

Chapter 2

Processing, Reporting, and Sensitivity of Cervical Cytology with an Emphasis on Glandular Lesions

Obtaining the Cervical Cytology Sample

Identification of glandular lesions in cervical cytology begins with an adequate sampling of the cervical transformation zone and endocervical canal. This is achieved by performance of a speculum examination of the cervix which entails opening of the vaginal canal with a speculum in order to visualize the ectocervix and cervical os. Cells are obtained from the exposed external cervix, the ectocervix, and from the endocervical canal, in order to assure evaluation of the transformation zone, the area of highest risk for preneoplastic and neoplastic squamous and glandular lesions. Several devices are available for cytology sampling. Cotton/dacron swabs had been widely used in the past but have been found to fail to release abnormal cells for examination so their use is no longer recommended [1]. Wooden spatulas should not be used with liquid-based fixatives, but may be used to make direct smears. Currently the most commonly used devices are the plastic spatula, the endocervical brush, and the cervical broom (Fig. 2.1). All devices are intended to circumferentially scrape the cervix. When using the endocervical brush, the bristles nearest the operator should be at the level of the external os of the cervix in order to obtain the best sample; the brush should be rotated 90–180° in one direction being



FIG. 2.1 Devices used to obtain a cervical cytology sample: Plastic spatula (*top*), Cervical broom (*center*) and Endocervical brush (*bottom*)

careful not to over-rotate. The use of both the plastic spatula for the ectocervix and the endocervical brush for the endocervix has been shown to provide more endocervical cells than the spatula alone [2]. The cervix broom is a device designed to sample both the ecto- and endocervix simultaneously. The central longer bristles are inserted into the endocervical canal while the shorter, more peripheral bristles are splayed over the ectocervix. The bristles of the broom are angled in such a way that an adequate sample can only be obtained if the broom is turned *clockwise* for 360° five times.

For conventional smears (CS), the cell samples are smeared from the spatula (either wooden or plastic) and rolled from the brush onto a glass slide and immediately fixed in alcohol, either by direct immersion into a solution of ethanol or by the use of aerosol spray fixation (Fig. 2.2). The CS is now ready for staining with the Papanicolaou (Pap) stain, the stain mandated by the Clinical Laboratory Improvement Amendment of 1988. The Pap stain uses hematoxylin to color the nucleus and several other colors to stain the cytoplasm. The stain was originally developed to analyze the degree of keratinization of the squamous cell cytoplasm but also adequately stains endocervical cells.

Alternatively, the sampling device (only plastic) can be directly immersed into a proprietary vial of alcohol-based fixative in order to capture and release the cells; this method constitutes “liquid-based cytology” (LBC). In North America,

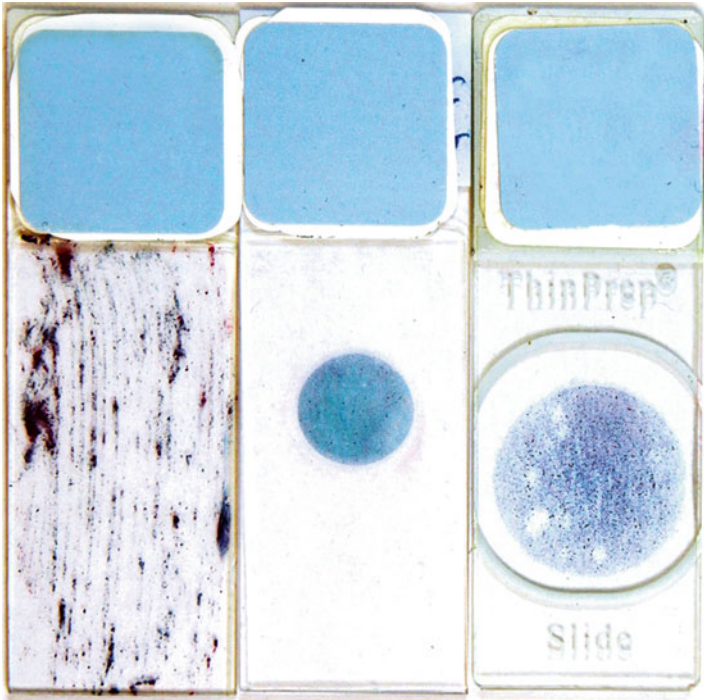


FIG. 2.2 Slide preparations used for cervical cytology: Conventional smear (*left*), SurePath™ (*center*) and ThinPrep® (*right*)

two types of LBC are commercially available: ThinPrep® Pap Test (Hologic, Inc., Marlborough, MA) and BD SurePath™ Pap Test (Becton-Dickinson) (Fig. 2.2). The tests have features that produce subtle but important differences in cervical cytology, especially relative to glandular epithelium.

