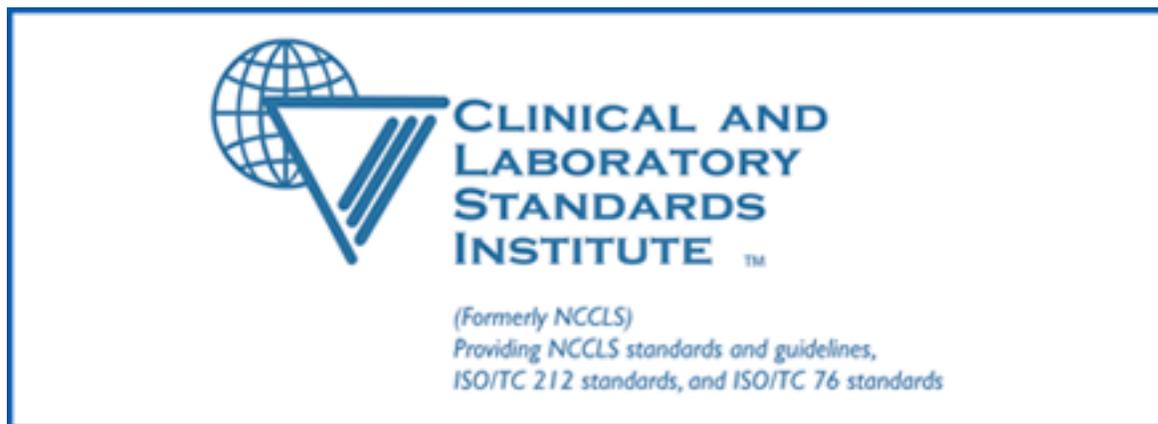


August 1999

Nongynecologic Cytologic Specimens: Collection and Cytopreparatory Techniques; Approved Guideline



This document provides recommended procedures for the collection, handling, transport, and processing of cytologic specimens from nongynecologic sources.



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Nongynecologic Cytologic Specimens: Collection and Cytopreparatory Techniques; Approved Guideline

Abstract

Nongynecologic Cytologic Specimens: Collection and Cytopreparatory Techniques; Approved Guideline (NCCLS document GP23-A) was developed for use by clinical personnel responsible for the collection and processing of cytologic specimens. This guideline provides recommendations for the collection and handling of specimens from nongynecologic sources for transport to the cytology laboratory. Also included are procedures for processing the specimens (i.e., smear preparation, fixation, and staining) for cytologic evaluations. This document does not address issues related to the interpretation of the slide preparation.

(NCCLS. *Nongynecologic Cytologic Specimens: Collection and Cytopreparatory Techniques; Approved Guideline*. NCCLS document GP23-A [ISBN 1-56238-380-9]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 1999.)

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Foreword

Modern cytopathologic techniques, when carried out by a healthcare practitioner on a cooperative patient and followed by “quality controlled” laboratory techniques, can produce results with high diagnostic accuracy. Adequate patient preparation and education are required.

The emphasis on cytopathologic techniques of nongynecologic specimens is on effectiveness and quality. This guideline is directed specifically at specimen collection and processing. If the predictive value of a nongynecologic cytology specimen can be increased, a major achievement in medical care and cost effectiveness will have been accomplished.

This “user” guideline is the beginning of a process of improvement in the nongynecologic smear production chain. The intent is to provide guidance for this process up to and including the preparation of the nongynecologic cytologic specimen, in order to provide a specimen suitable for accurate diagnosis.

Standard Precautions

Because it is often impossible to know what might be infectious, all human blood 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, 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

Cytologic specimen collection, nongynecologic cytology

Nongynecologic Cytologic Specimens: Collection and Cytopreparatory Techniques; Approved Guideline

1 Introduction

The primary purpose of cytologic examination of body fluid samples is to detect malignancy, but the method is also appropriate for the detection of inflammatory or infectious disorders. Reliable cytodiagnosis of body fluids depends on sufficient patient history and excellent technical slide preparations — at the very least. To that end, this document provides guidance for a number of clinical and laboratory procedures for the collection and processing of body fluid specimens. Followed skillfully and properly, these techniques will provide an indispensable foundation for high diagnostic accuracy.

While using the following guideline, the laboratorian and clinician are cautioned to remember that the diagnostic technique is being done for the patient. Most people preparing for a physical examination of any type experience anxiety over the possibility of an abnormal finding. Since body fluid procurement often involves some sort of invasive maneuver, the situation can be even more distressing. There are many ways to decrease the anxiety surrounding the procedure. A comfortable setting with pleasing surroundings helps to allay fear. Courteous, well-trained personnel and an efficient system for handling patients also help to decrease apprehension. A thoughtful, concerned healthcare provider who takes time to explain the procedure, answers questions, and interacts with the patient in a professional manner can do a lot to decrease the anxiety of a patient about to undergo a procedure for the sake of obtaining a cellular sample.

Cytologic samples are collected and prepared by a variety of methods, all of which have an effect on cytomorphology. The goals of standardizing specimen collection and processing are to minimize unwanted artifacts; and to obtain, well distributed, well-preserved, and well-stained cells that can be sharply imaged lending themselves to accurate diagnoses.

2 Scope

This document provides relevant information about various aspects of the performance of nongynecologic cytologic specimen preparation in order to evaluate and assess disease processes. It considers both immediate processing and complex handling. It is recognized that new developments in the art may augment or supplant the suggestions provided herein.

3 Preparation of the Patient, Collection and Handling of the Specimen ^{1,2}

The life cycle of the nongynecologic cytology specimen begins with the test request and ends when a cytologic diagnosis is reported to the ordering physician and documented (see Figure 1).

For optimal cytologic preparations, patients should be adequately prepared and the specimens should be properly collected. The methods for patient preparations will vary according to the sampling site. The following sections address common specimens requiring patient preparation. Standard aseptic technique should be followed. Copies of procedural protocols should be present wherever the procedures are performed as well as at the cytology laboratory.

In the practice of nongynecologic cytology, samples are derived from:

- cerebrospinal tract
- gastrointestinal tract
- joint spaces
- ocular area
- pericardium
- peritoneum
- pleura
- respiratory tract
- skin and mucosal samples
- urinary tract
- breast/nipple
- breast secretions.

They may be collected by:

- brushings and washings
- direct puncture and drainage
- touch preparations and scrapings
- patient voiding
- patient expectoration.

This guideline will address a number of techniques as commonly practiced in the cytopreparatory laboratory. Cell suspensions may be hypercellular or hypocellular, requiring either dilution or concentration. Dilutions are generally performed with balanced salt solutions if the specimen is fresh. Cell suspensions may be concentrated by conventional centrifugation, cytocentrifugation, direct deposition on filters with whole-mount processing, or deposition on filters with subse-

quent transfer to glass slides by touch-imprint or gravity sedimentation. For material obtained via fine needle aspiration, please see the most recent version of NCCLS document [GP20— Fine-Needle Aspiration Biopsy \(FNAB\) Techniques](#), for cervical vaginal smears to be stained by the Papanicolaou Method, please see the most recent version of NCCLS document [GP15— Papanicolaou Technique](#).

Cytologic samples may contain blood, mucus, inflammatory cells, microbial agents, crystals, proteinaceous material, or other debris. These elements may require hemolysis, mucolysis, or coarse filtration. Cytologic samples may be processed immediately, stored, refrigerated, placed in fixative and/or transported great distances to specialty laboratories for processing.

