CSIRO PUBLISHING
Sexual Health
http://dx.doi.org/10.1071/SH12003

Screening for anal neoplasia: anal cytology – sampling, processing and reporting

Teresa M. Darragh[A,C] *and Barbara Winkler*[B]

[A]UCSF Mt. Zion Medical Center, Departments of Pathology and Obstetrics, Gynecology & Reproductive Sciences, 1600 Divisadero Street, Room B618, San Francisco, CA 94115, USA.
[B]Department of Pathology, Mount Kisco Medical Group, 110 South Bedford Road, Mount Kisco, NY 10549, USA.
[C]Corresponding author. Email: teresa.darragh@ucsf.edu

Abstract. Anorectal cytology (ARC) is increasingly accepted as a valid screening tool for the diagnosis of squamous intraepithelial lesions in populations at increased risk for anal cancer. As with cervical cancer screening protocols, proper patient preparation, specimen collection and specimen processing are essential for obtaining an optimal cytological sample. With attention and experience, the clinician can collect the best possible ARC specimen for laboratory evaluation. The incorporation of repeated interval anal cytology into standard surveillance practices for high-risk individuals is a valuable tool for the early detection of human papillomavirus-related anal squamous epithelial lesions and the prevention of anal squamous cell carcinomas.

Received 9 January 2012, accepted 1 May 2012, published online 20 August 2012

Introduction

Anal cytology or anorectal cytology (ARC) is becoming a more common tool for the evaluation of human papillomavirus (HPV) related anal squamous intraepithelial lesions (ASIL) and anal squamous cell carcinomas (SCCs), particularly in populations at increased risk for anal cancer, including men who have sex with men (MSM) and HIV-seropositive patients. Because the aetiology and pathogenesis of anal squamous neoplasias are analogous to cervical disease, most of the screening and diagnostic protocols have been adapted from the knowledge base of cervical cancer screening and management.

As ARC gains wider acceptance as a screening tool for anal cancer and its precursors, techniques to optimise its performance need to be delineated. This review focuses on specimen collection and processing, specimen adequacy, and the sensitivity and specificity of anal cytology, and briefly reviews the cytomorphologic features of HPV-related abnormalities and other findings.

Specimen collection

As with the cervix, the technique of obtaining the anal cellular sample is critical for maximising the quality of the sample and, ultimately, the success of cytological screening. The goal of specimen collection is to harvest a cellular sample that adequately represents the morphology of the entire epithelial lining of the anal canal. The anal canal is ~3.5–4 cm long in men and shorter in women.[1] It extends from the distal rectal vault to the anal verge. Its mucosal surface is apposed due to the resting tone of the anal sphincters, resulting in a plicated surface that is challenging to sample thoroughly.

As with gynaecological cytology, instructing the patient is important to obtaining an optimal sample. Enemas and receptive anal intercourse should be avoided for 24 h before the procedure.[2] The rectum should be emptied before obtaining the cytology sample.[3] Patients can be positioned in various ways for collection of ARC specimens. The left lateral decubitus position is preferred when the ARC is followed by high-resolution anoscopy. For female patients who are also having a pelvic exam, the dorsal lithotomy position is typically used.

For sampling, a tap water-moistened Dacron or synthetic polyester fibre swab is the most commonly used sampling device, in the manner first described by Palefsky *et al.*[4] A variety of other sampling devices have been used, although direct comparisons on the performance of the devices are few in number. Arain *et al.* reported use of the Rovers EndoCervex brush (Rovers Medical Devices, Oss, The Netherlands), the Digene cervical sampler brush (Qiagen, Inc. USA, Valencia, CA, USA) and the brush from the SurePath sample collection kit (Franklin Lakes, NJ, USA),[5] although they did not report how these devices compared to each other. Others have also used cervical brushes,[6–8] although they can cause unnecessary discomfort and bleeding.[2] In another study, flocked nylon swabs collected slightly more cells than the Dacron swab, though this was not statistically significant and the flocked nylon swabs were considerably (≥6 times) more expensive.[9] Roka *et al.*[10] found that the Dacron swab provided better

sampling for HPV DNA testing than using the cervical brush; however, cytology results were not reported.

