This document discusses procedures for cervicovaginal specimen collection, as well as the preparation, fixation, staining, and storage of Papanicolaou-stained cervicovaginal cytology slides.

A guideline for global application developed through the NCCLS consensus process.
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Papanicolaou Technique; Approved Guideline—Second Edition

Abstract

Papanicolaou Technique; Approved Guideline—Second Edition (NCCLS document GP15-A2) is intended for healthcare providers who are responsible for collecting cervicovaginal cytology specimens and preparing conventional Papanicolaou smears and liquid-based preparations. The guideline focuses on quality collection and processing of specimens, addressing all steps, including patient assessment, test requisition, specimen collection, specimen transport, and specimen receipt and processing. Illustrations of the techniques are described, and a sample requisition form is also included.


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Foreword

Cervicovaginal cytology, which was developed by Dr. George N. Papanicolaou for detection of preneoplastic lesions of the uterine cervix, is a routine screening procedure for women. Routine cervical screening is recognized as the most effective method of reducing the incidence and mortality of cervical cancer. The test has become a means for the medical profession to regularly review the cervical health status of women.

For cervicovaginal cytology to produce optimal results, the specimen collection procedure must be carried out by a healthcare provider on an adequately prepared, educated, and cooperative patient. The specimen must be evaluated using quality-controlled laboratory techniques.

The intent of this second-edition, approved guideline is to provide updated recommendations. These recommendations include: patient assessment, test requisition, cervicovaginal specimen collection, specimen transport, specimen receipt, specimen processing, and storage of slides. Cytologic interpretation is outside the scope of this document and is not addressed.

Standard Precautions

Because it is often impossible to know what might be infectious, all human specimens are to be treated as infectious and handled according to “standard precautions.” Standard precautions are new guidelines that combine the major features of “universal precautions and body substance isolation” practices. Standard precautions cover the transmission of any pathogen and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (Guideline for Isolation Precautions in Hospitals. Infection Control and Hospital Epidemiology. CDC. 1996;Vol 17;1:53-80.), [MMWR 1987;36(suppl 2S):2S-18S] and (MMWR 1988;37:377-382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure, refer to NCCLS document M29—Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue.

Key Words

Cervical cancer, cervicovaginal specimen collection, diagnostic cervicovaginal cytology, liquid-based preparations, Papanicolaou stain, Papanicolaou technique, Pap smear, preneoplastic lesions
Papanicolaou Technique; Approved Guideline—Second Edition

1 Introduction

The primary purpose of obtaining a sample of cells from the uterine cervix is to detect precursor lesions of cervical cancer. The goal of this guideline is to provide recommendations for optimal specimen collection and processing. This is essential for accurate cytologic interpretation.

2 Scope

The scope of this document addresses the collection and processing of the cervicovaginal cytology specimen, including the conventional and liquid-based methods. The intent of this document is not to address interpretation of the cervicovaginal specimen, but to prepare a guideline that deals with the process up to and including the preparation of the slide.

This guideline will be useful to healthcare providers, laboratory directors, supervisors, and others who have responsibilities for quality control in cytopathology laboratories.

Various manufacturer devices are available for collecting and processing cervicovaginal cytology specimens. The manufacturer’s instructions for each product should be checked before instituting the use of that product.

3 Definitions

Cervical intraepithelial neoplasia (squamous intraepithelial lesion) (CIN), n - Precancerous cellular changes in the cervix which encompass a spectrum of cellular abnormalities, including CIN 1 (mild dysplasia, low-grade squamous intraepithelial lesion), CIN 2 (moderate dysplasia, high-grade squamous intraepithelial lesion), and CIN 3 (severe dysplasia/carcinoma in-situ, high-grade squamous intraepithelial lesion).

Colposcopy, n - A procedure used to view the cervix with a long, focal-length, dissecting-type microscope after a solution of dilute acetic acid has been applied to the cervix; NOTE: The acetic acid solution removes and dissolves the cervical mucus and causes CIN lesions to become whiter (acetowhite) than the surrounding epithelium; the coloration allows the colposcopist to identify and biopsy intraepithelial lesions and cancer.

Conventional smear, n - A method of slide preparation utilizing the smearing and fixation of a sample of cells from the cervix/vagina onto a glass slide.

Diethylstilbestrol (DES), n - Synthetic, nonsteroidal estrogens that were administered to gravid women who were thought to be at high risk for early pregnancy loss during the 1940s through the 1960s; NOTE: Subsequently, DES-exposed daughters developed non-neoplastic (adenosis) and neoplastic (adenocarcinoma) changes in the female genital tract.

Human papillomavirus (HPV), n - A sexually transmitted virus implicated in the pathogenesis of cervical cancer and its precursor lesions; NOTE: HPV infections of the genital tract are thought to be the most common sexually transmitted viral disease.

a Some of these definitions are found in NCCLS document NRSCL8—Terminology and Definitions for Use in NCCLS Documents. For complete definitions and detailed source information, please refer to the most current edition of that document.
Liquid-based Pap preparations, n - An alternative method to the conventional smear whereby the collection device(s) with the cervical/vaginal cellular sample is/are rinsed into a vial of preservative fluid rather than smeared onto a glass slide; NOTE: An automated processing device produces a thin layer of evenly distributed cervicovaginal cells onto the slide.

Papanicolaou stain technique, n - A method of polychromatic staining fashioned to exhibit differences in cellular morphology, maturity, and metabolic activity; NOTE: Developed by George N. Papanicolaou.

Pap smear, n - A sample of cells from the cervix obtained to test for cervical cancer and precancerous lesions of the cervix; NOTE: See also Papanicolaou stain technique.

Precursor lesions, n - Changes in cervical/vaginal tissue that are potentially premalignant.

Transformation zone (TZ)/Endocervical component, n - The region of metaplastic squamous epithelium that lies between the original (anatomic) squamocolumnar junction and endocervical epithelium of the cervix; NOTE: It is in the TZ that the vast majority of squamous epithelial abnormalities of the cervix arise.

4 Patient Assessment

The woman should be advised to schedule her gynecological examination two weeks after the first day of her last menstrual period and preferably not when she is menstruating. Women should not use vaginal medication, vaginal contraceptives, or douches during the 48 hours before the appointment. Intercourse is not recommended the night before the examination. Patient assessment includes a pertinent clinical history and physical examinations. (See Section 5.2.)

5 Test Requisition

It is important for the person obtaining the cervicovaginal cytology specimen to complete the cytology requisition form completely and accurately. A patient name or a unique identifier, the date of collection, and all other pertinent requested information should be provided.