ThinPrep (TP) employs a methanol-based fixative. The clinician must vigorously agitate the sampling device to extract the maximum amount of cells prior to removing and discarding the device. SurePath (SP) uses an ethanol-based fixative of relatively low concentration. The sampling devices used with SP have detachable heads which are simply dropped into the fixative.

Sample processing differs for the two products leading to a different presentation of the sample on the cytology slide. The TP sample is drawn up through a membrane without any preliminary extraction of unwanted material (mucus, red blood cells, or leukocytes) to which the cells adhere while fluid passes through to be discarded. When the cellularity is sufficient, the cells are pressed to a glass slide. The TP process gives the cell sample a relatively flattened presentation on the slide. SP processing begins with centrifugation with density gradient material to remove excess blood and leukocytes. An aliquot of sample is then allowed to settle by force of gravity onto the slide producing a more three-dimensional quality to the presentation especially prominent in tightly cohesive groups of glandular cells.

The decision to use CS vs. LBC depends on several factors including the rate of fully adequate samples, the reliability of the screening process, the ability to perform additional concurrent tests, and the cost. The cost of LBC is more than CS due to the need for disposables and additional laboratory equipment which is mitigated when additional tests are performed as a second separate sample is unnecessary for the other tests. Despite these differences between CS and LBC, the American College of Obstetricians and Gynecologists does not advocate for one method over the other citing a meta-analysis report of eight studies and a randomized trial that failed to show a significant difference [3–5]. Regarding the unsatisfactory rates between TP and SP, a recent systematic review and meta-analysis of 14 SP studies and 28 TP studies found significantly fewer unsatisfactory SP samples as compared to TP [6].

Performance of CS as compared to LBC has not found significant differences in the rate of detection of high grade squamous intraepithelial lesions [5, 7–9]. On the other hand, observational studies suggest that CS does not perform as well as LBC in the detection of glandular abnormalities [7, 10–14].

Obscuring blood may result in an unsatisfactory CS sample so the first few days of the menstrual cycle should be avoided [15]. Appropriately processed LBC and HPV testing

results do not appear to be affected by the timing of the menstrual cycle [16]. Likewise gel lubricant on the speculum does not appear to interfere with the cytology [17, 18].

The Requisition

The cervical cytology sample must be accompanied by a requisition form which should be completed by the clinician in order to provide the basic patient information needed to interpret the sample. Required elements by US Federal Regulations include patient name, age, date of birth, ordering clinician, and whether the patient is undergoing routine screening or is at high risk for cervical disease. The most important elements for the specific cytologic evaluation of glandular cells are the patient's age, the last menstrual period, gestational history, use of an intrauterine device (IUD) as a method of contraception, history of diethylstilbestrol exposure in utero, and any history of prior abnormal cytology samples or gynecologic pathology (Table 2.1). During the analysis of the slide, the cytologist should correlate the cytologic findings with the clinical information provided. If a discrepancy is identified between the information provided and the cytologic findings, the cytologist must make an attempt to explain the differences. If the electronic medical record and laboratory information system are available, the records can be searched for clarification. As an example, changes in glandular cells suggestive of the presence of an IUD may be

TABLE 2.1 The cytology requisition form

Patient demographics: Name; Age; Date of Birth; Medical record number
Date of procedure
Ordering Clinician
Sample obtained for : routine screening; follow-up of high risk
Patient history: gestational history; last menstrual period; contraception; other history

identified, but the clinician has not indicated the placement of an IUD. Not infrequently in searching the medical record the history of IUD placement will be found.