Generally, wooden cotton-tipped swabs should be avoided for two main reasons. First, cells cling to the cotton fibres more than to the Dacron swab, reducing transfer of the cellular sample to the glass slide or liquid cytology medium.[2] Second, the wooden handle of cotton-tipped swabs may break and splinter when using the pressure required for collection of an adequate sample.[11] For this same reason, prescored sampling devices of any type should be avoided. Ultimately, however, the type of collection device is probably less important than the skill and experience of the clinician in collecting a representative sample.[3]

Studies have also reported two basic approaches for collecting cells from the anal canal: 'blind' sampling (i.e. without direct visualisation of the canal) and anoscope-guided sampling. In a paired random sequence clinical trial, Vajdic *et al*. found that 'blind' sampling was superior to the anoscope-guided method, and resulted in more adequate samples and an increased detection of cytological abnormalities.[12] Plausible hypotheses for the superiority of 'blind' sampling are that the anoscope may mechanically interfere with sampling or cover the target mucosa, or the use of a lubricant, even water, with insertion of the anoscope before collection, may hinder cell harvest or interfere with slide preparation.

Self-collected ARC samples are more feasible than those for cervicovaginal cytology. Four studies have looked at various aspects of patient-collected anal specimens. In the first published study of patients that had previously experienced clinician-collection of ARC, high specimen adequacy rates were reported: 91% for self-collected specimens *v.* 99% for clinician-collected ones.[13] In a population naïve to anal cancer screening, Lampinen *et al*. showed similar, albeit lower, adequacy rates, with 83% of self-collected and 92% of provider-collected samples being deemed adequate for cytological evaluation.[14] In another population of MSM, the majority (93%) who had not previously been screened had specimen adequacy rates of 80%.[15] In this study, in HIV-seropositive MSM, the sensitivity of ARC, for the detection of biopsy-proven anal intraepithelial neoplasia, was 75% when self-collected and 90% when clinician-collected; the values were lower in HIV-negative MSM at 48% and 62%, respectively, presumably due to the lower burden of HPV-associated disease in immunocompetent MSM. In addition, self-collected swabs have been integrated into an existing community venue-based HIV surveillance system for MSM. Although only 62% of the samples were adequate for cytological evaluation, the study demonstrated the feasibility of community-based monitoring for HPV-related anal disease.[16] Illustrated instructions for self-collection of anal specimens are available,[17] and may increase the acceptability and accessibility of anal cancer screening.

Although they are not standardised, there are multiple protocols for collection of anal cytology samples, available in the literature and online.[2,17–19]

Collecting an ARC is a simple procedure but requires diligence on the part of the clinician to collect an adequate sample (Table 1). Typically, the patient lies on their side with the knees drawn to the chest. Remembering that the anal canal is ~3.5–4 cm long in men (though shorter in women), the Dacron swab should be gently inserted into the distal rectal vault, ensuring sampling of the anorectal junction (ARJ) and the anal transformation zone, the location of origin of most high-grade lesions.

Sampling only the perianal skin is not an 'anal cytology' and cytological sampling of keratinised skin cells does not provide sufficient cellularity for diagnosis, misses the target zone of the ARJ and will result in an unsatisfactory sample.

Specimen preparation

Cytological specimens, either liquid-based cytology or conventional smears, are stained in the laboratory with the Papanicolaou (Pap) stain. Two studies have compared conventional smears and ThinPrep (Hologic Corporation, Bedford, MA, USA) anal cytology. In a split-sample study, with experienced clinicians collecting the samples, both preparations yielded similar diagnoses, but the ThinPrep technique reduced the faecal contamination and air-drying artefacts that frequently hinder a cytological evaluation of conventional anal smears.[20] In another study with clinicians who had no prior experience in obtaining cytological specimens from the anus, ThinPrep liquid-based samples were twice as likely to be satisfactory, with adequate cellularity, and detected nearly eight times as many ASILs when compared to conventional cytology.[21]