5.1 Demographic Information

The requisition form should include the following identifying information:

(1) Name of patient/unique identifier;
(2) Date of birth and/or age;
(3) Date of collection;
(4) Source of material (cervix, endocervix, vagina);
(5) Number of slides submitted; and
(6) Healthcare provider’s name and phone number or other means of contact.
5.2 Clinical Information

(1) Last menstrual period;

(2) Hormonal status (e.g., gravid, postmenopausal);

(3) Exogenous hormone therapy (including birth control pills, estrogen/progesterone replacement treatment [HRT], treatment for endocrine responsive malignancy, estrogen creams);

(4) Presence of an intrauterine device;

(5) DES exposure;

(6) History of cervicovaginal intra-epithelial neoplasia, cervicovaginal malignancy, or any other genital or extragenital malignancy;

(7) History of systemic chemotherapy, pelvic radiotherapy, gynecologic surgery, cryosurgery, electrocautery, or laser surgery;

(8) Date of last gynecological smear and history with dates of any previous pertinent surgical pathology or cytopathology reports;

(9) Any current abnormal clinical findings or patient symptoms; and

(10) Risk-factors for cervical cancer (e.g., multiple sexual partners, sexually transmitted diseases including human papillomavirus [HPV], sexual activity at an early age, and smoking) if obtainable.

5.3 Design

The form should be designed in such a way that adequate space is allowed for each of the identifying and clinical items requested. “Yes/No” prompters with additional space for details can aid in obtaining complete information in the clinical history section. (See Figure 1.)
<table>
<thead>
<tr>
<th><strong>PATIENT INFORMATION - Please print</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient name:</td>
</tr>
<tr>
<td>( Last)</td>
</tr>
<tr>
<td>Patient ID#:</td>
</tr>
<tr>
<td>Date of birth:</td>
</tr>
<tr>
<td>Sex:</td>
</tr>
<tr>
<td>Room #:</td>
</tr>
<tr>
<td>Lab reference:</td>
</tr>
<tr>
<td>Healthcare provider name:</td>
</tr>
<tr>
<td>Healthcare provider telephone #:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>PATIENT HISTORY</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age:</td>
</tr>
<tr>
<td>Last menstrual period:</td>
</tr>
</tbody>
</table>

Check if patient is at increased risk for cervical cancer □

<table>
<thead>
<tr>
<th><strong>CLINICAL INFORMATION - Check all that apply</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Pregnant</td>
</tr>
<tr>
<td>□ Postpartum</td>
</tr>
<tr>
<td>□ Postabortion</td>
</tr>
<tr>
<td>□ Menopause</td>
</tr>
<tr>
<td>□ Other</td>
</tr>
</tbody>
</table>

Additional clinical information (i.e., pertinent clinical history, physical findings, gynecological surgery and colposcopic findings):

__________________________________________________________________________________
__________________________________________________________________________________
__________________________________________________________________________________

<table>
<thead>
<tr>
<th><strong>Source of Specimen – check all that apply</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Cervix</td>
</tr>
<tr>
<td>□ Endocervix</td>
</tr>
<tr>
<td>□ Vagina</td>
</tr>
<tr>
<td>□ Vulva</td>
</tr>
</tbody>
</table>

Collection date:  # Slides:  or Liquid based

Figure 1. Required Information for a “Pap” Requisition
6 Conventional Smear

6.1 Specimen Collection

6.1.1 Position of the Patient

Although it is possible to perform cervicovaginal cytology sampling with the patient in a variety of positions, it is usually performed with the patient in the dorsolithotomy position.

6.1.2 Preparation of the Cervix

Once the patient is positioned, a sterilized or single-use disposable bivalve speculum of appropriate size should be gently inserted into the vagina, avoiding direct pressure on the sensitive anterior structures (e.g., urethra). Water may be used to lubricate and warm the speculum; however, lubricant jellies should not be used. Several sizes of specula should be available so that an appropriate device may be chosen for the patient. Very young patients, patients with little sexual experience, and elderly patients with vaginal atrophy require the use of a smaller, narrower speculum than women who are sexually active. The speculum must be positioned so that the entire face of the cervix appears at the end of the instrument, because a sample from this area is necessary for adequate specimen collection. A large, cotton-tipped swab is often useful for helping to position the cervix.

Visual inspection of the lower genital tract and cervix through the speculum is a prerequisite for optimal sample collection. Squamous epithelium of the ectocervix is smooth, pink, and opaque. Native columnar epithelium of the endocervix is dark pink with a “cobblestone” surface. The transformation zone (where native endocervical columnar epithelium has undergone conversion to metaplastic squamous epithelium) has an intermediate, variegated appearance. (See Figure 2.)

Figure 2. View of the Cervix Through the Speculum

The location and configuration of the active transformation zone is variable, depending on factors such as vaginal pH, pregnancy, hormonal milieu, menopause, prior therapy, and individual anatomy. The upper
(endocervical) limit of the transformation zone is dynamic, defined by the leading edge of the migrating squamocolumnar junction. In postmenopausal women, the squamocolumnar junction is often high in the endocervical canal and no longer visible. (See Figure 3.)

![Figure 3. Variations in Mucosa of the Cervix. A: narrow transformation zone; B: broader transformation zone; C: broadly everted transformation zone—parous type; D: squamocolumnar junction high in endocervical canal—postmenopausal or posttreatment type. Modified from Thompson DW. Adequate "Pap" Smears, A Guide for Sampling Techniques in Screening for Abnormalities of the Uterine Cervix. Ontario, Canada: Laboratory Proficiency Testing Program, 1989. Modified with permission from the Quality Management Program—Laboratory Services (formerly Laboratory Proficiency Testing Program), a Department of the Ontario Medical Association.]

An optimal cervicovaginal specimen includes sampling of the squamous and columnar epithelium, encompassing in particular the transformation zone where the majority of cervical neoplasias arise. The specific sampling instrument(s) and sampling technique used should be based on a consideration of individual patient anatomy, particularly the location and configuration of the transformation zone as determined by visual inspection.

It is important to obtain a specimen that is not obscured by blood, mucus, or inflammatory exudate. Following correct positioning of the speculum in the vagina, if there is excess mucus or other discharge present, it should be gently removed with ring forceps holding a folded gauze pad. The cervix should not be cleaned by washing with saline, as it may result in a relatively acellular specimen. The specimen should be obtained before the application of acetic acid or Lugol’s iodine solution.