Following receipt of a test request by a physician:

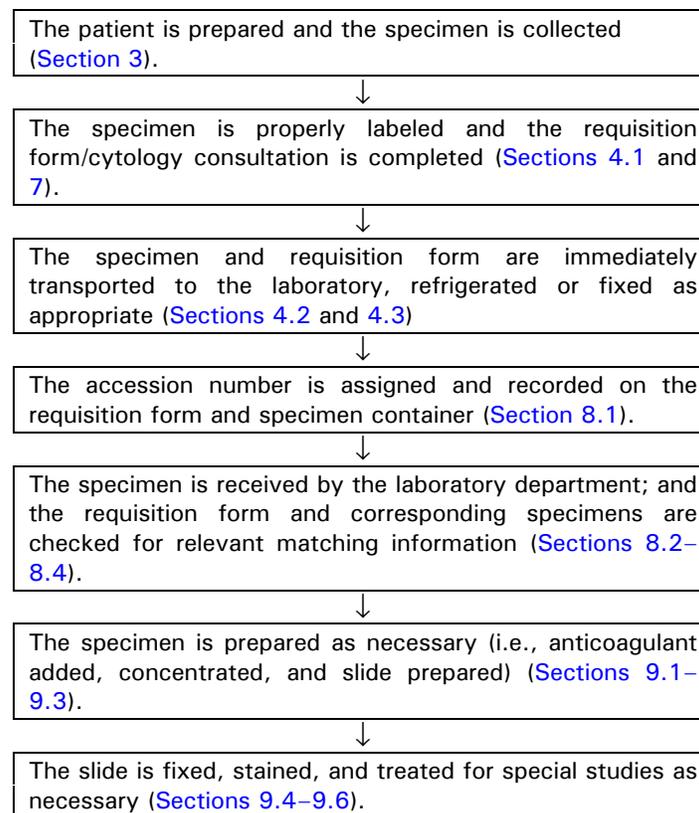


Figure 1. Nongynecological Specimen Life Cycle. A cytopathologist/pathologist would then evaluate microscopically, at times with the assistance of a cytotechnologist, and report findings to the ordering physician.

3.1 General Techniques

Nongynecologic specimens must be processed in such a manner as to maximize potential for cell preservation, cytologic detail, and accuracy of diagnosis. The number and type of preparations and the order in which they are made varies according to specimen type and the nature of the specimen.

Fresh specimens are preferred. If specimen transport is to be delayed for hours, then refrigeration is recommended. Refrigeration temporarily preserves cells by suspending their metabolic processes. At room temperature (22-25 °C), sample integrity is maintained for approximately four hours before it is significantly compromised. Exceptions include CSF and urine which degenerate within one hour, even with refrigeration. If laboratory processing cannot be performed within four hours, then a specimen can be refrigerated for up to 72 hours if it is anticoagulated and evenly suspended. If the specimen processing is further delayed, the specimen should be fixed. The addition of fixative should be noted on the requisition form.

Specimens may be collected in proprietary liquid fixatives for automated thin layer processing.

3.2 Body Fluids (Pleural [Thoracic], Peritoneal [Ascites], Pericardial, Cyst, and Synovial)

Each fluid specimen sent for cytologic examination must be submitted in a separate, clearly labeled, leakproof container. Most body fluids contain a significant amount of protein which is precipitated by alcohol and hampers cytopreparation. Body fluids should be sent fresh and unfixed if at all possible. Heparin is commonly used as a fluid anticoagulant by clinicians and pathologists in collecting cytology specimens. To keep body cavity fluids uniformly suspended, 3-5 IU of heparin per mL is added to the collection vessel before the specimen is introduced.^{3,4} The heparinized specimen should be mixed well and sent to the cytology laboratory immediately or refrigerated, if a delay is likely.

In some cases there is no alternative and fixation must be done. In that case, although certainly not ideal, a volume of 50% ethyl alcohol equal to specimen volume should be added and the addition should be noted on the requisition form. A cytology laboratory can usually work with a minimum of 1 to 2 mL but 20 to 50 mL are preferred. For large fluid collections from pleural or peritoneal taps, a well-mixed aliquot on the order of 50 mL to 200 mL is favored. Subdivision of specimens into aliquots can be accomplished in the laboratory. Transport of liter bags or bottles of body fluid material can be a biohazard problem: and thus, should be handled with care.

3.2.1 Washings

Bronchial, body cavity, and genitourinary washings should be collected in balanced salt solutions (e.g., Ringer's). Optimally, they are sent unfixed to the laboratory. However, if immediate transport is not possible then they should be either refrigerated or a volume of 50% ethyl alcohol equal to specimen volume should be added immediately to the container and the addition of fixative should be noted on the request form.

3.2.2 Brushings (Endoscopic, Bronchial, Peritoneal, Urinary Tract, Gastrointestinal Tract)

In preparation for a brushing procedure, the patient's name or other unique identifier should be written on the frosted end of the glass slide using a pencil or permanent marker. After the brushing is performed, an assistant will obtain the brush from an operator and roll the brush across the glass slide, pressing firmly in a small area in a circular motion. (Using a small area helps prevent air-drying.) If Papanicolaou staining is to be used, the sample should be *fixed immediately* with a spray fixative or immersed in 95% ethanol for 15 minutes. Poorly fixed or air-dried material seriously impairs the evaluation. In addition, many practitioners clip the end of the brush (approximately 1.5 inches) and send it to the laboratory for further cell harvest. The brush should be transported in a separate, labeled, leakproof

container with enough balanced salt solution or fixative to cover the brush.

3.3 Site-Specific Techniques

3.3.1 Breast Secretion

Breast cancer may be the reason for an unexpected nipple discharge, especially if the discharge contains blood. Examination of the discharge may yield a rapid diagnosis. The

patient's name should be written in pencil on the frosted end of a glass slide. Fluid is then expressed directly onto the slide and *fixed immediately* with spray fixative or immersed in 95% ethanol. Poorly fixed or air-dried material seriously impairs evaluation (see [Figure 2](#)). To obtain the specimen, the clinician or the patient may gently express the secretions onto a slide. The method illustrated will express secretion without trauma.

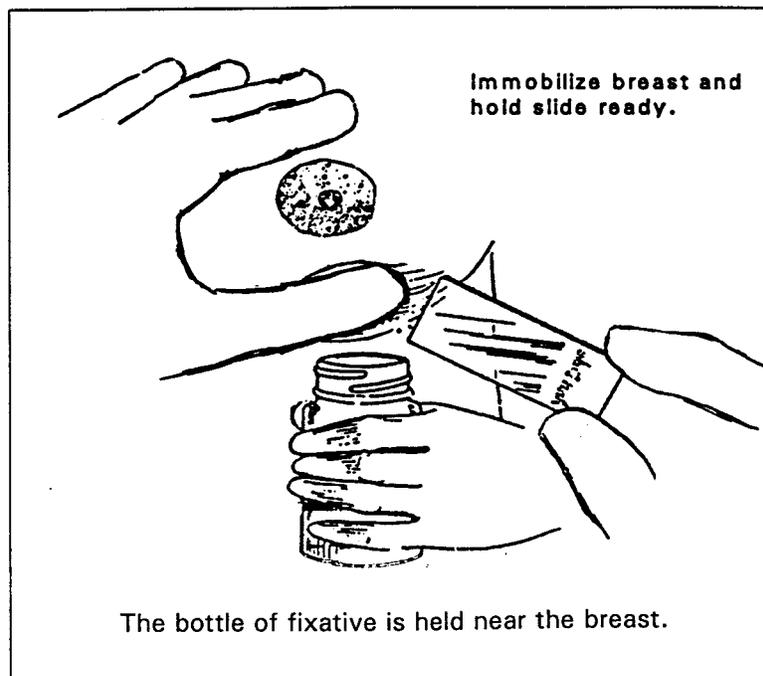


Figure 2. Collection of a Breast Secretion

3.3.1.1 Materials Needed

- Glass slides, labeled with patient's name on frosted end.
- Slotted glass/plastic jars or paper clips to hold slides apart in the fixative. (Place a paper clip on one end of each glass slide.)
- Bottle of fixative (95% ethyl alcohol).

