



## Protocol for the Reporting of Anal Cytology Specimens

**Version:** 1.0.0.0

**Protocol Posting Date:** September 2023

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:**

Procedure	Description
Anal cytology	Includes cytobrush, non-cotton swab, broom, and brush collection methods
Specimen Type	Description
PAP stained anal cytology	

**The following should NOT be reported using this protocol:**

Specimen
Non-anal cytology specimens

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With guidance from the CAP Cancer and CAP Pathology Electronic Reporting Committees.

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### 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.

CAP Approved

Cyto.Anal\_1.0.0.0.REL\_CAPCP

**Summary of Changes**

**v 1.0.0.0**

- New protocol

**Reporting Template****Protocol Posting Date: September 2023****Select a single response unless otherwise indicated.****CASE SUMMARY: (ANAL CYTOLOGY)**

*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: \_\_\_\_\_

**Prescription Drugs (select all that apply)**

\_\_\_ None

\_\_\_ Unknown

\_\_\_ Hormone replacement therapy (estrogen / progesterone)

\_\_\_ Androgen therapy

\_\_\_ Immunosuppressive therapy (other than chemotherapy)

\_\_\_ Chemotherapeutic agents

\_\_\_ Other (specify): \_\_\_\_\_

**Clinical History (select all that apply)**

\_\_\_ Unknown

\_\_\_ Pregnant

\_\_\_ Anal receptive intercourse (including MSM)

\_\_\_ Genital warts

\_\_\_ Prior radiation therapy

\_\_\_ Anorectal bleeding

\_\_\_ Transplant

*Select all that apply*

\_\_\_ Solid organ: \_\_\_\_\_

\_\_\_ Bone marrow

\_\_\_ Other immunocompromised conditions (specify): \_\_\_\_\_

\_\_\_ Other (specify): \_\_\_\_\_

**History of Anorectal and Perianal Dysplasia or Malignancy**

\_\_\_ Unknown

\_\_\_ Negative

\_\_\_ Positive

*Select all that apply*

\_\_\_ Dysplasia, NOS

\_\_\_ Low-grade squamous intraepithelial lesion (LSIL / AIN1)

\_\_\_ High-grade squamous intraepithelial lesion (HSIL / AIN2-3)

\_\_\_ Squamous cell carcinoma

- Carcinoma, NOS
- Other (specify): \_\_\_\_\_

**History of Other Lower Urogenital Tract Dysplasia or Malignancy**

- Unknown
  - Negative
  - Positive (specify site): \_\_\_\_\_
- Select all that apply*
- Dysplasia, NOS
  - Condyloma acuminatum
  - Low-grade squamous intraepithelial lesion (LSIL)
  - High-grade squamous intraepithelial lesion (HSIL)
  - Squamous cell carcinoma
  - Endocervical adenocarcinoma in situ (AIS)
  - Endocervical adenocarcinoma, invasive
  - Carcinoma, NOS
  - Other (specify): \_\_\_\_\_

**High-Risk Human Papillomavirus (HPV) History (select all that apply)**

- Not performed
- Unknown
- Negative
- Positive for high risk
- Positive for genotype 16
- Positive for genotype 18
- Positive for genotype 16/18
- Positive for genotype 18/45
- Other high-risk types (specify, if known): \_\_\_\_\_
- Date of first positive (specify, if available): \_\_\_\_\_
- Date of most recent HPV testing: \_\_\_\_\_

**HPV Vaccination History (Note [C](#))**

- Unknown
  - Unvaccinated
  - Vaccinated
- Select all that apply*
- +  Completed
  - +  Incomplete
  - +  Quadravalent
  - +  Nonavalent
  - +  Other (specify): \_\_\_\_\_

**+Human Immunodeficiency Virus (HIV) Status (Note [D](#))**

- Unknown
- Negative
- Positive
- Positive but undetected

**PREANALYTICAL EXAMINATION OF THE SPECIMEN**

**Source**

- Anorectal, clinician-collected
- Anorectal, self-collected
- Other (specify): \_\_\_\_\_

**+Sampling Device**

- Broom
- Brush
- Spatula
- Swab
- Unknown
- Other (specify): \_\_\_\_\_

**Test(s) Ordered**

- Anal Pap test only
- Anal Pap test with concurrent HPV test
- HPV test following abnormal diagnosis