6.1.3 Collection Procedure for Conventional Smear Using Spatula and Cervical Brush or Cervical Broom

(1) Observe standard precautions for collecting and handling specimens. Label the frosted end of the glass slide (3- x 1-inch [7.6- x 2.5-cm] glass slide with a thickness of approximately 1 mm) with the patient's name before sample collection. Before the smear is obtained, slides must be labeled to ensure correct identification of the patient smear(s) (e.g., the patient’s name, hospital/patient

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b The use of both instruments is recommended for optimal sampling. The preferred order of spatula and brush sampling has not been subjected to large-scale studies. Obtaining the spatula specimen first diminishes the possibility of blood contamination due to trauma by the brush. However, some speculate that performing the brush collection first may increase the yield of exfoliated abnormal cells by the spatula.
identifier or bar code). The identifier should be legibly printed on the frosted end of the slide using a hard lead pencil. (Inks tend to run in processing.) If the specimen consists of more than one slide, it is mandatory that the source of the specimen be indicated.

2. Insert the speculum, which may be slightly moistened with water or saline if necessary. No other lubricants should be used.

3. Visually inspect the cervix for abnormalities. Identify the transformation zone, if visible, and direct sampling efforts to encompass this area.

**NOTE:** If an elevated, ulcerated, necrotic, or exudate-covered lesion is observed, arrangements should be made for biopsy following cytology sampling. The smear may not contain viable representative material and a biopsy should be taken of the suspected lesion. Similarly, a superficial scrape of an elevated keratotic plaque of the cervix may not be representative, and a biopsy is recommended for such lesions.

4. Choose the contoured end of the spatula that best conforms to the anatomy of the cervix and the location of the transformation zone. A plastic spatula is recommended. Rotate the spatula at least 360° about the circumference of the cervical os and ectocervix, while maintaining firm contact with the epithelial surface.  

**NOTE:** A clockwise rotation beginning and ending at 9 o’clock (or counter-clockwise rotation from 3 o’clock to 3 o’clock) will position the spatula so that the collected material is retained on the upper horizontal surface as the instrument is removed.

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![Sampling of the Cervix with Three Different Instruments: Spatula, Brush, and “Broom.”](image)

The solid black area is mature squamous epithelium; the hatched area is the transformation zone; and the stippled area is the original endocervical zone. Modified from Thompson DW. *Adequate "Pap" Smears, A Guide for Sampling Techniques in Screening for Abnormalities of the Uterine Cervix*. Ontario, Canada: Laboratory Proficiency Testing Program, 1989. Modified with permission from the Quality Management Program—Laboratory Services (formerly Laboratory Proficiency Testing Program), a Department of the Ontario Medical Association.

5. Do not smear the sample at this time, unless the specimen is to be immediately fixed (see steps 7b and 7c). Hold the spatula between the fingers of the nonsampling hand (or rest it on the glass slide) with the specimen face-up, while the cervical brush material is collected without delay.

6. Insert the cervical brush into the os; some bristles should still be visible. This will minimize inadvertent sampling of the lower uterine segment. With gentle pressure, rotate the brush only 90 to 180° to minimize bleeding.  

**NOTE:** Brushes have circumferential, radiating bristles that come in contact with the entire surface...
of the endocervix upon insertion. This is in contrast to the edge of a spatula, which is in contact with only a fraction of the epithelial surface at any one time. Therefore, the brush need only be rotated one quarter turn (90°), while the spatula must be rotated at least one full turn (360°).

(7) Spread the material collected on the spatula evenly over the glass slide with a single, smooth stroking motion. Roll the brush across the glass slide by rotating the handle and slightly bending the bristles. (See Figure 5.)

**NOTE:** The object is to quickly but evenly spread the cellular material in a thin layer on the glass slide. Thin out large clumps of material as much as possible, while avoiding excessive manipulation, which can damage cells. To avoid the development of air-drying artifact, transfer the material from both sampling instruments to the slide within a few seconds and fix immediately.

To transfer material from the spatula, smear the sample with a single stroking motion using moderate pressure to thin out clumps of cellular and mucus material. Avoid excessive force or manipulation, which will damage cells.

To transfer material from the brush, roll the bristles across the slide by rotating the brush handle.

To transfer material from the broom, smear the sample with a painting action, using both sides of the broom.

**Figure 5.** Transferring the Sample(s) to the Slide. Modified from Thompson DW. *Adequate "Pap" Smears, A Guide for Sampling Techniques in Screening for Abnormalities of the Uterine Cervix.* Ontario, Canada: Laboratory Proficiency Testing Program, 1989. Modified with permission from the Quality Management Program—Laboratory Services (formerly Laboratory Proficiency Testing Program), a Department of the Ontario Medical Association.

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* Do not apply the specimen to the end of the slide that is or will be labeled.
Following are options for transferring the material to the glass slide (see Figure 6):

(a) Spread the spatula sample over the left side of the slide and fix while covering the right side. Roll the brush over the right side of the slide and fix.

(b) In rapid succession, spread the spatula sample lengthwise over one half of the labeled surface of the slide, then roll the brush sample lengthwise over the remaining half of the labeled surface and fix.

(c) Smear the spatula sample across the slide; roll the brush directly over top and fix. (With this method, the ability to localize the origin of the cells may be lost.)

(d) Two-slide method: Collect, transfer, and fix each sample separately.

Figure 6. Options for Transferring the Sample(s) to the Glass Slide(s). NOTE: With the technique illustrated in Figure 6a or 6d, the spatula specimen may be spread and fixed before obtaining the endocervical brush sample.

(8) Another collection instrument is a plastic, “broom-like” brush that simultaneously samples the endocervix and ectocervix (see Figure 4). To use the “broom,” the long central bristles are inserted into the os until the lateral bristles bend against the ectocervix and are rotated a total of three to five times in a clockwise direction. To transfer material, each side of the “broom” is stroked once across the slide. (See Figure 5.)

(9) Immediately fix the specimen by either immersing the slide in 95% ethanol or coating the slide with a surface fixative. If using spray fixation, hold the container at the appropriate distance per manufacturer’s instructions from the slide to avoid “blasting” the cells (see Section 6.1.5, Slide Fixation). Spray-fixed or liquid-coated slides must be allowed to dry completely before packaging for transport.
6.1.3.1 Other Collection Instrument

Although not recommended, the cotton swab (saline-moistened) is another collection instrument that may be used. Use of a cotton-tipped applicator usually provides less cellular samples, possibly because of trapping cellular material in the cotton fibers.

6.1.4 Special Collection Procedures

6.1.4.1 Hormonal Evaluation

Samples for hormonal evaluation should be obtained separately using a spatula to gently scrape the epithelium from the upper third of the lateral vaginal wall. The separate slide should be labeled with the site information. The requisition should indicate a request for hormonal evaluation and provide relevant patient information.

