NON-GYNECOLOGICAL CYTOLOGY PRACTICE GUIDELINE

I Introduction
II Specimen Collection and Submission
III Laboratory Sample Processing
IV Nongynecological Cytology Analysis
V Nongynecological Cytology Reporting
VI Quality Control and Quality Assurance Practices
VII Data Management and Laboratory Information Systems
VIII Archiving Testing
IX Archiving and Interlaboratory Slide Review
X Laboratory Cost Accounting and Financial Management
XI Respiratory Tract Supplementary Information
XII Bibliography

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NON-GYNECOLOGICAL CYTOLOGY PRACTICE GUIDELINE

prepared by the American Society of Cytopathology, Cytopathology Practice Committee.

I. INTRODUCTION

I.A. Purpose, Intended Audience, and Limitations

"Clinical practice guidelines are systematically developed statements to assist practitioner and patient decisions about appropriate health care for specific clinical circumstances." 1,2 Practice guidelines are used by diverse segments of the medical community to define and communicate standards of performance and care. To date, the American Medical Association has catalogued nearly 2,000 clinical practice guidelines or equivalent documents.3 An additional source on practice Guidelines is the web site www.guideline.gov (National Guideline Clearinghouse) that is sponsored by the Agency for Health Care Research and Quality in partnership with the American Medical Association and the American Association of Health Plans.

The Nongynecological Cytology Practice Guideline is a document for laboratories and is intended for use primarily by cytologists – pathologists and cytotechnologists – who perform non-gynecologic cytology analyses and report their findings to clinicians. It is not intended to represent the standard of care for use in medico legal proceedings. Thus, this document focuses on laboratory processes and related topics such as techniques of sample procurement, slide staining and analysis, and cytology laboratory management. Although this guideline is intended for use by laboratorians, clinicians, and patients, others involved in healthcare may find this document to be a resource when correlating nongynecological cytology results with clinical care decisions. Information that is unique for specific organs, organ systems or body sites is contained in sections entitled supplementary information.

The Nongynecological Cytology Practice Guideline is not intended for use as or a substitute for a procedure manual for cytology technical protocols. Readers may use references cited at the end of this Guideline, or other sources, for information such as preparation methodology or staining formulations.

The process of creating a guideline represents consensus building within a specialty with subsequent endorsement by national professional organizations. In light of rapidly evolving science and technology, a guideline devoted to nongynecological cytology requires timely review and revision. This guideline serves not as a specific blueprint or set of dictates, but as a device to assist standardization and continuous quality improvement efforts. It is with this understanding that the American Society of Cytopathology promulgates this Nongynecological Cytology Practice Guideline.
I.B. **Context and Scope**

The emphasis of this guideline is on nongynecological cytology specimen procurement, analysis, reporting and management. Specific microscopic criteria for interpretation are not included since these are well described in textbooks, symposia and workshops. A detailed analysis of related clinical topics such as patient care algorithms for follow up of abnormal nongynecological cytology results are also beyond the scope of this document.

An important general limitation is that this guideline, in many respects, is applicable for laboratories in the United States that are subject to the provisions of the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88) and for laboratories that participate in the Laboratory Accreditation Program of the College of American Pathologists (CAP). Many of the elements of the guideline are defined or specified in the CLIA regulations and/or in the CAP laboratory accreditation checklists. In the text of this guideline the word “must” is used to distinguish regulatory requirements from other best practice recommendations.

I.C. **Variability of Practice**

This document highlights procedural and interpretive areas where there are variations in practices, and areas where there is consensus for "best practices". Where the literature is conflicting, absent, or consists only of case reports rather than more comprehensive studies, this document describes different laboratory practices.
II. Specimen Collection and Submission

Nongynecological cytology specimens are collected from a variety of sites for the detection of malignant and benign processes. The site from which the sample is collected dictates the method of collection. The method of collection affects the morphology of the cellular samples. The importance of proper specimen collection and submission is essential. Clinical personnel should be trained in the appropriate submission of samples as well as in procedural techniques. The laboratory must provide instructions for proper collection of all nongynecological specimens. The instructions must be available to personnel at the location where the specimens are collected. The laboratory provides feedback on the adequacy of the sample via individual reports and may elect to provide summary information regarding patient sampling to its clients.

II.A. Collection

II.A.1 Fluids
Serous or “body cavity fluids” are usually collected with aseptic technique by needle puncture and aspiration of the body cavity fluid. Methods for obtaining the specimen vary depending upon the site. Fluids are best collected into a dry container and submitted in the fresh state to the laboratory. Adding 3-5 IU heparin/mL to a container prior to obtaining a bloody sample will usually inhibit clotting and not adversely affect morphology. If delay in transportation to the laboratory is unavoidable, most fluids may be kept refrigerated (4°C) up to 72 hours. (The exception to this is cerebrospinal fluid, which begins to degrade shortly after collection if stored at room temperature or refrigerated.) A fluid sample intended for cytologic analysis should never be frozen. If a longer delay is expected, preservation at the time of collection with 50% ethanol equal to the volume of the specimen is suggested. Alternatively, proprietary transport medium supplied by the manufacturers of liquid based systems may be used. Any added fixative should be noted on the requisition. For small fluid accumulations the entire specimen is submitted for laboratory evaluation. For larger effusions, 50-200 mL of well-mixed fluid should be sent for cytologic examination; however, the entire specimen is also acceptable. All fluid specimens must be transported in accordance with OSHA regulations for biohazardous substances. Three separate specimens collected at different times are optimal for the diagnosis of malignancy.

II.A.2 Washings
Washings can be collected from various body sites. Small aliquots of balanced saline solution are washed over a directly visualized area and removed immediately with suction. These washings are usually submitted unfixed to the laboratory. If a delay is expected, they may be partially fixed in 50% ethanol equal to the volume of the specimen, or in the proprietary transport medium supplied by the manufacturers of liquid based systems. Any added fixative should be noted on the requisition.

II.A.3 Brushings
Brushing specimens may be taken from any surface of the body. Direct smears, brush contents or a brush tip may all be submitted as brushing cytology.
IIA.3.a Direct smears
Prior to the procedure, slides should be labeled with the patient’s full name or other unique identifier, using pencil or solvent resistant marker. Some laboratories may require a second identifier such as date of birth, medical record number, social security number or collection date. The laboratory must have a written procedure that specifies the requirements for proper specimen identification.11

After the brushing is performed, the brush is rolled across the slide in an area approximately 2.5 centimeters in diameter (the size of a quarter) to produce a thin evenly layered smear.8 The slide should be fixed immediately. Immediate fixation of the cellular sample is necessary to prevent air-drying which obscures cellular detail and compromises specimen evaluation. Fixation can be by immersion (preferably) or spray fixation. Immersion fixation consists of placing the smear into 95% alcohol or its equivalent.12 If the specimen is immersed in alcohol, it may remain in the alcohol for transport to the laboratory. Alternatively, the specimen can be immersed in alcohol for 20-30 minutes, removed and allowed to air dry, then placed in a container/mailer to be transported to the laboratory. The immersion technique requires use of a separate fixative container for each body site sampled.

If a specimen is spray fixed, only quality controlled cytology fixatives should be used. Hair spray should NOT be used due to the fact that most modern products contain no alcohol. In the past it was the alcohol content of hair spray that allowed its use as a cellular preservative. The manufacturer’s instructions for fixation should be followed. Generally, spray fixatives should be held 6-10 inches (15-25 cm) from the glass slide when applied.13

Alternatively, air-dried brushing smears may be submitted for staining with one of the Romanowsky stains or for rehydration prior to Papanicolaou staining14,15,16

IIA.3.b Brush contents in liquid media
Material obtained from a brushing should be submitted in a physiologic transport solution, 50% ethanol equal to the volume of the specimen, or in the proprietary transport medium supplied by the manufacturers of liquid based systems. The brush may be submitted in the solution or discarded after vigorously removing the adherent cellular material into the medium. The container must be clearly labeled with the patient’s name or other unique identifier. It should be a leak proof container with enough fluid to cover the brush if submitted.8

IIA.4 Fine Needle Aspiration Biopsy (FNAB)
Fine needle aspiration biopsy in this document is distinct from core or cutting biopsies and refers to a procedure that procures cellular material for diagnosis using suction or non-suction techniques. Fine needle aspiration biopsy may be performed on any body site that can be reached with a fine needle. A fine or thin needle is defined as 22 or higher gauge. FNAB is a minimally invasive, cost-effective technique with high diagnostic accuracy (in the range of 90 to 99%).17

The accuracy of FNAB depends upon the site and type of lesion sampled, the operator’s experience, the quality of specimen preparation, and the pathologist’s diagnostic skills. Fine
needle aspiration biopsy may be performed on palpable (superficial) lesions or with radiological, endoscopic or bronchoscopic guidance (deep lesions).

The aspirator should be aware of the pertinent history and clinical information, significant radiological studies and the clinical question that FNAB may answer. The procedure as well as the minor complications of bruising and bleeding should be explained to the patient. Site-specific complications for deep needle aspiration biopsy should be described to the patient if image guided FNAB is performed. Informed consent should be obtained from the patient, guardian or legal representative. Written consent should be obtained if the institution requires it where the procedure is performed.

The operator should be prepared to obtain material for ancillary tests, such as cell block preparations, molecular studies, flow cytometric studies or microbiologic studies if indicated. Prior to the procedure, slides should be indelibly labeled with the patient’s name or other unique identifier. Individual laboratories may require other information such as site of biopsy, date of birth, medical record number, social security number or collection date.

After a limited physical examination, palpable FNAB may be performed as clinically indicated. The operator should wear examination gloves as described in standard precautions. The skin should be cleansed with an alcohol swab prior to puncture for superficial FNAB. For percutaneous biopsy of deep lesions, sterile or aseptic technique is used.