With the widespread adoption of liquid-based cytology in the United States and the ready availability of gynaecological cytology supplies in many clinics, most ARC samples are prepared using one of the two United States Food and Drug Administration-approved methods for cervical Pap tests: SurePath or ThinPrep. Once the collected ARC sample is

Table 1. Steps in collecting an anal cytology specimens (adapted from Jay[2])

Step	Procedure
1	Moisten synthetic swab with tap water or saline
2	Separate buttocks gently so anal opening is clearly viewed
3	Insert swab slowly until it bypasses the internal sphincter; be certain to find an angle that is not painful or immediately resistant; adjust angle and reinsert if needed
4	Insert as far as possible, usually 5–7 cm (2–3 inches), until resistance is met and the swab abuts the distal wall of the rectum
5	Slowly remove swab in a spiral motion applying firm, consistent lateral pressure to sample all aspects of the mucosa of the anal canal
6	Count slowly to 10 or more while removing the swab and collecting the cellular sample
7	When reaching the anal verge (i.e. distal end of the anal canal), release hold on the buttocks so that the verge is sampled
8	Transfer sample to liquid-cytology vial by vigorously swirling swab in the preservative fluid or prepare smear on glass slide for immediate fixation

transferred to the preservative vial (SurePath Preservative Fluid or PreservCyt for ThinPrep), the processing procedures in the laboratory are identical to those for the specific liquid-based Pap test preservative vial used. However, this is an 'off-label' use of these products and requires validation by the laboratory. Both techniques result in a slide with a thin layer of cells displayed in a circular area of 13 mm for SurePath and 20 mm for ThinPrep. No published data are available for the use of computer-assisted screening for ARC.

Alternatively, ARC can also be processed in the laboratory as are other nongynaecological specimens. For ThinPrep ARC, the samples are collected in CytoLyt (Hologic Corporation) and processed according to the manufacturer's instructions. Samples collected in CytoLyt require additional concentration and washing steps in the laboratory before slide preparation. For SurePath specimens processed using the PrepStain System, the sample is submitted in CytoRich Red Fixative (BD Diagnostics – TriPath, Burlington, NC, USA) and processed using standard nongynaecological sample procedures.[11]

Specimen adequacy

Specimen adequacy protocols for ARC have not been evaluated critically but certain important assumptions have been carried over from the cervical cytology model. It is critical that all ARC have sufficient numbers of nucleated squamous cells. As a guide, the minimum cellularity required for an adequate sample is ~2000–3000 nucleated squamous cells.[22] For liquid-based ARC, this equates to 1–2 nucleated squamous cells per high-power field (HPF) for ThinPrep (with a diameter of 20 mm) and 3–6 nucleated squamous cells per HPF for SurePath (with a diameter of 13 mm). In their study of 200 ARCs using SurePath, Arain *et al*.[5] found that only ARCs with an average of six or more nucleated squamous cells per HPF had diagnostic cells and recommend that SurePath preparations have an average of six or more nucleated squamous cells per HPF for adequacy.

ARC is reported in the same format as cervical cytology using modified Bethesda terminology. The presence or absence of transformation zone components – rectal columnar cells or squamous metaplastic cells – may be reported as a qualify indicator but the relationship to the finding of diagnostic abnormalities is controversial, similar to gynaecological cytology.[23] Using conventional smears, Palefsky *et al*. found the absence of columnar cells did not affect the sensitivity, specificity or predictive value of anal cytology.[4] Using ThinPrep cytology, Vadjic also found that the absence of rectal glandular cells did not reduce the detection of anal neoplasia.[12] In a SurePath study, although most ARC with high-grade cytological abnormalities had transformation zone components present, their absence did not correlate statistically to the detection of high-grade disease.[5] However, in a study of conventional smears, Bakotic *et al*. found a statistically significant association between the presence of columnar cells and anal intraepithelial lesions.[24]

