COLLEGE of AMERICAN PATHOLOGISTS

December 2024 Changes

Cytopathology Checklist

CAP Accreditation Program

College of American Pathologists
325 Waukegan Road
Northfield, IL 60093-2750
www.cap.org

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Using the Changes Only Checklist

This document contains new checklist requirements, major and minor requirement revisions, and changes to explanatory text. **Changes appear in a track changes format that compares the previous checklist edition to the December 26, 2024 edition.** Requirements with significant revisions will display a “Revised” flag. These changes may affect your laboratory operations. Requirements with minor revisions will not display a “Revised” flag. They are editorial changes that are not likely to affect your laboratory operations.

Information regarding requirements that are new or have been combined, moved, resequenced or deleted, as applicable, appears in table format below.

2024 CHECKLIST EDITION CHANGES
NEW, DELETED, MERGED, AND MOVED REQUIREMENTS *

2023 Requirement		Action Taken	2024 Requirement	
		New	CYP	04335
		New	CYP	07620

*Deleted – Removed the requirement from the checklist edition
*Merged – Combined the requirement with a similar requirement in the same or different checklist
*Moved – Relocated the requirement to another checklist or resequenced it within the same checklist

ON-LINE CHECKLIST DOWNLOAD OPTIONS

Participants of the CAP accreditation programs may download the checklists ~~from the CAP website (cap.org)~~ by logging into cap.org and going to e-LAB Solutions Suite - Accreditation Checklists. They are available in different checklist types and formatting options, including:

- Master — contains ALL of the requirements and instructions available in PDF, Word/XML or Excel formats
- Custom — customized based on the laboratory's activity (test) menu; available in PDF, Word/XML or Excel formats
- Changes Only — contains only those requirements with significant changes since the previous checklist edition in a track changes format to show the differences; in PDF version only. Requirements that have been moved or merged appear in a table at the end of the file.

INTRODUCTION

This checklist is used in conjunction with the All Common and Laboratory General Checklists to inspect a cytopathology laboratory section or department.

Laboratories that do not file slides on-site (eg, "read-only" laboratories) must retain a sample of slides on-site for review by the inspector on all days when the laboratory is subject to its regular on-site inspection. The sample must, at minimum, include all slides accessioned over a continuous two-week period within the previous two years.

If telepathology is used by the pathologist or cytotechnologist to review slides or images for primary diagnosis of cytology or real time evaluation of FNA specimens for adequacy or triaging, refer to the Telepathology section of the Laboratory General Checklist for additional requirements. Telepathology occurs when a pathologist views digitized or analog video or still image(s), and renders an interpretation that is included in a formal diagnostic report or recorded in the patient record. This also includes the review of images by a cytotechnologist when a judgment of adequacy is recorded in the patient record.

Policy/Procedure icon - The placement of this icon next to a checklist requirement indicates that a written policy or procedure is required to demonstrate compliance with the requirement. The icon is not intended to imply that a separate policy or procedure is required to address individual requirements. A single policy or procedure may cover multiple checklist requirements.

Laboratories not subject to US regulations: *Checklist requirements apply to all laboratories unless a specific disclaimer of exclusion is stated in the checklist. When the phrase "FDA-cleared/approved test (or assay)" is used within the checklist, it also applies to tests approved by an internationally recognized regulatory authority (eg, CE-marking).*

GENERAL CYTOPATHOLOGY

QUALITY MANAGEMENT

CYP.01650 Cytopathology Exclusion **Phase I**

The institution defines specimens that may be excluded from routine submission to the cytology department for examination.

NOTE: This policy may be made in conjunction with the hospital administration and appropriate medical staff departments. The laboratory director should have participated in or been consulted by the medical staff in deciding which cytology specimens are to be sent to the laboratory for examination.

~~*This checklist item is not applicable if 1) All specimens are submitted to pathology, or 2) The laboratory is not part of an institution that provides cytologic services.*~~

(No policy is needed for fluids such as urines and CSF that do not routinely undergo cytologic examination.)

CYTOLOGY STAINS AND SLIDE PREPARATIONS

*****REVISED** 12/26/2024***

CYP.04150 Cross-Contamination **Phase ~~I~~II**

The laboratory prevents cross-contamination of cytologic specimens during processing and staining.

NOTE: Procedures must prevent cross-contamination between the following:

- *Gynecologic and non-gynecologic specimens.*
- *Non-gynecologic cases that have high potential for cross-contamination from other non-gynecologic specimens.*

Laboratories must define what is considered a specimen that has a high potential for cross-contamination. Methods to prevent cross-contamination between specimens may include cytocentrifuge, filter, air dried preparations, and monolayer preparations. Direct smears made

from the sediment of highly cellular cases should be stained after the other cases, and the staining fluids must be changed or filtered between each of the highly cellular cases. One procedure to detect highly cellular specimens is to use a toluidine blue, or other rapid stain, on a wet preparation. ~~One procedure to detect possible contamination is to insert a clean blank slide in each staining run and examine it for contamination.~~

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* ~~2003(Jan 24):7169~~2023(Dec 28): [42CFR493.1274(b)(2-3~~)]~~)].

IMMUNOCYTOCHEMISTRY (ICC)

***REVISED** 12/26/2024*

CYP.04330 QC - Antibodies Phase II

Positive controls are used for each antibody.

NOTE: Positive controls assess the performance of the ~~primary antibody. They are performed on sections~~immunocytochemistry (ICC) assay (including impact of ~~tissue or cells known to contain the target~~ fixation and antigen~~, using the same epitope~~ retrieval) and can assess the sensitivity of the assay. They should, whenever possible, be subjected to the same processing, antigen retrieval, and immunostaining ~~protocols~~protocol as ~~the~~ patient ~~specimen.~~ specimens.

Results of controls must be recorded, either in internal laboratory records, or in the patient report. A statement in the report such as, "All controls show appropriate reactivity" is sufficient.

~~Ideally (but not essential to satisfy this requirement), the positive~~The ideal control ~~would be~~is of the same specimen type~~/fixative~~ as the patient ~~test~~specimen and is processed and fixed in the same manner as the patient specimen (eg, air-dried touch imprints, air-dried and/or alcohol fixed smears, cytocentrifuge or other liquid-based preparations, and cellular materials fixed in alcohol blends or other fixatives). However, for most laboratories, it is not practical to maintain separate positive control samples to cover every possible combination of fixation, processing and specimen type. Thus, it is reasonable for a laboratory to maintain a bank of formalin-fixed tissue samples as its positive controls; these controls can be used for patient specimens that are of different type, or fixed/processed differently, providing that the laboratory can show that these patient specimens exhibit equivalent immunoreactivity. This can be accomplished by parallel testing a small panel of common markers to show that specimens of different type, or processed in a different way (eg, alcohol-fixed cytology specimens) have equivalent immunoreactivity to routinely processed, formalin-fixed tissue.

A separate tissue section may be used as a positive control, but cytology specimens may contain normal elements that express the antigen of interest (internal controls). Internal positive controls are acceptable for these antigens, but the laboratory manual must clearly state the manner in which internal positive controls are used.

A positive control ~~section~~ included on the same slide as the patient specimen is optimal practice because it helps identify failure to apply primary antibody or other critical ~~reagent~~reagents to the patient test slide; however, one separate positive control per staining run for each antibody in the run (ie, batch control) may be sufficient provided that the control slide is closely scrutinized by a qualified reviewer.

Ideally, positive controls ~~possess~~have low levels of antigen expression, as is often seen in neoplasms. Different expression level controls are suggested if related to companion diagnostic clinical decision points (ie, HER2; 0, 1+, 2+, 3+). Exclusive use of normal tissues that have high levels of antigen expression may result in failure to identify assays of insufficient sensitivity, leading to false-negative results.

Synthetic materials (eg, microbeads) and cell lines containing ICC analytes of interest may be run as controls in addition to positive tissue controls. Synthetic controls and cell lines should

contain the target epitope of the ICC assay. Controls that assess the ICC protocol should be sensitive to the antigen retrieval step.