6.1.4.2 Vaginal Pool Sample

The blunt end of a spatula may be used to collect secretions from the posterior vaginal fornix. This “vaginal pool” sample may fortuitously collect abnormal cells of upper genital tract origin and is sometimes obtained in peri- or postmenopausal women.

6.1.4.3 Diethylstilbestrol (DES)-Exposed Patients

Four-quadrant vaginal samples for DES exposure should be obtained using a spatula to scrape the epithelium from the upper-third of the vaginal wall for the detection of adenosis (negative) and/or clear-cell carcinoma (positive). Four separate slides should be labeled with the site information. The requisition should provide relevant clinical information.

6.1.4.4 Ancillary Studies

If multiple specimens are collected for cytopathology, as well as other ancillary studies, then the first sample obtained should be allocated for cytopathology. (See Section 6.4.8).

6.1.4.5 Self-Collection

Self-collection of a cervicovaginal specimen is not recommended, because the sample is often inadequate.

6.1.5 Slide Fixation

Fixatives are agents that are used on gynecologic smears to prevent cell distortion and to maintain true morphologic structure. Distortion due to improper fixation nearly always prevents proper and accurate evaluation of the cell population.

6.1.5.1 Timing

After the specimen has been spread evenly on the slide, the slide should be fixed immediately (within seconds). The slide(s) must not be allowed to air dry.
6.1.5.2 Types of Fixatives

6.1.5.2.1 Coating Surface Fixatives

Coating fixatives (alcohol with polyethylene glycol) are those that cover the surface of the prepared smears. The coating fixative may be applied by pressure spraying from a commercial cytofixative spray can, by a dropper from a dropper bottle, or by pouring from an individual envelope from a commercially available kit. When coating or spray fixatives are used, the nozzle of the spraying apparatus should be held at the appropriate distance from the slide per the manufacturer’s instructions. (See Figure 7.) Holding the pump-spray fixative container too close to the slide can result in the development of cellular artifacts, while holding the spray fixative container too far from the slide may result in drying artifacts or uneven fixation. Holding the spray fixative too close to the slide can also result in flooding the slide and washing or blowing away the cells. (The use of commercially available hairspray as a fixative is not recommended.)

![Figure 7. Fixation of the Spread Sample Using a Pump Spray.](image)


6.1.5.2.2 95% Ethanol (Wet Fixation)

An alternative cellular fixative for cervicovaginal smears is 95% ethanol. Place 95% ethanol in an appropriate container and immerse the freshly prepared smear immediately into the fixative. If the fixative is to be reused, it should be filtered.

6.1.5.2.3 Air Drying

Air drying is the artifact that occurs in the absence of fixation. Air drying produces cellular distortion and may lead to misinterpretation of smears. Air drying of a cervicovaginal smear is not recommended. Rapid fixation is imperative to avoid air-drying artifact.
6.2 Specimen Transport

A variety of containers (cylindrical plastic-slotted, rectangular plastic-slotted, and cardboard or plastic slide booklets) can be used for transport and mailing of slides. Appropriate slide containers should (a) have a means to stay closed and also be easily opened; (b) provide shock-resistant housing; and (c) prevent the slide surface from contacting the holder. Prefixed smears that have been allowed to dry may be mailed to a designated cytopathology laboratory in an appropriate slide container. The slides should be adequately packaged for transport to prevent breakage. Use of cardboard slide holders is not recommended. If used, care should be taken to properly dry the slides before transport. It is recommended that slide containers be discarded after a single use.

Care should be taken to tighten the lids of vials containing liquid-preservative solutions before shipment. Liquid preservatives that contain flammable materials such as alcohol should be shipped according to regulatory requirements (i.e., in the U.S., the Department of Transportation guidelines) for flammable materials.

6.3 Specimen Receipt

6.3.1 Criteria for Slide Rejection

Criteria for slide rejection include the following:

(1) Any slide that is lacking appropriate patient identifiers;

(2) A requisition form that is lacking patient identifiers or contains discrepant information;

(3) Any slides not accompanied by a requisition form; and

(4) Broken slides that cannot be reconstructed.

It is within the authority of the laboratory to reject any specimen because of insufficient information on the requisition form or probable misidentification (e.g., discrepancy between the patient name and number or disagreement between the requisition identification and the specimen identification).

6.3.2 Specimen Accession

Upon receipt of the specimen in the laboratory:

(1) The date of collection and receipt should be noted, and

(2) A laboratory number is assigned.

6.4 Staining

6.4.1 Papanicolaou Stain

The Papanicolaou stain employs a standard nuclear stain—hematoxylin and two cytoplasmic counterstains: OG-6 and EA. (OG-6 stands for orange-G-6 and consists of Orange G stain plus phosphotungstic acid in 95% ethanol; EA is a mixture of Light Green SF Yellowish and Eosin Y. Various preparations of EA may add other reagents including: Bismarck brown, phosphotungstic acid, lithium carbonate, and acetic acid.) Hematoxylin formulations that employ mercury should be avoided.
6.4.1.1 Papanicolaou Stain Technique

The Papanicolaou stain technique is a polychrome method fashioned to exhibit differences in cellular morphology, maturity, and metabolic activity. Of special importance, since the cells in a cytologic smear tend to overlap, is that this stain produces transparent cytoplasm, which allows the examiner to see through layers of cells, debris, and mucus. The different modifications display some variation in intensity of nuclear and cytoplasmic staining. The choice of which modification to use is largely a matter of personal preference.

6.4.2 Objectives of the Papanicolaou Staining Method

The modified Papanicolaou technique (see Figure 8) is the recommended method for staining cytologic preparations from the female genital tract, especially cervicovaginal smears, because it provides:

(1) Well-stained chromatin and definition of nuclear detail;

(2) Differential counterstaining (i.e., staining the cytoplasm of different cell types in different colors, reflecting the maturity and activity of the cells); and

(3) Cytoplasmic transparency.

6.4.3 Hematoxylin and Eosin Staining Techniques

Hematoxylin and eosin staining techniques are not recommended due to decreased ability to resolve cellular differentiation, as compared to the Papanicolaou stain.4

6.4.4 Stain Recommendations1,2

(1) Stains are light sensitive and will oxidize when exposed to light; therefore, staining dishes should be covered with opaque covers whenever possible. Stored stains should be placed in brown, light-retardant, airtight bottles.

(2) Stains should be filtered daily before use.