Local anesthesia may be used for palpable lesions at the preference of the operator and the patient, but is usually used for percutaneous radiologically guided needle biopsies. Sedation is rarely needed for adult superficial FNAB, but may be used for pediatric patients, or deep-seated FNAB when the patient is uncomfortable or anxious. Rarely FNAB may be performed under general anesthesia, usually during surgery.

For solid lesions multiple passes with separate needles are performed. Staying within the lesion, the needle is moved in a cutting motion withdrawing cells into the needle hub. The force of the cutting motion needed to obtain an adequate sample must be adjusted for the body site and characteristics of the lesion. These biopsies may be performed with suction or by the “non-suction” technique. There are many styles of handles, syringes and needles that may be used, depending upon the operator’s preference. Once the cellular material is seen in the needle hub, suction is released and the needle is withdrawn.

The cellular material is expressed onto one or more slides and is smeared with a second slide or a cover slip. Often, a “paired” smear is used, by smearing the material between two slides, to make a mirror image pair. One of the slides is often fixed and the other air-dried. Alternatively, push smears, pull smears and one or two-step smearing techniques are used. Fixation techniques and fixatives vary, but should be stipulated by the laboratory that accepts the specimen for analysis. Smears are prepared from each needle pass. Two or more aspirates are often required for adequate sampling.
For subsequent analysis of the specimen, the needles may be rinsed into a physiologic solution, tissue culture medium, transportation medium or proprietary fixative for liquid based processing. Needles should not be re-sheathed using a two handed technique and instead should be discarded directly into an OSHA approved sharps disposal container.9

For cystic lesions, remove as much fluid as possible. The cyst fluid can be handled as a liquid specimen. If there is a residual mass, the procedure for solid lesions as described above should be followed. Ancillary testing can be performed as indicated. See section VIII.

Specimen adequacy may be assessed during FNAB by using a rapid stain such as a toluidine blue stained wet film, a Romanowsky stain or a modified Papanicolaou stain for microscopic analysis. Although there are no universal criteria for adequacy, a multiparameter assessment is usually performed which includes cellularity, site-specific architectural features, and cellular elements. Intraprocedural evaluation should be documented. At this time, additional material (or the needle rinses) may be designated for ancillary testing.

Local pressure is usually adequate to control post procedural bleeding for superficial sites. Patients who have undergone deep FNAB should be followed for complications as clinically appropriate. All patients who undergo FNAB should be observed during and following the procedure until they are stable.

A written report should follow the procedure. Depending on local practice this report may include a description of the procedure, any preliminary information obtained, complications and the disposition of the patient when released.

II.B. Test Requisition

A manual or electronic requisition must be generated for each specimen. The requisition should include the:

- Full patient name
- Unique identifier such as medical record number
- Date of birth and age
- Date and time of specimen collection
- Source or site of the specimen
- Type of specimen or examination requested
- Collection method
- The ordering clinician’s full name, and the name and address or other suitable identifier of the authorized provider ordering the test19
- Methods of rapid communication such as telephone or FAX numbers of the ordering clinician
- Clinical history, including pertinent physical findings, radiographic findings, history of environmental exposure, possible exposure to infectious agents, immunosuppression, chemotherapy, radiation therapy, surgical operations, history of cancer, previous histological or cytological abnormalities
- ICD-9 code
Diagrams of the site sampled may be helpful, especially for fine needle aspiration biopsies.\(^8\)

**II.C. Specimen Submission**

**II.C.a Slides**

Fixed smears should be submitted in containers that protect against breakage. Slide containers are available in a variety of shapes, volumes and material. Optimal design features include easily opened containers which stay closed during transport, shock resistant material, and enough room to prevent slides from adhering to one another or the container. The slides should be marked clearly with the patient’s name, as well as other identifiers if possible. If more than one site is sampled, the slides must be clearly marked as to their site. Enclosure in a transport bag indicating biohazardous contents is prudent if a courier system or manual delivery is planned. Fixed slides may be mailed according to transport guidelines.\(^20\) A paper or electronic requisition must accompany the specimen.\(^21\) Slides in fixative should be submitted in leak proof containers that protect against breakage and are clearly labeled with the patient’s name and specimen site(s). The slides in fixative may be placed in a secondary container or transport bag, to avoid leakage of fixative during transport. Paper requisitions that accompany slides in fixative should be placed in an outside pocket to avoid exposure to any leakage of fixative.

**II.C.b Liquid transport**

Each fluid specimen should be placed in a clearly labeled container that is leak proof. Enclosure in a transport bag indicating biohazardous contents is prudent if a courier system or manual delivery is planned. Paper requisitions that accompany slides in fixative should be placed in an outside pocket to avoid exposure to any leakage of fixative. Needles should never be transported with fluid specimens.\(^9\) Large glass collection containers should be avoided.
III. Laboratory Sample Processing

Laboratory sample processing includes steps from the receipt of the specimen in the laboratory to the delivery of a stained slide ready for microscopic examination. The information for achieving this is based upon practices cited in standard cytology references. Throughout processing, the identity and integrity of the specimen must be maintained, and the principles of universal precautions followed.

III.A. Receipt and Identification of the Specimen

The laboratory should confirm the identity and integrity of the specimen received. Specimens are accepted only when ordered by physicians or other persons authorized by law. Each sample must have a request completed by the authorized provider prior to processing. The laboratory must have a procedure in place for handling verbal requests.

III.A.1. Requisition

The specimen requisition (whether electronic or manual) must provide space for the patient’s first and last name or other unique identifier and the date of birth (or age.) The requisition must also provide space for the date the sample was collected, the test to be performed, the source of the material, and the name and address or other suitable identifiers of the authorized person requesting the test. Methods of rapid communication such as telephone or FAX numbers are also recommended. These elements ensure that specimen results are linked with the appropriate patient.

Ideally, the following information should also be provided on the requisition form as applicable: pertinent physical findings, radiographic findings, history of environmental exposure, possible exposure to infectious agents, immunosuppression, chemotherapy, radiation therapy, surgical operations, history of cancer, previous surgical or cytologic abnormalities.

Clinical history is important and should be correlated with the type of specimen submitted. For example, if the history states that the patient has a left lung mass and the specimen is a right bronchial washing clarification and resolution of the discordance should be undertaken before interpretation of the specimen is attempted. All available patient information should be included in the demographic and clinical history sections of the report and archived database for current and future use.

A written procedure must be in place to handle specimens that are received without adequate information on the request form.

III.A.2. Accessioning

When the specimen and requisition are removed from the transport container, the specimen identifiers on the requisition form and sample must match. The requesting physician or designee may rectify variations; the laboratory must keep a record of all changes made, according to the laboratory's standard operating procedure. When all specimen identifiers match, the specimen is accessioned. Accessioning assigns a unique laboratory identifier linking the sample to the patient.
The laboratory identifier may be generated manually or electronically and may be numeric or alphanumeric and may also be bar coded. This unique identifier is placed on the slide and on the requisition with a material or marking device that will withstand subsequent processing.

III.A.3. Specimen Condition
Written criteria for the rejection of specimens must be available in each laboratory.6,28

III.A.3.a. Glass Slides
Criteria for rejection of glass slides should address unlabeled slides, slides labeled with non-permanent writing utensils or paper labels and broken slides. For slides that can be salvaged, a comment regarding the sample condition should be noted in the report. The number of slides received, their method of fixation, and body site should be documented.

III.A.3.b. Liquid Specimens
Liquid specimens should be received in tightly closed containers. Patient identification must be on the specimen container. If the sample has leaked into the transport container, a reasonable effort should be made to salvage the sample and this should be documented in the report. The volume, color, clarity, other pertinent gross characteristics, presence of fixative, presence of heparin, and other additives should be documented.

III.A.3.c. Brushing Specimens
Brushing samples may be received as direct smears, fluid-filled containers with brushes enclosed, or liquid specimens in which the sample was removed from the brush. III.A.2 and III.A.3 should be followed as dictated by the sample(s) submitted. The number of slides and the variety of specimens should be documented.

III.A.3.d. Fine Needle Aspirations
Fine needle aspirates are usually received as direct smears and may be accompanied by a liquid specimen collected as a needle rinse. Alternatively, needle aspiration biopsies may be submitted entirely in liquid. III.A.2 and III.A.3 should be followed as dictated by the sample(s) submitted.29

III.B. Specimen Preparation

III.B.1. Smears
The preparation objective of direct smears is a slide with an evenly and thinly applied cellular specimen that is free of mechanical distortion and free of drying artifact when the slide is fixed in alcohol. Smears fixed in alcohol (wet fixation) are usually stained by the Papanicolaou method; air-dried smears are usually stained with a Romanowsky stain. Smears preserved with spray fixatives that contain Carbowax™ should be soaked in 95% alcohol. Carbowax™ left on the slides will impede stain uptake and must be completely removed before beginning the staining process.12

III.B.2. Liquid Specimens
Liquid specimens should be processed according to the manner in which they are submitted. Liquid specimens may be received fresh, with heparin, with preservative (alcohol or other
fixative), or with physiologic solution or tissue culture medium. Additional processing should be considered for grossly bloody specimens prior to slide preparation. Blood clots should be removed and processed as a cell block. The addition of red blood cell lysing agents and density gradient centrifugation are both methods that sufficiently remove blood while retaining diagnostic cells.12,30,31

III.B.2.a. Liquid specimens received without preservative
Specimens of low cellularity and low volume may be cytocentrifuged directly. High volume specimens are usually concentrated prior to preparation. Centrifugation is frequently used with the re-suspended pellet used for direct smears, preparation of slides using automated liquid-based methods, filtration or subsequent cytocentrifugation. A cell block can also be prepared from the centrifuged sample.