Synthetic and cell line-based controls can be particularly useful to assess assay performance at low expression levels, such as detecting low levels of expression in breast cancer. Synthetic and cell line-based controls are not ideal for optimizing digital pathology algorithms, which are optimally tuned to ICC expression in human tumors.

Evidence of Compliance:
✓ Patient reports or worksheet with control results **AND**
✓ Immunochemical-stained slides with positive controls

REFERENCES
~~1)~~ ~~O'Leary TJ. Standardization in immunohistochemistry. *Appl Immunohistochem Molecul Morphol* 2001;9:3-8~~
~~2~~1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. ~~2003(Jan 24~~2023(Dec 28): [42CFR493.1273(a)~~)~~)].
~~3~~2) Cheung CC, D'Arrigo C, Dietel M, et al; From the International Society for Immunohistochemistry and Molecular Morphology (ISIMM) and International Quality Network for Pathology (IQN Path). Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 4: Tissue Tools for Quality Assurance in Immunohistochemistry. *Appl Immunohistochem Mol Morphol.* 2017;25(4):227-230.
~~4~~3) Cheung CC, Taylor CR, Torlakovic EE. An Audit of Failed Immunohistochemical Slides in a Clinical Laboratory: The Role of On-Slide Controls. *Appl Immunohistochem Nol Morphol.* 2017;25(5):308-312.
~~5~~4) Torlakovic EE, Nielsen S, Francis G, et al. Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee. *Appl Immunohistochem Mol Morphol.* 2015;23(1):1-18.
5) Clinical and Laboratory Standards Institute (CLSI). *Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline - Second Edition.* CLSI document I/L28. Clinical and Laboratory Standards Institute, Wayne, PA; 2011.
6) ISO 20166-4:2020 Molecular in vitro diagnostic examinations. Specifications for pre-examination processes for formalin-fixed and paraffin-embedded (FFPE) tissue. Part 4: In situ detection techniques. International Organization for Standardization. 2020.

***NEW** 12/26/2024*

CYP.04335 Synthetic and Commercial Control Range Establishment or Verification Phase II

If synthetic or commercial controls are used for quantitative testing, the laboratory establishes or verifies an acceptable control range for each lot of synthetic or commercial control material.

NOTE: The laboratory must verify control ranges supplied by the manufacturer if provided and establish an acceptable range by repetitive analysis if control ranges are not provided by the manufacturer.

Control values supplied by the manufacturer may be used without verification for qualitative (eg, positive or negative) testing.

Evidence of Compliance:
✓ Records for control range establishment or verification of each lot, as applicable

REFERENCES
1) Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Precision of Quantitative Measurement Procedures. Approved Guideline.* 3rd ed. CLSI document EP05-A3. Clinical and Laboratory Standards Institute, Wayne, PA; 2014.
2) Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures, Principles and Definitions.* 4th ed. CLSI guideline C24. Clinical and Laboratory Standards Institute, Wayne, PA; 2016.

CYP.04340 QC - Antibodies Phase II

Appropriate negative controls are used.

NOTE: Negative controls must assess the presence of nonspecific staining in patient specimens as well as the specificity of each antibody with the exception listed below. Results of controls must be recorded, either in internal laboratory records, or in the patient report. A statement in the report such as, "All controls show appropriate reactivity" is sufficient.

For laboratories using older biotin-based detection systems, it is important to use a negative reagent control to assess nonspecific or aberrant staining in patient specimens related to the

antigen retrieval conditions and/or detection system used. A separate section of patient specimen is processed using the same reagent and epitope retrieval protocol as the patient test slide, except that the primary antibody is omitted, and replaced by <u>any one</u> of the following:

- *An unrelated antibody of the same isotype as the primary antibody (for monoclonal primary antibodies)*
- *An unrelated antibody from the same animal species as the primary antibody (for polyclonal primary antibodies)*
- *The negative control reagent included in the staining kit*
- *The diluent/buffer solution in which the primary antibody is diluted*

In general, a separate negative reagent control should be run for each specimen being immunostained; however, for cases in which there is simultaneous staining of multiple specimens from the same specimen with the same antibody, performing a single negative control on one of the specimens may be sufficient provided that all such specimens are fixed and processed identically. This exception does not apply to stains on different types of tissues or those using different antigen retrieval protocols or antibody detection systems. The laboratory director must determine which cases will have only one negative reagent control, and this must be specified in the department's procedure manual.

The negative reagent control would ideally control for each reagent protocol and antibody retrieval condition; however, large antibody panels often employ multiple antigen retrieval procedures. In such cases, a reasonable minimum control would be to perform the negative reagent control using the most aggressive retrieval procedure in the particular antibody panel. Aggressiveness of antigen retrieval (in decreasing order) is as follows: pressure cooker; enzyme digestion; boiling; microwave; steamer; water bath. High pH retrieval should be considered more aggressive than comparable retrieval in citrate buffer at pH 6.0.

Immunochemical tests using polymer-based detection systems (biotin-free) are sufficiently free of background reactivity to obviate the need for a negative reagent control and such controls may be omitted at the discretion of the laboratory director following appropriate validation. ~~The Centers for Medicare and Medicaid Services (CMS) recognizes the use of polymer-based detection systems (biotin free) may preclude the use of a negative reagent control. However, there have been no changes to the histopathology regulations. The CMS will be looking into an alternate QC method for these types of stains.~~

It is also important to assess the specificity of each antibody by a <u>negative cellular/tissue control,</u> which must show no staining of cells/tissues known to lack the antigen. The negative control is processed using the same fixation, epitope retrieval and immunostaining protocols as the patient tissue. Unexpected positive staining of such tissues indicates that the test has lost specificity, perhaps because of improper antibody concentration or excessive antigen retrieval. Intrinsic properties of the test tissue may also be the cause of "non-specific" staining. For example, cells/tissues with high endogenous biotin activity such as liver or renal tubules may simulate positive staining when using a detection method based on biotin labeling.

A negative cellular sample or tissue control must be processed for each antibody in a given run. Any of the following can serve as a negative cellular/tissue control:

1. *Multitissue blocks. These can provide simultaneous positive and negative tissue controls, and are considered "good practice" (see below).*
2. *The positive control slide or patient test slides, if these slides contain cellular or tissue elements that should not react with the antibody.*
3. *A separate negative cytologic preparation or tissue control slide.*

The type of negative cellular/tissue control used (ie, separate sections, internal controls or multitissue blocks) must be specified in the laboratory manual.

Multitissue blocks or tissue microarrays (TMAs) can have a major role in maintaining quality. When used as a combined positive and negative tissue control as mentioned above, they can serve as a permanent record of the sensitivity and specificity of every stain, particularly when mounted on the same slide as the patient tissue. When the components are chosen

appropriately, multitissue blocks may be used for many different primary antibodies, decreasing the number of different control blocks needed by the laboratory. Multitissue blocks are also ideal for determining optimal titers of primary antibodies since they allow simultaneous evaluation of many different pieces of tissue. Finally, they are a useful and efficient means to screen new antibodies for sensitivity and specificity or new lots of antibody for consistency, which should be done before putting any antibody into diagnostic use.

Evidence of Compliance:

✓ Patient reports or worksheet with control results **AND**

✓ Immunochemical-stained slides with appropriate negative controls

REFERENCES

1) Leong AS-Y, Cooper K, Leong FJW-M. Manual of Diagnostic Antibodies for Immunohistology. 2nd ed. London: Greenwich Medical Media; 2003
2) Dabbs DJ, ed. Diagnostic Immunohistochemistry: Theranostic and Genomic Applications. Philadelphia: Saunders/Elsevier; 2010
3) Burry RW. Specificity controls for immunocytochemical methods. *J Histochem Cytochem* 2000;48:163-166
4) Weirauch M. Multitissue control block for immunohistochemistry. *Lab Med.* 1999;30:448-449
5) Miller RT. Multitumor "sandwich" blocks in immunohistochemistry. Simplified method and preparation and practical uses. *Appl Immunohistochem* 1993;1: 156-159
6) Chan JKC, Wong CSC, Ku WT, Kwan MY. Reflections on the use of controls in immunohistochemistry and proposal for application of a multitissue spring-roll control block. *Ann Diagn Pathol* 2000;4: 329-336
7) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. ~~2003(Jan 24~~2023(Dec 28): [42CFR493.1273(a~~)]~~)].
8) Torlakovic EE, Francis G, Garratt J, et al. International Ad Hoc Expert Panel. Standardization of negative controls in diagnostic immunohistochemistry recommendations from the international ad hoc expert panel. *Appl Immunohistochem Mol Morphol.* 2014;22(4):241-52.
9) Allen M, Gown, MD. Diagnostic Immunohistochemistry: What Can Go Wrong and How to Prevent it. *Arch Pathol Lab Med.* 2016;140(9):893-898.