(3) Stains should be filtered again at the end of the day, if being returned to storage bottles. This step is designed to help keep the solutions cell free. Busy laboratories may need additional filtrations during the working day. Filtration through a medium-speed filter paper (grade 202 or equivalent) removes most cells; however, total cell removal can be accomplished by filtration through a membrane filter of controlled, small-pore size in a cross-contamination control and stain storage system.

(4) Unused solutions are to be covered throughout the staining procedure. When finished for the day, it is preferable that all solutions be transferred to airtight containers to retard evaporation. At the very least, solutions should be kept in covered containers overnight.

(5) A schedule for changing the stains should be established. The changes should occur in proportion to the laboratory's volume and experience.

• Spray fixatives or fixatives other than 95% ethanol tend to contaminate and degrade hematoxylin; they should be soaked off in 95% ethanol before starting the staining process.

• If cells appear drab and gray, the stains are exhausted and need to be replaced.

4 In the U.S., CLIA regulations mandate a Modified Papanicolaou Stain.
(6) Staining dishes should be washed at least once a week.

(7) Tap water is usually satisfactory for the water rinses of the Papanicolaou staining method. It is not necessary to use distilled water. (Distilled water tends to be acidic and will need to have its pH raised).

- Water rinses that come after progressive hematoxylin staining should be replaced after every use. The final water-rinse dish should be nearly colorless.
- All other water rinses, acids, and bluing solutions should be changed at least once daily.
- Tap water should be slightly alkaline to function well. Acidic tap water will result in nuclear fading. The pH of tap water can be checked with litmus paper.
- Excess chlorine levels in tap water will bleach hematoxylin. In that event, it may be necessary to use deionized water.

6.4.5 Process

Surface-coating fixatives must be removed before staining can be accomplished. To totally remove the coating, the slides should be immersed in 95% alcohol for at least ten minutes. If the coating is not totally removed, staining will be irregular, spotty, and of poor quality.

Nuclear staining in the staining method may be achieved by either a regressive or a progressive method.

6.4.5.1 Regressive Method

In the regressive method, the specimen is overstained with hematoxylin and the excess removed by immersion in dilute hydrochloric acid (HCl). The specimen is then subjected to a running water bath.

6.4.5.2 Progressive Technique

In the progressive technique, the specimen is submerged in hematoxylin only long enough to reach the desired intensity of nuclear stain. Decolorization with HCl and a running water bath are not required in the progressive method. Because the running water bath of the regressive method may produce cell loss, the progressive method is usually preferred. The stain intensity is also more easily controlled and reproducible with the progressive method. An outline of the Papanicolaou staining procedure is presented in Figure 8.

6.4.5.3 Staining Scheme Documentation

The staining scheme used in a particular laboratory should be documented in the laboratory procedure manual, and a chart exhibiting the progression of reagents and staining times should be displayed close to the staining workbench.
Figure 8. Principles of the Papanicolaou Staining Procedure

An NCCLS global consensus guideline. ©NCCLS. All rights reserved.
6.4.5.4 Manual Staining Procedure

(1) The staining area should be clean and free of clutter. Ample space should be available for the staining equipment. Solution dishes should be laid out in a manner that facilitates easy transfer of specimen racks or holders.

(2) Clean staining racks and solution dishes are recommended.

(3) Covers are needed for all solution dishes if stain is stored in the dish for any length of time.

(4) All solution dishes should be clearly labeled.

(5) Specimens are placed in solutions for defined time periods or numbers of “dips.” Strict adherence to the standard operating procedure is recommended to minimize batch-to-batch and day-to-day variation of stain quality.

(6) Dipping is performed by totally submerging a specimen slide (or rack) into a solution, without touching the bottom of the solution container, and then elevating the specimen(s) completely out of the solution. Each dip should last approximately one second.

(7) Dipping should be performed gently so that:
   - Cell loss will be minimized, and
   - Cross-contamination of specimens with cells from other specimens will not occur.

(8) Fluids are drained from the samples between immersions but are never allowed to dry.

(9) Staining racks should be blotted with paper towels when progressing from one type of solution to another.

(10) After cytoplasmic counterstaining, specimens should not be allowed to remain in alcohol for longer than the time indicated by the method, because the cytoplasmic colors will fade.

6.4.5.5 Automated Staining Procedure

Automated Papanicolaou staining methods are available. Such procedures should be followed according to vendor’s directions. The use of an automated method does not obviate the need for routine microscopic evaluation of the specimens for staining quality. As is the case with any other laboratory instrument, the operator’s manual should be available, maintenance logs should be kept, and preventive maintenance should be performed in accord with the manufacturer's recommendations.

6.4.6 Reagent Use and Maintenance

6.4.6.1 Alcohols (Ethanol, Methanol, and Isopropanol)

6.4.6.1.1 Changing

The alcohol rinses should be changed before they become too stain-laden or hydrated.

6.4.6.1.2 Safety Precautions

(1) Following are some general safety precautions:
In the staining method, ethanol is extensively used in various aqueous concentrations and as absolute alcohol. It is volatile and presents certain physical and toxic hazards. It can act as an irritant to the eye, skin, and mucous membranes. It is flammable and can be explosive under certain conditions.

Certain ethanol distillation processes use benzene, and even absolute alcohol produced by these methods can have enough remnant benzene to present an exposure hazard. For this reason, ethanol used in the cytology laboratory should be benzene-free.

Some liquid preservative solutions contain methanol, which is flammable and may form toxic and/or explosive vapors when heated.

Alcohol blends including ethanol, methanol, and isopropanol may be used during staining of cervicovaginal smears. The blend is also flammable and can be explosive under certain conditions.

(2) Appropriate laboratory clothing and protective equipment are recommended according to regulatory requirements when using alcohols.

- The worker should wear appropriate laboratory clothing or other equipment to prevent repeated or prolonged skin contact.
- Protective barriers should be in place to prevent eye contact.
- Contact lenses are not to be worn when working in a cytopreparatory laboratory (in the event of splashes to the eye, contact lenses can trap dangerous substances and keep them in contact with the cornea).

(3) Following are some precautions that pertain to quantities of solvent:

- Alcohol containers should be tightly closed when not in use and protected from heat, corrosion, mechanical damage, and ignition sources.
- Consult the appropriate agency for safe storage conditions of combustible solvents. (In the U.S., the National Fire Protection Association (NFPA) and Occupational Safety and Health Administration (OSHA))

6.4.6.2 Clearing Agents

After smears have been stained, they must undergo dehydration and removal of alcohol before cover slip mounting. The step of replacing the alcohol is also known as “clearing.” The clearing agent must render the cytoplasm of cells transparent. It must be colorless, and its refractive index should be close to that of cover slips, slides, and mounting medium. Xylene (dimethylbenzene) is the most commonly used agent. Xylene vapors are toxic; therefore, xylene must be used with appropriately controlled ventilation.