III.B.2.b. Liquid specimens received with preservative
Specimens submitted in commercially available preservative products should be processed as indicated by the manufacturer.30,31 Specimens submitted in alcohol should be processed as indicated in III.B.2.a. Cell blocks can be prepared from these specimens.32,33,34,35

III.C. Staining Procedure

The Papanicolaou stain is recommended for the staining of alcohol fixed cytology slides.24,36,37 Romanowsky stains may also be used for wet fixed slides, but are primarily applied to air-dried smears.14

III.C.1. Papanicolaou Stain
The Papanicolaou stain uses a standard nuclear stain, hematoxylin, and two cytoplasmic counterstains, OG-6 and EA. The outcome of this method is crisp nuclear detail and transparency of the cytoplasm, which allows the examiner to clearly visualize cellular morphology. Either a progressive or regressive technique may be used for nuclear staining. Several automatic programmable stainers are available. Each laboratory must develop a written staining protocol for manual, automated, or for both methods, which results in the optimal staining of the specimen.6,38

III.C.2. Romanowsky Stain
A Romanowsky stain is recommended for air-dried smears. Romanowsky stains, mixtures of eosin and methylene blue, are a family of polychrome stains that produce their effect by the production of azure dyes as a result of demethylation of thiazines and the acidic component eosin. Unlike the Papanicolaou stain they are metachromatic.39 Most Romanowsky stains used in cytology are aqueous stains as opposed to the methyl alcohol based stains of hematology. Many commercial stains are available, and most consist of a methanol-based fixative, and two dyes which result in differentiation of cytoplasmic and nuclear components. Most Romanowsky stains are rapid and are useful in enhancing pleomorphism, and distinguishing extracellular from intracytoplasmic material.
III.C.3. Stain Quality Control
Maintenance of good staining requires that the stains are filtered and replaced on a regular schedule, determined either by the number of slides processed or the time elapsed since stains were last replaced.\textsuperscript{40,41} Furthermore, the quality of the stain should be monitored daily and the results documented. Deviations from optimal quality should be addressed immediately, the problem identified and corrective action(s) taken. The laboratory must document all problems and corrective action taken.\textsuperscript{6}

To prevent cross-contamination, non-gynecologic preparations are usually stained separately from gynecologic specimens. If only one Papanicolaou staining setup is used, solutions should be changed or filtered between gynecological and nongynecological staining runs. Samples with a high potential for cross-contamination must be stained separately from the remainder of the laboratory’s cases.\textsuperscript{6,42}

III.D. Dehydration, Clearing and Coverslipping

III.D.1. Dehydration and Clearing
After staining, the sample is dehydrated by a series of increasing concentrations of alcohol followed by rinsing in clearing solutions. The last clearing solution should be colorless and its refractive index should be close to that of the coverslip, slides and mounting medium. While xylene (dimethyl-benzene) is the most commonly used clearing agent, others derived from citrus terpenes and other sources have found some use. Xylene clearing must be performed in a well-ventilated area or fume hood to limit exposure to xylene fumes.\textsuperscript{43} Slides should remain in the clearing agent until coverslipping is performed.

III.D.2. Coverslipping
Mounting medium used to bond the slide and the coverslip should be compatible with the clearing agent, transparent, and have a refractive index similar to the glass slide and the stained specimen. Glass slides have a refractive index of 1.515, according to the American Society for Testing and Materials (ASTM) specifications.\textsuperscript{44} The refractive index of cells is similar to that of glass. Ideally the refractive index of the mounting medium should be 1.52-1.54. Most commercially available mounting media have refractive indices that fall within the range of 1.49-1.57.

Adequate mounting medium should be applied to protect the cellular material from air-drying and shrinkage, and to prevent fading of the cell sample. The cellular material should be covered by a suitably sized coverslip or covering material of appropriate quality. The ASTM requires that coverslips have a refractive index of 1.523\textpm.005. Mounting medium and coverslip should have a total thickness of 0.17 to 0.18 mm according to microscope manufacturers and this number is usually indicated on the microscope objective. No. 1 coverslips (0.13-0.17 mm) meet these specifications.

Coverslipping requires good light, ventilation and eye protection. Slides should be removed from the clearing agent one at a time to avoid drying of the cell surface and the resultant brown “corn flaking” artifact. Different methods used to coverslip include placing the mounting medium on
the coverslip, then inverting the coverslip onto the slide surface, or lowering the slide onto a coverslip containing adequate mounting medium. Glass coverslips, coverfilm and automated coverslippers are available. Ideally, the mounting medium should be allowed to dry before the slides are reviewed to reduce movement of cellular material during the slide examination.

Chemical waste collected throughout the staining, dehydration, clearing and coverslipping processes must be disposed of or recycled according to OSHA, state and local regulations.43

III.E. Destaining and Restaining

Destaining a slide is a stepwise process, beginning with removal of the coverslip and mounting medium, and proceeding backward through the staining steps, omitting the stains themselves. Alternatively, once the coverslip and mounting medium are removed, the slide can be soaked in acid alcohol until the cells are colorless. The process is completed by thoroughly rinsing the slide in water baths. Once destaining is complete, restaining can begin at the nuclear stain step. Error! Bookmark not defined.

III.F. Confirming Identification of the Specimen

The stained and labeled slide(s) should be matched with its requisition or other laboratory document that displays the same information. The information on the slide must correspond to the information on the requisition or laboratory document. If there are any discrepancies, these must be noted and resolved before the report is released.11

III.G. Configuration of Laboratory Space According to Function

The laboratory must have adequate space to ensure that the quality of preparatory work, interpretive services, and the safety of laboratory personnel are not compromised.
IV. Nongynecological Cytology Analysis

IV.A. Individual Qualifications

According to the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88) a qualified pathologist must examine all non-gynecological cytology specimens. In laboratories with cytotechnologists there must be a qualified pathologist serving as laboratory director or technical supervisor, and a general supervisor as defined by CLIA 88. Adequate support personnel should be available to minimize clerical duties.

IV.B. Environment and Equipment

Elements of the working environment and equipment used to analyze nongynecological cytology specimens are identical to those used for cervical cytology analysis. These were reported under the guidelines for cervical cytology practice and are reiterated below. Examination of all cytology slides should be performed in a comfortable area of the laboratory with minimal distraction. Adequate space, facilities and equipment must be available to the pathologist and cytotechnologist to perform their duties. Ergonomics play a vital role in the pathologist’s and cytotechnologist's workstation to minimize the risk of repetitive motion injury and musculoskeletal strain. Regular monitoring and maintenance of all equipment and instruments is essential. Proper equipment and resources include: sufficient desk or bench space, a cushioned chair with seat and height adjustment as well as adjustable back support, and a microscope in good working order. Arm rests that fit the desktop, tilting microscope heads, rubber focus knob adapters and devices that adjust microscope height are available options that increase the comfort of the pathologist and cytotechnologist. Other factors include diffuse, moderate room illumination, a non-reflective desk surface, and a comfortable, quiet, draft-free room separate from the processing area where protective equipment is required. Clerical and record-keeping areas of the laboratory should be conveniently located.

IV.C. Analysis Time

The amount of time a cytotechnologist spends screening a slide is variable. Factors which influence all types of cytologic specimen screening time include method of sample preparation (liquid based vs. conventional), overall sample cellularity, blood, inflammation or other obscuring factors, clinical history, complexity of findings and the cytotechnologist’s experience and state of mind. For cytotechnologists workload limits must be set for each individual based upon an evaluation of the individual’s capability and must not exceed the limits set by CLIA '88. Individual workload limits apply to slides screened per hour and in any given 24-hour period. Screening rates must be monitored to ensure compliance with the workload limits established for each individual.
In settings where cytology cases are screened and interpreted solely by a pathologist, the same factors listed above are applicable. Nongynecological specimens should be reported in a timely fashion.6

IV.D. Screening Process

As reported for the cervical cytology practice guidelines54 certain procedures should be followed. The process of screening should always begin with a check of slide identification (name and/or identifying number) against the accompanying accession slip, test request or pertinent laboratory document. The examiner must consider available patient history provided by the ordering clinician.

The screening process usually begins with a low power scan of the specimen to assess background and overall adequacy. The actual screening process is usually performed with a 10X objective. Higher magnification is used for more detailed observation of potentially abnormal areas. The slide should be screened in a systematic and thorough process. The cytotechnologist is responsible for locating and identifying reportable findings. To facilitate review the location of any abnormal cells or reportable findings should be marked in a consistent pattern by all cytotechnologists within the laboratory. When marking slides, care should be taken to avoid covering other significant cellular material.

IV.E. Recording Results and Hierarchical Review

After examining and marking the slide, the cytotechnologist records his or her findings. All findings must be recorded accurately, legibly and precisely for future reviewers and data entry personnel. Cytotechnologists should be able to discuss the basis of their interpretations as well as demonstrate them at the microscope. All nongynecological slides must be referred to a qualified pathologist for final interpretation.

