

UCSF

UC San Francisco Previously Published Works

Title

A comparison of human papillomavirus genotype-specific DNA and E6/E7 mRNA detection to identify anal precancer among HIV-infected men who have sex with men.

Permalink

<https://escholarship.org/uc/item/5h515896>

Journal

Cancer Epidemiology, Biomarkers and Prevention, 22(1)

Authors

Castle, Philip
Follansbee, Stephen
Borgonovo, Sylvia
et al.

Publication Date

2013

DOI

10.1158/1055-9965.EPI-12-0984

Peer reviewed



Published in final edited form as:

Cancer Epidemiol Biomarkers Prev. 2013 January ; 22(1): 42–49. doi:10.1158/1055-9965.EPI-12-0984.

A comparison of human papillomavirus genotype-specific DNA and E6/E7 mRNA detection to identify anal precancer among HIV-infected men who have sex with men

Philip E. Castle^{1,*}, Stephen Follansbee², Sylvia Borgonovo², Diane Tokugawa³, Lauren M. Schwartz⁴, Thomas S. Lorey³, Brandon LaMere⁵, Julia C. Gage⁴, Barbara Fetterman³, Teresa M. Darragh⁶, Ana Cecilia Rodriguez^{1,7}, and Nicolas Wentzensen^{4,#}

¹Albert Einstein College of Medicine, Bronx, NY

²Kaiser Permanente Medical Center, San Francisco, CA

³Kaiser Permanente TPMG Regional Laboratory, Berkeley, CA

⁴Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, MD

⁵Women's Health Research Institute, Division of Research, Kaiser Permanente, Oakland, CA

⁶University of California, San Francisco, CA

⁷Proyecto Epidemiológico Guanacaste, Fundación Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud, San José, Costa Rica

Abstract

Background—Human papillomavirus (HPV) RNA detection is reportedly more specific for the detection of anogenital precancer than HPV DNA but it is unknown whether this is due to detection of RNA or due to HPV genotype restriction.

Materials and Methods—363 human immunodeficiency virus (HIV)-positive men who have sex with men had two anal cytology samples taken and were evaluated using high-resolution anoscopy and biopsies of visible lesions. Anal specimens were tested for E6/E7 RNA for 5 carcinogenic HPV genotypes (HPV16, 18, 31, 33, and 45) and tested for the DNA of 13 carcinogenic HPV genotypes.

Results—DNA testing was more likely to be positive than RNA testing (53% vs. 48%, $p = 0.02$) for the same 5 HPV genotypes in aggregate. When restricted to 5 HPV genotypes targeted by the RNA test, the sensitivity to detect anal precancer was the same for DNA and RNA (81%) while RNA was more specific than DNA (65% vs. 58%, $p = 0.007$). By comparison, DNA detection of all 13 carcinogenic HPV genotypes was more sensitive (96% vs. 81%, $p = 0.001$) but much less specific (65% vs. 33%, $p < 0.001$) compared to RNA detection of the 5 HPV genotypes.

Conclusion—After controlling for HPV genotypes, RNA was only slightly more specific than DNA detection for anal precancer.

*To whom correspondence should be addressed: Nicolas Wentzensen M.D., Ph.D., M.S., Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd, Room 5024, Rockville, MD 20852-7234, wentzenn@mail.nih.gov.

#Dr. Castle is a Visiting Professor in Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, New York

Disclosures: Dr. Castle is compensated for his service on a data and safety monitoring board for Merck. He has received HPV tests and testing for research at a reduced or no cost from Qiagen and Roche. For this study, Norchip and Roche provided testing at no cost.

Impact—DNA or RNA testing for a subset of the most carcinogenic HPV genotypes may be useful for distinguishing between those HPV-positive men at higher and lower risk of anal precancer and cancer.