CYP.04360 Control Slide Review **Phase II**

The laboratory director or designee reviews batch control slides for acceptability before reporting patient/client results.

NOTE: Records of this daily review must be retained and clearly show that positive and negative controls for all antibodies stain appropriately. Batch control records must be retained for two years.

Immunochemical tests using polymer-based detection systems (biotin-free) are sufficiently free of background reactivity to obviate the need for a negative reagent control and such controls may be omitted at the discretion of the laboratory director following appropriate validation. ~~The Centers for Medicare and Medicaid Services (CMS) recognizes the use of polymer-based detection systems (biotin free) may preclude the use of a negative reagent control. However, there have been no changes to the histopathology regulations. The CMS will be looking into an alternate QC method for these types of stains.~~

The batch control slides must be readily available to pathologists who are signing out cases. The location of the slides should be stated in the procedure manual.

Evidence of Compliance:

✓ Records of control slide review

REFERENCES

1) Shellhorn N. IHC troubleshooting tips. Advance/Lab. 2000;9(1):33-37
2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. ~~2003(Jan 24~~2023(Dec 28): [42CFR493.1273(f~~)]~~)].

*****REVISED** 12/26/2024***

CYP.04370 ~~Antibody~~ Validation/Verification - Non-Predictive Antibody Marker Testing **Phase II**

The laboratory has records of validation/verification of new antibodies, including introduction of a new clone, prior to use for patient diagnosis or treatment.

NOTE: The performance characteristics of each assay must be appropriately validated/verified before being placed into clinical use. The initial goal is to establish the optimal antibody titration, detection system, and antigen retrieval protocol. Once optimized, a panel of cellular samples or tissues must be tested to determine the assay's sensitivity and specificity. The scope of the validation/verification is at the discretion of the laboratory director and will vary with the antibody.

Means of validation/verification may include, but are not limited to: 1) correlating the results using the new antibody with the morphology and expected results; 2) comparing the results using the new antibody with the results of prior testing of the same cellular samples or tissues with a validated/verified assay in the same laboratory; 3) comparing the results using the new antibody with the results of testing the same cellular samples or tissue in another laboratory with a validated/verified assay; or 4) comparing the results using the new antibody with previously validated/verified non-immunocytochemistry tests or testing previously graded tissue challenges from a formal proficiency testing program.

For an initial validation/verification, laboratories should achieve at least 90% overall concordance between the new test and the comparator test or expected results.

*For validation~~/verification of a~~ of **laboratory-developed or modified FDA-cleared/approved nonpredictive** ~~assay~~**assays**, the validation~~/verification should test~~ must be performed on a minimum of 10 positive and 10 negative cellular samples or tissues.*

*For verification of **unmodified FDA-cleared/approved nonpredictive assays**, the laboratory must follow the instructions provided by the manufacturer. If the instructions do not list a minimum number of samples for assay verification, the verification must be performed on a minimum of 10 positive and 10 negative cellular samples or tissues.*

If the laboratory director determines that fewer validation/verification cases are sufficient for a specific marker (eg, a rare antigen or cell/tissue), the rationale for that decision needs to be recorded. Positive cases in the validation/verification set should span the expected range of clinical results (expression level), especially for those markers that are reported quantitatively.

For p16/Ki67 dual stain testing performed on gynecologic cytopathology specimens using FDA-cleared/approved kits, the laboratory must verify that test performance is consistent with the manufacturer's validation data.

When possible, laboratories should use cellular samples or tissues that have been processed using the same fixative and processing methods as cases that will be tested clinically. If immunocytochemistry is regularly done on specimens that are not fixed or processed in the same manner as the cellular samples or tissues used for validation/verification (eg, air-dried touch imprints, air-dried and/or alcohol fixed smears, cytocentrifuge or other liquid-based preparations, and cellular materials fixed in alcohol blends or other fixatives), the laboratory should test a sufficient number of such cellular samples or tissues to ensure that assays consistently achieve expected results with the alternative fixative/processing conditions. The laboratory director is responsible for determining the number of positive and negative cases and the number of ~~predictive and nonpredictive~~ markers to test.

Refer to the subsection "Predictive Markers" in the ~~Anatomic Pathology~~Cytopathology Checklist for specific validation/verification requirements for tests that provide independent predictive information (eg, HER2 and ER testing in breast carcinoma).

Evidence of Compliance:

✓ Records of validation/verification, if applicable

REFERENCES

1) Hsi ED. A practical approach for evaluating new antibodies in the clinical immunohistochemistry laboratory. *Arch Pathol Lab Med.* 2001;125:289-294
2) Clinical and Laboratory Standards Institute (CLSI). *Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline~~—~~ - Second Edition.* CLSI document I/~~LA28-A2 (ISBN 1-56238-745-6).~~L28. Clinical and Laboratory Standards Institute, ~~940 West Valley Road, Suite 1400,~~ Wayne, ~~Pennsylvania 19087 USA,~~PA; 2011.
3) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):~~7166~~ [42CFR493.1256(e)(2~~)] and [42CFR493.1273(a)~~].

4) ~~Fitzgibbons PL, Bradley LA, Fatheree LA, et al. Principles of Analytic Validation of Immunohistochemical Assays. *Arch Pathol Lab Med.* doi: 10.5858/arpa.2013-0610-CP.~~Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2023(Dec 28): [42CFR493.1273(a)].
5) Uhlen M, Bandrowski A, Carr S, et al. A proposal for validation of antibodies. *Nat Methods.* 2016; 13(10):838-7.
6) ~~Fitzgibbons PL, Bradley LA, Fatheree LA~~Goldsmith JD, Troxell M, Roy-Chowdhuri S, et al. ~~College of American Pathologists Pathology and Laboratory Quality Center.~~ Principles of analytic validation of immunohistochemical assays~~. Guideline from the Pathology and Laboratory Quality Center.~~: guideline update. *Arch Pathol Lab Med.* ~~2014;138(11):1432-43.~~2024. https://doi.org/10.5858/arpa.2023-0483-CP
7) Allen M, Gown, MD. Diagnostic Immunohistochemistry: What Can Go Wrong and How to Prevent it. *Arch Pathol Lab Med.* 2016;140(9):893-898.

Predictive Markers

~~This checklist~~The term predictive marker as used in this section ~~applies only~~refers to immunocytochemical ~~tests used to predict responsiveness to a specific treatment~~biomarkers used independent of ~~other~~histologic and cytopathologic findings to identify individuals who are more likely to experience a favorable or unfavorable effect from a specific (targeted) therapy compared to individuals with the same diagnosis lacking the biomarker. Rather than confirming a specific diagnosis (such as B-cell lymphoma or gastrointestinal stromal tumor), these ~~tests should differentiate predicted~~biomarkers predict responsiveness to a ~~targeted therapy~~specific treatment among cases of the same diagnosis. For example, this section applies to estrogen receptor testing used to determine eligibility for hormonal treatment of breast carcinoma but does not apply to estrogen receptor testing used solely to assist in determining the primary site of origin of a metastatic neoplasm.

The current CAP guidelines (https://www.cap.org/protocols-and-guidelines/current-cap-guidelines) relating to predictive marker testing (eg, ASCO/CAP HER2 and ER testing in breast cancer) may be found at cap.org in the Protocols and Guidelines section. The guidelines are periodically updated based on new evidence. Laboratories should review updated predictive marker guidelines and promptly implement changes for items relating to requirements in the checklists (eg, validation, fixation, scoring criteria).