**Gross Description (select all that apply)**

- Number of conventional smear slides: \_\_\_\_\_
- Liquid-based in fixative

**Color (specify):** \_\_\_\_\_

**Approximate Volume in Milliliters (ml):** \_\_\_\_\_ ml

- Other (specify): \_\_\_\_\_

**Preparation Type**

- Conventional
- Liquid-based ThinPrep
- Liquid-based SurePath
- Other (specify): \_\_\_\_\_

**Number of Slides Prepared (specify):** \_\_\_\_\_

**INTERPRETATION**

**Specimen Adequacy (Note E)**

- Satisfactory for evaluation
- Unsatisfactory for evaluation
- Processed and examined
  - Select all that apply*
  - Insufficient nucleated squamous cellularity
  - Obscuring blood
  - Obscuring inflammation
  - Obscuring acellular material
  - Other (specify): \_\_\_\_\_
- Not processed (explain): \_\_\_\_\_

**Quality Indicators**

- Transformation zone present
- Transformation zone absent
- Not applicable
- Cannot be determined

**Results (select all that apply)**

- Negative for intraepithelial lesion or malignancy (NILM)
- 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 glandular cells
- Adenocarcinoma
- Other (specify): \_\_\_\_\_
- Other malignant neoplasms (specify): \_\_\_\_\_

**+Other Significant Findings (select all that apply)**

- Amoeba species
- Trichomonas vaginalis
- Fungal organisms morphologically consistent with Candida species
- Enterobius vermicularis (pinworm)
- Cellular changes consistent with herpes simplex virus
- Cellular changes consistent with cytomegalovirus
- Inflammation (includes typical repair)
- Therapy related change (radiation): \_\_\_\_\_
- Other (specify): \_\_\_\_\_

**ANCILLARY TESTING**

*Please complete all available test results associated with the current Pap test*

**HR-HPV (select all that apply)**

- Not performed
- Negative
- Positive, NOS
- 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

**+Treponema pallidum**

- Negative
- Positive

**+Herpes Simplex Virus (HSV) (select all that apply)**

- Negative
- Positive, NOS
- 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

**COMMENTS**

**Comment(s):** \_\_\_\_\_

## Explanatory Notes

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### A. Introduction

The aim of this protocol is to improve the completeness, clarity, and portability of Anal 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 terminology from the Bethesda System for Reporting Cervical Cytology as anal cytology is backed by our knowledge of HPV infection in the cervix.<sup>1,2</sup> That terminology is widely used and standardized.<sup>3,4</sup> Although there are no well-established screening and management guidelines, this protocol incorporates clinical and ancillary testing results that have been already incorporated in daily practice.<sup>5,6,7</sup> It also takes into consideration the introduction of additional testing modalities in the future.

The protocol is based upon input from 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 Anal Pap tests in their practice. The committee hopes this is a first step in providing a general framework for more standardized quality Anal Pap test 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

1. Nayar R, DC Wilbur, Eds *The Bethesda System for Reporting Cervical Cytology*, 3rd. Edition. New York, NY: Springer 2015.
2. Nayar, R. and Wilbur, D.C. (2015), The Pap test and Bethesda 2014. *Cancer Cytopathology*, 123: 271-281. <https://doi.org/10.1002/cncy.21521>.
3. Morency EG, Harbert T, Fatima N, Samolczyk J, Maniar KP, Nayar R. Anal Cytology: Institutional Statistics, Correlation With Histology, and Development of Multidisciplinary Screening Program With Review of the Current Literature. *Arch Pathol Lab Med*. 2019 Jan;143(1):23-29. doi: 10.5858/arpa.2017-0242-RA. Epub 2018 Apr 13. PMID: 29652190.
4. Roberts JM, Ekman D. The reporting of anal cytology and histology samples: establishing terminology and criteria. *Sex Health*. 2012 Dec;9(6):562-7. doi: 10.1071/SH10140. PMID: 22951231.
5. Darragh, T.M. and Winkler, B. (2011), Anal cancer and cervical cancer screening: Key differences. *Cancer Cytopathology*, 119: 5-19. <https://doi.org/10.1002/cncy.20126>.
6. Ortoski RA, Kell CS. *Anal Cancer and Screening Guidelines for Human Papillomavirus in Men* JAOA, Supplement 2, Vol 111, No 3 March 2011 S35 [https://www.acofp.org/acofpimis/acofporg/PDFs/PM/HPV\\_in\\_Men.pdf](https://www.acofp.org/acofpimis/acofporg/PDFs/PM/HPV_in_Men.pdf).
7. NYS Guidelines recommendations on anal pap smears [https://www.natap.org/2010/HIV/032510\\_01.htm](https://www.natap.org/2010/HIV/032510_01.htm).