IV.F. Variability in Practice

Depending on the practice setting, nongynecological cytology cases may be initially screened by a cytotechnologist with final interpretation rendered by a pathologist, or screened by a cytotechnologist, reviewed by a supervisory level cytotechnologist and the final interpretation rendered by a pathologist. Although all cytotechnologists are trained to evaluate specimens from all body sites, how specimens are assigned for evaluation may vary among laboratories. In laboratories without cytotechnologists the pathologist both screens and makes the final cytologic interpretation. Laboratories may also vary in methods used for cytologic preparation including filters, cytocentrifugation specimens, liquid based preparations and/or conventional smears. These various types of preparations may result in one-half or less of the total available slide area covered with cells. According to CLIA ’88 such slides may be counted as half a slide, which may become relevant for a cytotechnologist’s workload calculation.55 Which preparations count as whole or
half slides may vary from laboratory to laboratory. However, within an individual laboratory whole or half slide determination should be consistent.
V. Nongynecological Cytology Reporting

The nongynecological cytology report must include the patient’s name and/or unique identifier, patient’s age and/or date of birth, the name of the ordering physician (where appropriate) and the laboratory’s accession number. If the collection date is provided, it should be noted on the report. Information regarding the date and time of receipt of the sample and release of the report must be readily accessible if it is not printed on the report itself. The final report must include useful data that are legible and accurate, and must be released only to persons authorized by law to receive and use the information.

The nongynecological cytology report should include the type of specimen and quantity submitted, as well as a gross description of the specimen received (fixed or air-dried for smears, color and turbidity for fluids). The source/site and method of collection should be noted as well as the preparation method (automated liquid-based, cytocentrifugation, membrane filtration, or cell block.)

V.A. Reporting Specimen Adequacy and Cytological Findings

The laboratory must indicate on the patient report any information regarding the condition of a sample if it fails to meet the criteria for acceptance, such as inappropriate specimen submitted for the test requested, inadequate patient identification, lack of proper fixation, etc.

It is important to consider the adequacy of each sample and to note in the report if a specimen is suboptimal. “Insufficient for diagnosis”, “Non-diagnostic specimen”, or “Unsatisfactory”, are comments that can mean less than adequate samples. Commenting on the material that is submitted and its limitations is important to assist the clinician in patient follow-up. Factors limiting the interpretation of the specimen, such as degeneration or obscuring elements should be noted.

If the sample is diagnostic, the report should be as specific as possible using histopathological terms. If a specific interpretation cannot be rendered then the report should include a differential diagnosis as appropriate.

Laboratories must decide if any nongynecological cytology results qualify as critical values (i.e. immediate impact on patient care) for their respective institutions. If a laboratory has established critical values for nongynecological cytology then procedures must be in place for notification of the clinician who submitted the cytology specimen and this notification should be documented in a laboratory record. Any other results that are communicated to the clinician should also be documented.

Incorporating into the report any patient history and clinical information provided on the requisition may aid the clinician in correlating cytological with clinical findings. The laboratory should attempt to correlate cytological findings with histological material, if available.
V.B. Storage and Retrieval of Laboratory Records

See Section X.

V.C. Referral Laboratories

The laboratory director is responsible for selecting referral laboratories. All referral laboratories must be CLIA ’88 certified for high complexity testing. It is the responsibility of the laboratory director to monitor the quality of testing received from all referral laboratories. The name and address of the laboratory performing the test must be available to the requestor if it does not appear on the report. The referring laboratory must report all test results from the referral lab without alterations that could affect clinical interpretation. The referring laboratory must retain a record of all reports from testing performed by a referral lab.61

V.D. Variability of Practice

Nongynecological cytology cases may be screened by a cytotechnologist prior to examination by a pathologist or cases may be sent directly to a pathologist after processing. Cell blocks can be prepared for processing in the cytology or histology departments, while the cytology staff usually prepares cytology slides. If two separate reports are issued on a case for cytological slides and a cell block, respectively, then the same pathologist should interpret the two samples or the reports at least should reference each other. Any diagnostic discrepancies between the two specimen preparation methods should be explained.

The descriptive nomenclature used to report nongynecological cytology results varies. Laboratories can assign categorical terminology for use within an individual laboratory. Information regarding the adequacy of the sample should be conveyed to the clinician in the report. Some laboratories call clinicians with the results of all malignant cases; however, this is not required. In some laboratories, certain diagnoses require an additional pathologist review prior to releasing the report. Laboratories may document this additional review in the report or keep internal documentation only. For multiple reviews and consultations, refer to Section VI.
VI. Quality Control and Quality Assurance Practices

Quality control (QC) and quality assurance (QA) can be considered as the first two levels, respectively, in the hierarchical stages of quality. Quality control is defined as a system for verifying and maintaining a desired level of quality using operational techniques for an individual test or process. Quality control activities span the testing process, from pre-analytic (specimen collection and processing) through analytic (interpretive) and post-analytic (receipt of the report and analysis of results) phases. Quality assurance (QA) is defined by the College of American Pathologists as systematic monitoring of quality control results and quality practice parameters to assure that all systems are functioning in a manner appropriate to excellence in health care delivery. Quality assurance is a coordinated system designed to detect, control and prevent the occurrence of errors and, ultimately, to further a clinician’s ability to appropriately care for his or her patient. The third stage, quality system, consists of the comprehensive and coordinated efforts to meet quality objectives including the organizational structure and resources. Quality management, the fourth stage includes the first three and also the cost of quality. The hierarchy culminates with total quality management, which is management centered on quality, and aimed at long-term success through customer (patient, physician and payer) satisfaction.

A number of quality control and quality assurance measures for cytopathology have been specified by the Clinical Laboratory Improvement Amendments of 1988. All QC and QA processes must be described and documented in the laboratory.

VI.A. Pre-analytical Quality Control

Each laboratory must perform and maintain records of routine quality control relating to specimen collection, receipt and preparation. Most of these activities are required by lab accreditation agencies and include such things as:

- Preparation and distribution of clinical specimen collection and handling instructions,
- Assurance of properly labeled specimens,
- Use of a requisition that provides space for all pertinent demographic and clinical data,
- Accessioning and assignment of a unique specimen identifier,
- Criteria for specimen rejection,
- Review of stain quality and maintenance of stain quality records,
- Procedure for preventing nongynecological specimen cross contamination,
- Microscope and instrument maintenance,
- Instrument calibration records.

VI.B. Analytical Quality Control

VI.B.1 Screening of Nongynecological Cytology Specimens

Federal regulations require that the individual examining a cytology specimen be a qualified cytotechnologist or pathologist in a certified laboratory. These individuals may examine up to 100 slides (gynecological and nongynecological) per 24 hours (average 12.5 slides/ hour) and in not less than eight hours. This number is not a performance target but a maximum allowed by law.
Pathologists are limited by this ceiling when they perform primary screening. This includes nongynecological slides that have not been previously screened. Each laboratory must establish individual workload limits for each cytotechnologist. The Technical Supervisor of the laboratory must review these limits every six months and re-assess using lab defined performance standards. The record of slides reviewed by the primary screening cytotechnologist or pathologist must be documented and retrievable for inspectors during the retention period prescribed by CLIA ’88 or applicable state law. Cytotechnologists and pathologists must also maintain work logs for any primary screening site (in cases of multiple site employment), again, for the applicable retention period. As discussed in section VI, all specimens must be reported using descriptive nomenclature; use of a numerical reporting system alone is unacceptable.

VI.B.2 Review and Reporting of Nongynecological Cases
All nongynecological specimens must be referred to a pathologist for final interpretation and final report. Discordance between pathologist and cytotechnologist interpretation, if the cases are screened prior to pathologist examination, can be used as a basis for identifying areas for continuing education. Peer review is often included in a quality assurance program. Multiple people may review difficult or interesting cases for educational and interpretive purposes. Laboratories may require a second pathologist opinion for specific diagnoses and/or type of specimen. See Section VI for variability of documenting intralaboratory consultations. Seeking the opinion of an outside consultant may be considered for unusually difficult cases with significant clinical implications. Documentation of all reviews is essential for quality assurance monitoring.

VI.B.3 Rescreening of Negative Cases
Quality control rescreening of nongynecological cases is not required by CLIA or accreditation agencies. However, re-examination of a subset of cases by a second pathologist prior to release of the final report may be incorporated into the anatomic pathology quality assurance program. The re-examined cases may be randomly chosen or may be selected based on volume and complexity of workload and cytopathology resources.

VI.C. Post-analytical Quality Control

VI.C.1. Cytological-Histological Correlation and Clinical Follow Up
The laboratory must make an effort to correlate nongynecological cytopathology findings with histology and clinical findings. This can be for all specimens or for a focused subset of specimens. It is suggested that if significant disparities exist they should be reconciled. Cytological-histological correlation can be an educational tool used to refine methods of evaluation for both cytology and tissue specimens. The correlation process should be documented in the laboratory quality assurance program. If a nongynecological cytology specimen is collected concurrently with a tissue specimen, cytological-histological correlation is best performed prospectively. Ideally, the cytology and tissue reports should each refer to the other with integration of the correlation statement into either report. Reporting cytological-histological discordance may be helpful in directing further patient management. If an abnormal or nondiagnostic nongynecological cytology result is subsequently followed with tissue sampling, and
retrospective correlation is performed, then the result of the correlation should be documented. If histological material is not available, the laboratory may attempt to obtain patient follow-up by sending a letter to the ordering physician requesting this information.

VI.C.2 Retrospective Reviews
There are no federal or accrediting agency requirements for retrospective review of nongynecological cytology specimens. In certain clinical situations, review of previously examined specimens may affect current patient care by determining subsequent management protocols. Retrospective comparison of specimens from multiple body sites within a relatively short time span may be required for clinical staging, or comparison of a current specimen with one from the remote past may distinguish a metastasis from a second primary neoplasm. Amended reports are not indicated in these situations. Results of the review can be incorporated in the current cytology or tissue report or in a separate document. Retrospective reviews are subject to the biasing effect of knowledge of outcome, and this fact should be kept in mind during any such review.