*****REVISED**** ***08/24/2023***

CYP.04510 Report Elements **Phase II**

For immunocytochemical tests that provide independent predictive information, the patient report includes information on specimen processing, the antibody clone, and the scoring method used.

NOTE: The laboratory processing the cytology specimen must record the cold ischemia time (if applicable) and the length of time in fixative. If the cytopathology laboratory refers immunocytochemistry or ISH studies, this information must be provided to the laboratory(ies) performing these studies.

For immunocytochemical studies used to provide predictive information independent of diagnosis or other cytopathologic findings (eg, ~~hormone~~estrogen receptors and HER2 in breast carcinoma, PD-L1 and lung adenocarcinoma predictive immunostains), the laboratory must include the following information in the patient report:

1. *The type of specimen fixation and processing (eg, formalin-fixed paraffin-embedded sections, air-dried imprints, etc.)*
2. *The antibody clone and general form of detection system used (eg, LSAB, polymer, proprietary kit, vendor name, etc.; information on the type of equipment used is not necessary)*
3. *Criteria used to determine a positive vs. negative result, and/or scoring system (eg, percent of stained cells, staining pattern)*
4. *Laboratory interpretation of predictive marker testing is reported according to the manufacturer's instructions, or when available, following the structure, format, and criteria set forth in the current CAP guidelines relating to predictive marker testing (eg, ASCO/CAP*

HER2 and ER testing in breast cancer and CAP/ASCP/ASCO HER2 in gastroesophageal carcinoma)

5. *Limitations relating to suboptimal preanalytical factors that may impact results, such as prolonged cold ischemia time, unknown ischemia time, or over- or under-fixation.*

Evidence of Compliance:

✓ Report template containing all required elements **AND**
✓ Copies of patient reports confirming inclusion of the required elements **AND**
✓ Established guidelines used by the laboratory

REFERENCES
1) Fischer AH, Schwartz MR, Moriarty AT, et al. Immunohistochemistry practices of cytopathology laboratories: a survey of participants in the College of American pathologists Nongynecologic Cytopathology Education Program. *Arch Pathol Lab Med.* 2014;138(9):1167-72.
2) Fisher ER, *et al.* Solving the dilemma of the immunohistochemical and other methods used for scoring ER and PR receptors in patients with invasive breast cancer. *Cancer.* 2005;103:164-73
3) Collins LC, *et al*. Bimodal frequency distribution of estrogen receptor immunohistochemical staining results in breast cancer: an analysis of 825 cases. *Am J Clin Pathol.* 2005;123:16-20
4) Allred DC, *et al.* ER expression is not bimodal in breast cancer. *Am J Clin Pathol.* 2005;124:474-5
5) Wolff AC, Somerfield MR, Dowsett M, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Pathol Lab Med.* Published online June 7, 2023. doi: 10.5858/arpa.2023-0905-SA.
6) Allison KH, Hammond EH, Dowsett M, et al. Estrogen and Progesterone Receptor Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update *Arch Pathol Lab Med.* 2020; 144(5):545-63.
7) Bartley AN, Washington MK, Ventura CB, et al. HER2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma: Guideline from the College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology. *Arch Pathol Lab Med.* 2016:140(12):1345-1363.

***REVISED** 12/26/2024*

CYP.04520 Annual Result Comparison - Breast Carcinoma **Phase ~~II~~I**

For HER2 and ER immunocytochemical tests performed on breast carcinoma that provide independent predictive information, the laboratory at least annually compares its patient results with published benchmarks, ~~and evaluates interobserver variability among the pathologists in the laboratory~~if applicable to the patient population tested.

~~NOTE:~~ NOTE: This checklist requirement is not applicable if the laboratory director determines that the population of breast carcinoma patients tested is not representative of the overall population of breast carcinoma patients.

For estrogen receptor studies: in general, the overall proportion of ER-negative breast cancers (invasive and DCIS) should not exceed 30%. The proportion is somewhat lower in postmenopausal than premenopausal women (approximately 20% vs. 35%). The proportion of ER-negative cases is considerably lower in well-differentiated carcinomas (<10%) and certain special types of invasive carcinomas (<10% in lobular, tubular, and mucinous types). Investigation is warranted if the proportion of ER-negative cases varies significantly from the published benchmarks.

For HER2 studies, the overall proportion of HER2 positive breast cancers is 10-25%. Laboratories must monitor their results. Investigation is warranted if the proportion of HER2 positive cases varies significantly from published data.

~~Individuals interpreting the assay must also have their concordance compared with each other and this concordance should also be at least 95%.~~

Evidence of Compliance:

✓ Records of annual result comparison~~ and evaluation of interobserver variability~~

REFERENCES
1) Wolff AC, Somerfield MR, Dowsett M, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Pathol Lab Med.* Published online June 7, 2023. doi: 10.5858/arpa.2023-0905-SA.
2) Allison KH, Hammond ME, Dowsett M, et al. Estrogen and progesterone receptors in breast cancer: American Society of Clinical Oncology/College of American Pathologists Guideline update [published online ahead of print January 2020] *Arch Pathol Lab Med.* doi: 10.5858/arpa.2019-0904-SA.

3) Fitzgibbons PL, Murphy DA, Hammond ME, *et al.* Recommendations for validating estrogen and progesterone receptor immunohistochemistry assays. *Arch Pathol Lab Med* 2010;134:930-935
4) Dunnwald LK, Rossing MA, Li CI. Hormone receptor status, tumor characteristics, and prognosis: a prospective cohort of breast cancer patients. *Breast Cancer Research* 2007;9:R6
5) Rüschoff J, Lebeau A, Kreipe H, et al. Assessing HER2 testing quality in breast cancer: variables that influence HER2 positivity rate from a large, multicenter, observational study in Germany. *Mod Pathol.* 2017;30:217-26.

*****NEW** 12/26/2024***

CYP.04525 Predictive Marker Interpretation Phase I

Each pathologist interpreting immunocytochemistry predictive markers participates in an annual analyte-specific quality assessment for each of the following predictive markers, as applicable:

- **Breast HER2**
- **Breast ER**
- **Gastric HER2**
- **Lung highly sensitive ALK**
- **Lung PD-L1 tumor proportion score (TPS)**

NOTE: This requirement applies to all pathologists in the laboratory that interpret one or more of these markers, whether in laboratories that perform both staining and interpretation or interpretation only. An Individual pathologist need participate only once for each predictive marker used by that pathologist in patient care evaluation, regardless of the number of locations where the pathologist performs interpretations. This requirement can be met by the use of laboratory-developed programs for sharing stained cytology slides or images thereof.

The quality assessment for each predictive marker must include a comparison of each pathologist's interpretation against the intended results. The laboratory director must define criteria for acceptable results and ensure follow up on each unacceptable result.

Evidence of Compliance:

✓ Records of annual assessment of each pathologist for predictive marker interpretation (performed on site or at another laboratory), where applicable

*****REVISED** 12/26/2024***

CYP.04530 Validation/Verification - Predictive Marker Testing Phase II

Predictive marker testing by immunocytochemistry is validated/verified and records of validation/verification are retained.

*NOTE: For validation of **laboratory-developed or modified FDA-cleared/approved predictive assays**, validation must be performed on a minimum of 20 cases (10 positive and 10 negative samples).*

*For verification of **unmodified FDA-cleared/approved predictive assays**, the laboratory must follow the instructions provided by the manufacturer. If the instructions do not list a minimum number of samples for assay verification, the verification must be performed on a minimum of 10 positive and 10 negative cellular samples or tissues.*

If the laboratory director determines that fewer validation/verification cases are sufficient for a specific marker (eg, a rare antigen or tissue), the rationale for that decision must be recorded. Positive cases in the validation/verification set should span the expected range of clinical results (expression levels). Only definitively positive and negative cases may be used for validation/verification.

The validation/verification data must clearly show the degree of concordance between assays or methods. Minimum acceptable concordance levels are 90% for positive and negative results.