Obtaining an adequate sample for ARC can be challenging, particularly for clinicians inexperienced with the procedure. Common causes of ARC that are unsatisfactory for evaluation include: insufficient cellularity, predominance of anucleate squames, and contamination with heavy faecal material and debris. Poor cellular preservation in conventional cytology smears may also yield an unsatisfactory result. Failure to insert the swab far enough into the anal canal is one of the more common clinical errors that leads to a nonrepresentative sample. Anal cytology collected when sampling devices were inserted at least 4 cm into the canal showed statistically significantly more abnormalities than those inserted only 2 cm.[25] Applying insufficient lateral pressure while retracting the swab can also lead to hypocellular samples, as can failure to sample adequately while retracting the swab slowly and methodically. Common causes of unsatisfactory and limited ARC are summarised in Table 2.

Accuracy of ARC

There is a wide reported range of sensitivity and specificity for ARC, depending, in part, on the type of preparation, the definition of an abnormal result and the population studied. In a systematic review of anal cancer screening in HIV-infected individuals, the sensitivity of ARC ranged from 69% to 93% and specificity ranged from 32% to 59%.[26] This range of sensitivity is comparable to the rates seen with cervical cytology. Sensitivity is higher in HIV-infected MSM, presumably due to the larger burden of HPV-related disease in immunosuppressed individuals leading to an increased chance of adequately sampling an abnormality. Conversely, specificity is higher in HIV-uninfected MSM.[4]

It is important to consider two essential points when evaluating the operating characteristics of ARC. First, accuracy

Table 2. Causes of unsatisfactory and limited anal cytology

Problem	Cause
Insufficient or scant nucleated squamous cells	Inadequate lateral pressure used when collecting sample Canal not sampled for sufficient time (swab retracted too quickly)
Anucleate squames predominate	Swab not inserted far enough into the canal (sampling incorrectly directed to the distal, keratinised portion) Swab retracted too quickly and primarily sampled the distal canal
Heavy or obscuring faecal material	Failure to evacuate stool before sample collection Insufficient lateral pressure applied during retraction of swab, failing to sample walls of the anal canal
Absent transformation zone components	Failure to insert swab far enough into anal canal
Air-drying and mechanical artefact (for conventional smear preparations)	Excessive pressure used to smear sample on glass slide Lack of rapid fixation Inadequate fixation

calculations are based on the imperfect 'gold standard' of HRA-guided biopsy, which itself is subject to sampling and measurement error.[27] Second, similar to the Pap test for cervical cancer screening, the success of ARC in anal cancer screening should be based on repeated testing over time. Palefsky *et al.* showed that sensitivity for the detection of ASIL increased from 69% to 81% when looking at repeat testing on a second visit.[4]

Basic cytomorphology

Interpretive review of ARC utilises an adaptation of the Bethesda System (TBS). Detailed information on the cytomorphology and classification of ARC can be found in several references.[5,22,28,29]

Normal findings

Normal cellular components in an ARC include epithelial cells from the entire anal canal: rectal columnar cells from the distal rectum and ARJ, squamous metaplastic cells from the transformation zone, and nucleated squamous cells and anucleated squames from the distal canal and anal verge. Faecal material is frequently seen, but typically does not limit interpretation, especially with liquid-based cytology.

Squamous epithelial abnormalities include: atypical squamous cells of uncertain significance (ASC-US), low-grade squamous intraepithelial lesions (LSIL), atypical squamous cells cannot exclude a high-grade squamous intraepithelial lesion (ASC-H) and high-grade squamous intraepithelial lesions (HSIL).

ASC-US is represented on ARC by rare, scattered mature squamous cells (superficial and intermediate type)with enlarged, wrinkled, hyperchromatic nuclei, or by parakeratotic cells with slight nuclear pleomorphism and atypia but lacking changes sufficient to render a diagnosis of LSIL. Dyskeratotic cells may also be seen. Even minor cytological changes should be reported because of the high incidence of disease in the populations targeted for screening. LSIL is characterised by the presence of koilocytes, and by superficial and high intermediate cells with an increased nuclear to cytoplasmic ratio and frequent binucleation (Fig. 1). Compared to ASC-US, the nuclei are more atypical, with angulation and irregularity, as well as chromatin clumping and hyperchromasia. Atypical parakeratotic cells may be numerous.