Image Analysis-Assisted Screening

Screening of cervical cytology slides is often assisted by image analysis (also known as automated review) prior to the review by the cytotechnologist. Two systems are FDA approved and available for image analysis: the ThinPrep Imager (TPI) and the SurePath FocalPoint family (Slide Profiler, Guided System [GS] and Location Guided Screening (LGS)) of which the GS is nearly exclusively used in North America. Both systems (TPI and FP GS) evaluate LBC slides and select fields of view (FOV) most likely to harbor abnormal cells for presentation to the cytotechnologist for initial review. The FOV make up approximately one third of the total slide area for both devices. If cytologic abnormalities are not identified by the cytotechnologist on initial review, the case is signed out as negative. If a potential abnormality is identified, the full slide is manually screened by the cytotechnologist. Both systems rely on robotic instrumentation that incorporates automated microscopy with high-speed image analysis. Both systems use complex computer algorithms to analyze morphologic findings. At the heart of the ThinPrep system is the use of a modified “Feulgen-like” hematoxylin stain which enables the image analysis software to calculate the relative stoichiometric DNA content of the epithelial cells. FOVs are presented in a geographic fashion for each slide. The FocalPoint family uses traditional Papanicolaou-stained slides and hierarchically “scores” each slide and the FOVs on each slide, presenting the FOVs to the reviewer in order of probability of abnormality (highest probability FOVs first). In addition, the slides within a given run are ranked according to their potential to harbor abnormal cells.

Both the TPI system and FP GS system have been found to accurately triage slides with glandular cells abnormalities to full manual review [19, 20]. However, as compared to the

ranking of slides containing high grade squamous lesions (HSILs are routinely ranked at the highest scores), the FPGS system may not always assign a slide containing a glandular lesion to the highest scoring ranks (slides with glandular lesions can occur at any score level) and hence all slides need to be carefully reviewed for their presence [21].

Specimen Reporting Format and Terminology for Reporting Glandular Lesions Identified on Cervical Cytology

In 1988 a new nomenclature, the Bethesda System (TBS), for reporting cervical cytology results was devised in order to standardize terminology, to produce clinically useful reports improving communication with clinicians and other cytologists, and to improve the reproducibility of diagnostic criteria for each entity. The Bethesda terminology has undergone one revision which took place at a second consensus conference in 2001. In the newest edition of the terminology, recommended components of the report include specimen type (conventional smear vs. LBC), adequacy (satisfactory or unsatisfactory), interpretation (negative for intraepithelial lesion or malignancy (NILM), other (endometrial cells in a woman over 40 years of age) or epithelial cell abnormalities (squamous or glandular) or other malignant neoplasms), ancillary testing, and automated review. An optional quality indicator component allows the report to signal features such as the absence of an endocervical/transformation zone component in an otherwise satisfactory sample, which may have clinical utility in selected circumstances. Under the interpretative category of NILM are included reactive changes; for example, reactive endocervical cells resulting from the presence of an intrauterine contraceptive device. Epithelial cell abnormalities: glandular are subcategorized according to cell-type (endocervical, endometrial, or extrauterine) and as to severity (atypical not otherwise specified, atypical favor neoplastic, adenocarcinoma in situ (AIS), and adenocarcinoma (ACA))

TABLE 2.2 Recommended Bethesda System cytology report format
SPECIMEN TYPE: CS vs. LBC
SPECIMEN ADEQUACY: Satisfactory vs. Unsatisfactory (Reason)
GENERAL CATEGORIZATION:
Negative for intraepithelial lesion or malignancy (NILM)
Other: See interpretation/result (e.g., endometrial cells in woman >40 years of age)
Epithelial cell abnormality: Squamous vs. glandular
INTERPRETATION/RESULT:
NILM: NOS or REACTIVE or ATROPHY or ORGANISMS
SQUAMOUS CELL ABNORMALITIES
GLANDULAR CELL ABNORMALITIES
Atypical endocervical cell—NOS vs. favor neoplastic
Atypical endometrial cells—NOS vs. favor neoplastic
Atypical glandular cells—NOS vs. favor neoplastic
Endocervical adenocarcinoma in situ
Adenocarcinoma—endocervical vs. endometrial vs. extrauterine vs. NOS
OTHER MALIGNANT NEOPLASMS
QUALITY INDICATORS: e.g., obscuring blood, no endocervical cells
ANCILLARY TESTING: HPV test method and result
AUTOMATED REVIEW: device and result
CS conventional smear, LBC liquid based cytology, NOS not otherwise specified

(Table 2.2). The first edition of TBS used the umbrella term, atypical glandular cells (AGC) of undetermined significance (AGUS) and required qualification as to cell of origin (endocervical or endometrial). In the second edition, AGUS was abandoned for at the interpretive designation AGC.