Keywords

Human Papillomavirus (HPV); anal cancer; anal intraepithelial neoplasia; screening; Human Immunodeficiency Virus (HIV); men who have sex with men (MSM)

Introduction

Anal cancer is a relatively uncommon malignancy, with approximately 6,000 cases projected for 2012 [1]. Men who have sex with men (MSM), especially human immunodeficiency virus (HIV)-infected MSM, are at an elevated risk of anal cancer [2–4]. Human papillomavirus (HPV), the necessary cause of cervical cancer, is the primary cause of anal cancer. Although less well studied than the cervix, the natural history of HPV and anal cancer is believed to be analogous to that of HPV and cervical cancer. The key steps in HPV-induced carcinogenesis are HPV acquisition, HPV persistence (vs. clearance or HPV infection becoming undetectable), progression of persistent HPV infection to precancer, and development of invasive cancer from a precancer. MSM who participate in receptive anal intercourse expose susceptible squamous epithelium of the anal canal to HPV infection. HIV-infected MSM are at a higher risk of anal cancer than HIV-uninfected MSM presumably because of their impaired immune response reduces their ability to clear HPV infections. As a consequence, HIV-infected MSM have a very high prevalence of anal HPV, with a 73.5% prevalence for anal carcinogenic HPV [4], which is two-fold greater prevalence for anal carcinogenic HPV in HIV-negative MSM [4] and cervical carcinogenic HPV in young (<20 years) women [5].

In anal and cervical cancer screening, most subjects who screen positive by HPV DNA testing or cytology do not have concurrent precancer. The challenge is then differentiating the screen-positive subjects with benign, transient HPV infections (or cytologic abnormalities caused by those infections) from those that have a precancerous lesion. This is especially true in anal cancer screening of HIV-infected MSM with the very high background of anal HPV infection.

There are a number of new, promising HPV-related biomarkers that may better differentiate between high- and low-risk HPV infections but more validation studies are needed. One of these promising biomarkers is the high-level expression of HPV E6 and/or E7 oncoprotein. In numerous studies of HPV-related anogenital disease [6–15], detection of HPV E6/E7 messenger RNA (mRNA) for HPV16, 18, 31, 33, and 45 together has been shown to be more specific albeit less sensitive for precancer than DNA detection of all carcinogenic-HPV genotypes.

We conducted a study of HIV-infected MSM in Northern California to evaluate some of these promising new biomarkers for anal cancer screening including E6/E7 mRNA detection of HPV16, 18, 31, 33, and 45. We previously confirmed that mRNA detection of the 5 HPV genotypes is more specific but less sensitive for anal precancer than DNA detection for 13 carcinogenic HPV genotypes. Here we extended the analysis to differentiate the effects of mRNA detection vs. type restriction on clinical specificity of E6/E7 mRNA detection.

Materials and Methods

Study population

We conducted a cross-sectional screening study at an anal cancer-screening clinic in San Francisco, California run by Kaiser Permanente Northern California (KPNC). We enrolled men who were identified as HIV-infected through the Kaiser HIV registry, who were 18 years or older, who were not diagnosed with anal cancer prior to enrollment and who provided informed consent. In total, 363 HIV-positive men on anti-retroviral therapy were enrolled between August 2009 and June 2010. The study was reviewed and approved by the institutional review boards at KPNC and the U.S. National Cancer Institute (NCI). All participants were asked to complete a self-administered questionnaire to collect risk factor information. Additional information on HIV status and medication, sexually transmitted diseases, and histopathology results were abstracted from the KPNC clinical database. For 87 subjects of 271 without histologically confirmed high-grade anal intraepithelial neoplasia (anal intraepithelial neoplasia grade 2 [AIN2] or grade 3 [AIN3]) identified at the enrollment visit, follow-up information from additional clinic visits up to 12/2011 was available and included in the analysis to partially reduce the misclassification of disease status at enrollment due to the imperfect sensitivity of high-resolution anoscopy (HRA) [16–17].

High-resolution anoscopy, cytology, and histology

During the clinical examination, two specimens were collected by inserting a wetted flocked nylon swab [18] into the anal canal up to the distal rectal vault and withdrawing with rotation and lateral pressure. Each specimen was placed into separate vials with PreservCyt medium (Hologic, Bedford, MA, USA) for cytology and HPV DNA and biomarker testing. A third specimen was collected for routine *Chlamydia trachomatis* and *Neisseria gonorrhoea* testing. After specimen collection, participants underwent a digital rectal exam followed by HRA. Suspicious-appearing lesions visualized during HRA were biopsied and sent for routine histopathological review by KPNC pathologists. From the first specimen container, a liquid-based cytology slide was prepared for routine Pap staining and read independently by two pathologists, who demonstrated moderate agreement [19]. Cytology results were reported analogous to the 2001 Bethesda classification for cervical cytology [20]: negative for squamous intraepithelial lesion (SIL) or malignancy (“negative”), atypical squamous cells of undetermined significance (ASC-US), low-grade SIL (LSIL), high-grade SIL (HSIL), and atypical squamous cells cannot rule out HSIL (ASC-H). The study pathologists made a further distinction of HSIL by categorizing them as either HSIL-AIN2 or HSIL-AIN3 based on severity.