NFPA standards indicate that safe storage of combustible solvents is limited to 1 gallon per 100 square feet outside of approved storage cabinets and safety cans and 2 gallons per 100 square feet, including amounts in approved storage cabinets and safety cans. (National Fire Protection Association. NFPA-99, Standard for Health Care Facilities. Quincy, MA: NFPA, 1993.) Any amount of solvent in excess of that quantity should be stored in an OSHA-approved cabinet for the storage of explosive flammable material. The size of the cabinet should be appropriate for the amount of solvent present. Because cytology laboratories vary in square footage, the amount of flammable solvent (mainly ethanol and xylene) kept outside of an approved cabinet will vary.
6.4.6.3 Xylene

6.4.6.3.1 Recommendations for Use of Xylene in the Staining Method

(1) Xylene can be kept water-free by filtering the solution through laboratory-grade filter paper. Water-absorbent beads may also be used. Periodic replenishment of xylene is necessary due to evaporation and/or contamination.

(2) All alcohol and xylene rinses should be filtered daily to remove cellular debris.

6.4.6.3.2 Xylene Safety Precautions

(1) In the U.S., the Occupational Safety and Health Administration (OSHA) sets the exposure limit of xylene at 100 parts per million (ppm) air by volume as a time-weighted average (TWA). OSHA recommends that time-weighted averaging occur over an eight-hour exposure time period. At a concentration of 200 ppm, OSHA recommends a 15-minute short-term exposure limit (STEL). Public and private laboratories are available to test the air concentration of xylene in the workplace.

(2) The materials safety data sheet (MSDS) for xylene states that the ventilation must provide local exhaust or process enclosure ventilation to meet the published exposure limits. The ventilation equipment must be explosion-proof (refer to the current MSDS for updated information). The ventilation scheme employed in a laboratory using xylene should be designed to draw fumes away from the worker and not draw fumes up toward his/her nose and mouth.

(3) The employee should wear appropriate laboratory clothing to prevent repeated or prolonged skin contact. Clothing that is contaminated with spilled xylene should be taken off and not put back on until the xylene is removed.

To prevent eye contact, workers should use splashproof or dust-resistant safety goggles when using xylene.

Contact lenses are not to be worn when working in a cytopreparatory laboratory; this is especially important when working with xylene. Plastic lenses can be dissolved by fumes. Furthermore, in the event of splashes to the eye, contact lenses can trap harmful substances and keep them in contact with the cornea.

Appropriate protective gloves should be worn to prevent skin contact. Solvent-resistant gloves are recommended. Every effort should be made to prevent prolonged skin exposure.

(4) The U.S. National Institute of Occupational Safety and Health (NIOSH) recommends that xylene containers should be closed tightly when not in use and protected from heat, corrosion, mechanical damage, and ignition sources. Xylene vapors are heavier than air and may travel considerable distances to ignition sources. [See also Section 6.4.6.1.2(3)) for regulations on storage quantities.]

(5) In dealing with xylene spills, it is important to make sure that there are no possible sources of ignition (e.g., flame sources or electric motors).

Small spills (less than 500 mL) may be wiped up with paper towels and placed in the fume hood to evaporate. The site of spillage must be well ventilated, and the paper towels disposed appropriately. Alternatively, small-spill pillows are available commercially. Personnel who are responsible for cleaning up the spill should wear personal protective equipment known to be resistant to penetration by xylene.
In the event of larger spills (liter quantities), superiors and the safety agency that is in place for the laboratory are to be notified. A face-shield or goggles and gloves are to be worn when dealing with the spill. (Respiratory protection may be mandated if there is a possibility that exposure limits will be exceeded during the clean-up operation.) A spill pillow or nonflammable dispersing agent is used to soak up the solvent. If a dispersant is not available, the solvent is absorbed on dry sand, the sand is shoveled into a bucket, and it is transported to a safe, open area for atmospheric evaporation or burial. The site of spillage should be well ventilated to allow the remaining liquid to evaporate and to disperse vapors.

(6) Rules for xylene disposal include the following:

- Xylene must not be discarded into standard sink drains.
- Fume build-up in confined spaces (e.g., drains and sewers) is potentially explosive.
- Agencies or firms contracted for xylene disposal should provide written guarantees of proper handling and procedures. (In the U.S., the Federal Environmental Protection Agency (EPA) and/or relevant state offices may be contacted for lists of qualified disposers.)

(7) Other dangers include:

- One of the isomers of xylene, p-xylene (present in cytologic-grade xylene), can oxidize if mixed with acetic acid and form an explosive compound, terephthalic acid.
- Xylene may detonate immediately on contact with concentrated nitric acid by a mechanism that also involves the formation of terephthalic acid from p-xylene.

6.4.6.4 Xylene Alternatives

Alternative clearing agents derived from citrus terpenes and other sources exist. These agents apparently function well and are less toxic than xylene; however, increased specimen drying times and specimen fading have been noted.

6.4.7 Other Recommendations

(1) Daily microscopic checks of the quality of the stained material are recommended. A well-stained slide should be available for reference to the person who is checking the freshly stained material. A log should document such quality checks with the date, time, and initials of the evaluator inscribed.

(2) Because of the possibility of cross-contamination, separate staining set-ups or some other method to prevent cross-contamination should be employed for gynecologic and nongynecologic specimens.

(3) Laboratory procedure manuals should have a clearly written safety section that includes details on what to do and whom to notify in the event of solvent spills, contamination, and fires.

6.4.8 Special Studies

If special procedures are requested on cytologic preparations from the female genital tract, at least two extra, identically collected slides should be provided. One can be prepared by the standard Papanicolaou method and serve as a baseline. The other slide(s) will be subjected to the requested special study(ies). Such special studies may include:

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(1) Genetic probes
   - Nucleic acid in situ hybridization studies

(2) Immunocytochemistry studies
   - Immunofluorescent studies
   - Immunoperoxidase studies

6.5 Mounting and Storage

6.5.1 Mounting Medium

The mounting medium acts as a permanent bond between the slide and the cover slip. It must be miscible with the clearing agent. It must be transparent so the cellular detail on the slide is not obscured and should have a refractive index that renders it free of optical distortion. The refractive index of the medium must be equal to the refractive index of the slide, cover slip, and the material on the slide. A low-viscosity medium is recommended in order to avoid entrapment of air bubbles. It should have a neutral pH and contain an antioxidant that will reduce fading or yellowing of the specimen in storage. Excess mounting medium should be removed from the slide or cover slip and air bubbles should be extruded. To avoid cross-contamination, the dropper of the mounting medium is not to touch the specimen. The cells are to be kept wet, and air-drying is to be avoided to prevent the development of brown, “cornflaking” artifact.