### B. Gender

In anal cancer screening, it is important to recognize the importance of using inclusive gender terminology within the pathology report. Individuals at risk for anal cancer may identify as women, men, or other non-binary or gender fluid terms. Pathology reports should avoid gendered terminologies unless the patient is known to identify with those terminologies.



Patients at risk of anal cancer include all genders. Evidence specific to anal cancer risks in transgender and non-binary patients is limited given the lack of available information in national databases.<sup>1</sup> Men who have sex with men are at increased risk of anal cancer; however, this risk is not uniform to all patients who engage in anal receptive intercourse. In cisgender women with cervical HPV infection, anal receptive intercourse is not associated with an increased risk of anal HPV, and the approach to screening should not be limited to patients with a history of anal receptive intercourse.<sup>2</sup>

Laboratory information systems should accurately convey the patient's gender identity on reports. At minimum, a nonbinary option 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.<sup>3</sup> 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/theirs" pronouns are recommended if patient-identified pronouns are not indicated.

#### References

1. Compton ML, Taylor SS, Weeks AG, Weiss VL, Hogan MM, Wang H, Ely KA. Cytology and LGBT+ health: establishing inclusive cancer screening programs. *Journal of the American Society of Cytopathology*. 2022;11(5):241-252. doi:10.1016/j.jasc.2022.06.003.
2. Moscicki AB, Darragh TM, Berry-Lawhorn JM, et al. Screening for Anal Cancer in Women. *J Low Genit Tract Dis*. 2015;19(3 0 1):S26-S41. doi:10.1097/LGT.000000000000117.
3. Gamelin M. Guide to LGBTQ+ *Inclusive Forms*. Accessed January 17, 2022. <https://denverptc.org/resource.php?id=231>.

### C. Human Papillomavirus (HPV) Vaccination

Human papillomavirus (HPV) is a common sexually transmitted viral infection that affects multiple sites. For both sexes, it causes cancer of the anus and oropharynx. In females, the virus causes cancer of the cervix, vulva, and vagina. In males, it causes cancer of the penis. 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 the two most common genotypes associated with HPV-related cancers and are considered high risk types.

Currently, there are three vaccines approved by the United States Food and Drug Administration. 9-Nonavalent, (Gardasil 9, 9vHPV), quadrivalent, (Gardasil, 4vHPV), and bivalent (Cervarix, 2vHPV).<sup>1</sup> 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 additional targets to HPV 6, 11, 31, 33, 45, 52, and 58. Currently, only nonavalent vaccine is distributed in the United States. Both bivalent and 9-valent vaccines are distributed in Canada and all three are distributed in Europe.<sup>2</sup>

The recommended dosing for the vaccination is based on the patient's age at administration and patient's history.<sup>3</sup> Two doses are required for patients who received the first dose before their 15th birthday. Three doses are required for a) patients who received two doses less than 5 months apart when they were between 9-14 years old OR b) patients are between 9-26 years old with weakened immune systems. Vaccination is not recommended for patients older than 26 years old.

#### References

1. Human Papillomavirus (HPV) Vaccination: What Everyone Should Know. <https://www.cdc.gov/vaccines/vpd/hpv/public/index.html>.

2. European Medicine Agency, Human papillomavirus vaccines, Cervarix, Gardasil, Gardasil 9, Silgard. [https://www.ema.europa.eu/en/documents/referral/hpv-vaccines-article-20-procedure-assessment-report\\_en.pdf](https://www.ema.europa.eu/en/documents/referral/hpv-vaccines-article-20-procedure-assessment-report_en.pdf).
3. National Advisory Committee on Immunization (NACI). Update on Human Papillomavirus (HPV) Vaccines. An Advisory Committee Statement (ACS). Canada Communicable Disease Report. January 2012; Volume 38, ACS-1:1-62.