3.3.1.2 Technique

- (1) Open the bottle of fixative and hold the bottle near the breast.
- (2) Gently express the nipple and subareolar area only using the thumb and forefinger.

- (3) Allow "pea-size" drop of fluid to collect upon the nipple tip.

NOTE: If no secretion appears at the nipple with this gentle compression, do not manipulate further.

- (4) Immobilize the breast and using the nipple, smear the material across a glass slide.
- (5) Immediately drop the slide into the fixative. The smearing of the material across the slide and the dropping of the slide into the fixative should be accomplished in one motion.

(6) Repeat the complete procedure until all available secretion is used.

(7) Complete the requisition form.

3.3.2 Cerebrospinal Fluid (CSF)

A minimum of 1 mL of CSF should be collected; 2 to 3 mL are preferred. If the specimen is collected late and will not be processed within 12-15 hours, it should be refrigerated. If specimen processing will be delayed beyond 72 hours, it should be fixed (with an equal volume of 50% ethanol). The addition of a fixative should be noted on the requisition form.

3.3.3 Gastrointestinal (GI) Specimens

3.3.3.1 GI Endoscopy/Rectal Brushings/Rectal Pap Smears

Patient preparation for endoscopy involves fasting overnight, or for a minimum of six hours. The patient may be premedicated with diazepam and/or atropine one hour prior to the procedure. Lidocaine hydrochloride throat spray can facilitate insertion of the tube. Patients should be reassured throughout the procedure. Following the procedure, the patient should be observed for two hours.

Punch or surgical biopsies, brushings, and washings are frequently done as part of GI endoscopy or as a separate procedure.

3.3.3.2 Gastric Washings

Gastric washings may be obtained as a blind technique as described below or under direct visualization at endoscopy. As an example, blind washings may be collected in small children to retrieve swallowed acid-fast organisms from productive cough or hemosiderin-laden macrophages in idiopathic pulmonary hemosiderosis.

3.3.3.3 Blind Washings

Patient preparation includes fasting overnight or for a minimum of six hours. Before the procedure, the patient's mouth and throat are cleaned of all secretions. A Levine gastric tube is passed without lubricant. The tube is

passed into the stomach to the 55-cm mark. The contents of the stomach are evacuated and discarded since they contain undigested food and are unsuitable for cytologic study. The position of the tube in the stomach can be checked by injecting air into the nasogastric tube and listening with a stethoscope or checking the pH of the evacuated material with litmus paper. Five hundred mL in 50-mL quantities of a balanced salt solution is introduced into the stomach. The fluid is withdrawn and forcibly reinjected to flush the gastric mucosa. This is done six or seven times in each of the different positions (back, abdomen, and right and left sides) with abdominal massage and the contents are aspirated. The specimen is sent to the cytology laboratory immediately.

3.3.3.4 Abrasive Balloon Collection

This technique is usually practiced as a screening technique for esophageal carcinoma in high-risk patients. A specially constructed catheter that incorporates a balloon is swallowed, then inflated. It is then pulled up through the esophagus. The surface of the balloon is designed to abrade the surface of the mucosa and collect cells. When the balloon is 18 cm from the front teeth, the balloon is deflated and quickly withdrawn. The cells are recovered by rolling the balloon surface on to two to five glass slides with immediate fixation of the slides in 95% ethanol. The balloon is then vigorously rinsed in balanced salt solution or 50% ethanol. This material may be centrifuged for preparation of cytocentrifuge or cell block material.⁵

3.3.4 Ocular Samples

Smears from ocular discharges, secretions, conjunctival lesions, or corneal lesions are handled in much the same manner as the Tzanck preparation described in [Section 3.3.6](#). Local anesthetic administered by drops will usually facilitate the acquisition of ocular samples.

The technique of intra-ocular aspiration for fluid cytology should be performed by an ophthalmologist. If small amounts of fluid are to be recovered then the material should be treated like a fine needle aspiration (see

NCCLS document GP20). If only milliliters of fluid are recovered, if at all possible, the specimen should be sent to the laboratory, for immediate processing; otherwise, it should be treated as a routine body fluid (see Section 3.2).

3.3.5 Respiratory Specimens

Early morning sputum specimens yield the greatest number of diagnostic cells. Separate specimens should be collected for cytologic study. Shared specimens (e.g., as with microbiology) are not recommended. Separate specimens should be collected for each analysis.

The patient must be able to produce a deep cough specimen that is confirmed by finding the presence of macrophages ("dust cells") on microscopy or the specimen will be deemed unsatisfactory. Patients unable to cough deeply cannot bring these characteristic cells up from the distal airways.

The patient should be instructed to clear the throat of postnasal secretions and to gargle and rinse the mouth to remove food residue. The patient then is encouraged to cough deeply. Expecterated material is collected in a wide-mouth, appropriately labeled container, fresh or with fixative depending on laboratory policy. Examples of fixatives include 70% ethyl alcohol, or 50% ethyl alcohol with 2% polyethylene glycol (Saccomanno's fixative).

Specimens collected after hours or on the weekend should have an equal volume of 50% alcohol added. The addition of fixative should be noted on the requisition form.

An adequate cytologic examination consists of a series of three-to-five consecutive daily sputum specimens. Alternatively, a three-day-pooled-sputum collection may be done.

Sputum specimens are laden with microorganisms. Refrigeration beyond 24 hours is not recommended because even with refrigeration, pathogenic organisms may multiply. This increases exposure-risk to laboratory personnel, activates degradation pathways in the cells, and contributes to background clutter of the preparation. Sputum that is held more than four hours benefits from fixation.

3.3.5.1 Routine, early morning sputum series

- (1) The patient receives a clean sputum cup the night before and is instructed not to use it until morning.
- (2) The patient is instructed to cough *deeply* ("from the diaphragm") upon awakening and expectorate all sputum into the cup. The patient should be encouraged to expectorate *deep sputum, not saliva*.
- (3) The patient continues the deep coughing and expectoration until the cup is collected, or for one hour.
- (4) The cup should be collected by early a.m. and *immediately* taken to the laboratory.
- (5) The procedure should be repeated once a day for three days.

3.3.5.2 Postbronchoscopy sputum

- (1) If possible, the patient is given a clean sputum cup *before* the bronchoscope is withdrawn.
- (2) The patient should cough deeply and expectorate *all* sputum into the cup for one to two hours.
- (3) The cup is collected after one-to-two hours and taken *immediately* to the laboratory.
- (4) The sputum series is continued the next morning, using the routine early morning sputum series, as explained in the previous section.