6.5.2 Cover slip

The cover slip should be No. 1 clear glass, 0.130 to 0.170 mm in thickness, and it must be big enough to cover all the cellular material on the slide (e.g., rectangular 24 x 60 mm). It must have a plane-like surface, be free from irregularities, and it must resist weathering on storage. Its refractive index must be equivalent to that of the mounting medium and the slide. Plastic cover slips and liquid cover slips may be used as an alternative. Such methods are frequently automated.

6.5.3 Storage

The mounting medium must dry completely before the slide is placed in storage. This can be accomplished by exposing the slide to ambient temperature or by placing it in a 37 °C-incubator at least overnight.

The slides should be kept for a minimum of five years. However, federal, state, or local regulations may require longer retention.

Slides should be stored in a secure area and must be filed in a manner that permits ready retrieval. Storage temperature must be controlled to prevent slides from sticking together because of overheating.

7 Liquid-Based Preparation

7.1 Aim of Liquid-Based Preparation Systems

Liquid-based preparation methods may decrease preanalytic errors by:
   - fixating more rapidly (eliminates air-drying and better preservation of cells);
• decreasing or eliminating sources of artifacts (mucus, cellular debris) and obscuring cells (rbc); and

• homogeneously mixing all the cells taken from the cervix.

Liquid-based preparation methods allow for homogeneous mixing of all the cells taken from the cervix into a uniform suspension of cells in the preservative fluid. Because a conventional Pap smear does not mix the cell sample, the conventional smearing process may not deposit a representative sample of all of the cells taken from the cervix.

7.2 Specimen Collection

The steps of patient assessment, patient positioning, cervical preparation, sample collection, and accurate completion of the test requisition remain the same as for a conventional Pap smear (Sections 4, 5, 6.1.1, and 6.1.2). However, instead of smearing the sample on a glass slide the collected material is placed in a liquid fixative as per the manufacturer’s instructions.

• Label specimen collection vial with appropriate patient identifiers.

• Collect the specimen according to manufacturer’s instructions as per the package insert.

7.3 Specimen Transport

Follow the manufacturer’s instructions per the package insert.

7.4 Specimen Receipt

7.4.1 Criteria for Liquid-Based Specimen Rejection

Criteria for liquid-based specimen rejection include the following:

(1) Any sample that is lacking appropriate patient identifiers;

(2) A requisition form that is lacking patient identifiers or contains discrepant information;

(3) Any sample not accompanied by a requisition form;

(4) A vial that has leaked and insufficient fluid remains from which to prepare a slide; and

(5) A vial with expired preservative.

7.4.2 Specimen Accession

Upon receipt of the specimen in the laboratory:

(1) The date of collection and receipt should be noted.

(2) A laboratory number is assigned.
7.5 Processing and Staining

7.5.1 Processing (transfer of cells from collection vial to glass slide)

7.5.1.1 Filtration/Vacuum Method

The filtration/vacuum method disperses any mucus and debris and draws the fluid through a filter. A gentle vacuum is applied across the membrane to collect an optimal number of cells. The pores of the filter are sized to prevent the loss of diagnostic cells but allow red cells, most white cells, bacteria, and fine debris to pass through the filter. The manufacturer’s instructions should be followed in detail. One must be vigilant in transcribing patient identifiers from the original specimen collection vial onto the glass slides. Once a specific number of cells are collected on the filter (about 70,000), the filter is inverted and touched to the glass slide. The cells are then transferred from the filter to the slide. Once the slide is made, it is placed into an alcohol fixative.

7.5.1.2 Density Gradient Method

The density gradient method uses several steps in order to transfer the cells from a liquid-based suspension to a microscope slide. The process includes removal of nonepithelial material, homogenization, enrichment of epithelial material, robotic pipetting, sedimentation, and automated individual staining of up to 48 slides. The manufacturer’s instructions should be followed in detail. One must be vigilant in transcribing patient identifiers from the original specimen collection vial onto the processing tube and ultimately the glass slide.

Coated slides are used and may be prepared up to a week in advance of use. The preserved sample is mixed by vortexing and is gently dispersed through the pores of a syringe-like device and transferred onto the density reagent in a centrifuge tube. After centrifugation a portion of the supernatant is aspirated and discarded. The remaining solution and pellet are recentrifuged, decanted, and vortexed before placing onto the system. The robotic system then transfers material to the settling chamber mounted on a coated slide. The cells are sedimented by gravity.

7.5.2 Staining

7.5.2.1 Filtration/Vacuum Method

With the filtration/vacuum method the slides are stained in the same manner as the conventional smear.

7.5.2.2 Density Gradient Method

With the density gradient method, the slides are discretely stained by a robotic system per the manufacturer’s package insert.

7.6 Mounting and Storage

Mounting and storage of liquid-based slides are the same as with conventional Pap slides. (See Section 6.5.) However, the laboratory must establish storage time for the residual specimen based on the laboratory’s need for special studies and the manufacturer’s recommendations of shelf life.

Liquid preservatives containing methanol should be stored according to regulatory requirements. (See Section 6.4.6.1.2(3).)

7.7 Special Studies

Residual specimens may be used for additional studies such as HPV testing or repeat smears according to the manufacturer’s guideline.
References


Additional References


NCCLS consensus procedures include an appeals process that is described in detail in Section 9 of the Administrative Procedures. For further information, contact the Executive Offices or visit our website at www.nccls.org.

Summary of Comments and Committee Responses

GP15-A: Papanicolaou Technique; Approved Guideline

General

1. The future of the Papanicolaou technique probably lies in liquid-based collections followed by a controlled “thin-smear” preparation. A commercially available apparatus for this technique is already in use.

• Section 7 on liquid-based methods has been added to the guideline.

2. Since the document was written, liquid-based collection has been FDA approved and is increasingly common. Although there is brief mention, perhaps more should be added.

• See response to Comment 1.

3. The advantages and disadvantages of automated methods should be discussed.

• Liquid-based collection methods have been added to the guideline. See response to Comment 1. If the commentor is referring to automated screening of the specimens, that issue is beyond the scope of this guideline.