#### **D. Human Immunodeficiency Virus (HIV)**

Human immunodeficiency virus (HIV) is a lentivirus that attacks the immune system. The virus destroys CD4 positive T-cells, a subset of lymphocytes, that are a key component of the immune system. Although there is currently no cure, treatment of HIV with antiretroviral therapy can lead to long term suppression of the virus. Left untreated, viral loads increase over time, and the disease progresses to the most advanced stage, acquired immunodeficiency syndrome (AIDS). AIDS is also the stage during which HIV is most easily transmitted to other people.<sup>1</sup>

Because of their weakened immune systems, patients with HIV are at an increased risk of contracting other infections and developing cancers. Opportunistic infections are those infections that occur more easily and progress more rapidly and extensively in immunocompromised individuals compared to those with an intact immune system. A few examples of opportunistic infections in HIV patients are Pneumocystis, Candidiasis, Mycobacterial organisms (tuberculous and atypical Mycobacterial infections), and Coccidiomycosis. HIV patients are at elevated risk for cancers, such as lymphoma and Kaposi sarcoma.<sup>2</sup>

Human papillomavirus infection (HPV) is the most common sexually transmitted infection. HPV infection in the anal region can cause benign genital warts, precancerous lesions, or anal cancers. Anal screening in HIV patients is strongly recommended due to their increased risk of infection and cancer from HPV.<sup>3</sup>

#### **References**

1. HIV. World Health Organization. <https://www.who.int/news-room/fact-sheets/detail/hiv-aids>.
2. Wang CJ, Palefsky JM. HPV-Associated Anal Cancer in the HIV/AIDS Patient. *Cancer Treat Res*. 2019;177:183-209. doi: 10.1007/978-3-030-03502-0\_7. PMID: 30523625.
3. Barroso II LF, Stier EA, Hillman R, Palefsky J, Anal Cancer Screening and Prevention: Summary of Evidence Reviewed for the 2021 Centers for Disease Control and Prevention Sexually Transmitted Infection Guidelines, *Clinical Infectious Diseases*, Volume 74, Issue Supplement\_2, 15 April 2022, Pages S179–S192, <https://doi.org/10.1093/cid/ciac044>.

#### **E. Specimen Adequacy**

The criteria for specimen adequacy of anal cytology samples are based on the Bethesda System recommendations.<sup>1</sup> For conventional smears, the minimal cellularity required for an adequate sample is approximately 2,000-3,000 nucleated squamous cells (NSC). For evaluating the adequacy of liquid-based preparations, the average number of NSC per high-power field (HPF) is estimated and varies with the type of preparation and the optical parameters of the microscope. For ThinPrep (Hologic, Marlborough, MA), an average of 1-2 NSC per HPF and for SurePath (Beckton Dickinson, Franklin Lakes, NJ), an average of 3-6 NSC per HPF correspond to the conventional smear requirement and are considered satisfactory. Specimens that are predominantly composed of anucleated squames without the minimal required number of NSC, should be designated as unsatisfactory.

Similar to cervical cytology, the presence of a transformation zone (TZ) component (i.e., at least ten rectal columnar cells/squamous metaplastic cells in this scenario) is not a requirement for deeming an anal cytology sample adequate. However, the presence of the TZ component does signify sampling of the anal

canal above its keratinized portion and is utilized as a quality indicator. The significance of the presence of the TZ component has been variably reported in literature. In a study involving conventional smears, the absence of rectal columnar cells in the sample did not impact the sensitivity, specificity, or predictive value of anal cytology.<sup>2</sup> However, in a relatively recent study based on the evaluation of ThinPrep, the lack of the TZ component was associated with a significantly higher number of false-negative results.<sup>3</sup> Obscuring factors such as fecal material, lubricant, bacteria and inflammation may interfere with the evaluation of an anal cytology sample and if the majority of the cells are obscured and the required minimal NSC cellularity cannot be well visualized, the specimen should be considered unsatisfactory. It is also important to note that any atypia encountered in the specimen makes it adequate regardless of the number of NSC.

#### References

1. Darragh TM and Palefsky JM. *Anal Cytology*. In: Nayar R, Wilbur DC (Eds.). The Bethesda system for reporting cervical cytology. Definitions, criteria, and explanatory notes. Springer; 2015. p. 263-85.
2. Palefsky JM, Holly EA, Hogeboom CJ, Berry JM, Jay N, Darragh TM. Anal cytology as a screening tool for anal squamous intraepithelial lesions. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1997; 14:415-422.
3. Roberts JM, Jin F, Thurloe JK, et al. The value of a transformation zone component in anal cytology to detect HSIL. *Cancer Cytopathol*. 2016;124(8):596-601.