VI.C.3 Measures of Performance
Nongynecological cytology can be both a screening test and a diagnostic procedure depending upon the clinical circumstances and specimen examined. Nongynecological cytology is limited (as are all laboratory tests) by both false positive (FP) and false negative (FN) results. As a screening test, a false positive is defined as a “positive” test result for a patient who does not have an abnormality. As a diagnostic procedure, a false positive could be defined as a malignant interpretation when in fact the patient has a benign neoplasm or perhaps as the presence of any neoplasm when the condition is reactive or inflammatory. Since “positive” results are variably defined in the medical literature, a standard definition for a false positive nongynecological cytology specimen does not exist.

A false negative is defined in this document as a negative or nondiagnostic nongynecological cytology result in a patient with an abnormality or lesion. False negative results may be a consequence of (a.) Sampling variance, (b.) Laboratory interpretation, or (c.) General limitations of the method. Sampling false-negatives occur when diagnostic cellular and noncellular material is not collected or is not transferred to the slide. A laboratory interpretive false negative is one in which diagnostic material is present on the slide, but is not identified during slide examination or is misinterpreted as to its significance.

The false negative rate is the sum of lesions missed in sampling plus the false negative proportion (FNP.) The FNP is the measure of the laboratory component of false negative results and is defined as the number of false negative reports divided by the total number of patients sampled who have an abnormality (False Negative Proportion = False Negative reports/True Positive reports + False Negative reports)._{72, 73, 74}

\[
\text{FNP} = \frac{FN}{TP + FN}
\]

The value of determining the FNP for a laboratory is widely acknowledged; however, precise calculation of the FNP requires 100% accurate determination of the true diagnosis. For
nongynecological cytology this requires exhaustive cyto-histologic and clinical correlation, which is impractical.

The College of American Pathologists Q-Probes studies provide a comprehensive resource for comparative laboratory data and performance benchmarks. These data are a good starting point for laboratory self-assessment since operational definitions, laboratory methods and statistical analyses are specified.66

VI.D. Proficiency Testing and Continuing Medical Education

Proficiency testing for nongynecological cytology is not mandated under CLIA ‘88 as it is for individuals who examine gynecologic specimens. However, a number of private institutions provide educational programs for self-assessment and performance improvement activities. Examples include:

- CytoQuest ® Glass Slide Program from Midwest Institute for Medical Education (MIME); www.cytoquest.com.
- CheckSample ®, CheckPath ® and STAR® Programs from the American Society of Clinical Pathologists; www.ascp.org.

These programs are not designed as proficiency tests nor should they be used as such.75

Ongoing education is a requirement for quality performance. This requirement can be fulfilled by participation in programs such as those listed above, intradepartmental slide review sessions, attending workshops and symposia, teaching cytotechnology students, pathology residents and fellows, independent study, and community outreach programs. Some states and professional societies have requirements for continuing medical education to maintain professional licensure.
VII. Data Management and Laboratory Information Systems

Manual methods as well as computerized systems exist for management of laboratory data. Manual methods may include logs and card files organized by date, patient name, specimen number or interpretation. Computerized systems, most often referred to as laboratory information systems (LIS), may stand alone, be part of an integrated anatomic pathology system, part of a multispecialty laboratory system, or integrated with a larger hospital or corporate information system. This section of the Guideline describes data management capabilities required for pre-analytic and analytic laboratory activities in addition to post-analytic information management.

VII.A. Security

All laboratory records are confidential. Access should be limited to authorized individuals. Locked cabinets for paper records and security codes for electronic systems are recommended. Limiting access may deter corruption of computer software or inadvertent change or release of results by unauthorized individuals. Electronic signatures are preferable for reports that are stored in electronic format. A procedure should be in place to assure that the electronic signature identifies the person who is responsible for the case and indicates that they approve of the content of the report. This procedure should prohibit interpretations that require pathologist review from being released by any other individual prior to the pathologist’s authorization.

VII.A.1. Health Insurance Portability and Accountability Act (HIPAA)

National health information privacy standards issued by the U.S. Department of Health and Human Services (DHHS), pursuant to the Health Insurance Portability and Accountability Act of 1996 were enacted in 2003. HIPAA was adopted to ensure health insurance coverage after leaving an employer and also to provide standards for facilitating healthcare-related electronic transactions. The HIPAA Privacy Rule provides the first national standards for protecting the privacy of health information. The Privacy Rule regulates how certain entities, called covered entities, use and disclose certain individually identifiable health information, called protected health information (PHI). PHI is individually identifiable health information that is transmitted or maintained in any form or medium (e.g., electronic, paper, or oral), but excludes certain educational records and employment records. Among other provisions, the Privacy Rule

- Gives patients more control over their health information;
- Sets boundaries on the use and release of health records;
- Establishes appropriate safeguards that the majority of healthcare providers and others must achieve to protect the privacy of health information;
- Holds violators accountable with civil and criminal penalties that can be imposed if they violate patients' privacy rights;
- Strikes a balance when public health responsibilities support disclosure of certain forms of data;
- Enables patients to make informed choices based on how individual health information may be used;
- Enables patients to find out how their information may be used and what disclosures of their information have been made;
• Generally limits release of information to the minimum reasonably needed for the purpose of the disclosure;
• Generally gives patients the right to obtain a copy of their own health records and request corrections; and
• Empower individuals to control certain uses and disclosures of their health information.76

VII.B. Accessioning and Work Flow

The laboratory must assign a unique accession number for each individual case. All patient demographic data required by regulatory agencies should be entered at accessioning. The unique accession number facilitates the tracking of a case through all stages of handling in the cytology laboratory from pre-analytic (accessioning and specimen preparation,) and analytic (screening and interpretation,) through post-analytic processing (reporting, and quality assurance follow up.)77 Labels for paperwork and slides may be handwritten, purchased, printed with a stand-alone printer or generated by the LIS as part of accessioning. Bar coded labels can increase the efficiency and accuracy of this process.

VII.C. Record Storage and Retrieval

The laboratory must have the ability to record and retrieve specimen information and patient reports for the periods specified by regulatory agencies.6, 78 The system, whether manual or automated, should allow access to all cytology reports and all available and related surgical pathology reports to facilitate cytologic/histologic correlation. Older data may be electronically archived or records may be stored offsite as long as retrieval does not hinder patient care or delay regulatory inspections. The ability of a system to correlate or merge records when there is an alteration in patient identifiers (such as name, hospital record number or other identifiers) without altering the data in the original records is also desirable. The use of unique identifiers, such as the patient’s hospital record number, allows for more accurate matching.

VII.D. Terminology

Standardized terminology (preferably Systematized Nomenclature of Medicine [SNOMED]) used in the LIS should be stored in the computer database and accessed by use of mnemonics or assigned codes. Free-text capability is necessary for rare or unusual interpretations, microscopic descriptions, and for comments and/or recommendations that are not routine. Manual reporting should be standardized to allow retrieval of data based upon interpretation.

VII.E. Data Transfer

Transfer of clinical information and interpretive data to the report must be precise. This may occur via a manual written report, by manual entry into the LIS, or by use of optical mark readers that are interfaced with the LIS. The accuracy of this information must be monitored through the
In addition to storing patient information and reports, laboratory information systems (LIS) may be used to generate billing statements or to transfer data to billing systems, clinician offices, hospital computer systems, Medicare, and other third party payers. Linkage of reports to interpretation and procedure codes [International Classification of Disease (ICD-9)], hospital procedure and billing codes [HCFA Common Procedure Coding System (HCPCS)] and Current Procedural Terminology (CPT) codes may be required for billing purposes. Linkage of reports to SNOMED (Systematized Nomenclature of Medicine) is desirable for statistical reporting.

VII.F. Quality Assurance

Laboratory data must be retrievable for quality assurance purposes and to generate statistical reports required by regulatory agencies and accrediting organizations within the retention period prescribed by CLIA ’88 or applicable state regulations. At a minimum the laboratory must document the number of nongynecological cytology cases. It is desirable for the LIS to facilitate random or directed selection of cases. The LIS should not allow release of results until the rescreen examination is complete. Patient information should be stored in a manner that easily allows for cytologic/histologic correlation.

VII.G. Variability of Practice

Differences between manual and electronic data management systems are discussed throughout this section and encompass most practice settings.
VIII. ANCILLARY TESTING

Ancillary testing is used in cytology at the discretion of the pathologist and according to the clinical circumstances. Light microscopy alone may not always yield sufficient information to render a specific diagnosis. Tumor cells may be poorly differentiated and their origin morphologically obscured. Microorganisms may be difficult to identify by routine cytologic stains. There are special tests that can assist in rendering the most specific diagnosis possible.

VIII.A. Cytochemical Stains

Cytochemical stains can help in the identification of and discrimination between

1. Organisms (e.g. bacteria, fungi)
2. Enzymes (e.g. chemical substances, such as acid phosphatase)
3. Other intra and extracellular substances (e.g. fat, mucin, amyloid, collagen, melanin)

The appropriate use of controls is of utmost importance in cytochemical stains. Refer to VIII.G. for further discussion.