Histology results were reported as negative, condyloma, and AIN grades 1–3.

HPV Testing

The test manufacturers performed HPV DNA (Roche Molecular Systems, Pleasanton, CA, USA) and RNA (NorChip AS, Klokkestua, Norway) testing on the second PreservCyt specimen according to their instructions, blinded to all study data.

Specimens were tested for the DNA of 37 HPV genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51–56, 58, 59, 61, 62, 64, 66–73, 81–84, 82v, and 89) using HPV (LA-HPV)[®] assay (Roche) [21–22]. HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 were classified as carcinogenic HPV genotypes [23].

Specimens were tested for the E6 and E7 mRNA of 5 HPV genotypes (HPV16, 18, 31, 33, and 45) by real-time multiplex nucleic acid sequence-based amplification using the PreTect HPV-Proofer assay (NorChip) as previously described [7–11]. DNA/RNA was first isolated

from 5 mL PreservCyt specimens by using the NucliSENS easyMAG system (bioMerieux, Marcy l'Etoile, France). In order to avoid false negatives due to degradation of mRNA, primers and probes against human U1A mRNA are included in the PreTect HPV-Proofer kit as a performance and integrity control.

Statistical analysis

We calculated the basic agreement statistics (percent agreement, percent positive agreement, and kappa value) for comparing paired DNA and RNA test results. We tested for differences in percentage of test positives and the number of HPV genotypes detected for paired tests using an exact version of a symmetry chi-square test. A non-parametric test [24] was used to assess the trends in percentage of test positives with increasing severity of the cytologic interpretation and older age, categorized into groups based on approximate quartiles in age (<46, 46–51, 52–59, and 60 and older). Standard clinical performance characteristics (sensitivity, specificity, negative (NPV) and positive (PPV) predictive value, negative (LR–) and positive (LR+) likelihood ratio, and odds ratio (OR) with 95% confidence interval (95% CI) were calculated for the two tests for identifying subjects with anal precancer.

Results

We restricted our analysis to the 334 of 363 (92.0%) participants who had complete HPV DNA and E6/E7 mRNA results; 20 were missing DNA results, 8 were missing RNA results, and 1 was missing both DNA and RNA. The median and mean age and the range of ages were 52 years, 53 years, and 32–79 years, respectively. The description of the population can be found in the Supplemental Table. Approximately 80% of the participants completed the short questionnaire. The population was predominantly white and well educated. Most MSM (74%) had 10 or more anal intercourse partners in their lifetime. Approximately 80% of the subjects had CD4 counts of 350 or greater per μL and more than 80% had HIV viral loads of less than 75 viral copies per mL of blood.

In Table 1, we present the agreement between HPV DNA and E6/E7 mRNA detection of HPV16, 18, 31, 33, and 45 individually and collectively. An HPV DNA assay including the five genotypes would have a positivity of 53% in this population (corresponding to the referral rate), while the HPV mRNA assay for the five genotypes had an overall positivity of 48%. For the 5 individual HPV genotypes, the overall agreement ranged from 92.8% (HPV31) to 96.4% (HPV18 and HPV33), the positive agreement ranged from 60.7% (HPV31) to 78.2% (HPV16), and the kappa values from 0.71 (HPV31) to 0.83 (HPV16). There were no statistically significant differences in the percent positive between measures for each genotype although the percent positive by DNA was consistently slightly higher than by RNA. For any of 5 HPV genotypes detected (regardless of whether was agreement for the specific HPV genotypes present), there was 85.9% total agreement, 75.6% positive agreement, and kappa value of 0.72 (95% confidence interval (95% CI) = 0.61–0.84) for the two measures. DNA testing for the 5 HPV genotypes in aggregate was more likely to be positive than RNA testing (53% vs. 48%, respectively, $p = 0.02$). Considering agreement for all 5 HPV genotypes individually (including agreement when multiple HPV genotypes are detected) ($n = 1,670$; 334 subjects and 5 possible infections per subject), there was 94.7% total agreement, 69.6% positive agreement, and kappa value of 0.79 (95% CI: 0.75–0.83) for the two measures. There were no significant differences in the individual detection for all 5 HPV genotypes ($p = 0.2$).

