Cervical disease screening and detection: emerging techniques in molecular diagnostic assays

By Dorothy L. Rosenthal, MD, FIAC

Current techniques in cervical disease detection

The detection of cervical neoplasia, including the detection and diagnosis of moderate-severe dysplasia, squamous cell carcinoma, and adenocarcinoma, is accomplished through the morphological assessment of cervical cells with the annual Pap screen. Historically, this has been accomplished through the collection of scrapings of both endocervical and ectocervical cells from the uterine-cervix (through the use of a brush, broom, or spatula). The specimen is smeared on a microscope slide, followed by specific staining of the deposited cells on the slide with the Papanicolaou stain. Microscopic examination searches for cells with abnormal morphology consistent with neoplasia.

Since the FDA approval in 1995 of the ThinPrep (Cytyc, Boxborough, MA), the adoption of liquid-based specimen preparations for the collection, preparation, and subsequent analysis of cytology specimens has become widely used in annual screening for cervical disease within the United States. Approximately 80% of the estimated 50 million cervical Pap smears performed annually in the United States are accomplished through the use of liquid-based cytology specimens. To date, there are two commercially available liquid-based preparation technologies to prepare cytology thin-layer specimens for subsequent examination for cervical malignancies and precursor lesions. Both technologies have received approval by the FDA and are used in routine clinical practice: ThinPrep technology (and associated sample preparation instrumentation) offered through Cytyc Corp. and SurePath thin-layer technology (and associated PrepStain sample preparation instrumentation) offered through TriPath Imaging Inc. (Burlington, NC).

Attributes of liquid-based specimens include sample uniformity, increased cellular density for the detection of rare high-grade intraepithelial lesion (HSIL) cells within the cytology specimen, and the ability to perform multiple adjunctive tests for infectious disease diagnosis from the residual cytology specimen. Human papillomavirus (HPV) has been the prototype organism for this application, but gonorrhea,
chlamydia, and herpes virus tests are currently available. The quality and cell integrity of cervical specimens collected in liquid (or fluid) makes this an ideal medium for morphology-based screening and diagnosis (see Figure 1). The typical clinical attributes for liquid-based cervical specimens are summarized in Table I. Similar, but not identical qualities can be expected from ThinPrep preparations.

**Image analysis for routine screening of cervical disease**

The manual screening and interpretation of both Pap smears and liquid-based cytology samples have been the standard in clinical laboratory practice since the 1940s. In the past decade, the application of computerized microscopy for the screening and identification of cervical neoplasia has been used in laboratories with high volumes of cervical Pap smears. For example, the FocalPoint slide profiler (TriPath Imaging Inc.) is an FDA-approved computerized imaging system for the primary screening of both conventional Pap smears as well as liquid-based cytology specimens. The advantages of such a system are that the algorithms are used to scan the entire slide and then score and rank the slides based upon the likelihood of an abnormality. The Cytose Image Processor uses a slightly different approach by automatically locating the possible abnormal cells, then automatically directing a human to 22 fields of view (FOV) on the slide, leaving the ultimate decision for complete slide review to the cytopathologist. FDA approval was obtained in 2003. Clinical studies have confirmed the utility and advantages in the use of these image-analysis systems to facilitate the laboratory detection and identification of cervical abnormalities in routine clinical screening.

**Dilemmas with current cytology-based diagnosis**

The identification of carcinoma and severe dysplasia, if present, is often challenging. Confounding factors include: rare-event detection; sample adequacy; and obscuring features, such as blood, mucous, and inflammation. Most daunting is the appearance of suspicious cells that can be mistaken for malignant/dysplastic cells yet arise from benign cellular changes due to inflammation, treatment, or repair processes. Finally, the diagnosis of cervical disease based solely upon cellular morphology results in a subjective analysis that is highly dependent upon the skills and experience of the cytopathologist and the cytopathologist. This subjectivity is responsible for the well-recognized and documented variability in diagnoses from different individuals. Hence, there is a need within the medical community to evaluate supplemental diagnostic markers to improve the sensitivity, specificity, reproducibility, and, therefore, utility, of current cytology-based diagnostics.