In HSIL, the abnormal squamous cells are of immature squamous metaplastic, low intermediate or parabasal types (Fig. 2). There is prominent nuclear enlargement with coarse chromatin and wrinkling, and irregularity of the nuclear membrane. Chromatin margination and clearing may also be noted in some nuclei. Nucleoli are inconspicuous. The high-grade cells are frequently numerous but are usually present as single dyshesive cells. A mixture with atypical parakeratotic cells is also common, representing a keratinising HSIL. Cellular sheets and syncitia are not a common feature of HSIL in ARC. The presence of any parabasal type atypical squamous cells should be considered significant, even if very scarce in number. When there are insufficient features to be diagnosed as HSIL, the use of the ASC-H classification is appropriate.

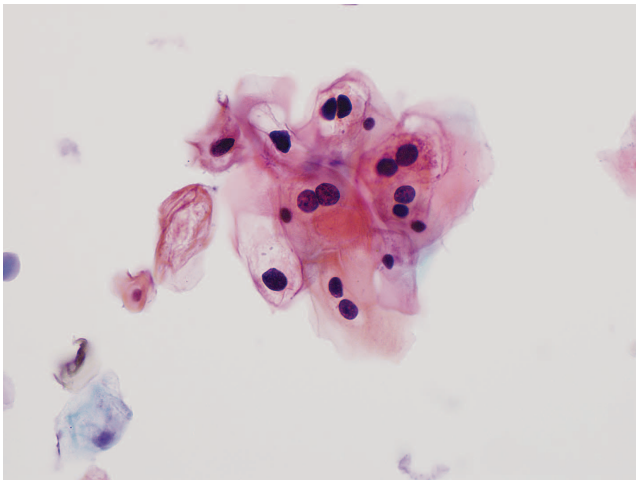

Fig. 1. Anal low-grade squamous intraepithelial lesion. A group of superficial squamous cells with the characteristic changes of HPV. Note the binucleation and koilocytosis (high magnification, ThinPrep).

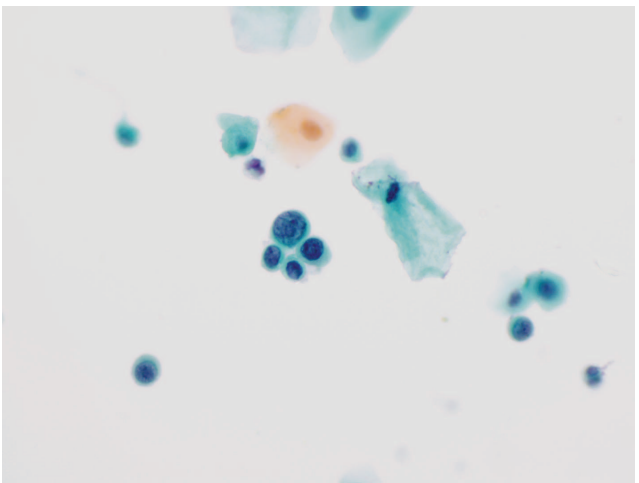

Fig. 2. Anal high-grade squamous intraepithelial lesion with parabasal type squamous cells with enlarged nuclei and increased nuclear : cytoplasmic ratio. Atypia in the nuclei is characterised by chromatin clumping and irregular nuclear membranes (high magnification, ThinPrep).

