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November 2008

GP15-A3

Cervicovaginal Cytology Based on the Papanicolaou Technique; Approved Guideline—Third Edition

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

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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For additional information on committee participation or to submit comments, contact CLSI.

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ISBN 1-56238-679-4
ISSN 0273-3099

GP15-A3
Vol. 28 No. 28
Replaces GP15-A2
Vol. 21 No. 17

Cervicovaginal Cytology Based on the Papanicolaou Technique; Approved Guideline—Third Edition

Volume 28 Number 28

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Abstract

Clinical and Laboratory Standards Institute document GP15-A3—*Cervicovaginal Cytology Based on the Papanicolaou Technique; Approved Guideline—Third Edition* is intended for health care providers who are responsible for collecting cervicovaginal cytology specimens and preparing conventional Papanicolaou smears and liquid-based preparations. The guideline focuses on quality collection and processing of specimens, addressing all steps, including patient assessment, test requisition, specimen collection, specimen transport, and specimen receipt and processing. Illustrations of the techniques are described, and a specimen requisition form is also included.

Clinical and Laboratory Standards Institute (CLSI). *Cervicovaginal Cytology Based on the Papanicolaou Technique; Approved Guideline—Third Edition*. CLSI document GP15-A3 (ISBN 1-56238-679-4). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2008.

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Suggested Citation

CLSI. *Cervicovaginal Cytology Based on the Papanicolaou Technique; Approved Guideline—Third Edition*. CLSI document GP15-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

Previous Editions:

December 1989, October 1991, July 1994, November 2001

Reaffirmed:

March 2016

Archived:

September 2016

ISBN 1-56238-679-4
ISSN 0273-3099

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SAMPLE

Foreword

Dr. George N. Papanicolaou developed cervicovaginal cytology to facilitate the detection of precancerous lesions of the uterine cervix during the routine screening procedure for female patients. Regularly scheduled cervical screening is recognized as the most effective method of reducing the incidence and mortality of cervical cancer.

For cervicovaginal cytology to produce optimal results, the specimen collection procedure must be carried out by a health care provider on an adequately prepared and informed patient. Specimens should be evaluated using quality-controlled laboratory techniques.

This third edition has been updated to provide additional information on education of the patient, design of the requisition, and specimen collection. Additional information regarding the handling and processing of liquid-based Pap tests and ancillary studies is also provided. The nomenclature of the second edition of The Bethesda System is incorporated in this edition, and handling of cases in the context of an electronic health record is discussed.

Key Words

Cervical cancer, cervical cancer screening, cervicovaginal specimen collection, diagnostic cervicovaginal cytology, human papillomavirus (HPV), liquid-based preparations, Pap test, Papanicolaou stain, Papanicolaou technique, precancerous lesions

SAMPLE

Cervicovaginal Cytology Based on the Papanicolaou Technique; Approved Guideline—Third Edition

1 Scope

This guideline provides the most current recommendations regarding standard precautions, patient assessment, test requisition, cervicovaginal specimen collection, specimen transport, specimen receipt, specimen processing, and storage of slides. Cytologic interpretation is outside the scope of this document and is not addressed.

This guideline is useful to health care providers, laboratory directors, supervisors, and others who have responsibilities for quality control in cytopathology laboratories.

2 Introduction

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

3 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to “standard precautions.” Standard precautions are guidelines that combine the major feature of “universal precautions and body substance isolation” practices. Standard precautions cover the transmission of all infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the US Centers for Disease Control and Prevention.¹ For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious disease, refer to CLSI document M29.²

4 Terminology

4.1 Definitions

colposcopy – a procedure where a dissecting-type microscope is used to view the cervix following an application of dilute acetic acid, which colors the cervical intraepithelial neoplasia (CIN) lesions transiently white (acetowhite) and/or accentuates abnormal vasculature to facilitate the identification of intraepithelial lesions and cancer for biopsy; **NOTE 1:** Colposcopy is done following an abnormal Pap test result, or in the investigation of symptoms of cervical pathology such as abnormal vaginal bleeding, even if the Pap test is reported as normal. Colposcopy allows illuminated examination of the lower genital tract to detect epithelial abnormalities and assess severity of these lesions; **NOTE 2:** Colposcopy is also done when the cervix is visually abnormal in appearance, and when a high-risk (HR) HPV test is positive in the following clinical situations: 1) postcolposcopy follow-up of women treated for CIN 2,3; 2) postcolposcopy follow-up of women not found to have CIN 2,3 or adenocarcinoma *in situ* (AIS) at initial colposcopy and referred for the evaluation of repeat atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesion (ASC-H), low-grade squamous intraepithelial lesion (LSIL), and atypical glandular cells not otherwise specified (AGC-NOS); and 3) follow-up of women age 30 and over having a normal Pap and a positive HR HPV

test on the initial screen, and either a positive HR HPV test and/or an abnormal Pap on the 12-month follow-up exam.