**Liquid-based specimens as platform for new molecular diagnostics**

The ability to detect cervical disease based upon objective clinical-diagnostic parameters would be a highly desirable improvement to cervical disease detection and diagnosis. Liquid-based specimens represent an opportune platform for the application of molecular-diagnostic assays. The uniformity of the sample as a homogenous suspension of cells makes this an ideal medium for the detection of nucleic acids and proteins associated with cervical disease development and progression. These new methods are potentially either nucleic acid-based using polymerase chain reaction (PCR) target amplification or protein-based assays utilizing immunochemistry formats. Liquid-based cytology samples have been shown to be compatible with both assay formats as described below.

**HPV testing**

Human papillomavirus (HPV) is recognized as the etiologic agent responsible for the initiation of cervical neoplasia. The oncogenic forms of HPV can be classified into high-risk and intermediate-risk viral subtypes. The high-risk HPV viral subtypes include 16, 18, 45, and 58; and the intermediate-risk HPV viral subtypes are 31, 33, 35, 39, 51, 52 and 69. Both the high-risk and the intermediate-risk HPV viral subtypes are detectable through the use of the hybrid capture (HC-2) assays (Digene Corp., Gaithersburg MD) as well as a variety of PCR-based methods. Most HPV infections are transient due to inflammation, treatment, or repair processes. Finally, the diagnosis of cervical disease based solely upon cellular morphology results in a subjective analysis that is highly dependent upon the skills and experience of the cytopathologist and the cytopathologist. This subjectivity is responsible for the well-recognized and documented variability in diagnoses from different individuals. Hence, there is a need within the medical community to evaluate supplemental diagnostic markers to improve the sensitivity, specificity, reproducibility, and, therefore, utility, of current cytology-based diagnostics.

**Table I**

<table>
<thead>
<tr>
<th>Clinical attributes of typical liquid-based cervical cytology specimens</th>
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<tbody>
<tr>
<td>Significant increase in detection of HSIL+ versus conventional Pap smear</td>
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<tr>
<td>Decrease in unsatisfactory cases compared to conventional Pap smear</td>
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<tr>
<td>100% of collected cells transferred to the collection device insuring improved sample adequacy</td>
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<tr>
<td>Sample processing that removes obscuring factors, such as blood and mucus, enabling a clear view for analysis</td>
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<tr>
<td>Proprietary ethanol-based preservative for enhanced cellular morphology and exquisite nuclear detail for morphology-based diagnosis</td>
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<tr>
<td>Nonhazardous ethanol-based preservative for safe laboratory handling and disposal</td>
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<tr>
<td>Sufficient residual material remaining for adjunctive tests such as Chlamydia trachomatis / Neisseria gonorrhoeae or HPV</td>
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<tr>
<td>Compatibility with emerging molecular tests</td>
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**Figure 1:** The detection of HSIL in a typical cytology liquid-based specimen

**Figure 2:** Detection of MCM-5 protein in cervical disease: CIN 3 lesion in tissue (above panel) and HSIL in typical cytology specimen (bottom panel).
Cytology information system helps improve patient care

By Betty Judge, CT(ASCP)

While much diagnostic testing produces numbers or metrics from which a diagnosis is rendered, cytology — the study of exfoliated cells — requires that the cytotecnologist screens slides, determining if changes in the cells are consistent with a certain type of abnormality, then flagging those patients for further testing. Other factors also come into play: slide preparation, work environment, and tools provided. Thus, qualitative cytology testing carries a relatively higher margin of error compared with quantitative-diagnostic tests.

Though charges of criminal negligence are rare, lawsuits springing from misdiagnosis are not — even if a laboratory’s findings are within the acceptable margin of error.

Challenges associated with the screening process can be compounded by a manual reporting system of hard-copy reports and file folders. If a significant Pap-smear abnormality is found, for example, the lab must re-screen the previous five years of negative slides. Sorting through paper files can be cumbersome. Simple clerical errors (like spelling mistakes in a patient’s name) and incomplete or misfiled reports can also make it difficult for a tech to review a patient’s history — essential in identifying re-screening needs and in determining the aggressiveness of treatment if abnormal cells are present.