Squamous cell carcinoma

Invasive SCC is difficult to diagnose on ARC because diathesis is often absent or may be difficult to distinguish from normal faecal flora. With the classic keratinising SCC, bizarre atypical squames, characterised by orangeophilic cytoplasm and hyperchromatic nuclei in a background of HSIL, should give rise to a suspicion of invasion, as should the presence of more obvious malignant squamous epithelial cells. Nucleoli are typically identified in cells derived from nonkeratinising SCCs. (Fig. 3)

Organisms

Although organisms may also be noted in ARC specimens as incidental findings, ARC should not be considered an accurate tool for diagnosing infection with any reliability. Herpes virus

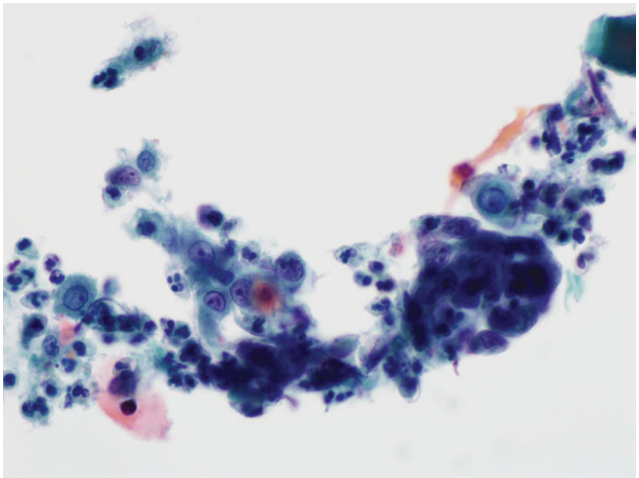

Fig. 3. Anal squamous cell carcinoma. Enlarged malignant squamous cells with chromatin clearing and nucleoli. Also present are atypical keratinised squamous cells with dyskeratosis. Clusters of inflammatory cells are seen near the sheets of malignant cells (high magnification, ThinPrep).

infection is characterised by the classic multinucleated cells with cleared chromatin and nuclear inclusions. Typical viropathic nuclear inclusions specific for cytomegalovirus may also be identified in the immunosuppressed patient. *Candida hyphae* and spores are similar in appearance to those seen in cytology of the female genital tract. Amoebae, both pathologic and nonpathogenic, can also be seen in ARC.

HPV testing

In the populations at increased risk for anal cancer targeted for screening, studies have demonstrated that high-risk HPV testing adds little value to anal cytology because of its low positive predictive value and poor specificity.[30–32] Type-specific testing for HPV-16 may prove to be more useful because of its specific association with high-grade AIN and SCC.[30] The excellent negative predictive value of high-risk HPV testing may be of value after high-resolution anoscopy and in post-treatment management.[33]

Summary

Using the model of cervical cancer screening protocols, ARC is developing into a valuable tool for the early detection of the HPV-related anal squamous intraepithelial lesions that are the precursors of anal SCCs. A thorough understanding of the proper technique to obtain an optimal ARC sample is important to maximise sensitivity when incorporating this procedure into a surveillance protocol for at-risk individuals over time. Patient preparation is the first step and should include instructions to avoid enemas and anal receptive intercourse for 24 h before the procedure. Emptying the rectum before the specimen is obtained will decrease contamination of the specimen by faecal contents. As with cervical cytology, attention must be paid to the methodical details of specimen collection, including the target anatomy, the appropriate technique for proper sampling and specimen preparation. With attention and experience, the clinician can collect the best possible ARC specimens for laboratory evaluation, and facilitate the diagnostic communication between practitioners and pathologists that is essential to patient care.

Conflicts of interest

Dr Darragh receives research supplies from Hologic Corporation. Dr Winkler declares no conflicts of interest.

References

1 Simpson JA, Scholefield JH. Diagnosis and management of anal intraepithelial neoplasia and anal cancer. *BMJ* 2011; 343: d6818. doi:10.1136/bmj.d6818

2 Jay N. Elements of an anal dysplasia screening program. *J Assoc Nurses AIDS Care* 2011; 22(6): 465–77. doi:10.1016/j.jana.2011.08.006

3 Leiman G. Anal screening cytology. *Cytojournal* 2005; 2: 5. doi:10.1186/1742-6413-2-5

4 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(5): 415–22. doi:10.1097/00042560-199704150-00004

5 Arain S, Walts AE, Thomas P, Bose S. The anal Pap smear: cytomorphology of squamous intraepithelial lesions. *Cytojournal* 2005; 2: 4. doi:10.1186/1742-6413-2-4

6 Moscicki AB, Hills NK, Shiboski S, Darragh TM, Jay N, Powell K, *et al*. Risk factors for abnormal anal cytology in young heterosexual women. *Cancer Epidemiol Biomarkers Prev* 1999; 8(2): 173–8.