VIII.B. Immunocytochemical Stains

Immunocytochemical stains use monoclonal and polyclonal antibodies to detect antigens. These antigens may indicate the tissue of origin for poorly differentiated neoplasms, may identify infectious organisms, and may aid in distinguishing malignant from benign processes. In rare instances it may be appropriate to use a single immunocytochemical stain; however, it is much more common to use a panel of immunocytochemical stains to arrive at the correct diagnosis. Immunocytochemical stains can be used on cytologic samples, including all forms of slide preparations and cell blocks, with appropriate controls (refer to section IXG). This is a rapidly evolving and growing field. Some stains are commercially available; some are available for research use only. 82,83,84

VIII.C. Transmission Electron Microscopy

Although used extensively in the 1970’s and 1980’s, immunocytochemical stains have supplanted transmission electron microscopy as an ancillary test, except in certain circumstances. Cytologic preparations are ideally suited for this study because of the large number of cells they contain and because they are often a single cell type. Certain neoplasms have ultrastructural qualities that may help in their identification. The high magnification of electron microscopy elucidates specific ultrastructures 85 such as,

1. Extracellular features (e.g. amyloid)
2. Cell surface features (e.g. microvilli, intercellular junctions)
3. Cytoplasmic features (e.g. mitochondria, lysosomes, neurosecretory granules, melanosomes)
4. Nuclear features (e.g. inclusions)
5. Other (e.g. viral particles)

Special preparation of the cells, such as fixation in glutaraldehyde, is needed.
**VIII.D. Flow Cytometry/ Image Analysis**

Flow cytometric analysis is used primarily in the analysis of suspected hematologic malignancy. Characterization of cell surface markers and intracytoplasmic markers are performed on live cells in single cell suspensions. Cells must be submitted in tissue culture medium, e.g. RPMI, to maintain viability necessary for processing.

DNA analysis can also be performed by flow cytometry on fixed or fresh cells, although this application is less popular than in past decades.

**VIII.E. DNA or RNA Amplification (Polymerase Chain Reaction (PCR))**

Another ancillary test available, but not widely used in cytologic preparations, is DNA or RNA amplification. This technique is used to identify genetic abnormalities or certain DNA or RNA markers. This is a highly specific ancillary study that can be performed with only a small amount of sample. Specific mutations or specific DNA gene sequences may identify certain disease processes. DNA or RNA amplification can be used to rapidly determine the presence of viruses or other organisms, such as Mycobacterium tuberculosis.86

**VIII.F. Fluorescence In Situ Hybridization (FISH)**

Fluorescence in situ hybridization (FISH) is an ancillary test that allows the visualization of genetic alterations on a cell-by-cell basis.87 Current cytopathology textbooks include sections regarding FISH applications.88,89 Monolayer cell preparations facilitate the application of FISH in cytopathology. Optimal results are obtained on specimens with moderate cellularity;90 however, successful application has been demonstrated on specimens of low cellularity. FISH can be applied to fine-needle aspirates, smears, tissue imprints and disaggregated whole cells from paraffin sections.91

**VIII.G. Ancillary Test Limitations**

Ancillary tests have their own limitations. Each of these studies should be used in conjunction with initial morphologic evaluation along with clinical information. False positive and false negative results can occur.

Immunocytochemical tests may cross-react with antigens that are not intended to be reactive, or may have high background staining, which renders the test less interpretable. Positive and negative controls should be performed with each sample. An antibody specific and method specific control tissue, cell block or cytology preparation simultaneously stained with the unknown or target specimen is required.92 The ideal control in cytology is a cytology sample; however, tissue or cell block controls are widely used for practical purposes.

Electron microscopy utilizes a minute sample of tissue and is subject to sampling error. Suboptimal specimen preservation and areas of necrosis can contribute to problems in diagnosis.93
Flow cytometry requires a sufficient number of viable cells and sometimes only limited panels are possible.

DNA quantitation by image analysis is time consuming and requires a skilled morphologist.\textsuperscript{93}

PCR is very sensitive; however, there is a high risk of contamination.\textsuperscript{93}

Although FISH is becoming more widely available, it is still expensive and time consuming.\textsuperscript{93}

**VIII.H. Variations in practice**

Several different techniques can be used in the preparation of the ancillary studies. The range of available ancillary tests will vary among laboratories. Some of these ancillary studies may not be available in every institution and may be referred to laboratories that offer these specialized services.
IX. Archiving and Interlaboratory Slide Review

IX.A. Slide Storage and Retrieval

Cytology laboratories must retain all nongynecological slide preparations, regardless of diagnosis, for five years from the date of microscopic examination, or for longer if state regulations require it. Fine needle biopsy glass slides must be retained for a minimum of 10 years, unless local regulations require longer retention. Slides may be stored on-site in the laboratory or on institutional premises, or may be stored off-site. Whether stored on-site or off-site, slides must be retrievable within a reasonable amount of time for histologic-cytologic correlation or as requested for external inspection procedures. Slide breakage and slide loss may occur on rare occasions. When breakage is discovered, there should be appropriate documentation of the incident and repair of the slide if possible.

IX.B. Records Storage and Retrieval

As is the case with storage and retrieval of slides, records may be stored on-site in the laboratory or on institutional premises, or may be stored off-site. Whether stored on-site or off-site, records must be retrievable within a reasonable amount of time if retrospective review is necessary or as requested for external inspection procedures. The storage and retrieval site should allow for appropriate patient care and quality assurance practices.

If reports are stored in a computerized information system with appropriate backup, (such as microfilm, microfiche or other electronic storage) laboratories are not required to retain paper copies of reports. Such stored report records must contain the same information ("exact copy") that is sent to the authorized individual who orders or utilizes the test report. However, it is not required that an "exact copy" be an exact duplicate of the report. Exact copies must also contain the signatures (electronic or manual) when required.

IX.C. Retention Requirements

Cytopathology records should be retained for an appropriate time period, allowing for optimum patient care and quality assurance practices. Laboratories must comply with local standards for retention.
• Test requisitions must be retained for 2 years from date received
• Test reports must be retained for 10 years from date of the report
• Logs and accession records for cytology specimens must be retained for 2 years from date of receipt.
• Quality control records for cytology specimens must be retained for 2 years from the date that they were created/generated.
• Documents pertaining to discontinued procedures for cytology specimens must be retained for 2 years from the date that they were discontinued.
• Maintenance records for instruments used in processing and analyzing cytology samples must be retained for 2 years after the instrument(s) has been out of use.
• All nongynecological cytology slides, regardless of diagnosis, must be retained for 5 years from date of examination.
• All fine needle biopsy slides must be retained for at least 10 years from date of examination according to the College of American Pathologists.

IX.D. Loaning of Slides for Proficiency Testing Programs and Interlaboratory Slide Review

Slides may be loaned to proficiency testing programs in lieu of maintaining them for this time period. The laboratory must receive acknowledgment of the receipt of slides by the proficiency-testing program and maintain documentation of the loan of such slides thereby allowing retrieval of the slide(s) if needed. Documentation of slides that are loaned or referred for purposes other than proficiency testing (such as for interlaboratory slide comparisons, consultation, or educational purposes) also must be maintained. ⁹⁶

IX.E. Discarding Slides and Records

Slides and records that are outside retention and retrieval requirements may be discarded. When discarding such materials, patient confidentiality must be insured. The disposal process must result in the inability to identify the patient. If outdated/expired materials are retained for educational or research purposes, then patient identifiers should be removed.

IX.F. Requirements for Cytology Materials Received from or sent to Secondary Laboratories (Reference or Referral Laboratories)

The laboratory in which the slides were actually examined for final interpretation must store the slides. A reference or referral (secondary) laboratory is responsible for storing slides interpreted in that laboratory for the retention period. Reports must clearly state which laboratory performed the interpretation. ⁹⁴ For cytological-histological correlation, the reference or referral laboratory may review previous cases stored in the laboratory's files or may request slides from another laboratory, carefully following procedures for documenting loaned material (Section X.D).
IX.G. Variability in Practice

- Slide retention requirements for state and federal regulations and professional accreditation organizations may vary. CLIA ’88, College of American Pathologists, and state regulations should be consulted.
- Academic and research goals may merit longer slide storage by individual laboratories.
- Restrictive slide storage and access policies may be necessary on the basis of federal regulations mandating slide storage and custody.
- The systems by which laboratories retain, store and retrieve slides and records vary. For example, laboratories may store these materials in accession number order, by patient name, by date received or reported, by interpretive categories or by other means.
X. Laboratory Cost Accounting and Financial Management

Clinical laboratories need to be accurate and realistic in accounting practices if they are to be viable in a competitive market. Financial accounting and cost accounting are separate entities. Financial accounting provides external financial reports for a business entity whereas cost accounting is a measure of the current internal economic state of the business. Cost accounting is an important laboratory management tool for determination of laboratory test pricing. The elements used to calculate the cost of a laboratory test are multifaceted and complex. More comprehensive reviews of cost accounting methods not covered in this document are available including NCCLS Approved Guidelines. Mathematical formulas and computer software programs are also available for this purpose.

Costs are divided into direct, indirect, variable and fixed categories. To ensure an appropriate cost per test all expenses must be captured including preanalytical, analytical and postanalytical components. Direct costs include those items that are readily measurable. Included in this group are salaries with fringe benefits, costs for reagents and consumable supplies, capital for testing equipment, and equipment maintenance expenses. Indirect costs (or overhead) can be more difficult to quantitate than direct costs. Indirect costs include administrative oversight, logistics (couriers and specimen transportation), facilities (real estate, building maintenance, utilities and furnishings), quality assurance, marketing, sales, malpractice and legal expenses and information technology. Indirect costs vary depending on the practice setting such as hospital versus independent laboratory. Variable costs are affected by specimen volume and market variation for suppliers’ costs. Fixed costs are not affected by change in volume and include such items as rent or mortgage and salaries.

NCCLS recommends that a cost analysis be performed on a biannual basis or when a new test is introduced. Cost analysis is one of many factors that are considered in determining laboratory test pricing. Price is often guided by what the market will bear and should be above the laboratory’s cost for each individual test.
XI. Respiratory Tract Supplementary Information

XI.A. Epidemiology and Public Health

XI.A.1. Incidence and Mortality
Lung cancer is now the most common fatal malignancy in both sexes and its incidence is second only to prostate cancer in men and breast cancer in women. In the year 2001, 169,500 new cases of lung cancer, with 157,400 deaths were expected (90,100 men; 67,300 women.) Cancer of the lung occurs at an average age of 60 years, and is rare before 40 years of age.