The characteristics of the cases used for validation/verification should be similar to those seen in the laboratory's patient population (ie, core biopsy vs. open biopsy, primary vs. metastatic tumor, etc.).

Samples used for validation/verification must be handled in conformance with the guidelines in this checklist. Laboratories should use cellular samples or tissues that have been processed using the same fixative and methods as cases that will be tested clinically.

If significant changes are made to the testing methods (eg, antibody clone, antigen retrieval protocol or detection system, or pretreatment protocol), revalidation/verification is required.

This requirement is applicable to both new and existing assays. If review of the initial validation/verification does not meet the current standard, it must be supplemented and brought into compliance. If no records exist from the initial validation/verification, the assay must be fully revalidated/verified.

Evidence of Compliance:

✓ Records of validation/verification data including criteria for concordance

REFERENCES

1) Wolff AC, Somerfield MR, Dowsett M, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Pathol Lab Med.* Published online June 7, 2023. doi: 10.5858/arpa.2023-0905-SA.
2) Fitzgibbons PL, Murphy DA, Hammond ME, Allred DC, Valenstein P. Recommendations for validating estrogen and progesterone receptor immunohistochemistry assays. *Arch Pathol Lab Med* 2010; 134:930-935.
3) Wolff AC, Hammond ME, Hicks DG, *et al.* Recommendations for human epidermal growth factor receptor 2 testing in breast cancer; American Society of Clinical Oncology/College of American Pathologists. *Arch Pathol Lab Med* 2014;138(2):241-256
4) Goldsmith JD, Troxell M, Roy-Chowdhuri S, et al. Principles of analytic validation of immunohistochemical assays: guideline update. *Arch Pathol Lab Med.* 2024. https://doi.org/10.5858/arpa.2023-0483-CP

ON-SITE MICROSCOPIC REVIEW

***REVISED** 12/26/2024*

CYP.05000 On-Site Slide Review **Phase II**

The findings from the on-site slide review are free of any issues or any significant diagnostic discrepancies as defined in the Inspector Instructions.

NOTE: If p16/Ki67 dual stain is performed, slides should be included in the on-site slide review.

RECORDS AND REPORTS

***REVISED** 12/26/2024*

CYP.05300 Cytopathology Report Elements **Phase II**

The cytopathology report includes all of the following elements:

1. **Name of patient and unique identifying number, if available**
2. **Age and/or birth date of patient**
3. **Date of collection**
4. **Accession number**
5. **Name of submitting physician and/or clinic**
6. **Name of the responsible reviewing pathologist, when applicable**
7. **Name and address of the laboratory location where the test was performed**
8. **Date of report**
9. **Test performed**
10. **Anatomic source and/or type of specimen**
11. **Basis for amendment (if applicable)**

NOTE: If slide screening is performed at one laboratory location and the interpreting pathologist is at a different location, the names and addresses of both laboratory locations must be on the report. If slide processing and staining are performed at one location and screening and

interpretation at a second location, only the name/address of the second location need be on the report.

For institutions utilizing integrated cytology reports (including primary HPV screening, reflex HPV, co-testing, and p16/Ki67 dual stain), the names and addresses of each performing laboratory with a different CLIA number must be on the report.

Refer to CYP.05316 below for additional details regarding the reviewing pathologist.

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* ~~2003(Jan 24):3711~~2023(Dec 28): [42CFR493.1274(e)(6~~)~~).
2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):3713 [42CFR493.1291(c)(1-6) and (k)(1,2~~)]~~).

***REVISED** 12/26/2024*

CYP.06100 Report - Morphologic Findings **Phase II**

The cytopathology report includes an interpretation of the morphologic findings, and as appropriate, standard descriptive terminology.

NOTE: Cytopathology reports must clearly communicate whether disease is present, absent, or uncertain. When a definite diagnosis cannot be rendered (ie, terms such as "inconclusive," "indeterminate" or "non-diagnostic" are used), the reason should be given.

Reports must include a concise descriptive diagnosis either in a format similar to a histopathology report, or standard descriptive terminology that includes a general categorization and descriptive diagnosis (as is recommended by the Bethesda System for gynecologic ~~cytopathology reports).~~/anal cytology, the Paris System for urinary cytology, or Bethesda System for thyroid cytology). The use of diagnostic ~~"classes"~~numerical categories alone is not recommended~~, as it does not reflect current understanding of neoplasia, has no comparable equivalent in diagnostic histopathologic terminology, and does not provide for diagnosis of non-neoplastic conditions~~.

A simple diagnosis of "Negative" is not an adequate descriptive diagnosis. However, a diagnosis such as, "Negative for malignancy" or "No malignant cells identified" is acceptable for non-gynecologic exfoliative cytology specimens (ie, urine, fluids, washings and brushings). When appropriate (particularly for fine needle aspiration samples of mass lesions), a statement regarding the adequacy of the specimen should be included, with a description of the limitations of the specimen when a specific diagnosis cannot be made.

Evidence of Compliance:
✓ Cytopathology reports including morphologic findings

REFERENCES
1) Solomon D, *et al*. The 2001 Bethesda system. Terminology for reporting results of cervical cytology. *JAMA.* 2002:287:2114-2119
2) Solomon D, Nayar, R, *eds*. The Bethesda System for Reporting Cervical Cytology; Definitions, Criteria, and Explanatory Notes. 2nd ed., 2004
3) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* ~~2003(Jan 24~~2023(Dec 28): [42CFR493.1274(e)(5~~)~~).

RETENTION OF SLIDES

***REVISED** 12/26/2024*

CYP.06900 Slide Retention - Cytopathology **Phase II**

All glass slides are retained for an appropriate period.

NOTE: Minimum requirements for laboratories rendering cytopathology services, providing these are not less stringent than national, federal, state (or provincial), or local laws and regulations, are:

1. *Gynecologic glass slides (including p16/Ki67 dual stain gynecologic cytology slides) -five years*
2. *Non-gynecologic glass slides (including fine needle aspiration (FNA) slides)-10 years*

The retention period for non-gynecologic (non-FNA) glass slides changed from five years to 10 years in the 2019 Checklist edition. Cases diagnosed prior to December 31, 2014 are not subject to the 10-year retention requirement.

Laboratories may utilize archived slides for the benefit of the patient, even if that use destroys the slide. In such cases, the laboratory policy on material and record retention must authorize the destruction of a retained slide for such purposes (eg, molecular testing).

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):~~7170~~ [42CFR493.1105(a)(7)(i)(A~~); 493.1274(f)(2) through (f)(4)]~~)].
2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2023(Dec 28): [493.1274(f)(2) through (f)(4)].
~~2~~3) College of American Pathologists. Retention of laboratory records and materials. Northfield, IL: CAP, current edition

GYNECOLOGIC CYTOPATHOLOGY

Content has been added to some requirements in this section for primary HPV screening. Primary HPV screening is a stand-alone HPV test that is performed as an initial cervical cancer screen, with reflex to additional testing as necessary. This is different than HPV/PAP co-testing where both tests are performed together.

***REVISED** 12/26/2024*

CYP.07452 Unsatisfactory Specimens - Gynecologic Cytopathology **Phase II**

The laboratory has written criteria for identification and reporting of unsatisfactory gynecologic specimens and slide preparations including p16/Ki67 dual stain.

NOTE: Cytopathology reports must clearly specify when a specimen and/or slide preparation is unsatisfactory for evaluation and state the reason in the cytopathology report. The criteria for categorizing a specimen and/or slide preparation as unsatisfactory (eg, scant cellularity, obscuring blood, obscuring inflammation, or quantity insufficient for reflex testing from primary HPV screening) must be defined by the laboratory. Unsatisfactory cases must not be reported as negative or normal. Gynecologic specimens with atypical cells are always "satisfactory," although the report may include comments on the quality of the preparation.

Adequacy criteria are consistent with manufacturer instructions; however, any p16/Ki67 dual stain with positive cell(s) is reported as adequate.