7 Garcia FU, Haber MM, Butcher J, Sharma M, Nagle D. Increased sensitivity of anal cytology in evaluation of internal compared with external lesions. *Acta Cytol* 2007; 51(6): 893–9. doi:10.1159/000325866

8 Calore EE, Nadal SR, Manzione CR, Horta SC, Santos RR, Nadal LM. Anal cytology in patients with AIDS. *Diagn Cytopathol* 2010; 38(4): 260–3.

9 Gage JC, Ghosh A, Borgonovo S, Follansbee S, Wentzensen N, Gravitt PE, *et al*. A comparison of Dacron versus flocked nylon swabs for anal cytology specimen collection. *Acta Cytol* 2011; 55(4): 364–7. Epub 2011 Jul 22. doi:10.1159/000329488

10 Roka F, Roka J, Trost A, Schalk H, Zagler C, Kirnbauer R, *et al*. Anal human papillomavirus testing with Digene's Hybrid Capture 2 using two different sampling methods. *Dis Colon Rectum* 2008; 51(1): 62–6. Epub 2007 Nov 21. doi:10.1007/s10350-007-9082-6

11 Darragh TM, Winkler B. The ABCs of anal-rectal cytology. *CAP Today* 2004; (May): 42–50.

12 Vajdic CM, Anderson JS, Hillman RJ, Medley G, Grulich AE. Blind sampling is superior to anoscope guided sampling for screening for anal intraepithelial neoplasia. *Sex Transm Infect* 2005; 81(5): 415–8. doi:10.1136/sti.2004.014407

13 Cranston RD, Darragh TM, Holly EA, Jay N, Berry JM, Da Costa M, *et al*. Self-collected versus clinician-collected anal cytology specimens to diagnose anal intraepithelial neoplasia in HIV-positive men. *J Acquir Immune Defic Syndr* 2004; 36(4): 915–20. doi:10.1097/00126334-200408010-00004

14 Lampinen TM, Miller ML, Chan K, Anema A, van Niekerk D, Schilder AJ, *et al*. Randomized clinical evaluation of self-screening for anal cancer precursors in men who have sex with men. *Cytojournal* 2006; 3: 4. doi:10.1186/1742-6413-3-4

15 Chin-Hong PV, Berry JM, Cheng SC, Catania JA, Da Costa M, Darragh TM, *et al*. Comparison of patient- and clinician-collected anal cytology samples to screen for human papillomavirus-associated anal intraepithelial neoplasia in men who have sex with men. *Ann Intern Med* 2008; 149(5): 300–6.

16 Gilbert M, Kwag M, Mei W, Rank C, Kropp R, Severini A, *et al*. ManCount Study Team. Feasibility of incorporating self-collected rectal swabs into a community venue-based survey to measure the prevalence of HPV infection in men who have sex with men. *Sex Transm Dis* 2011; 38(10): 964–9. doi:10.1097/OLQ.0b013e318222899d

17 BC Cancer Agency (BCCA). Cytology: anal specimens, specimen required/supplies. Vancouver: BCCA; 2011. Available online at: www.bccancer.bc.ca/HPI/labservices/diagcytology/referring/anal/anal_specimen_supplies_collection.htm [verified December 2011].

18 University of California. UCSF Anal cancer information: obtaining an anorectal cytology specimen. San Francisco: University of California; 2011. Available online at: www.analcancerinfo.ucsf.edu [verified December 2011].