XI.A.2. Risk Factors for Lung Cancer

XI.A.2.a Tobacco Smoke
Lung cancer is strongly associated with tobacco smoking, particularly cigarettes. There is also an increased risk associated with secondary smoke or “passive smoking”. It is estimated that 87% of lung cancers occur in tobacco smokers. The risk pertains to all major types of lung cancer, particularly squamous and small cell types, which rarely occur in patients who have never smoked, and also adenocarcinomas, although they are less strongly associated with smoking. Tobacco smoking is also associated with a wide-variety of other cancers, including head and neck, gastrointestinal, and bladder cancers, as well as other non-malignant conditions, such as chronic obstructive pulmonary disease.

Although many continue to smoke despite the well-known risks of tobacco usage, the good news is that smoking rates have been decreasing, particularly among males. A downward trend in smoking should be followed by a decrease in overall lung cancer mortality, which, if current trends continue, may in fact have begun in 1997. For those who have quit smoking for 10 years or more, the lung cancer rate approaches that of persons who never smoked. There also has been a shift in the histologic type and location of lung tumors. In men and women, the relative proportion of adenocarcinomas, particularly bronchioloalveolar carcinoma, and peripherally located tumors has increased markedly. These tumors are less strongly associated with smoking.

XI.A.2.b. Other Co-Factors
The pathogenesis of lung cancer is probably multifactorial, including environmental and genetic factors, such as radiation, air pollution, diet, and occupational exposure to certain metals (e.g., arsenic, nickel, chromate, cadmium) and chemicals (e.g., chloromethylesters, acrylonitrile, vinyl chloride). Radon is perhaps the second most common cause of lung cancer. Genetic or familial cases of lung cancer also occur. Lung cancer is the most common malignancy associated with asbestos exposure. Asbestos potentiates the ill effects of tobacco smoking, and in those exposed to asbestos, the risk of lung cancer in smokers is substantially increased compared with non-smokers. Pre-existing pulmonary disease, such as interstitial fibrosis and emphysema, may predispose to lung cancer. Viruses may also be associated with the development of some lung cancers.
The actual mechanism by which cells become malignant is thought to involve an accumulation of genetic alterations, including oncogenes, such as \textit{c-myc} in small cell carcinomas and \textit{K-ras} in adenocarcinomas, with loss or inactivation of tumor suppressor genes, such as \textit{p53}, and alterations of the short arm of chromosome 3.\textsuperscript{135,136} It is estimated that by the time a tumor has become clinically apparent, some 10 to 20 genetic mutations have occurred.\textsuperscript{137}

**XI.A.3 Screening**

The most common routine screening tests for lung cancer are chest radiograph (x-ray) and sputum cytology. However, these procedures are no longer recommended for routine screening of asymptomatic persons, because they lack sufficient sensitivity and specificity.

The accuracy of the chest x-ray is limited by the capabilities of the technology, suboptimal technique, and observer variation among radiologists. By the time lung cancer is suspected on chest x-ray, micrometastases often have occurred, limiting the effectiveness of early detection. Furthermore, the yield of screening chest radiography is low, largely due to the low prevalence of lung cancer in asymptomatic individuals, even those at high risk.

A significant reduction in the mortality rate is the gold standard for any cancer-screening test. A 20-year follow-up study of the Mayo Lung Project (MLP) reinforces the conclusion published in 1986 that screening for lung cancer by frequent chest x-rays does not save lives.\textsuperscript{138} Perhaps more importantly, these latest findings suggest that aggressive screening for lung cancer could do more harm than good, by detection of lesions that will never cause serious illness or death. In the absence of screening, it is likely that these incidental lesions would never be found. Such over-diagnosis can lead to unnecessary anxiety or, more seriously, to expensive and risky biopsies or surgery. These findings may diminish enthusiasm for newer, ever more sensitive screening technology.

Sputum cytology is an even less effective screening test than chest x-ray, largely due to its low sensitivity. In screening trials, the sensitivity of sputum cytology ranges as low as 10% to 20%. When combined with chest x-ray, only one in four cancers are detected by sputum cytology alone; most of those were squamous cell carcinomas found at a favorable stage. Mass screening to detect lung cancer with tests that lack a high sensitivity is inefficient. Moreover, the spectrum of lung cancer type has shifted over the last two decades. Squamous cell carcinoma used to be the most common type of lung cancer and is usually centrally located where it is more readily detected by sputum cytology. Now, adenocarcinoma is the most common type and it is usually peripherally located where it is less readily detected by sputum cytology.\textsuperscript{139,140} Another disadvantage of sputum cytology is that even when a cancer is detected, another method must be used to localize the lesion. Still, attempts to refine the use of sputum cytology for early cancer detection continue.\textsuperscript{141}

At present lung cancer is mainly treated in advanced stages. Identifying molecular markers that could detect the disease while still confined to the bronchial epithelium would potentially allow cures with local therapies. Preliminary data suggest that over-expression of a tumor-associated antigen, heterogeneous nuclear ribonucleoprotein (hnRNP), is found in normal appearing lung epithelium in patients with lung cancer. If biomarker-based screening were found to be accurate, this could lead to identifying disease at a much earlier and more treatable stage. Aerosol delivery of
therapeutic agents is a possible solution to the problem of treating multiple lesions in the
tracheobronchial tree.

In summary, there is no compelling evidence that screening for lung cancer using chest x-ray or
sputum cytology can reduce lung cancer mortality. No major medical organization currently
recommends routine screening of either the general population or of smokers for lung cancer with
either chest x-rays or sputum cytology. There are intensive efforts to improve
lung cancer screening with newer technologies (e.g., low-radiation-dose [spiral] computed
tomography) and molecular techniques which, although promising, have not been validated in
large controlled studies. Counseling people against the use of tobacco products is currently
the best method to reduce lung cancer mortality.

XI.B. Specimen Collection and Submission

The diagnostic reliability of respiratory cytology depends upon many factors that affect sensitivity
and specificity. Time and method of specimen collection, number of samples submitted, location
of the tumor, and tumor type affect sensitivity. Specificity is affected by state of preservation, lack
of clinical history, background necrosis and inflammation, as well as cellular differentiation.

XI.B.1. Sputum
Sputum has been a mainstay for the diagnosis of lung cancer with the first reports by Hampeln in
1876 and 1887, Menetrier in 1886 and Betschardt in 1895. Earlier reports of malignant cells in
sputum by Walshe in 1843 and Beale in 1860 represented exfoliated cells from upper airway
tumors.

Sputum is most easily obtained in patients who are symptomatic and have a productive cough.
Three to five sputum specimens will most likely detect malignant cells. Pooled morning
samples are the optimal specimens in which to detect cancer cells in symptomatic patients.

Induced sputum, collected by specially trained individuals, may be necessary in patients with a
lung mass who are not producing sputum. Nebulizing solution stimulates secretions in the
respiratory tract. Nebulized solutions are varied and may include: 15% nebulized saline, 15%
saline with 20% propylene glycol, or heated (115°F) 3-8% saline. Hypertonic solutions are
more successful in inducing sputum, but less well tolerated. While cytologists are most familiar
with induced sputum specimens used for diagnosis of cancers, in the last decade they have been
used for assessing the inflammatory components of patients with asthma, sarcoidosis and chronic
obstructive pulmonary disease. Induced sputum specimens are also used for the investigation
of opportunistic organisms in immunosuppressed patients.

Postbronchoscopy sputum is used in conjunction with bronchial washings and brushings for
diagnosis of carcinoma. It may have a higher diagnostic rate than standard sputum collection.

Fresh sputum submitted immediately to the laboratory is preferred. Preservation with 50% to 70%
ethanol may be necessary when specimens cannot be directly submitted. Saccamanno fixative
consisting of 50% ethanol with polyethylene glycol (Carbowax®) is also used. Proprietary liquid based cytology medium should be added if using liquid based cytology processors. Some laboratories use mucolytic agents in the preparation process for sputum specimens.159,160,161,162

XI.B.2. Bronchial Brushing
Bronchoscopy is performed when patients have respiratory symptoms or a radiologically evident lung mass. During fiberoptic bronchoscopy the operator may wish to sample a lesion by brushing. After brushing, the cellular material may be submitted as follows:
- The surface of the brush may be rotated on slide(s) and immediately fixed and/or
- The brush may be submitted in a physiologic transport solution or a proprietary solution used for liquid based techniques or
- The brush may be discarded after completely removing the adherent cellular material into a container of fixative or transport solution.

XI.B.3. Bronchial Washing
During bronchoscopy small aliquots of balanced saline solution are washed over a directly visualized area and removed immediately by using suction. These washings are usually submitted immediately to the laboratory. If a delay is anticipated, they may be partially fixed in an amount of 50-70% ethanol that is equal to the specimen volume, or in the proprietary transport medium supplied by one of the manufacturers of liquid based processors.

XI.B.4. Bronchoalveolar Lavage
Bronchoalveolar lavage (BAL) is a method of sampling the lower respiratory tract. A bronchoscope is advanced until it is “wedged” into a subsegmental bronchus. Saline or a balanced salt solution that is suitable for use in vivo is introduced and re-aspirated. The volume of fluid and the extent of the lavage depend upon the suspected disease and the training and preference of the bronchoscopist, as well as the patient’s tolerance. Usually 20-60 mL aliquots of fluid are used to separately sample up to three subsegmental areas of the lung. In this manner, 1% of the lung, or 1 to 3 million alveoli are sampled. The samples initially contain respiratory epithelium, but the latter portions of the aliquots are enriched for alveolar components. BAL is performed for the detection of microorganisms, interstitial lung disease, transplant rejection, pulmonary hemorrhage, acute inflammatory diseases, disorders in which lymphocytes predominate, and malignancy. BAL specimens are submitted fresh because microbiologic studies, immunologic studies or chemical analyses may be requested. When separate aliquots are provided for ancillary studies then the BAL specimen may be fixed as previously described for bronchial washings (section II.C.).