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* ~~2003(Jan 24):7169~~2023(Dec 28): [42CFR493.1274(e)(4)~~)~~)].
2) Davey DD, *et al.* Terminology and specimen adequacy in cervicovaginal cytology. *Arch Pathol Lab Med.* 1992;116:903-907
3) Ransdell JS, *et al.* Clinicopathologic correlation of the unsatisfactory Papanicolaou smear. *Cancer Cytopathol.* 1997;81:139-143
4) Renshaw AA, *et al.* Accuracy and reproducibility of estimating the adequacy of the squamous component of cervicovaginal smears. *Am J Clin Pathol.* 1999:11:38-42
5) Selvaggi SM. Is it time to revisit the classification system for cervicovaginal cytology? *Arch Pathol Lab Med.* 1999;123:993-994
6) Davey DD, *et al.* Atypical cells and specimen adequacy. Current laboratory practices of participants in the College of American Pathologists interlaboratory comparison program in cervicovaginal cytology. *Arch Pathol Lab Med.* 2000;124:203-211

7) ~~Nakhleh RE, Fitzgibbons PL, *eds.*~~Zhai Q, Siegal GP. Quality ~~management~~Management in ~~anatomic pathology. Promoting patient safety through systems improvement and error reduction.~~Anatomic Pathology. Northfield, IL: ~~College of American Pathologists, 2005~~CAP Press, 2017.
8) Solomon D, *et al.* The 2001 Bethesda system. Terminology for reporting results of cervical cytology. *JAMA.* 2002;287:2114-2119
9) Solomon D, Nayar, R, *eds*. The Bethesda system for reporting cervical/vaginal cytologic diagnoses: Definitions, criteria, and explanatory notes for terminology and specimen adequacy. New York, NY: Springer-Verlag; 2nd edition, 2004
10) Clinical and Laboratory Standards Institute. *Cervicovaginal Cytology Based on the Papanicolaou Technique; Approved Guideline*; 3rd ed. CLSI document GP15-A3. Clinical and Laboratory Standards Institute, Wayne, PA, 2008.

***REVISED** 12/26/2024*

CYP.07465 Pathologist Interpretation **Phase II**

All gynecologic slides in the following categories are interpreted by the pathologist.

1. **Malignant or suspicious for malignancy**
2. **Low and high-grade squamous intraepithelial lesions**
3. **Atypical squamous cells**
4. **Atypical glandular cells**
5. **Reactive or repair**
6. **Gynecologic slides with p16/Ki67 dual stain**

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):7169 [42CFR493. 1274(e)(1)(i) through (e)(1)(v), and (e)(2)]
2) Raab SS, *et al.* Interobserver variability of a Papanicolaou smear diagnosis of atypical glandular cells of undetermined significance. *Am J Clin Pathol.* 1998;110:653-659
3) Selvaggi SM. Is it time to revisit the classification system for cervicovaginal cytology? Arch Pathol Lab Med. 1999;123:993-994

***REVISED** 12/26/2024*

CYP.07478 10% Rescreen **Phase II**

At least 10% of each cytotechnologist's gynecologic cases, including cases reflexed from primary HPV cases, that have been interpreted to be negative are rescreened.

NOTE: The 10% rescreening is a CLIA requirement, and only applicable to US laboratories and other laboratories subject to those regulations. An individual who qualifies as a cytotechnologist supervisor and who performs initial screening must also have a minimum of 10% of his or her cases that are initially interpreted as negative subjected to rescreening. This rescreening must include some cases from high-risk patients, based upon criteria established by the laboratory director, as well as random negative cases. Cases screened by MDs or DOs who are certified in Anatomic Pathology by the American Board of Pathology or the American Osteopathic Board of Pathology, or who possess qualifications that are equivalent to those required for the above certifications are not subject to this rescreening requirement. If FDA-approved automated instruments are used for quality control rescreening case selection, the laboratory must ensure that the methods used meet the requirements of CLIA, and that manufacturer and FDA recommendations for quality control are followed.

Slides must be rescreened in their entirety, including slides processed by imaging instruments that select a limited number of microscopic fields for examination by the cytotechnologist.

Evidence of Compliance:

✓ Defined qualifications of the individual to perform rescreening and the criteria for case selection **AND**
✓ Records of rescreened cases with comparison to original screening results

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7169 [42CFR493.1274(c)(1)]
2) Krieger P, *et al.* Guest editorial: random rescreening of cytology smears: a practical and effective component of quality assurance programs in both large and small cytology laboratories. *Acta Cytol.* 1994;38:291-298
3) Krieger P, *et al.* A practical guide to Papanicolaou smear rescreens. How many slides must be reevaluated to make a statistically valid assessment of screening performance? *Cancer Cytopathol.* 1998;84:130-137

4) Renshaw AA, *et al.* Performance characteristics of rapid (30-second) prescreening. Implications for calculating the false-negative rate and comparison with other quality assurance techniques. *Am J Clin Pathol.* 1999;111:517-522
5) Intersociety Working Group for Cytology Technologies. Proposed method for evaluating secondary screening (rescreening) instruments for gynecologic cytology. *Am J Clin Pathol.* 1999;111:590-593
6) Raab SS, *et al.* Cost effectiveness of rescreening cervicovaginal smears. *Am J Clin Pathol.* 1999;111:601-609

***REVISED** 12/26/2024*

CYP.07582 ~~Pap~~Cervical Cancer Screening **Test - False Negative Notification** **Phase I**

There is a mechanism to educate providers ~~of cervicovaginal specimens~~ that ~~the Pap test is a screening test for~~ cervical cancer screening tests, including primary HPV and Pap tests, are screening tests with inherent false negative results.

NOTE: The preferred mechanism is an educational note on all negative Pap test reports~~.~~ and all primary HPV screening tests. Other mechanisms include sending periodic educational information to providers, conference presentations, specimen collection manual, etc.

REFERENCES
1) Robb JA. The Pap smear is a cancer screening test: why not put the screening error rate in the report? *Diagn Cytopathol.* 1993;9:485-486~~.~~
2) Mitchell, H. Report disclaimers and informed expectations about Papanicolaou smears; an Australian view. *Arch Pathol Lab Med.* 1997;121:327-330~~.~~
3) Ge Y, Mody RR, Olsen RJ, et al. HPV status in women with high-grade dysplasia on cervical biopsy and preceding negative HPV tests. *J Am Soc Cytopathol.* 2019;8(3):149-156. doi:10.1016/j.jasc.2019.01.001.

***REVISED** 12/26/2024*

CYP.07600 **Statistical Records - Gynecologic Cytopathology** **Phase II**

For gynecologic cytopathology cases~~,~~ (not including those reflexed from primary HPV screening), statistical records are maintained and evaluated at least annually, and include the following:

- **Total number of gynecologic cytology cases examined**
- **Number of cases reported by diagnosis for each specimen type (including the number reported as unsatisfactory for diagnostic interpretation)**
- **Number of cases with a diagnosis of HSIL, adenocarcinoma, or other malignant neoplasm for which histology results were available for comparison**
- **Number of cases where cytology and histology are discrepant**
- **Number of cases where any rescreen of a normal or negative specimen results in reclassification as low-grade squamous intraepithelial (LSIL), HSIL, adenocarcinoma, or other malignant neoplasms**
- **Number of negative cases rescreened before sign-out~~.~~**
- **Number of positive and negative p16/Ki67 dual stains performed.**

NOTE: The data must be evaluated by the laboratory director or designee and included in the annual cytopathology statistical report. Inclusion of AGC data is optional. Separate statistics for conventional and each type of liquid-based preparations are required.

If a p16/Ki67 dual stain is used as a follow-up to an HPV positive co-test with a negative Pap test, statistics should be maintained separate from p16/Ki67 dual stain results derived from a positive primary HPV screening test.

The benchmarking data listed in the table below are based on 2021 case volumes. These benchmarking data may not be applicable for laboratories that utilize primary HPV screening for a significant portion of cervical cancer screening. Results were excluded for laboratories that included primary HPV screening results in the interpretive totals when more than 25% of their cervical/gynecologic cytology slides were from positive primary HPV screening. In evaluating its statistics, the laboratory's patient population should be taken into consideration. Percentile-reporting rates refer to the distribution of individual laboratory responses from reporting rates in various categories. Responses are ranked from lowest to highest, and the 50th percentile-

reporting rate refers to the median response. A 25th percentile-reporting rate (which corresponds to 1.7% in the table) for the ThinPrep LSIL category means that a quarter of laboratories have LSIL rates of 1.7% or less. A 90th percentile-reporting rate (which corresponds to 11.7% in the table) for ASC-US in ThinPrep preparations means that 9 of 10 laboratories have an ASC-US rate of 11.7% or less.