3.3.5.3 Induced Sputum

The respiratory therapist usually provides this service. These trained individuals have a number of techniques to obtain sputum including the use of aerosol, mucolytic, and bronchodilating agents.

Sputum production is dependent on an open airway. If there is no productive sputum after thirty minutes of vigorous coughing, an induced specimen may be considered. A heated aerosol solution is administered by a

respiratory technician and after breathing the aerosol mist for three minutes, the patient then coughs deeply. The expectorated material is collected in an appropriately labeled, wide-mouth container. This procedure is repeated for approximately 30 minutes and all material is collected.

3.3.5.4 Fiberoptic Bronchoscopy

Patient preparation involves fasting overnight, or for a minimum of six hours. The patient may be premedicated with diazepam and/or atropine one hour prior to procedure. Other pharmacologic agents are available to the clinician and are used as needed. Local anesthetic is applied to the pharynx and upper respiratory tract prior to inserting the bronchoscope. The patient should be reassured throughout the procedure. Following the procedure the patient should be observed for two hours.

Biopsies, brushings, and washings are usually done in concert with fiberoptic bronchoscopy. The type of the specimen collected is dependent on the findings at bronchoscopy and the clinical judgement of the operator. Washings should be taken using a balanced salt solution (e.g., Ringer's, Hank's, RPMI). Bronchioalveolar lavage is a selective washing of the bronchial tree. It is performed by wedging the bronchoscope in a smaller airway so as to effect a seal and rinsing the distal airways with a balanced salt solution. Balanced salt solutions are preferred for the maintenance of cytologic morphology. The resultant fluid is recovered via suction and subjected to cytologic analysis.

3.3.6 Lesion Specimens

3.3.6.1 Touch Preparations

Sometimes there is a need to obtain information about a lesion on an urgent basis. Touch-imprints may be obtained from surgical specimens, lymph nodes, bone marrow core biopsies, or exposed body surfaces such as wounds, lesions, open vesicles, or sores. These preparations enable cells to be examined apart from their connective tissue matrix. When cells are obtained by touching a wet tissue with a glass slide, cells may adhere to

the glass in roughly the same orientation as they exist on the surface of the lesion that is touched. Slides may be air-dried, wet-fixed by immersion, or spray-fixed in the same manner as a concentrated smear of a fresh fluid specimen. Also, a brush preparation of a surface lesion (such as sores or vesicles) can be prepared by direct transfer to glass slides or by immersing the brush into a balanced salt or fixative.

3.3.6.2 Tzanck Smear Preparation: Direct Smear for Viral Screening⁶

Certain viral infections produce characteristic morphologic features that can be recognized on a cytologic preparation made directly from a lesion. Some of the viral agents include:

- Cytomegalovirus
- Herpes Simplex and Herpes Zoster
- Human Papillomavirus
- Measles Virus
- Respiratory Syncytial Virus.

A direct scrape procedure is preferred. A cotton swab or other similar material should not be used to obtain a sample because diagnostic cells will become trapped in the fiber matrix.

3.3.6.2.1 Procedure

- (1) The suspect lesion is premoistened with saline. If possible, a fresh vesicle should be chosen that has not ruptured and crusted.
- (2) With a disposable needle, a fresh vesicle is carefully opened or the crust from a ruptured lesion is removed.
- (3) Using the edge of a metal spatula, scalpel blade, or glass slide, the margin of the lesion is scraped. The edges of the lesion will have the best yield of cells with morphologically recognizable inclusions.
- (4) The obtained material is carefully spread on an alcohol moistened microscopic slide and fixed. It is imperative that the material be fixed immediately after smearing. Alternatively, the scraping tool may be rinsed in a preservative and the

resultant solution may be used for concentration procedures like filtration or cytocentrifugation.

3.3.7 Urinary Specimens

3.3.7.1 Urine Specimens

The first morning specimen is discarded. The patient is hydrated and the subsequent midstream clean catch sample is collected in a wide-mouth container and appropriately labeled. The specimen is sent fresh to the cytology laboratory, if at all possible. If significant delay is anticipated, or if refrigeration is not an option, the sample should be mixed with a volume of 50% ethanol or Saccamanno's fixative equal to specimen volume, and then transported to the laboratory as soon as possible. If a catheterized specimen is to be collected, the catheter should be passed with only just enough lubricant to effect placement. If too much lubricant is used, it will accumulate in the cytologic sample and obscure cellular features.

Any instrumentation should be noted on the requisition.

3.3.7.2 Bladder Washings

Bladder washings are typically performed by a urologist at cystoscopy by introducing a balanced salt solution via a large volume syringe which has been connected to the port of a cystoscopy device or catheter. The fluid is usually withdrawn and reinjected with a moderate amount of force to dislodge epithelial cells. The resultant fluid is then sent for study. The provisos on refrigeration and or fixation indicated in the above paragraph should be observed for this type of sample.

4 Specimen Handling and Transport

4.1 Specimen Identification and Labeling

Accurate identification of a specimen and its source are essential; otherwise, a laboratory's diagnostic effort has no purpose. A secure chain of identity must be maintained between a sample and the patient from whom it came.

- Specimens *must be labeled with the patient's full name* and it is strongly recommended that another unique identifier also be used (e.g., medical record number). (For slide submission, refer to [Section 4.3.](#))
- It is recommended that the specimen container be also labeled with
 - the specimen source,
 - the date of collection, and
 - the name of the ordering physician.
- If *more than one site is sampled, the source of the specimen must* be indicated on each specimen container.
- Upon receipt of the specimen and requisition form, a *laboratory identification number (accession number) must be added to the specimen container/slide and the requisition form.*
- *The date and time* that the sample was received must be added to the requisition form or otherwise recorded.^{7,8}
- The *condition of the specimen*, including quantity, color, turbidity, and other gross characteristics of the specimen *must be added to the form* or otherwise recorded by laboratory personnel receiving the sample. It should be noted whether the sample is fixed or not.
- Specimens can be received only from licensed authorized sources such as physicians or those persons authorized by a physician.

4.2 Liquid Specimen Transport

Ideally, each fluid specimen should be sent for cytologic examination in a separate, clearly labeled, leakproof container. This container may be placed inside a sealable plastic bag or other container as protection against leakage and contamination. The requisition form should not be placed within the plastic bag or secondary container. Standard precautions and all other applicable procedures in labeling and transporting biohazardous material should be followed. (See the most current editions of NCCLS

documents H5 *Procedures for the Handling and Transport of Diagnostic Specimens and Etiologic Agents*, and M29— *Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue.*)

4.3 Slide Submission

If slides are submitted to the laboratory they *must be labeled* to ensure correct identification of the patient sample (e.g., the patient's full name, hospital patient identifier or barcode). The identifier should be legibly printed on the frosted end of the slide using a diamond pencil, hard lead pencil, or other permanent marker. Pen-type inks tend to run in processing and should not be used. The number of slides sent must be recorded on the requisition form or otherwise recorded. If more than one site was sampled and slides produced, it is mandatory that the source of the specimen be indicated on each slide. If a code is used, this code must be explained on the requisition form.