XI.B.5. Transbronchial or Transesophageal Fine Needle Aspiration Biopsy
Transbronchial fine needle aspiration biopsy is performed during the bronchoscopic procedure to sample endobronchial or peribronchial lesions and peritracheal or peribronchial lymph nodes, usually for evaluation of malignancy. Transesophageal FNAB is usually performed to evaluate paraesophageal abnormalities in the chest cavity or mediastinal and lower thoracic lymphadenopathy. A small, sheathed needle is advanced during bronchoscopy or endoscopy, and under fiberoptic visualization is introduced into the lymph node or lesion. Suction is applied while vigorously sampling the site. Suction is released, the needle is re-sheathed and removed from the
bronchus. The material is expressed onto slides and direct smears are prepared as described in section II.A.4. Additionally or alternatively, the needle can be rinsed in transport medium and submitted as a liquid based sample. The needle should never be submitted.9

XI.B.6. Pulmonary Microvascular Cytology
Pulmonary microvascular cytology is not commonly used to evaluate pulmonary lymphatic carcinomatosis. A pulmonary artery is catheterized and the catheter is wedged into a small vessel. A blood sample from the wedged pulmonary catheter is collected into a heparinized tube. The heparinized blood sample is processed to separate red cells from any diagnostic cells, most frequently by gradient centrifugation.164 The residual white cell components are evaluated for the presence of carcinoma. Megakaryocytes, which signal an adequate specimen and which are normally seen in the pulmonary bed, must be distinguished from cancer cells.165

XI.B.7. Percutaneous Thoracic Fine Needle Biopsy
Percutaneous thoracic FNAB is performed for evaluation of any pulmonary abnormality, but is usually used for evaluation of suspected malignancy. The diagnostic sensitivity ranges from 75-95% and the specificity from 95-100%.166 Percutaneous transthoracic FNAB is usually radiologically guided. A variety of imaging modalities are used including computerized axial tomography (CT) scan, fluoroscopic guidance and ultrasound guidance. The lesion is entered with a hollow needle containing a stylet. The stylet is removed and the lesion is aspirated.

Pneumothorax is the most significant complication, but of those patients experiencing pneumothorax, 5-10% require treatment; most cases of pneumothorax resolve without intervention. Hemoptysis occurs in up to 8% of patients. Rare complications of air embolism do occur. Contraindications for performing the procedure may include an uncooperative patient unable to remain still during the procedure, anticoagulation therapy or bleeding diathesis, poor lung function, pulmonary hypertension, or a suspected vascular lesion.166

Because lung FNAB is associated with possible serious complications, a cytologist is often requested to perform intraprocedural adequacy evaluation to avoid multiple thoracic punctures. Assessment of adequate pulmonary specimens includes evaluation of: overall cellularity, architecture, presence of malignant features, presence of inflammatory features, and cellular elements that explain the lesion that is identified radiologically. Once adequacy has been established, the cytologist may direct triage of remaining material or subsequently obtained material for appropriate ancillary studies such as cultures, flow cytometry or immunologic studies. See section IX.

Post procedural x-rays are often obtained by the attending clinician to detect pneumothorax.166

XI.B.8. Pleural Fluid
Normally, not more than 15 mL of pleural fluid exist in the pleural space, although liters of blood plasma are filtered daily through the semipermeable membrane of endothelial capillaries of the mesothelial coverings. Clinically, pleural fluid accumulations of less than 250 mL are undetectable, but radiological imaging can usually detect smaller amounts.167,168 Pleural fluid specimens are usually obtained by thoracentesis or during thoracic surgery. Thoracentesis is
usually performed under local anesthesia with radiological guidance. Sterile technique is used to prepare the skin and the thoracic wall is punctured entering the thoracic cavity with a large caliber needle. For small fluid accumulations the entire specimen is submitted for laboratory evaluation. For larger pleural effusions, 50 mL of well-mixed fluid should be sent for cytologic examination; however, the entire specimen is also acceptable. All fluid specimens must be transported in accordance with OSHA regulations for biohazardous substances.

Pleural fluid is collected into a dry container and submitted in the fresh state to the laboratory. Adding 3-5 IU heparin/mL to a container prior to obtaining a bloody sample will not adversely affect morphology and will usually prohibit clotting. If delay in transportation to the laboratory is unavoidable, fluids may be kept refrigerated at 4°C until the specimen can be processed. The chemical composition of pleural fluid is such that it usually maintains cellular integrity for up to a week or more with refrigeration. However, some laboratories opt to partially fix specimens at the time of collection with 50% ethanol equal to the volume of the specimen. Alternatively, proprietary transport medium supplied by the manufacturers of liquid based processors may be used. Any added fixative should be noted on the requisition.

XI. C. Laboratory Sample Processing

XI.C.a. Sputum

Sputum samples received fresh can be processed by the pick and smear technique. Less widely used is the Saccamanno collection technique. In this technique, sputum samples are collected in 50% ethanol and 2% polyethylene glycol (Carbowax\textsuperscript{®}). The sample is homogenized in a blender, concentrated by centrifugation, and smears are prepared from the cell pellet. Sputum specimens can be collected in alcohol or proprietary transport medium supplied by the manufacturers of liquid based systems. Alcohol or proprietary transport medium supplied by the manufacturers of liquid based systems may also be added to fresh specimens that are received in the laboratory. Slides can then be prepared using cytocentrifugation, manual or automated filtration, sedimentation and/or cell block.

XI.C.b. Bronchial Brushing

Bronchial brushings may be collected from multiple sites in the respiratory tree during a single procedure. Bronchial brushings may be received as direct smears, fluid-filled containers with brushes enclosed, or liquid specimens in which the sample was removed from the brush. The type of sample received dictates processing. By convention, slides prepared from bronchial brushings are stained using a Papanicolaou stain. However, if submitted as air-dried preparations, they are amenable to Romanowsky staining. The number of slides, the variety of specimens, and the site(s) of brushing should be documented.
XI.C.c. Bronchial Washing

Bronchial washings are recovered under bronchoscopic visualization from a number of sites. They are usually low volume, fresh specimens. If a delay is expected in transportation to the laboratory, bronchial washings may be received preserved in 50-70% ethanol equal to specimen volume, or in the proprietary transport medium supplied by the manufacturers of liquid based processors. Depending upon their volume and cellularity, they may first need to be centrifuged (III.B.2) with the re-suspended pellet being used for direct smears, preparation of slides by using automated liquid-based methods, filtration or subsequent cytocentrifugation. A cell block can also be prepared from the centrifuged sample. Specimens of low cellularity and low volume may be cytocentrifuged directly or processed using automated liquid based systems. Specimens submitted in commercially available preservative products should be processed as indicated by the manufacturer. Cell blocks can be prepared from these liquid based processed specimens. Most bronchial washing preparations are stained by the Papanicolaou technique.

XI.C.d. Brochoalveolar Lavage

Bronchoalveolar lavage (BAL) specimens are often received fresh and may be submitted as separate aliquots or as a single specimen. Volumes vary depending upon the preference of the bronchoscopist, the disease process and the site(s) of abnormality. Additional microbiologic studies, immunologic studies or chemical analyses may be requested on BAL specimens, and therefore, these specimens should not be fixed, unless those studies are requested separately and separate aliquots are provided for the ancillary studies a priori. Depending upon the local laboratory practice, BAL specimens may first be sampled by microbiologic laboratories, or for cell counting prior to submission to cytology. BALs should be processed as detailed in III.B.2. Slide preparations are often stained by the Papanicolaou stain, Romanowsky stain (to detect organisms) and a variety of special stains for the evaluation of specimens from immunosuppressed patients. Coordination and communication among multiple laboratory sections is important in the analysis of BAL specimens.

XI.C.e. Pleural Fluid

Pleural fluid specimens should be accessioned, processed and stained as outlined in section III, depending upon their volume, presence of fixation, and gross characteristics.

XI.C.f. Fine Needle Biopsy

Fine needle biopsies of the respiratory tract may be obtained by percutaneous transthoracic fine needle aspiration, or by endoscopically or bronchoscopically directed fine needle aspiration. The material may be received as direct smears, needle rinses in transport medium or entire specimens submitted in a proprietary transport medium supplied by the manufacturers of liquid based processors. These specimens should be processed and stained as indicated in section III.
XI.C.g. Pulmonary Microvascular Cytology

These specimens are rarely used for evaluation of pulmonary lymphatic carcinomatosis. Density gradient centrifugation is used to concentrate the nucleated cells from a heparinized sample of pulmonary artery blood.\textsuperscript{164} The buffy coat is used for smears or other preparations. Megakaryocytes indicate that the specimen is adequate for evaluation.\textsuperscript{165} Papanicolaou or Romanowsky stains may be used for morphologic assessment. Ancillary immunologic stains may be necessary to separate megakaryocytes from malignant cells.

XI.D. ANCILLARY TESTING

Immunocytochemistry in respiratory tract cytopathology can be useful in supporting a diagnosis of cancer, subclassifying a tumor, or identification of an infectious agent. It can help in the identification or differentiation of:\textsuperscript{173}

1. Anaplastic/pleomorphic carcinoma
2. Adenocarcinoma versus mesothelioma
3. Neuroendocrine tumors versus other neoplasms
4. Primary versus metastatic tumor
5. Unusual primary tumors
6. Microorganisms (e.g. Pneumocystis carinii)

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