Regulatory bodies, which set standards for various areas of the lab, recognize that screening tests are not 100% accurate and have their own measurements for acceptable standards of care. Laboratories that wish to maintain certifications from standards organizations must carefully track, record, and compare the incidence of lab error over time as well as that of the individual techs working in the lab. These reports and the quality certifications granted by such organizations can help protect the laboratory and its staff in lawsuits, minimizing potential for damage. A cytology-information system can help the lab assure higher quality testing and better case-history tracking as well as ensure appropriate follow up with the ordering clinician, resulting in improved patient care.

Potentially serious consequences

Though charges of criminal negligence are rare, lawsuits springing from misdiagnosis are not — even if a laboratory’s findings are within the acceptable margin of error. In an extreme case concerning the deaths of two Wisconsin women from cervical cancer, criminal charges were brought against a lab, a technician, and a doctor who, prosecutors contended, each had missed unmistakable signs of cancer during Pap-smear screenings. A $4 million settlement awarded in 2000 to the family of a 37-year-old woman who died from cervical infection in the population.12-16 Recently, the FDA has approved the use of HPV testing in combination with Pap screening for primary screening and detection of cervical disease.17 This new utility for HPV testing in a primary diagnostic setting has been recommended in the 2001 Consensus Guidelines from the American Society for Colposcopy and Cervical Pathology (ASCCP), published in 2002.18,19 Recent studies have demonstrated that liquid-based cytology specimens can be used to support HPV analysis including PCR-based methods.20-22

The ability to detect cervical disease based upon objective clinical-diagnostic parameters would be a highly desirable improvement to cervical disease detection and diagnosis.
Emerging molecular diagnostics

The clinical utility of HPV-based screening for cervical disease is in its negative predictive value. An HPV-negative result in combination with a history of normal Pap smears is an excellent indicator of a disease-free condition and a low risk of cervical neoplasia development during the subsequent one to three years. A positive HPV result, however, is not diagnostic of cervical disease; rather, it is an indication of infection. Although the majority of HPV infections is transient and will spontaneously clear within a 12-month period, a persistent infection with a high-risk HPV viral subtype indicates a higher risk for the development of cervical neoplasia. To supplement HPV testing, a number of molecular markers have been evaluated in order to improve upon the specificity of HPV testing when used in combination with the Pap screen.

p16INK4A

p16INK4A (p16) is an inhibitor of cyclin-dependent kinases (CDK 4 and CDK6) and functions in the progression from G1 to S phase of the cell cycle. In response to infection by high-risk HPV infectious, p16 is overexpressed in cervical keratinocytes, including HSIL and carcinoma lesions. Overexpression of p16 has been shown to correlate with HPV type 16 and 18 infections and can be detected in both squamous cell carcinoma and adenocarcinoma. The specificity of p16 overexpression has been examined and is not only with carcinoma, but also with biopsy confirmed CIN 2+ lesions and a significant number of LSIL – CIN 1 lesions.23-27

The p16 protein can be detected in liquid-based specimens using immunoassay techniques.7-10 Characteristics of p16 detection of cervical disease are summarized in Table II.

MCM-5

MCM proteins function in the early stages of DNA replication through loading of the pre-replication complex onto DNA and functioning as a helicase to help unwind the duplicated DNA strand. Early publications have shown that the MCM proteins, and in particular, MCM-5, are useful for the detection of cervical disease11 as well as other cancers.12 The published literature indicates that antibodies to MCM-5 are capable of detecting cervical neoplastic cells. The specificity for the detection of MCM-5 expression is not restricted to high-grade cervical disease, but also identifies low-grade dysplasia and proliferative cells that have re-entered the cell cycle following infection with high-risk HPV. Figure 2 shows the detection of MCM-5 positive cervical disease cells within a histology specimen and from a liquid-based cytology specimen. Other transport media may be used in place of the liquid if properly validated. Table III summarizes the published performance of MCM-5 in the detection of cervical disease.

