Protocol for the Reporting of Cervicovaginal Cytology Specimens

Version: 1.0.0.0
Protocol Posting Date: June 2022
The use of this protocol is recommended for clinical care purposes but is not required for accreditation purposes.

This protocol may be used for the following:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervicovaginal cytology</td>
<td>Includes broom, spatula, and endocervical brush collection methods</td>
</tr>
<tr>
<td>Specimen Type</td>
<td>Description</td>
</tr>
<tr>
<td>PAP stained cervicovaginal cytology</td>
<td></td>
</tr>
</tbody>
</table>

The following should NOT be reported using this protocol:

<table>
<thead>
<tr>
<th>Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-cervicovaginal cytology specimens</td>
</tr>
</tbody>
</table>

Authors
Sana O. Tabbara, MD*; George G. Birdsong, MD; Christine N. Booth, MD; Jennifer Brainard, MD; Stuart E. H. Cameron, MD; James Dvorak; Abha Goyal, MD; Lananh Nguyen, MD; Kaitlin Sundling, MD, PhD.
With guidance from the CAP Cancer and CAP Pathology Electronic Reporting Committees.
* Denotes primary author.

Accreditation Requirements
The use of this case summary is recommended for clinical care purposes but is not required for accreditation purposes. The core and conditional data elements are routinely reported. Non-core data elements are indicated with a plus sign (+) to allow for reporting information that may be of clinical value.

Includes The Bethesda System (TBS) 2014 terminology for reporting cervicovaginal cytology specimens.
Summary of Changes

v 1.0.0.0

- New Protocol
Reporting Template
Protocol Posting Date: June 2022
Select a single response unless otherwise indicated.

CASE SUMMARY: (Protocol for the Reporting of Cervicovaginal Cytology Specimens)
This case summary may be useful for clinical care purposes but is not required for accreditation purposes. Core data elements are bolded to help identify routinely reported elements. (Note A)

PATIENT INFORMATION

Age: _________________

Gender (Note B)
___ Male
___ Female
___ Other (specify): _________________

Collection Date: _________________

Date of Last Menstrual Period (if applicable): _________________

Indication for Examination
___ Screening, routine
___ Screening, high-risk
___ Diagnostic
___ Reflex cytology following positive primary HPV screening result

Prescription Drugs (select all that apply)
___ None
___ Unknown
___ Hormone replacement therapy (estrogen / progesterone)
___ Androgen therapy
___ Oral contraceptive drugs
___ Chemotherapeutic agents
___ Other (specify): _________________

Clinical History (select all that apply)
___ Unknown
___ Pregnant
___ Post-partum
___ Hysterectomy
___ Total
___ Supracervical
___ Prior radiation therapy
___ Diethylstilbesterol (DES) exposure
___ Intrauterine device (IUD)
___ Post-menopausal bleeding
___ Abnormal bleeding
___ Vaginal discharge
___ Other (specify): ______________________

**History of Dysplasia or Malignancy**
___ Unknown
___ Negative
___ Positive
   ___ Abnormal Pap tests / Dysplasia, NOS
   ___ Low-grade squamous intraepithelial lesion (LSIL)
   ___ High-grade squamous intraepithelial lesion (HSIL)
   ___ Endocervical adenocarcinoma in situ (AIS)
   ___ Squamous cell carcinoma
   ___ Adenocarcinoma
   ___ Other (specify): ______________________
___ Carcinoma, NOS

**High-Risk Human Papillomavirus (HPV) History (select all that apply)**
___ Unknown
___ Negative
___ Positive for high risk
___ Positive genotype 16
___ Positive genotype 18
___ Positive for genotype 16/18
___ Positive for genotype 18/45
___ Other high-risk types (specify, if known): ______________________
___ Date of first positive (if available): ______________________
___ Date of most recent HPV testing: ______________________

**HPV Vaccination History (Note C)**
___ Unknown
___ Unvaccinated
___ Vaccinated
   + ___ Completed
   + ___ Incomplete
   + ___ Quadravalent
   + ___ Nonavalent
   + ___ Other (specify): ______________________

**Human Immunodeficiency Virus (HIV) Status**
___ Unknown
___ Negative
___ Positive
___ Positive but undetected
PREANALYTICAL EXAMINATION OF THE SPECIMEN