A variety of containers (cylindrical plastic-slotted, rectangular plastic-slotted, and cardboard slide booklets) can be used for transport and mailing of slides. Appropriate slide containers should: have the means to stay closed and also be easily opened; provide shock-resistant housing; and prevent the slide surface from contacting the holder. Prefixed smears that have been allowed to dry may be mailed to a designated cytology laboratory in an appropriate slide container. The slides should be adequately packaged for transport to prevent breakage. (See the most current version of NCCLS Document H5— *Procedures for the Handling and Transport of Diagnostic Specimens and Etiologic Agents.*) Use of cardboard slide holders is not recommended. Care should be taken to properly dry the slides before transport.

5 Procedure Manual

All matters pertaining to specimen collection and handling must be documented in the laboratory's procedure manual and that manual must be reviewed, modified as appropriate, and signed annually by the laboratory's technical supervisor. (Refer to

the most current version of NCCLS document [GP2](#)— *Clinical Laboratory Technical Procedure Manuals* for more information.)

6 Turnaround Time

Unless special studies are requested, it is recommended that results be reported within two working days.

7 Requisition Form

For effective communication between clinician and laboratory, to protect the patient, and to maximize the specimen evaluation, a well-designed, easily read requisition form should be used. (See [Figure 3](#).) The form must contain necessary identifying information and accompany the appropriately labeled specimen to the laboratory.

7.1 Submission of Form

A completed requisition form must accompany every specimen. In the case of electronic ordering, pertinent information must be entered in the computer. Clinical data is an inherent component of cytologic evaluation and diagnostic sensitivity is hindered by incomplete or inadequate information. Information essential to the submitted specimen which is to be included on the requisition form is described in [Section 7.2](#).

7.2 Demographic Information

The requisition form should transmit in a comprehensible fashion the following information:

- The full name of the patient.
- The identification number or other unique identifier if applicable.
- The date of birth and/or age.
- The date and time of collection.
- The source of the specimen.
- The number and type of specimens submitted (containers of fluid, slides, etc.).
- The type of examination requested.
- The ordering physician's full name and phone number.

- The relevant clinical history, especially history of cancer, cancer therapy, immunosuppression and differential diagnoses (ICD-9 Code).
- The physical, radiologic, and endoscopic findings.
- The collection method (e.g., whether the genitourinary specimen is from voided or catheterized urine, bladder wash, or ureteral brushing).

8 Specimen Processing

8.1 Accession Number

Once the laboratory is in receipt of the specimen and requisition form, a *laboratory identification number (accession number) must be added* to the sample container or slide, and to the requisition form.

8.2 Date and Time Stamp

The date and time that the sample was received must be added to the requisition form or otherwise recorded.

8.3 Specimen Condition on Receipt

The condition of the specimen, including quantity, color, turbidity, and other identifying information should be recorded on the form by laboratory personnel. It should be noted whether the sample is fixed or not.

Improper or incomplete identification of samples can interfere with the accuracy of a diagnosis. The following problems can arise:

- Multiple specimens from a single patient that are not properly labeled can be interchanged.
- Without unique identifiers, such as patient number and/or birth date, records of patients with similar names can be interchanged.
- Delay in reporting results is inevitable if the submitting physician's name, address, and phone number are not on the request form.

- Failure to report relevant history broadens the differential diagnosis and makes the result less accurate. Especially important are known history of cancer, cancer therapy, infections, systemic disease, asbestos exposure, and current clinical findings.

8.4 Specimen Rejection Criteria

Written protocols must exist, defining the criteria for rejection of a sample and defining the procedure that notifies the clinician that a sample has been rejected.

Specimens are rejected after documented efforts have been made to contact the clinician and obtain information.

Rejection criteria should include features that absolutely impair specimen processing.

8.4.1 Improper Documentation

Specimens are rejected for one or a combination of the following reasons.

- Discrepant or lack of patient demographics on container and requisition form.
- Incorrect or lack of requisition form.
- No specimen submitted with the cytology form.
- Requisition form lacking the following:
 - Full name of patient
 - Chart number or other unique identifier
 - Birth date
 - Physician's full name
 - Date and time of collection
 - Source of specimen and manner of collection.

8.4.2 Improper Specimen

Specimens are rejected for one or a combination of the following reasons.

- The specimen has been improperly submitted:
 - The full name of patient, or other unique identifier is missing from container/slide.
 - The name on specimen and the requisition form do not match.
- Shared specimens (although not recommended) are received with insufficient amount of specimen for cytologic preparations.
- The specimen submitted is not in compliance with standard precautions. See the most current version of NCCLS document [M29](#)— *Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*.
 - The container is leaking.
 - The glass container is greater than 15 mL.
 - The slides are broken or shattered beyond reasonable repair.
- Specimen deterioration:
 - The transport of fresh specimen is delayed.
 - The specimen is not fixed properly.
 - The specimen is not stored properly.
- The specimen is not received from a licensed, authorized source. These authorized sources may include: physicians; nurse practitioners/midwives; physician's assistants; medical clinics under the direction of a physician; hospitals; and medical laboratories.

9 Cytopreparation of Specimens

9.1 Anticoagulation

Regardless of source or cellularity, anticoagulation of the specimen may be preferred.

Some cell suspensions contain active components of the coagulation system. Coagulation results in fibrin polymers that entrap cells in a clot. This expedites cell block submission but interferes with cell isolation. Common laboratory anticoagulants include heparin, EDTA, citrate, and oxalate. Because oxalate forms insoluble solids on combining with divalent cations, it is not recommended for anticoagulation of cytology specimens.

Heparin is commonly used as a fluid anticoagulant in cytology. To keep body cavity fluids uniformly suspended, 3-5 IU of heparin per mL is added to the collection vessel before the specimen is introduced.^{3,4} When a cell suspension is collected in a syringe, it is convenient to transfer it into commercially prepared test tubes filled with premeasured amounts of anticoagulant. The manufacturer codes the anticoagulant using various stopper colors.

Some anticoagulants interfere with ancillary studies such as flow cytometry or cell culture. For example, lithium heparin can interfere with cell culture and EDTA can interfere with flow cytometry. Therefore, the choice of anticoagulant should be determined by the desired outcome and complexity of the diagnostic work-up.

9.2 Specimen Concentration

Fresh or fixed cell suspensions need to be concentrated before they can be stained and examined. Concentration may entail conventional centrifugation; cytocentrifugation; filtration with whole-mount processing of the filter or with imprint-transfer to a glass slide; or gravity sedimentation.

9.2.1 Conventional Centrifugation

Packed sediment is produced at the bottom of the centrifuge tube. The bulk of the supernate is removed; and either a portion of the supernate may be retained or another solution may be added to the packed sediment, which is then resuspended for further processing. An aliquot of the concentrated sediment may be directly applied to a glass slide or transferred to electrolyte, nutrient, or fixative solutions.

Conventional centrifugation using a swinging bucket centrifuge for a constant time (5 to 15 minutes) at a constant centrifugal force (400 to 1600 RCF; where $RCF = 1.118 \times 10^{-5} \times \text{radius of centrifuge head in cm} \times \text{rpm}^2$) is the most common method of specimen concentration. Low centrifugal forces are used for urines and high centrifugal forces are used for proteinaceous solutions.

If graduated conical or nipple-type test tubes are used to centrifuge the specimen, the nature of the sediment can be assessed and its volume can be directly measured. The nature of the sediment determines the need for erythrocyte or mucus lysis, and its volume (cell mass) can be used to gauge the subsequent dilution of the specimen. This is especially useful if cytocentrifugation or filter preparation is to follow because both the cell density and the physical characteristics of the carrier solution can be optimally adjusted, reducing preparative variability between specimens.