4. Has the FDA approved the use of the Medscand cervical brush for pregnancy?

• A device approved by FDA for use in the first trimester of pregnancy is available and may be used at the discretion of the caregiver. A stiff bristle cervical brush is not recommended for use in pregnancy.

5. There is no reference to “cytostain” Richard Allen combination of OG & EA as a viable choice.

• Trade names are generally not used in NCCLS documents. Using a product’s trade name can subject it to unfair criticism or give it an unfair advantage over other equivalent products. The following footnote has been added to Figure 8: “A combination of OG and EA is available and can be used as an alternative.”

Section 6.1 (Formerly Section 1.3)

6. Plastic spatulas (not wooden) are recommended with liquid sampling collection. The portion on wooden spatulas should be revised to reflect other types of spatulas.

• Liquid-based technology has been added to the guideline (see response to Comment 1). This section addresses recommended collection devices for this method.
Section 6.1.4 (Formerly Section 1.4)

7. In future revisions, under Special Collection Procedures, a Section 1.4.6 should probably be added regarding evaluation of DES patients for the diagnosis of adenosis. The standard of practice here is a smear from each of four quadrants.

- A new Section 6.1.4.3, entitled Diethylstilbestrol (DES)-Exposed Patients, has been added.

Figure 5 (Formerly Figure 4)

8. First slide figure: The angle of the spatula should be flatter on the slide, because the material spreads better if the whole side is used.

- Figure 5 has been modified as suggested.

Section 5.1(6) (Formerly Section 2.2.1(6))

9. “Physician” should be changed to “clinician” and the doctor’s office phone number should not have to be written on each order.

- Section 5.1(6) has been revised as follows: “Healthcare provider’s name and phone number or other means of contact.”
Summary of Delegate Comments and Working Group Responses

GP15-A2: *Papanicolaou Technique; Approved Guideline—Second Edition*

Section 3

1. In the definition for Diethylstilbestrol, replace “non neoplastic” with “neoplastic.”
   - The NOTE in the definition has been revised to: “Subsequently, DES-exposed daughters developed non-neoplastic (adenosis) and neoplastic (adenocarcinoma) changes in the female genital tract.”

Section 5.3

2. Figure 1 should include the type of testing, either liquid-based preparation or number of slides.
   - Figure 1 has been modified as suggested.

Section 6

3. Replace “spatula” with “plastic spatula” throughout the section.
   - Because this is a global document, the working group realizes that “plastic spatulas” are still not in universal use; therefore, they decided not to incorporate the suggested revision. However, the following sentence has been added to Section 6.1.3(4): “Choose the contoured end of the spatula that best conforms to the anatomy of the cervix and the location of the transformation zone. A plastic spatula is recommended.”

Sections 6.1.3.1 and 6.1.4.5

4. Why mention this when it is not an acceptable collection method?
   - Because this is a global document and unsatisfactory procedures need to be pointed out as unsatisfactory, the working group has agreed to retain these sections. Idiosyncratic, remote, odd, or antiquated clinical practices have not been abolished and need to be emphasized as NOT the best method of practice.

The working group deleted the last two sentences in Section 6.1.3.1 regarding pregnant patients.

Sections 6.1.4.1 and 6.1.4.2

5. Bethesda 2001 recommendations are to eliminate hormonal cytology as part of the Pap smear report. “Vaginal pool” specimens are very rarely collected/evaluated. These could both be omitted and better reflect today’s practice.
   - The working group has agreed to keep these sections, because the Bethesda 2001 recommendations have not yet been published; also, this is a global document, and Bethesda is not used universally.
6. Parenthetically add “negative” after adenosis and “positive” after carcinoma.

- The revision has been incorporated as suggested.

7. Why mention the last parenthetical sentence? It should be deleted.

- Because this is a global document and the recommended use of commercial hairspray as a fixative was a standard aphorism in cervical cytology for decades, the working group has retained this sentence in an effort to stamp out the last vestiges of the practice.

8. Why mention the last sentence? It should be deleted.

- Because the sentence stresses the importance of rapid fixation, the working group agreed to retain it; however, it has been modified to: “Rapid fixation is imperative to avoid air-drying artifact.”

9. This section should have a footnote that regulations in the United States require that all cervicovaginal specimens be stained using a modified Pananicolaou stain.

- The following footnote was added: “In the U.S., CLIA regulations mandate a modified Pananicolaou stain.”

10. The wording is confusing.

- The Working Group on Papanicolaou Technique reviewed the text and agreed it is stated correctly.

11. Delete 24 x 60 mm. Stating that cover glass should cover cellular material is sufficient.

- This parenthetical phrase was originally inserted, because laboratories were using half cover slips (approximately 25 x 30), wiping off the excess cellular material outside the cover slip and screening the remainder. To discourage this practice, the working group has agreed to retain the text.

12. Add as the fourth sentence: “The manufacturer’s instructions should be followed in detail. One must be vigilant in transcribing patient identifiers from the original specimen collection vial onto the glass slides.”

- The text has been added as suggested.
Section 7.5.1.2

13. Eliminate in the first paragraph, last sentence, the phrase, “…processing tube and ultimately....”

- The patient identifiers do not go from original collection vial directly to a glass slide (as indicated by the suggested correction). The vial and slide are never directly linked. The identifiers are placed on the vial and then the processing tube. The processing tube is placed on the straining machine and matched to the slide. All three items must be correctly labeled.

This identification step is a crucial watch point in this method. It is such a crucial step that company training manuals state that a second technician should double check the numbers on the slides versus the numbers on the processing tubes.
Related NCCLS Publications

**GP2-A3**  **Clinical Laboratory Technical Procedure Manuals—Third Edition; Approved Guideline (1996).** Provides guidance for the patient-testing community that addresses the design, preparation, maintenance, and use of paper or electronic technical procedure manuals.

**GP5-A**  **Clinical Laboratory Waste Management; Approved Guideline (1993).** GP5-A offers guidance on the safe handling and disposal of chemical, infectious, radioactive, and physical waste generated in the clinical laboratory.

**GP17-A**  **Clinical Laboratory Safety; Approved Guideline (1996).** *American National Standard.* General guidelines for implementing a high-quality laboratory safety program. The framework is adaptable to any laboratory.

**M29-A**  **Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline (1997).** A consolidation of M29-T2 and I17-P, this document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.

**NRSCL8-A**  **Terminology and Definitions for Use in NCCLS Documents; Approved Standard (1998).** This document provides standard definitions for use in NCCLS standards and guidelines, and for submitting candidate reference methods and materials to the National Reference System for the Clinical Laboratory (NRSCL).

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* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.

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