Source
___ Cervical
___ Vaginal
___ Other (specify): _________________

+Sampling Device
___ Broom
___ Spatula / Endocervical Brush
___ Unknown
___ Other (specify): _________________

Test(s) Ordered
___ Pap test only
___ Cotesting
___ Reflex cytology following positive primary HPV screening result

Gross Description (select all that apply)
___ Number of conventional smear slides: _________________
___ Liquid-based in fixative
   Color (specify): _________________
   Approximate Volume: _________________ ml
___ Other (specify): _________________

Preparation Type
___ Conventional
___ Liquid-based ThinPrep
___ Liquid-based SurePath
___ Other (specify): _________________

Number of Slides Prepared (specify): _________________

+Liquid-based Imaging System Type
___ ThinPrep Imaging System
___ BD FocalPoint GS
___ Other (specify): _________________

INTERPRETATION

Specimen Adequacy (Note D)
___ Satisfactory for evaluation

Quality Indicators
___ Transformation zone present
___ Transformation zone absent
___ Not applicable
___ Cannot be determined
__ Unsatisfactory for evaluation
__ Processed and examined
  __ Insufficient squamous cellularity
  __ Obscuring blood
  __ Obscuring inflammation
  __ Obscuring acellular material
  __ Other (specify): ___________________
__ Not processed (explain): ___________________

Results (select all that apply)
__ Negative for intraepithelial lesion or malignancy (NILM)
__ Negative for squamous intraepithelial lesion
+Non-Neoplastic Cellular Variations (select all that apply)
  __ Squamous metaplasia
  __ Keratotic changes
  __ Tubal metaplasia
  __ Atrophy
  __ Pregnancy-associated changes
  __ Other (specify): ___________________
Reactive Cellular Changes (Note E)
__ Present
  + ___ Inflammation (includes typical repair)
  + ___ Lymphocytic (follicular) cervicitis
  + ___ Radiation
  + ___ Intrauterine device (IUD)
  + ___ Glandular cells status post-hysterectomy
  + ___ Other (specify): ___________________
__ Absent

__ Squamous cell abnormalities

Squamous Cell Abnormalities
  __ Atypical squamous cells - undetermined significance (ASC-US)
  __ Atypical squamous cells cannot exclude HSIL (ASC-H)
  __ Low-grade squamous intraepithelial lesion (LSIL)
  __ High-grade squamous intraepithelial lesion (HSIL)
  __ High-grade squamous intraepithelial lesion (HSIL) with features suspicious for invasion
  __ Squamous cell carcinoma
  __ Other (specify): ___________________

__ Glandular cell abnormalities

Glandular Cell Abnormalities
  __ Atypical endocervical cells (NOS or specify): ___________________
  __ Atypical endometrial cells: ___________________
  __ Atypical glandular cells (NOS or specify): ___________________
  __ Atypical glandular cells, favor neoplastic
  __ Atypical endocervical cells, favor neoplastic
  __ Endocervical adenocarcinoma in situ
  __ Endocervical adenocarcinoma
  __ Endometrial adenocarcinoma
___ Extrauterine adenocarcinoma
___ Adenocarcinoma NOS
___ Other (specify): _________________
___ Other malignant neoplasms (specify): _________________

Other Significant Findings (select all that apply)
___ Endometrial cells present (in patients 45 years of age or older)
___ Trichomonas vaginalis
___ Fungal organisms morphologically consistent with Candida species
___ Shift in flora suggestive of bacterial vaginosis
___ Bacteria morphologically consistent with Actinomyces species
___ Cellular changes consistent with herpes simplex virus
___ Cellular changes consistent with cytomegalovirus
___ Other (specify): _________________
___ None identified

ANCILLARY TESTING
Please complete all available test results associated with the current Pap test

HR-HPV (select all that apply)
___ Not performed
___ Negative
___ Positive (not otherwise specified)
___ Positive for genotype 16
___ Positive for genotype 18
___ Positive for genotype 18/45
___ Positive for other high-risk types (specify): _________________
___ Positive for unknown subtype
___ Pending at the time of cytologic evaluation

HR-HPV Test Platform
___ BD Onclarity TM HPV Assay
___ Hologic Cervista
___ Hologic Aptima
___ Qiagen Digene Hybrid Capture 2 (HC2)
___ Roche cobas 4800
___ Roche cobas 6800/8800
___ Laboratory-developed method
   ___ DNA
   ___ RNA
   ___ Other (specify): _________________
   ___ Other (specify): _________________