9.2.2 Cytocentrifugation

Whereas conventional centrifugation produces a cell sediment, cytocentrifugation is intended to produce a cell monolayer on a glass slide. The cytocentrifuge applies a constant centrifugal force at right angles to the surface of the glass slide causing cells to deposit on the slide.

Many manufacturers recommend conventional centrifugation of most specimens with or without prior microscopic previewing before cytocentrifugation. If the cell mass is uniformly adjusted to produce a monolayer, the cytocentrifuged volume depends on the size of the sample chamber and the area of the

glass slide receiving the sediment. Among several manufacturers, chamber volumes vary from about 0.5 mL up to several mL.

Depending on the instrument, the supernate may be removed in the course of centrifugation by absorbent filters; or after centrifugation, by decanting or aspiration.

9.2.3 Cell Filters

Cell filters are comprised of either nitrocellulose, polycarbonate, or other materials. They are of two broad functional categories: (1) whole mount filters (upon which cells are collected, processed, stained, and examined), and (2) touch-transfer filters (from which cells are transferred to glass slides by touch-imprint). Whole mount filters are especially suited for very hypocellular unfixed specimens; however, both whole mount and touch-transfer filters may be used with all degrees of cellularity and volume. Polycarbonate touch-transfer filters may be used with fixed specimens.

Automated devices are commercially available and have been designed to process samples collected in a fluid fixative and prepare thin layer slides. The methodology reduces the presence of blood, mucus, and protein in the specimen during transport and processing.

9.2.4 Cell Blocks

Cell blocks, even those that occur during sample acquisition, are useful for morphologic analysis as well as ancillary techniques such as immunoperoxidase or in situ hybridization. Cell blocks may be prepared by embedding centrifuged cell samples in agar, thrombin, or other gels. Alternatively, a centrifuged cell sample may be placed directly in paraffin and processed. The sample is then processed as a histologic sample.

9.3 Adhesion

9.3.1 Introduction

The degree of cellular adhesion to the glass slide depends on the body site from which the specimen is derived and on the condition of the cells derived from the body site.⁹ It

also depends on the condition in which the specimen was received in the laboratory.

Plain glass slides are suitable for adhesive specimens which naturally adhere well to glass; and for specimens that adhere weakly, frosted slides or slides precoated with an adhesive are helpful.³ Adhesion is not absolutely predictable; therefore, it can never hurt to use adhesive-coated glass slides.

9.3.2 Frosted Glass Slides

The surface of frosted slides poses the problem of distracting, refractive granularity. Frosted slides should be mounted in a suitable medium whose refractive index is similar to that of glass.^{2,3}

9.3.3 Coated Glass Slides

Slides may be coated with albumin (a mixture of egg white and glycerin), water-soluble glue, chrome alum (a mixture of gelatin and chromium potassium sulfate), or poly-L-lysine.^{2,3,10} Most laboratories use poly-L-lysine which is commercially available and should be used in accordance with the manufacturer's recommendations. If large numbers of slides are racked for immersion into adhesive, it takes little extra effort to detergent wash, thoroughly rinse, and then dip them into a weak (0.01N) ammonium hydroxide solution to ensure their cleanliness immediately before coating them with adhesive.

9.3.4 Activated Glass Slides

Slides may be treated with aminoalkylsilane which is preferred for in situ hybridization studies. Clean slides are dipped in clean acetone, dried, soaked for two minutes in a 2% solution of 3-aminopropyltriethoxysilane in acetone, rinsed in distilled water, dried at 60 °C for 30 minutes, and stored.^{10,11}

9.4 Fixation and Staining

Fresh specimens offer several important advantages justifying the efforts taken to obtain them: (1) ease of handling, (2) greater cell recovery, (3) better cell flattening, and (4) "crisp" nuclear morphology.³ Fresh specimens can be triaged to a number of fixatives and stains; and special studies such as flow cyto-

metry, cell culture, and electron microscopy can be applied thereto.

The morphology that cells possess at the time of specimen collection will be altered by fixation. The morphology of fixed cells is partially natural and partially artificial. Diagnostic criteria of cell health and disease constitute the application of observations originally based on alcoholic wet-fixation dependent features of cytomorphology.⁴

The purpose of the cytologic fixative is to maintain, in a reproducible fashion, the cytomorphology and diagnostically essential cytochemistry of the cell. The characteristics of a good cytology fixative are the ability to: 1) penetrate cells rapidly; 2) minimize cell shrinkage; 3) maintain cell morphology; 4) inactivate autolytic enzyme activity; 5) replace cell water; 6) allow stain permeability across cell boundaries; 7) permit cell adhesion to glass surfaces; 8) kill pathogens; and 9) afford a permanent cellular record.⁹

Commercial preservatives manufactured for automated cell processors can be used as or general fixation/concentration in accordance with manufacturer's recommendations.

9.4.1 Wet Mounted Fresh Specimens

Cell samples may be examined in the native state by direct microscopy or with supravital stains. Temporary, rapid examination is possible on any nonfixed cellular fluid. After conventional centrifugation, a drop of the sediment is mixed with a stain such as toluidine blue and examined microscopically. This technique is useful to identify highly cellular and/or malignant samples in order to take steps to prevent cross contamination.

9.4.2 Air-Dried Specimens

Air-drying is the absence of fixation and is desirable for "Wright" and/or "Romanowsky," and ultrafast Papanicolaou staining techniques. Although air-dried, Romanowsky-stained slides minimize variability in the fixation of cellular material and have the advantage of metachromatic staining of certain aspects of the cellular and extracellular matrix.

9.4.3 Conventionally Fixed Specimens

When spray or immersion fixatives are used, fixation must be completed within seconds of smearing or cells may prove unsuitable for evaluation. Even minimum air-drying of the sample alters cellular features. The most frequent artifact of Papanicolaou-stained slides is poor fixation with autolysis of cellular material. Wet-fixed smears, stained with the Papanicolaou stain or with hematoxylin and eosin, ensure maximum resemblance between the cell in cytology preparations and corresponding cells in tissue sections.

When spray or immersion fixatives are used, fixation must be completed within seconds of smearing or cells may prove unsuitable for evaluation. Even minimum air-drying of the sample alters cellular features.

9.4.3.1 Immersion Fixatives

Immersion fixation is performed by obtaining unfixed fresh cells, and spreading them as a thin layer on a clean glass micro slide. The slide is then immersed in 95% ethanol. Air-drying is to be avoided both before and after fixation.^{3,4} Alternative immersion fixatives include absolute methyl alcohol, reagent grade alcohol, denatured or proprietary alcohols, 80% isopropyl alcohol, 80% n-propanol, and 90% acetone.^{3,4,9}

9.4.3.2 Coating Fixatives

Coating fixatives are probably more popular than immersion fixatives and comprise an alcohol that fixes cells and a wax-like substance (polyethylene glycol m.w. 1540) that forms a protective coat over the cells. Protecting fixed cells during air-drying is polyethylene glycol's only demonstrable role.

Coating fixatives can be sprayed or dripped over slides or slides may be dipped into coating fixatives. Spray fixation is more common. Only fixatives prepared specifically for cytology should be used—not alcohol based hair sprays.

Coated preparations need to be soaked in order to remove their coating. Some manufacturers recommend removing polyethylene glycol with water; but most laboratories use 95% ethanol.