The reporting rates for ASC-US, ASC-H, AGC, LSIL, HSIL, and UNSATISFACTORY are given as percentages of total case volume. An ASC-US rate of 2.0% means 2/100 cases in the lab are designated ASC-US. The ASC/SIL figure is a calculated ratio: the percentage or number of a laboratory's ASC-US and ASC-H cases divided by the percentage or number of LSIL, HSIL, and malignant cases. A laboratory with 4% ASC cases and 3% SIL cases has an ASC/SIL ratio of 1.3, as compared to the median ASC/SIL ratio of 1.5 for conventional Paps, 2.0 for ThinPrep® and 1.8 for SurePath.

CONVENTIONAL*
Laboratory Percentile-Reporting Rate

CATEGORY	5th	10th	25th	Median	75th	90th	95th
Unsatisfactory (%)	0.0	0.0	0.4	1.3	2.2	5.2	7.1
LSIL (%)	0.0	0.0	0.3	0.8	1.6	2.0	2.8
HSIL (%)	0.0	0.0	0.1	0.3	0.5	0.9	1.1
ASC-US (%)	0.1	0.3	1.0	1.8	3.6	5.3	6.7
ASC-H (%)	0.0	0.0	0.1	0.1	0.4	0.8	1.1
AGC (%)	0.0	0.0	0.0	0.1	0.2	0.6	1.2
ASC/SIL	0.4	0.5	1.0	1.5	2.7	4.2	5.6

ThinPrep**
Laboratory Percentile-Reporting Rate

CATEGORY	5th	10th	25th	Median	75th	90th	95th
Unsatisfactory (%)	0.2	0.4	0.9	1.7	2.9	4.8	5.7
LSIL (%)	0.4	0.9	1.7	2.4	3.3	4.8	6.6
HSIL (%)	0.1	0.1	0.2	0.4	0.6	1.0	1.3
ASC-US (%)	1.0	1.9	3.6	5.4	7.9	11.7	15.2
ASC-H (%)	0.0	0.1	0.2	0.4	0.6	1.1	1.5
AGC (%)	0.0	0.0	0.1	0.2	0.4	0.7	1.1
ASC/SIL	0.7	1.1	1.6	2.0	2.7	3.6	4.4

SurePath**
Laboratory Percentile-Reporting Rate

CATEGORY	5th	10th	25th	Median	75th	90th	95th
Unsatisfactory (%)	0.0	0.0	0.2	0.4	0.8	1.2	1.6
LSIL (%)	0.2	0.5	1.0	2.2	3.0	4.3	5.9
HSIL (%)	0.0	0.0	0.2	0.3	0.5	1.0	1.4
ASC-US (%)	0.3	0.7	2.1	4.2	6.6	9.2	10.6
ASC-H (%)	0.0	0.1	0.1	0.3	0.5	0.8	1.3
AGC (%)	0.0	0.0	0.1	0.2	0.5	0.7	1.6
ASC/SIL	0.5	0.9	1.2	1.8	2.9	3.6	4.2

**Includes conventional annual test volume of >60.*

***Includes SurePath and ThinPrep annual test volume of >300.*

Evidence of Compliance:

✓ Records of statistical data for defined categories **AND**

✓ Records of data review and evaluation against benchmark data by the laboratory director or designee

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* ~~2003(Jan 24):7169~~2023(Dec 28): [42CFR493.1274(c)(5)(i) through (c)(5)(vi~~)~~)].
2) Davey DD, Souers RJ, Goodrich K, Mody DR, Tabbara SO, Booth CN. Bethesda 2014 implementation and human papillomavirus primary screening: practices of laboratories participating in the College of American Pathologists PAP Education Program. *Arch Pathol Lab Med.* 2019;143:1196-1202
3) Genest DR, *et al.* Qualifying the cytologic diagnosis of "atypical squamous cells of undetermined significance" affects the predictive value of a squamous intraepithelial lesion on subsequent biopsy. *Arch Pathol Lab Med.* 1998;122:338-341
4) Raab SS, *et al.* Interobserver variability of a Papanicolaou smear diagnosis of atypical glandular cells of undetermined significance. *Am J Clin Pathol.* 1998;110:653-659
5) Schiffman M, *et al*. HPV DNA testing in cervical cancer screening results for women in a high risk province in Costa Rica. *JAMA*. 2000;283:87-93
6) Solomon D, *et al*. Comparison of three management strategies for patients with ASCUS. *J Natl Cancer Inst.* 2000;93:293-299
7) Juskevicius R, *et al*. An analysis of factors that influence the ASCUS/SIL ratio of pathologists. *Am J Clin Pathol.* 2001;116:331-335

*****NEW** 12/26/2024***

CYP.07620 Statistical Records - Reflexed Gynecological Cytopathology Phase I

For gynecologic cytopathology cases reflexed from primary HPV screening, statistical records are maintained and evaluated at least annually, and include the following:

- **Number of primary HPV screening tests performed, if available**
- **Number of Paps reflexed from primary HPV screening**
- **Number of reflexed Paps reported by diagnosis for each specimen type (including the number reported as unsatisfactory for diagnostic interpretation)**
- **Number of cases with a diagnosis of HSIL, adenocarcinoma, or other malignant neoplasm for which histology results were available for comparison**
- **Number of cases where cytology and histology are discrepant**
- **Number of cases where any rescreen of a normal or negative specimen results in reclassification as low-grade squamous intraepithelial (LSIL), HSIL, adenocarcinoma, or other malignant neoplasms**
- **Number of positive and negative p16/Ki67 dual stains performed.**

NOTE: The data must be evaluated by the laboratory director or designee and included in the annual cytopathology statistical report.

If a p16/Ki67 dual stain is used as a follow-up to an HPV positive test with a negative Pap test, statistics should be maintained separate from p16/Ki67 dual stain results derived from a positive HPV screening test.

Evidence of Compliance:

✓ Records of statistical data for defined categories **AND**

✓ Records of data review and evaluation against benchmark data by the laboratory director or designee

*****REVISED** 12/26/2024***

CYP.07655 Screening Performance Phase II

The laboratory evaluates and records the ongoing performance of individuals who do cervicovaginal cytology screening against the overall statistics for the laboratory as a whole.

NOTE: Mechanisms can include evaluation of rescreening and interpretive discrepancies and detection rates for abnormalities. This includes screening performance of p16/Ki67 dual stain gynecologic slides.

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* ~~2003(Jan 24):7169~~2023(Dec 28): [42CFR493.1274(c)(6)and (d)(1)(i)(B)~~)~~)].
2) Jones BA, Davey DD. Quality management in gynecologic cytology using interlaboratory comparison. *Arch Pathol Lab Med.* 2000;124:672-681
3) Cibas, ES, *et al.* Quality assurance in gynecologic cytology: the value of cytotechnologist-cytopathologist discrepancy logs. *Am J Clin Pathol.* 2001;115:512-516
~~4) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24): [42CFR493.1274(c)(6)]~~
~~5~~4) ~~Nakhleh RE, Fitzgibbons PL, *eds.* College of American Pathologists.~~Zhai Q, Siegal GP. Quality ~~improvement manual~~Management in ~~anatomic pathology, second edition.~~Anatomic Pathology. Northfield, IL: CAP~~, 2002~~ Press, 2017.

PERSONNEL

***REVISED** 12/26/2024*

CYP.07700 Section Director/Technical Supervisor - Cytopathology **Phase II**

The cytopathology laboratory has a qualified pathologist as the section director/technical supervisor.

NOTE: The section director/technical supervisor of the cytopathology laboratory must be a doctor of medicine or a doctor of osteopathy licensed to practice medicine in the jurisdiction in which the laboratory is located.