Prior to the revised 2001 TBS, the presence of cytologically benign exfoliated endometrial cells out of phase (present in the second half of the menstrual cycle) were reported for women of all ages. However, because studies showed that in women less than 40 years of age, this finding was not found to harbor significant endometrial pathology when further investigated, the terminology was modified. Women aged 40 years or more are occasionally found to have endometrial neoplasia, and as such, reporting of benign-appearing endometrial cells is now restricted to women aged 40 years or older, and in practice is often further restricted to only postmenopausal or premenopausal women over 40 only if the endometrial cells are present in the second half of the menstrual cycle.

Sensitivity of Cervical Cytology for the Presence of Glandular Abnormalities

Fewer studies concerning the sensitivity of cervical cytology for glandular abnormalities have been published than have been for the far more common squamous lesions. The problem of sensitivity has been approached in two ways, either as a retrospective review of patients with histologically diagnosed AIS/ACA or as a follow-up study of glandular lesions identified on cervical cytology.

Through follow-up studies the frequency of the cytologic diagnosis of AGUS or AGC has been found to vary from 0.11 to 0.46 % [12, 22–28]. Follow-up studies also permit determination of the rate of subsequent histologic abnormalities overall and by subcategorization of AGUS or AGC or by associated cytology findings.

The incidence of significant pathology following the AGUS or AGC interpretation varied widely, from as low as 9 % to as high as 58.6 % [29, 30] but almost all studies show a rate of over 20 % [23, 28, 31]. This level of variation may be due in part to uneven application of diagnostic criteria to the cytology sample. Subclassification of AGC as favor neoplasia results in a higher incidence of significant abnormalities on follow-up histology [28]. Association of AGC interpretation with a squamous lesion (ASCUS, ASC-H, LSIL, or HSIL) will more often be associated with a significant squamous lesion than a glandular lesion [25]. Interpretation of AGC in younger (<40 years) or premenopausal women is more likely to be associated with HSIL [25, 31].

Retrospective reviews of prior cytology following the diagnosis of adenocarcinoma in situ (AIS) or endocervical adenocarcinoma (ECA) on histology have provided insights into the sensitivity of cervical cytology for glandular lesions. By analyzing data from a cervical cytology registry in Australia, a retrospective review of ECA and AIS revealed several findings. The sensitivity of cervical cytology preceding the diagnosis of AIS was found to be about 50 % [32]. Examination of cytology samples preceding cases of ECA and AIS revealed

a significant rate of sampling errors (22.2 % and 35 %, respectively) [33,34]. Screening/interpretive errors accounted for 19.4 % of errors in cases of ECA and 10.4 % of AIS cases.

Another retrospective review of ECA found the sensitivity of a single cervical cytology between 45 and 76 % after review of the available smears [35]. The authors attributed a significant number of the false negatives to misinterpretation of preneoplastic/neoplastic glandular epithelium as normal lower uterine segment, tubal metaplasia or reactive endocervical cells. Other studies have noted a lower sensitivity for AIS or ECA when HSIL is also present [36]. The location of glandular neoplasia higher in the endocervical canal rather than at the transformation zone has been associated with a greater false negative rate for AIS [37].

The use of LBC rather than conventional smears has been associated with improved sensitivity for glandular lesions of the cervix [12, 23].

Certainly the move to new sampling devices that obtain more endocervical epithelium from higher in the canal has been found to be more sensitive in the detection of glandular lesions; however, increased recognition of the cytologic features of these processes undoubtedly also plays a role in sensitivity improvements that have been noted over time.

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