9.4.3.3 Fixing the Cell Suspension

Sputum. Sputum may be collected in Saccomanno's preservative.^{2,3,4,9} The specimen is subsequently homogenized in a blender, concentrated by conventional centrifugation, applied to a glass microslide, air-dried, postfixed in 95% ethanol for at least ten minutes, and otherwise processed like spray-fixed material. The blender produces potentially infectious aerosols. The blender should be constructed for laboratory use; its cap should prevent leakage; the cap should not be opened for one hour after the blending operation (unless a biohazard hood is available); and the blender should be decontaminated after each use.⁹ Alternatively, a magnetic stirring device can be used. Universal precautions should be followed.

Urine. Urine is best processed within four hours of collection. If this is not possible, its sediment should be concentrated by conventional centrifugation, decanted of its supernate, washed (optional), and suspended in at least 10x its cell mass of electrolyte solution to which an equal volume of either 50% ethyl alcohol or other fixative may be added.

Other Body Fluids. Conventionally centrifuged cell preparations, decanted of their supernate and cleared of blood by saponization (see below) contain little extracellular protein. These sediments can be suspended in at least ten volumes of balanced electrolyte solution to which an equal volume of either 50% ethyl alcohol or fixative may be added. Likewise, urines can be diluted in a similar fashion if they are to be transported off site for processing; or, if processing is to be delayed (see above). Reasonable cell preservation is achieved; and the specimen can be stored against the need for further processing. Other commercial preservatives manufactured for automated cell processors can be used in such situations in accordance with manufacturer's recommendations. Commercial preservatives may contain small amounts of formaldehyde at concentrations that do not interfere substantially with cytomorphology or staining.

9.5 Erythrocyte Lysis

Hemolytic agents may be used to reduce the effect of blood contamination. Such agents include acid elution of air-dried preparations,

Carnoy's fixative (and its variants), acidic alcohols, and saponin.

9.5.1 Conventionally Prepared Fixed Smears

Blood may contaminate freshly produced cell spreads. In such cases, blood may be hemolyzed by immersing cell spreads in Carnoy's fixative or its modifications (e.g., Methacarn)¹⁰ for 3 to 30 min. Fixation of all cells occurs, producing greater shrinkage and darker staining than occurs with 95% alcohol. Slides should be subsequently transferred to 95% alcohol. Carnoy's-type fixatives should be discarded after each use. Other methods include: 1) initially placing the slide in 50% to 70% ethyl alcohol and then transferring it to 95% ethyl alcohol; 2) placing the slide in 95% ethyl alcohol for 5 minutes and then in 12% aqueous urea for 20 to 30 minutes, then again in 95% ethyl alcohol; or, 3) placing the slide in acidified 95% ethyl alcohol (Clarke's solution or one drop HCl per 500 mL alcohol), then in 95% ethyl alcohol.^{2,9,10}

9.5.2 Fresh Cell Sediments

Conventionally centrifuged cell sediments, decanted of their supernate, can be suspended in at least ten volumes of electrolyte solution to which may be added 1 mL glacial acetic acid per 100 mL suspension. The specimen is mixed by several inversions, held for ten minutes, and centrifuged. The process may be repeated until the cell sediment no longer contains colored erythrocytes. The final cell sediment can be prepared by smear or cytocentrifuge methods.⁴

Saponins may be used for excessively bloody samples. One must be careful when using saponin because excess exposure may destroy the nonerythrocyte component of the cell sample. Time is critical and saponin's action must be stopped after exactly one minute by addition of calcium ions. Some damage to all cell types is seen. Saponin solution should be prepared under a hood to prevent inhalation of saponin powder.⁹

9.6 Mucolysis

Concentrating cells dispersed in mucus without ruining their morphology has been partially achieved by Saccomanno's method or its variations (see above). Homogenization using chemical methods is not generally

avored; although commercial mucolytic solutions are available. Homogenization of sputum with dithiothreitol (DTT) for early diagnosis of pulmonary malignancies has been discussed.¹²

10 Quality Control and Quality Assurance Programs⁷

The wide diversity of body sites and biologic processes exhibited in nongynecological cytologic specimens requires a simplifying, unifying approach to quality control (QC) and quality assurance (QA).

Under the overall umbrella of QC/QA relative to the laboratory's product, there are two sets of activities that have separate and equally valuable quality control and quality assurance components: 1) personnel proficiency and 2) cytopreparation.

Personnel Proficiency. The first set of activities relates to the training, education, and professional performance of the laboratory personnel. For additional information, refer to the most current version of NCCLS document [GP21](#)— *Training Verification for Laboratory Personnel*.

Cytopreparation. The second set of activities involves cytopreparation in the broadest definition. That is, cytopreparation is the science of collecting, preparing, and analyzing cytologic preparations in ways that optimize and standardize the detection of, and accurately interpret the cytomorphology of abnormal cells.

A program of quality improvement must reflect the philosophy and policies of each cytology laboratory in accordance with accrediting agencies. Its implementation at the procedures and practices level is the responsibility of the technical staff. Everyone who examines cytologic specimens is not only an interpreter of cytomorphology, but also an inspector of the outcome of the contributory processes. Cytotechnologist and pathologist should be sufficiently skilled to microscopically assess the quality of specimen collection and preparation. For a systematic approach that can be applied to cytopathology laboratories, refer to the most current version of NCCLS document [GP26](#)— *A Quality System Model for Health Care*.

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Appendix. Determination of Specimen Cellularity

It is best to determine the cellularity of the specimen prior to cytocentrifugation either by directly inspecting the specimen or by centrifuging the specimen in one or several graduated test tubes and directly measuring the sediment volume or cell mass. Several preprocessing steps can be applied before cytocentrifugation:

- Very hypocellular specimens can be pooled and their supernate volume reduced.
- Very cellular specimens can be diluted and their sediment volume reduced.
- The erythrocytes of bloody specimens can be removed by lysis.
- Unfixed specimens destined for air-drying and tetrachrome staining can have their suspending medium adjusted with "plasma-like" solutions including anticoagulated patient plasma, patient serum, balanced electrolyte solutions enhanced with bovine albumin, etc.
- Specimens can be "cleaned up" of mucus and fixed in suspension with Saccomanno-like preservatives prior to processing.

Summary of Comments and Subcommittee Responses

GP23-P: *Nongynecologic Cytologic Specimens: Collection and Cytopreparatory Techniques; Proposed Guideline*

General

1. New proprietary processors of liquid-based, thin-layer preparations work well with a variety of nongynecologic specimen sources and types. Urine, body cavity fluids, sputum, bronchial washes, bronchial brushes, gastrointestinal/biliary brushes, Tzanck smears, anal swabs have been processed with this method with good results. It would probably be worthwhile to mention them.
 - **The use of proprietary liquid fixatives for automated thin layer processing has been incorporated into Sections 3.1, 9.2.3, and 9.4.**
2. The subcommittee should review the terminology used and consider incorporating a glossary of terms.
 - **Upon review of the guideline, the subcommittee concluded that pertinent terminology is already well defined in the text and there would be no added benefit with the addition of a glossary.**

Foreword

3. Under Universal Precautions: do you want to say just blood specimens in the first line or expand to include body fluids?
 - **The precautions statement in the Foreword has been updated with a standard precautions statement which states that *all* patient specimens are to be treated with standard precautions.**

Introduction

4. It is true that there are common threads in the diagnostic criteria for malignancy in specimens from various body sites, but I wouldn't make the statement "Cytologic diagnoses are made by applying uniform criteria to cell samples."
 - **The first sentence of the third paragraph in Section 1 has been deleted.**

Section 3

5. Cytologic samples may be contaminated. "Contaminated" seems to be the wrong word as blood and mucus inflammatory cells and microbial agents may be part of the disease process reflected in the specimen; so they are in effect not contamination, but rather the disease.
 - **The first sentence of the last paragraph in Section 3 has been revised and contaminated has been deleted.**
6. The third paragraph indicates various specimen types. Samples are also derived from gastrointestinal brushings, not just fluids, and they may also be collected by direct puncture and drainage (or puncture and aspiration).