For laboratories subject to US regulations, the section director must also be certified in anatomic pathology by the American Board of Pathology or the American Osteopathic Board of Pathology ~~or possess qualifications equivalent to those required for board certification~~.

Individuals qualified and serving as a technical supervisor for high complexity testing in a CLIA-certified laboratory as of December 28, 2024, may continue to fill this role if they have done so continuously since December 28, 2024.

If more stringent state or local regulations are in place for supervisory qualifications, including requirements for state licensure, they must be followed.

For laboratories not subject to US regulations, ~~education, experience, and/or certification qualifications~~individuals must meet ~~or be equivalent to US qualifications or meet,~~ national, state or provincial, or local laws and regulations, and education must be equivalent to US qualifications.

Evidence of Compliance:

✓ Records of section director/technical supervisor qualifications including degree or transcript, certification/registration, current license (if required) and work history in related field

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* ~~1992(Feb~~2023(Dec 28):~~7179~~ [42CFR493.1449(~~k)(1)~~ e)].

***REVISED** 12/26/2024*

CYP.07800 Non-Supervisory ~~Personnel~~Cytotechnologists **Phase II**

All non-supervisory cytotechnologists meet at least one of the following qualifications.

1. **Graduated from a school of cytotechnology accredited by the Commission on Accreditation of Allied Health Education Programs ~~or other organization approved by Health and Human Services (HHS~~(CAAHEP); or**
2. **Certified in cytotechnology by a certification agency approved by HHS (eg, American Society of Clinical Pathology); or**

~~3. Before September 1, 1992, have successfully completed two years in an accredited institution (12 semester hours in science, eight of which are in biology) and have 12 months training in an approved school of cytotechnology; or have received six months formal training in an approved school and six months full-time experience; or~~
~~4. Before September 1, 1992, have achieved a satisfactory grade in an HHS proficiency test for cytotechnologists~~
~~5. Before September 1, 1994, have two years full-time experience or equivalent within the preceding five years examining slides under the supervision of a physician certified in pathology and before January 1, 1969, be a high school graduate with six months cytotechnology training in a laboratory directed by a physician and completed two years fulltime supervised experience in cytotechnology before 1/1/69; or~~
~~6. On or before September 1, 1994, have two years full-time experience or equivalent within preceding five years in the US and on or before September 1, 1995, have either graduated from a CAHEA-approved school or be certified as a cytotechnologist~~

3. Qualified and served as a cytotechnologist in a CLIA-certified laboratory as of December 28, 2024, and have done so continuously since December 28, 2024*.

NOTE: If more stringent state or local regulations are in place for cytotechnologist qualifications, including requirements for state licensure, they must be followed.

For laboratories not subject to US regulations, ~~education, experience, and/or certification qualifications~~individuals must meet ~~or be equivalent to US qualifications or meet~~ national, state or provincial, or local laws and regulations, and education must be equivalent to US qualifications.

**Cytotechnologist personnel qualifications prior to December 28, 2024, may be found in the CAP Personnel Guidance Document located in e-LAB Solutions Suite on cap.org (log-in required) under Accreditation Resources - Accreditation Checklists.*

Evidence of Compliance:

✓ Records of qualifications including degree or transcript, certification/registration, current license (if required) and work history in related field

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* ~~1992(Feb~~2023(Dec 28):~~7182~~ [42CFR493.1483~~]~~].
2) ~~Department of Health and Human Services, Centers for Medicare and Medicaid Services. Extension of certain effective dates for clinical laboratory requirements and personnel requirements for cytologists. *Fed Register.* 1994(Dec 6):62608~~

CYP.08100 General Supervisor Qualifications - Cytopathology Phase II

The cytopathology laboratory has a general supervisor who meets the qualifications defined by CLIA (for laboratories subject to US regulations) and other applicable national, federal, state (or provincial), or local laws and regulations.

NOTE: The supervisor can be a pathologist boarded in anatomic pathology. Alternatively, the supervisor can be qualified as a cytotechnologist, with at least three years of full-time experience as a cytotechnologist within the preceding 10 years. The section director/technical supervisor may also serve as the general supervisor.

For laboratories not subject to US regulations, appropriate national, state or provincial, or local laws and regulations also apply.

Evidence of Compliance:

✓ Records of qualifications including degree or transcript, certification/registration, current license (if required) and work history in related field

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 1992(Feb 28):~~7182 [42CFR493.1469],~~ [42CFR493.1467~~]~~.
2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2023(Dec 28): [42CFR493.1469].

CYTOLOGY WORKLOAD

***REVISED** 12/26/2024*

CYP.08500 Manual Screening - Laboratories Subject to US Regulations Phase II

Workload data are recorded for cytotechnologists and pathologists who manually screen previously unscreened gynecologic (including p16/Ki67 dual stain gynecologic cytology) and non-gynecologic (including FNA) slides.

NOTE: This checklist requirement applies only to laboratories subject to US regulations. The final rule implementing CLIA requires that each individual evaluating cytology preparations by manual microscopic technique must examine no more than 100 slides (gynecologic and non-gynecologic or both) in 24-hours. In addition, if there are different state regulations for cytology workload, the most stringent regulation must be followed (eg, workload for cytotechnologists manually screening gynecologic smears under a California state laboratory license is limited to 80 gynecologic slides in a 24-hour period, and reduced proportionately based on other duties performed).

Gynecologic slides include new routine slides, 10% rescreen slides, and five-year look-back negative slides. Records must be maintained showing the total number of slides examined by each individual during each 24-hours.

For primary manual screening of non-gynecologic liquid-based slide preparations, each slide may be counted as one-half slide for the purpose of workload recording, provided that cells are dispersed over one-half or less of the total available slide area.

For primary manual screening of all other slide types (including gynecologic liquid-based preparations), each slide must be counted as a single slide for the purpose of workload recording.

The maximum workload can be completed in no less than an eight-hour workday. These total limits apply regardless of the number of laboratories in which an individual works on a given day. For employees screening less than eight hours at an individual laboratory, this workload maximum must be prorated according to the formula: number of hours spent screening X 100/8.

Pathologists who screen previously unscreened gynecologic slides (including p16/Ki67 dual stained gynecologic cytology slides) and previously unscreened non-gynecologic slides (including FNA slides) must adhere to the above workload limit and retain records of compliance.

For all screening personnel, adequacy assessment of fine needle aspiration (FNA) smears or rapid on-site evaluation (ROSE) is not considered primary cytology screening; however, the time spent performing adequacy assessments must be used to prorate the maximum number of slides the individual can screen in a 24-hour period.

The following are not subject to the workload limit for pathologists:
1. *Previously screened reactive/repair, atypical, premalignant and malignant gynecologic, and p16/Ki67 dual stained gynecologic cytology slides*
2. *Rescreened five-year look-back slides*
3. *10% rescreen of negative gynecologic slides*
4. *Previously screened non-gynecologic slides*
5. *Previously screened FNA slides*

Evidence of Compliance:

✓ Records of workload recording for each individual

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. ~~Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and~~Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* ~~2003(Jan 24):5232~~2023(Dec 28): [42CFR493.1274(d~~)~~)].
2) Kline TS. The challenge of quality improvement with the Papanicolaou smear. *Arch Pathol Lab Med.* 1997;121:253-255
3) ~~Nakhleh RE, Fitzgibbons PL, *eds.* College of American Pathologists.~~Zhai Q, Siegal GP. Quality ~~improvement manual~~Management in ~~anatomic pathology, second edition.~~Anatomic Pathology. Northfield, IL: CAP~~, 2002~~ Press, 2017.
4) Moriarty AT. Cytology workload calculation—Has anything really changed? *Cancer Cytopath.* 2001;119(2):77-79.
5) Centers for Medicare and Medicaid Services. *Clarification Regarding Fine Needle Aspiration (FNA) Specimen Adequacy Assessment, Rapid On-Site Evaluation (ROSE) and Workload Limits.* March 16, 2018. Baltimore, M: Department of Health and Human Services; Ref: QSO18-14-CLIA.