Shown in Figure 1 is the prevalence (percent positive) by age group for DNA and RNA detection for the 5 HPV genotypes and DNA detection for all carcinogenic HPV. The age-group specific prevalence as measured by RNA was similar to that of DNA but slightly, non-significantly lower in all age groups. The DNA prevalence for all carcinogenic HPV

was approximately 20%–30% greater than for the 5 HPV genotypes for all age groups. The trend of decreasing test positivity with older age groups was significant for RNA detection of 5 HPV genotypes ($p_{\text{trend}} = 0.04$) but was not for DNA detection of 5 HPV genotypes ($p_{\text{trend}} = 0.2$) and of 13 HPV genotypes ($p_{\text{trend}} = 0.06$).

The distribution for the number of HPV16, 18, 31, 33, and 45 genotypes detected by DNA and RNA testing is shown in Table 2. There was no significant difference ($p = 0.1$) in the number of HPV genotypes detected between tests. The main discrepancy between the two tests was that DNA was more likely to be positive for a single HPV genotype and RNA negative for any of the 5-targeted HPV genotypes than the converse (29 vs. 12, respectively).

We then compared the DNA and RNA test results for the pool of 5 HPV genotypes to cytology and histology results as shown in Table 3. For both DNA and RNA, the percent positive increased with increasing severity of cytology or histology. The percent positive for DNA and RNA for cytologic categories as read by the two pathologists was as follows: 1) 35%–37% and 27%–30%, respectively, for negative cytology; 50%–57% and 46%–53%, respectively, for ASC-US or LSIL; and 82%–84% and 76%–81%, respectively, for high-grade cytology (ASC-H or HSIL cytology). The trend of increasing test positivity with increasing severity of the cytologic interpretation was significant for all 4 combinations of test and cytology results ($p_{\text{trend}} < 0.001$). The percent positive also increased with greater certainty of the presence of anal precancer. The percent DNA or RNA positive for AIN2 ($n = 40$) and AIN3 ($n = 22$) was approximately 85% and was 100% for AIN2/3 with both cytologic interpretations read as high-grade ($n = 25$). HPV DNA testing was more likely to be positive than RNA for the same 5 HPV genotypes among subjects whose cytology was read as negative by pathologist 1 (37% vs. 30%, $p = 0.1$) or negative by pathologist 2 (35% vs. 27%, $p = 0.03$), or who had histologic diagnosis of less severe than AIN2 or did not have a diagnosis (46% vs. 40%, $p = 0.01$).

Using a *post hoc* definition of anal precancer as anyone with histology of AIN2/3 and/or with high-grade cytology as read by both pathologists ($n = 96$), we calculated the clinical performance statistics for HPV DNA and RNA (Table 4). Comparing RNA and DNA detection for the common 5 HPV genotypes, we observed identical sensitivity (81%) for both while RNA detection was slightly more specific than DNA detection (65% vs. 58%, respectively, $p = 0.007$) for high-grade anal disease. Thus, RNA detection had a slightly better overall performance than DNA for detection of the same 5 HPV genotypes. Positive and negative predictive values were very similar for both assays. Including the DNA detection of other carcinogenic HPV genotypes into the definition of the test resulted in an incremental improvement in sensitivity (from 81% to 96%) but reduced the specificity by almost half (from 58% to 33%) compared to the DNA detection of only the 5 HPV genotypes. Consequently, the DNA test for all 13 carcinogenic HPV genotypes was more sensitive ($p = 0.001$) but much less specific ($p < 0.001$) for high-grade anal disease than the RNA test for HPV16, 18, 31, 33, and 45. Conversely, restricting the tests to HPV16 and HPV18, the two most carcinogenic HPV genotypes, resulted in a less sensitive but more specific test, and the DNA test for HPV16 and HPV18 was significantly less sensitive but more specific test than the RNA test for 5 HPV genotypes ($p < 0.001$ for both). Similar results were observed using different definitions of cases (data not shown).