19 Lindsey K, DeCristofaro C, James J. Anal Pap smears: should we be doing them? *J Am Acad Nurse Pract* 2009; 21(8): 437–43. doi:10.1111/j.1745-7599.2009.00433.x

20 Darragh TM, Jay N, Tupkelewicz BA, Hogeboom CJ, Holly EA, Palefsky JM. Comparison of conventional cytologic smears and ThinPrep preparations from the anal canal. *Acta Cytol* 1997; 41(4): 1167–70. doi:10.1159/000332840

21 Sherman ME, Friedman HB, Busseniers AE, Kelly WF, Carner TC, Saah AJ. Cytologic diagnosis of anal intraepithelial neoplasia using smears and Cytyc Thin-Preps. *Mod Pathol* 1995; 8(3): 270–4.

22 Darragh TM, Birdsong GG, Luff RD, Davey DD. Anal–rectal cytology. In: Solomon D, Nayar R, editors. The Bethesda system for reporting cervical cytology: definitions, criteria and explanatory notes. 2nd ed. New Work: Springer; 2004. pp.169–175.

23 Davey DD, Austin RM, Birdsong G, Buck HW, Cox JT, Darragh TM, *et al*. American Society for Colposcopy and Cervical Pathology: ASCCP patient management guidelines: Pap test specimen adequacy and quality indicators. *Am J Clin Pathol* 2002; 118(5): 714–8. doi:10.1309/6GBF-EGH8-WXDE-ANGX

24 Bakotic WL, Willis D, Birdsong G, Tadros TS. Anal cytology in an HIV-positive population: a retrospective analysis. *Acta Cytol* 2005; 49(2): 163–8. doi:10.1159/000326126

25 Nadal SR, Horta SH, Calore EE, Nadal LR, Manzione CR. [How far into the anal canal should the brush be introduced for more efficient cytological evaluation?] *Rev Assoc Med Bras* 2009; 55(6): 749–51. doi:10.1590/S0104-42302009000600022 [In Portuguese]

26 Chiao EY, Giordano TP, Palefsky JM, Tyring S, El Serag H. Screening HIV-infected individuals for anal cancer precursor lesions: a systematic review. *Clin Infect Dis* 2006; 43(2): 223–33. doi:10.1086/505219

27 Mathews WC, Cachay ER, Caperna J, Sitapati A, Cosman B, Abramson I. Estimating the accuracy of anal cytology in the presence of an imperfect reference standard. *PLoS ONE* 2010; 5(8): e12284. doi:10.1371/journal.pone.0012284

28 Darragh TM. Anal cytology. In Wilbur DC, Henry MR, editors. College of American Pathologists practical guide to gynecologic cytopathology: morphology, management and molecular methods. Northfield: CAP Press; 2008. pp 171–180.

29 Bean SM, Chhieng DC. Anal-rectal cytology: a review. *Diagn Cytopathol* 2010; 38(7): 538–46. doi:10.1002/dc.21242

30 Berry JM, Palefsky JM, Jay N, Cheng SC, Darragh TM, Chin-Hong PV. Performance characteristics of anal cytology and human papillomavirus testing in patients with high-resolution anoscopy-guided biopsy of high-grade anal intraepithelial neoplasia. *Dis Colon Rectum* 2009; 52: 239–47. doi:10.1007/DCR.0b013e31819793d9

31 Salit IE, Lytwyn A, Raboud J, Sano M, Chong S, Diong C, *et al*. The role of cytology (Pap tests) and human papillomavirus testing in anal cancer screening. *AIDS* 2010; 24(9): 1307–13.

32 Goldstone SE, Enyinna CS, Davis TW. Detection of oncogenic human papillomavirus and other predictors of anal high-grade dysplasia in men who have sex with men with abnormal cytology. *Dis Colon Rectum* 2009; 52: 31–9. doi:10.1007/DCR.0b013e31819736aa

33 Goldstone SE, Moshier E. Detection of oncogenic human papillomavirus impacts anal screening guidelines in men who have sex with men. *Dis Colon Rectum* 2010; 53(8): 1135–42. doi:10.1007/DCR.0b013e3181e10842