conventional smear – a method of slide preparation where a sample of cells collected from the cervix/vagina is smeared and fixed onto a glass slide in the patient examination room.

diethylstilbestrol (DES) – synthetic, nonsteroidal estrogens administered during the last century to gravid women at risk for early pregnancy loss; **NOTE:** There is evidence that administration may have caused adenosis (non-neoplastic) and clear cell adenocarcinoma (neoplastic) in the female genital (cervix and vagina) tract of some of the daughters who were exposed *in utero*.

dysplasia – precancerous cellular changes in the cervix that include a spectrum of cellular abnormalities, described as mild, moderate, and severe or marked dysplasia; **NOTE:** Dysplasia terminology has been replaced with Bethesda terminology³ for Pap tests; however, it is still used for histologic specimens in many laboratories.

epithelial cell abnormalities – precancerous cellular changes in the cervix that include a spectrum of cellular abnormalities such as atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesion (ASC-H), atypical glandular cells (AGC), low-grade squamous intraepithelial lesion (LSIL), koilocytosis, HPV effect, and high-grade squamous intraepithelial lesion (HSIL), adenocarcinoma *in situ* (AIS), and all varieties of epithelial neoplasms; **NOTE:** These terms are part of the Bethesda 2001 System³ nomenclature. The Bethesda System is in current use in the United States and several other countries.

human papillomavirus (HPV) – the most common sexually transmitted virus and causative agent in the pathogenesis of cervical cancer and its precursor lesions in almost all cases.

intraepithelial lesion – Bethesda System terminology for a subgroup of epithelial cell abnormalities that include low-grade squamous intraepithelial lesion (LSIL), koilocytosis, HPV effect, and high-grade squamous intraepithelial lesion (HSIL); **NOTE:** ASC-US, ASC-H, AGC, invasive carcinomas, sarcomas, and other neoplasms are not included among intraepithelial lesions.

liquid-based Pap preparations – a method of slide preparation where a sample of cells from the cervix/vagina is collected and rinsed into a vial of preservative fluid in the patient examination room, then transported to the laboratory where an automated processing device produces a thin layer of evenly distributed cervicovaginal cells onto a glass slide.

original squamocolumnar junction (OSCJ) – the junction between the squamous and columnar epithelium at birth.

Pap test – a sample of cells collected from the cervix (or vaginal cuff in posthysterectomy patients), prepared by conventional or liquid-based methods, and stained using the Papanicolaou staining technique for the purpose of screening for cervical cancer and precancerous lesions of the cervix and vagina.

Papanicolaou stain technique – a method of polychromatic staining developed by George N. Papanicolaou to identify differences in cellular morphology, maturity, and metabolic activity.

precursor lesions – precancerous changes in the cervical/vaginal epithelium.

squamocolumnar junction (SCJ) – the current junction between the squamous and columnar epithelium.

The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The approach is based on the model presented in the most current edition of CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care*. The quality management system approach applies a core set of “quality system essentials” (QSEs), basic to any organization, to all operations in any health care service’s path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager’s guide. The QSEs are:

- | | | | |
|--|--|--|--|
| Documents & Records
Organization
Personnel | Equipment
Purchasing & Inventory
Process Control | Information Management
Occurrence Management
Assessments—External & Internal | Process Improvement
Customer Service
Facilities & Safety |
|--|--|--|--|

GP15-A3 addresses the QSEs indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessments—External & Internal	Process Improvement	Customer Service	Facilities & Safety
GP02					X M29 MM03			MM03			M29

Adapted from CLSI/NCCLS document HS01—*A Quality Management System Model for Health Care*.

Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, CLSI/NCCLS document GP26—*Application of a Quality Management System Model for Laboratory Services* defines a clinical laboratory path of workflow, which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

GP15-A3 addresses the clinical laboratory path of workflow steps indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Examination ordering	Preexamination			Examination			Postexamination	
	Sample collection	Sample transport	Sample receipt/processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
X	X MM03	X MM03	X MM03	MM03	MM03		MM03	X

Adapted from CLSI/NCCLS document HS01—*A Quality Management System Model for Health Care*.

Related CLSI Reference Materials*

- GP02-A5** **Laboratory Documents: Development and Control; Approved Guideline—Fifth Edition (2006).** This document provides guidance on development, review, approval, management, and use of policy, process, and procedure documents in the medical laboratory community.
- M29-A3** **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.
- MM03-A2** **Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline—Second Edition (2006).** This guideline addresses topics relating to clinical applications, amplified and nonamplified nucleic acid methods, selection and qualification of nucleic acid sequences, establishment and evaluation of test performance characteristics, inhibitors, and interfering substances, controlling false-positive reactions, reporting and interpretation of results, quality assurance, regulatory issues, and recommendations for manufacturers and clinical laboratories.

* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most current editions.

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