___ Neisseria gonorrhoeae
___ Negative
___ Positive
+Chlamydia trachomatis
  ___ Negative
  ___ Positive

+Trichomonas vaginalis
  ___ Negative
  ___ Positive

+Herpes Simplex Virus (HSV) (select all that apply)
  ___ Negative
  ___ Positive (not otherwise specified): _______________________
  ___ Positive for HSV-1
  ___ Positive for HSV-2

+Immunocytochemistry (select all that apply)
  ___ P16: __________________
  ___ Ki-67: __________________
  ___ Other (specify): __________________

+Other Tests Performed (specify): __________________

+Concurrent Biopsy
  ___ Yes
  ___ No

COMPUTER-ASSISTED INTERPRETATION OF CERVICAL CYTOLOGY
  +Specify Device: __________________
  +Specify Results: __________________

COMMENTS

Comment(s): __________________
Explanatory Notes

A. Introduction
The aim of this protocol is to improve the completeness, clarity, and portability of Pap test reporting, while being mindful of the wide range of practice settings in which the data in the report is generated and disseminated. This report includes the Bethesda System for reporting Cervical Cytology which is widely used standardized terminology and incorporate clinical and ancillary testing results that have already been integrated into daily practice, as outlined in the ASCCP guidelines. It also takes into consideration the introduction of additional testing recommendations and modalities in the future.

The protocol is based upon input from past and present members of the CAP Cytopathology Committee and prepared in conjunction with the CAP Pathology Electronic Reporting Committee.

This reporting format is meant to replace the final report and will be adapted to laboratory information systems to facilitate utilization and provide more easily reproducible and extractable data. The construction of this protocol does allow for the insertion of pertinent additional information when available. It may be used as a guide for trainees and pathologists who may only perform a limited number of Pap tests in their practice. The committee hopes this is a first step in providing a general framework for more standardized quality Pap reporting practice.

The content of the protocol represents the consensus opinion of the CAP Cytopathology Committee and the CAP Pathology Electronic Reporting Committee. It is the Committees’ recommendation that all available elements be included.

References

B. Gender
In cervical cancer screening, it is important to recognize the importance of using inclusive gender terminology within the pathology report. Individuals at risk for cervical cancer may identify as women, men, or other non-binary or gender fluid terms.

Laboratory information systems should accurately convey the patient’s gender identity on reports. At minimum, a nonbinary option such as “Other” is recommended to be included, ideally allowing the patient to self-describe their gender identity. Laboratory information systems may also record the sex assigned at birth, which would be kept separately from the gender identity. Ideally, patients would be offered the opportunity to update this information at any time they choose, such as through an online patient portal or at appointments. If pronouns are used in the pathology report, “they/them/their” pronouns are
recommended if patient-identified pronouns are not indicated. Care should be taken not to assume “she/her/hers” pronouns on Pap test reporting.

While this form is developed specifically for cervical and vaginal sources (of natal organs), it is also recognized that patients with a neovagina are also at risk of HPV infection, and Pap test screening is recommended. Neovaginal specimens may be considered non-gynecologic in origin; however, many aspects of this reporting form may still apply. A neovaginal specimen source should be specifically indicated, when known to the collecting clinician.

References

C. HPV Vaccination
Human papillomavirus (HPV) is a common sexually transmitted viral infection that affects multiple sites, commonly the reproductive organs. In females, the virus causes cancer of the cervix, vulva, and vagina whereas in males, it causes cancer of the penis. For both genders, it causes cancer of the anus and oropharynx. HPV infection also causes benign lesions, such as anogenital warts and respiratory papillomatosis. Although there are many types of HPV, studies have identified key genotypes associated with disease. HPV 16 and 18 are two specific genotypes associated with cancer and are considered high risk types.

Currently, there are three vaccines approved by the United States Food and Drug Administration. Nonavalent, (Gardasil 9, 9vHPV), quadrivalent, (Gardasil 4, 4vHPV), and bivalent (Cervarix, 2vHPV). All three vaccines protect against high risk HPV types 16 and 18 with specific targets to the L1 protein. Quadrivalent vaccine includes additional targets to HPV 6 and 11. Nonavalent vaccine includes targets to HPV 6, 11, 31, 33, 45, 52, and 58. Nonavalent vaccine is only distributed in the United States. Both bivalent and nonavalent vaccines are distributed in Canada and all three are distributed in Europe.

