Williams & Wilkins.
- Operator's Manual-PrepStain Slide Processor. TriPath Imaging, Inc. Burlington, NC.
- Solomon, D., Nayer R. (2004) The Bethesda System for Reporting Cervical Cytology, 2<sup>nd</sup> Ed. Springer-Verlag, New York.
- ThinPrep 2000 Operator's Manual. CYTYC Corporation. Boxborough, MA.