Emerging diagnostic assays for the detection of cervical disease

The application of transcriptional profiling using DNA microarrays has identified a number of genes that are overexpressed within cervical disease samples. Genes overexpressed in cervical carcinoma and in response to HPV infections have been described in the literature.13-16 Recently, translational research on the genes identified using these approaches has yielded interesting advances in both the understanding and the application of these disease-specific markers for the detection of cervical disease.17 It is anticipated that the additional development and investigation within a clinical trial will further define the clinical utility of these molecular-based diagnostics with a significant improvement in the detection of cervical disease. The dilemmas of morphology-based diagnosis will benefit from the development and use of more objective molecular–diagnostic methods. Objective clinical diagnostics that specifically detect cervical disease without relying on the subjective interpretation of an individual cytopathologist will represent a significant advance in the clinical detection of cervical disease. The compatibility of these new molecular–diagnostic tests with the current morphology-based diagnostics using liquid-based cytology specimens are expected to significantly improve the current state of cervical disease screening and diagnosis within the healthcare system.

References


Dorothy L. Rosenthal, MD, FIAC, has been practicing cytopathology since 1970, working in both private and academic sectors. Currently, she is director of pathology, Johns Hopkins Bayview Medical Center, Baltimore, MD, and a paid member of the clinical advisory board for TriPath Oncology, a wholly owned subsidiary of TriPath Imaging.
cancer in 2000 brought about a St. Louis lab’s closing, even though defense experts testified that the presence of abnormal cells in the patient’s Pap smear was very low and their presentation unique, thus suggesting that the lab was not negligent.

A similar case at a Rhode Island hospital, in which the lab missed pre-cancerous cells in the Pap smear of a woman suffering from advanced cervical cancer, spurred another local hospital, Kent County Memorial, to invest in a computer system for the cytology lab so its section chief in charge of cytology could begin tracking its 25 to 50 monthly quality assurance (QA) statistical reports and keep a record of QA metrics with Microsoft Excel. Though improved from the manual methods, the reporting was still very time-consuming using the Excel worksheets. Additionally, Kent had a manual paper-based system for cytology-case reporting, consisting of a two-part requisition form, along with a card file to document patient histories of both cytology and surgical case results, hard copies of which were stored off-site.

Better patient care

Seven years ago, with a grant from a local foundation, Kent upgraded its legacy anatomic pathology (AP) information system (IS) to a newer version of AP software, implementing a cytology module. The IS cuts the QA report-generation time from two weeks to a couple of hours of Excel data entry, since the information is already tracked in the system. Tracking the cytotechs’ work to ensure that the cases are completed appropriately, signed out, and sent to the clinicians is also made easier.

In addition to productivity and management improvements, another benefit of Kent’s cytology system implementation is better patient care. Now, histories are easily accessible, even with clerical errors or patient-name changes, because the system links patients’ records by a number of fields. As a courtesy, it generates a monthly list of patients with abnormal Paps for each clinician, ensuring appropriate follow up.

Additionally, the lab is required to obtain patient follow-up information in the event of an abnormal diagnosis. The cytology system automatically produces follow-up letters to clinicians requesting the course of action taken. For QA purposes, the lab uses the IS to track who performed the original screening, the re-screening, and the final screening in order to aid in identifying re-training or additional education needs for a particular tech.

Highly skilled staff, easily accessed patient history, thorough reporting, and follow-up capabilities enable the lab to more quickly and accurately diagnose the presence of potentially life-threatening disease, which empowers the clinician to determine the best and most aggressive treatment possible.

At the end of the day, laboratories want to have given good patient care and to have provided the most accurate diagnosis in the most timely manner to help that patient receive good care. That really is the bottom line.

Editor’s Note: After her implementation of the software at Kent County Memorial Hospital and because of her experience with the product, Betty Judge, CTA(ASCP), joined Psyche Systems in 2000 after a 30-year career as a cytotechnologist and is presently its director of WindoPath product marketing.

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