The recommended dosing for the vaccination is based on the patient’s age at administration and patient’s history. Two doses are required for patients who received the first dose before they turn 15. Three doses are required for a) patients who received two doses less than 5 months apart when they are between 9 and 14 years old OR b) patients are between 9 and 26 years old with weakened immune systems. Vaccination is not recommended for patients older than 26 years old.

References

D. Specimen Adequacy

Adequacy criteria were set forth in The Bethesda System (TBS)\(^1\) to provide criteria and protocols to promote the consistent assessment of the adequacy of cervical and anal cytology specimens. In the absence of such criteria, individual laboratories would need to develop their own adequacy criteria which would probably lead to greater inconsistency in the percent of cases flagged as unsatisfactory or suboptimal across laboratories.

**Specimen Cellularity**

Cellularity thresholds are based on limited scientific evidence. One study has suggested that liquid-based preparations (LBP) with fewer than 5000-20,000 cells may have a higher risk of being false negative (FNP)\(^2,3\), however this has not been confirmed. TBS states that an LBP from a woman with a cervix should have an estimated minimum of 5000 well-preserved, well-visualized, nucleated squamous cells to be considered adequate.\(^4\) This threshold is provided to promote the usage of consistent criteria across laboratories however, it is not meant to be rigidly applied. Exceptions to this guideline are women with a history of chemo or radiation therapy for cancer, or who are post-hysterectomy or post-menopausal with atrophic changes. The patient’s history must be taken into account in assessing adequacy, but specimens with less than or equal to 2000 cells should usually be considered unsatisfactory.

Cytologists should not attempt to manually count cells to determine cellularity.\(^1\) The cellularity of LBP can be estimated by counting the number of cells in multiple high-power fields (HPFs), usually 40X, across the diameter of the preparation. The two commercially available LBPs, ThinPrep (Hologic), and SurePath (BD) deposit the cells in circles of different diameters and have different cellular densities. ThinPrep deposits cells in a 20 mm diameter circle whereas the SurePath circle is 13 mm. The number of cells/HPF that correspond to the 5000 cell minimum is therefore different. This number is also affected by the field number (FN) of the eyepieces of the microscope. Table 1 displays the number of cells/HPF for different combinations of eyepiece field number, objective power, and circle diameter.\(^1\)
Table 1. Guidelines for Estimating Cellularity of Liquid-Based Preparations

<table>
<thead>
<tr>
<th>Prep. diameter (mm)</th>
<th>Area (mm²)</th>
<th>Number of fields at FN20, 10X</th>
<th>Number of cells/field for 5K total</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>132.7</td>
<td>42.3</td>
<td>118.3</td>
</tr>
<tr>
<td>20</td>
<td>314.2</td>
<td>100</td>
<td>50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prep. diameter (mm)</th>
<th>Area (mm²)</th>
<th>Number of fields at FN20, 40X</th>
<th>Number of cells/field for 5K total</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>132.7</td>
<td>676</td>
<td>7.4</td>
</tr>
<tr>
<td>20</td>
<td>314.2</td>
<td>1600</td>
<td>3.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prep. diameter (mm)</th>
<th>Area (mm²)</th>
<th>Number of fields at FN22, 10X</th>
<th>Number of cells/field for 5K total</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>132.7</td>
<td>34.9</td>
<td>143.2</td>
</tr>
<tr>
<td>20</td>
<td>314.2</td>
<td>82.6</td>
<td>60.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prep. diameter (mm)</th>
<th>Area (mm²)</th>
<th>Number of fields at FN22, 40X</th>
<th>Number of cells/field for 5K total</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>132.7</td>
<td>559</td>
<td>9</td>
</tr>
<tr>
<td>20</td>
<td>314.2</td>
<td>1322</td>
<td>3.8</td>
</tr>
</tbody>
</table>

FN=field number.