- **This section has been revised to indicate the source of the specimen as an alternative to indicating the many different types of specimens.**

Section 3.2

7. While the use of heparin is nice, the reality of the matter is that it is impractical. I would make that use optional.
- **This section has been revised to be consistent with Section 9.1. The guideline indicates that heparin is commonly used and provides recommendations for its use. The guideline does not, however, state that it *should* be routinely used and the guideline also provides options if heparin is not available.**
8. Heparin should be added, not “can” be added.
- **See response to Comment 7.**

Section 3.2

9. We utilize 200 ml of large volume body cavity fluid for cytopathology.
- **The last sentence in Section 3.2 has been revised: a well-mixed aliquot on the order of 50 mL to 200 mL is favored.**

Section 3.2.1

10. Other sites include peritoneum, and in future, possibly pleural.
- **The first sentence in Section 3.2.1 has been revised: Bronchial, body cavity, and genitourinary washings should**

Section 3.2.2

11. “Papanicolaou” should be capitalized.
- **The error has been corrected.**
12. When a brush is sent in fluid, we have found it preferable to send it in salt solution rather than fixative. It is easier to remove cellular material from brush.
- **The subcommittee agrees with the commentor. The guideline emphasizes in many places that fresh specimens are preferred; however, the subcommittee agreed that both salt solutions and fixatives should be mentioned in the guideline.**
13. I would replace the statements on smear preparation rolling the brush across the slide with rolling the brush in a small area about the size of a nickel or dime. There is much less drying that way and patterns of cells are retained. Stating that “rolling the brush across the slide,” I think, promotes the idea of covering large areas of the slide— another bad idea. In the last line, should we be more specific about the word fixative? It is rather open-ended to just say “fixative.”

- **The second sentence of Section 3.2.2 has been revised: After the brushing is performed, an assistant will obtain the brush from an operator and roll the brush across the glass slide, pressing firmly in a small area in a circular motion. (Using a small area helps prevent air-drying.)**

Section 3.3.1

14. Delete material in the last sentence. Should there be further emphasis in this paragraph that fixation with 95% alcohol, which is still commonly done, is bad practice as cell shrinkage is extreme?
 - **The subcommittee agreed that this information would remain in the text because it is the common and correct practice used for fixation of breast secretions.**
15. There is no evidence for the statement that vigorous manipulation of the breast dislodges and spreads malignant cells, at least not in a prognostic sense. I agree it is not a good idea, but primarily from the point of view of discomfort to the patient.
 - **The subcommittee agrees with the commentor and this statement has been deleted.**

Section 3.3.3.4

16. How are cells recovered from the balloon? Should we provide more specific directions?
 - **This section has been expanded and a reference has been added.**

Section 3.3.5.4

17. "Balanced" has been misspelled in the last paragraph in this section.
 - **The spelling of balanced has been corrected.**

Section 3.3.7.1

18. The recommendations in this section and in 9.4.3.3 are not consistent.
 - **The subcommittee could not identify any inconsistencies and emphasizes to the commentor that Section 3.3.7.1 deals with handling/collecting the specimen and Section 3.3.7.1 deals with specimen processing.**

Section 3.3.7.2

19. The first sentence needs to be changed to "balanced salt solution," not "saline," which shrinks cells. Most urologists need to be cautioned about that.
 - **Saline has been deleted from this sentence.**

Section 4.3

20. Second paragraph: I would not list cardboard slide holders in the first sentence, and I would not recommend them in the last sentence. I would not recommend them period.
 - **Cardboard slide holders are only mentioned in the first sentence. The next to last sentence states that cardboard slide holders are not recommended.**

Section 6

21. One working day turnaround for non-gyns is nice, but impractical for many centers to prepare the material properly, screen it, and report it. Forty-eight hours is better and more realistic.
- **The guideline has been revised and now recommends that results be reported within two working days.**

Section 7.3

22. I would try not to combine non-gyn, gyn and FNA in the same form. At least FNA should stand alone and non-gyn in today's cytology practice should stand alone also.
- **The subcommittee combined nongynecologic, gynecologic, and fine needle aspiration because one form is less expensive and simpler to provide to clinicians. It was also the perception of the subcommittee that one form was preferred by clinicians.**

Section 8.4

23. Regarding "specimen rejection," I suggest adding a statement that, as a rule, the laboratory should not discard a rejected specimen, but should retain it under conditions appropriate for preservation of the specimen, pending discussion with the clinician who submitted the specimen. Often, additional information from the clinician can make an "unacceptable" specimen "acceptable" (if not optimal).
- **The following sentence has been added: Specimens are rejected after documented efforts have been made to contact the clinician and obtain information.**

Section 9.1

24. Sodium heparin works well as anticoagulant.
- **The subcommittee agrees with the commentor. In Section 9.1, heparin is used generically in the second paragraph. The fifth paragraph specifically states that lithium heparin can interfere with cell culture.**
25. No mention is made of preparation techniques for cell blocks. These are often critical for evaluation of body cavity fluids, providing the easiest source for immunocytochemical analysis.
- **Section 9.2.4, entitled Cell Blocks, has been added to the guideline.**

Section 9.4

26. It is not clear what is meant in (4). The sentence does not seem to make sense.
- **Number (4) in the third paragraph of Section 9.4 has been revised to: inactive autolytic enzyme activity.**

Related NCCLS Publications*

- GP2-A3** **Clinical Laboratory Technical Procedure Manuals Third Edition; Approved Guideline 1996).** Guidelines that address design, preparation, maintenance, and use of technical procedure manuals in the clinical laboratory.
- GP15-A** **Papanicolaou Technique; Approved Guideline (1994).** Discusses procedures for cervical specimen collection, as well as the preparation, fixation, staining, and storage of Papanicolaou slides.
- GP20-A** **Fine-Needle Aspiration Biopsy (FNAB) Techniques; Proposed Guideline (1996).** This document contains recommended procedures for performing fine-needle biopsies of superficial (palpable) and deep-seated (nonpalpable) lesions, from patient preparation through staining the smear.
- GP21-A** **Training Verification for Laboratory Personnel; Approved Guideline (1995).** This document provides background and recommends an infrastructure for developing a training verification program that meets quality/regulatory objectives.
- GP26-P** **A Quality System Model for Health Care; Proposed Guideline (1998).** This document provides a model for providers of healthcare services that will assist with implementation and maintenance of effective quality systems.
- H5-A3** **Procedures for the Handling and Transport of Diagnostic Specimens and Etiologic Agents Third Edition; Approved Standard (1994).** Gives proper packaging, handling, and transport requirements for medical specimens and governing regulations.
- 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).** 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).

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

NOTES

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