Conventional preparations should contain a minimum of 8000-12,000 well-preserved, well-visualized cells. As with LBP, cellularity should be estimated and not considered a rigid threshold. It is not necessary to count cells on conventional preparations. Cellularity can be estimated by comparing representative fields with the computer edited reference images in the Bethesda System for Reporting Cervical Cytology.1

Endocervical/Transformation Zone Component
TBS states that an adequate T-zone sample requires at least 10 well preserved endocervical or squamous metaplastic cells, singly or in clusters. The presence of a transformation zone or endocervical component (T-zone) is not necessary for a specimen to be considered adequate. Theoretically, it might be expected that the risk of an FNP specimen would be elevated if a T-zone component were absent since squamous intraepithelial lesions are thought to arise in the T-zone. Some studies have shown that endocervical cells are more likely to be present in specimens with squamous intraepithelial lesions,4,5,6 however other studies have shown that specimens which lack endocervical cells are not significantly more likely to be FNP, and in fact, some studies show a trend toward a lower risk of FNP.7,8,10,11 While these observations may be somewhat confusing, the co-presence of endocervical cells and dysplastic cells in specimens does not by itself indicate that specimens which lack endocervical cells have a higher risk of being FNP. Nevertheless, the presence of the T-zone component is considered an important quality indicator. Lack of endocervical cells indicates that the endocervical region has not been well sampled, possibly increasing the risk of missing an endocervical lesion such as adenocarcinoma-in-situ or adenocarcinoma.1

Obscuring Factors
Excessive blood or inflammation may obscure epithelial cells in Pap tests. A specimen should be deemed unsatisfactory when more than 75% of the squamous cells are obscured unless abnormal cells are
identified. If 50-75% of cells are obscured, a comment describing the specimen as partially obscured should be added following the satisfactory term. The percentage of cells obscured, not the area of the slide, is what is assessed. Minimal cellularity criteria should also be applied.¹

Interfering Substances
Excessive blood or lubricants that contain carbomers or carbopol polymers can interfere with ThinPreps by clogging the filter thereby reducing the cellularity of the specimen.¹²¹³ Some specimens which are unsatisfactory due to blood can be successfully reprocessed utilizing dilute glacial acetic acid.¹⁴¹⁵ The unsatisfactory rate can be reduced by 50% or more with this technique, but it interferes with some types of HPV tests. Interfering substances have little or no effect on SurePath specimens.¹⁶¹⁷¹⁸

References
E. Reactive Cellular Changes

The Bethesda 2014 classification system for cervical cytology includes the subcategory of “Reactive Cellular Changes” under the Pap test reporting category “Negative for Intraepithelial Lesion or Malignancy”.

The cells in the Pap test can undergo reactive changes associated with inflammation (including typical repair and lymphocytic cervicitis), radiation, and changes associated with intrauterine contraceptive devices. These changes can be a part of normal reactive changes and do not represent dysplastic or precancerous changes.

The reactive cells seen in repair or associated with inflammation can show an increase in nuclear size, presence of nucleoli, binucleation, cytoplasmic vacuolization, and polychromasia. A majority of reactive cells are of metaplastic origin but can also be seen in mature squamous cells or columnar epithelial cells. The nuclei are usually non-overlapping and have an even and uniform fine granular chromatin. Small perinuclear halos can also be present but do not have peripheral thickening. The reactive changes in repair are often cohesive sheets of enlarged cells that can form a “school of fish” appearance.

In lymphocytic cervicitis, a polymorphous population of lymphocytes is present and can be seen with or without tingible body macrophages.

The changes seen in radiation can include markedly enlarged cells which maintain a normal nuclear to cytoplasmic ratio. Binucleation or multinucleation is also commonly seen. Chronic radiation-induced cellular changes can be seen indefinitely in the Pap test.

Changes associated with intrauterine devices can be either endometrial or endocervical columnar cells that undergo irritation and then exfoliation. These cells can be in small groups or be seen as single cells with a clean background. The cytoplasm frequently has large vacuolization that may even displace the nucleus. Actinomyces-like organisms are frequently also seen.

Reactive cellular changes seen on a Pap test have been found to show an increased risk of CIN2-3 but no significant increased risk of cancer. In some patients with a previous history of CIN, benign cellular changes (BCC) may be of some significance, however, in patients with no significant prior cervical abnormalities, a Pap test classified as BCC represents a reactive process.

References
1. The Bethesda System for Reporting Cervical Cytology. 3rd edition