Discussion

We found that the patterns of HPV detection by DNA and RNA were much alike. While we observed that there was a consistent pattern of lower positivity of RNA than DNA detection, the differences in detection for individual HPV genotypes were minor so that only pooling

of the 5 targeted HPV genotypes showed a statistically significant difference in relative specificity of ~10% for identifying HIV-positive MSM with anal precancer. Inclusion of all the carcinogenic HPV genotypes into the definition of a positive DNA test incrementally increased sensitivity while drastically reducing the specificity compared to a HPV E6/E7 mRNA test targeting 5 HPV genotypes, as previously observed.

We previously showed that mRNA detection of the 5 HPV genotypes is more specific but less sensitive for anal precancer than DNA detection for 13 carcinogenic HPV genotypes [25]. Based on our data, we suggest that the improved specificity of RNA testing compared to DNA testing observed in the previous studies of anogenital disease [6–15] can be explained in a large part to restricting detection to 5 highly carcinogenic types. However, the RNA test showed a small significant increase in specificity compared to the type-restricted DNA test, suggesting that there is a small independent effect of RNA testing leading to improved specificity. The PreTect HPV-Proofer assay targets the most carcinogenic HPV genotypes, HPV16, 18, 31, 33, and 45, which together are responsible for causing 80–85% of all cervical cancer and a similar percentage of cervical intraepithelial neoplasia grade 3 (CIN3). HPV16, 18, 31, 33, and 45 cause similar or more anal cancer [26–27] and AIN2/3 [27–29], although the exact percentage of AIN2/3 caused by these types in HIV-infected MSM populations is difficult to estimate precisely because of the frequent presence of multiple HPV infections [26, 28] and the challenges associated with attribution in the context of multiple infections[30]. Adding weaker carcinogenic HPV genotypes (e.g., HPV59 and HPV68), which rarely cause cervical cancer [23, 31–32]and may never cause anal cancer [27], into the test provides better sensitivity and improved NPV for CIN3 and AIN3, desirable for a screening test to rule out who is at risk, but reduces the diagnostic accuracy because of poorer specificity and PPV. By contrast, restricting DNA and RNA detection to HPV16 and HPV18, the two most carcinogenic HPV genotypes, further improved specificity and PPV compared to detection of the 5 HPV genotypes targeted by PreTect HPV-Proofer but reduced sensitivity.

Based on the effects observed for 5 carcinogenic types, we would predict that if the PreTect HPV-Proofer targeted all 13 carcinogenic HPV genotypes we would observe significantly lower specificity vs. RNA detection of the 5 HPV genotypes and a 10% relative improvement in the specificity vs. the DNA detection of the 13 carcinogenic HPV genotypes. This would be consistent with previous reports comparing carcinogenic DNA detection and Aptima [33–34], a recently FDA-approved E6/E7 mRNA test for 14 carcinogenic HPV genotypes¹, for the detection of cervical precancer.

The additional ~10% in relative DNA positivity to RNA positivity for the 5 HPV genotypes did not appear to be associated with any disease. These DNA-positive, RNA-negative HPV infections appeared to be of low viral load: single infections that were DNA positive but RNA negative were more likely to have concurrent negative cytology and had a lower signal strength on a real-time PCR test for HPV (cobas4800, Roche) than those that were DNA- and RNA-positive (data not shown). It is probable that these are DNA detectable-only HPV infections are transient and low risk for incident anal precancer and cancer.

We acknowledge several limitations of this study. First, there was limited number of histological diagnoses of AIN3, which by analogy to CIN3 in cervix, is the most rigorous definition of precancer and therefore the best surrogate for anal cancer risk [35]. In addition, there were a number of men (n = 34) without AIN2 or worse histology and yet had high-grade cytology as read by both pathologists, suggesting that HRA had missed some anal

¹<http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/Recently-ApprovedDevices/ucm278520.htm>

precancer. We therefore included in our case definition of anal precancer those men with histologic AIN2 and/or confirmed high-grade cytology. It was reassuring that the patterns of HPV detection in these different groups included in our case definition were very similar to one another.

Second, our participants were from a single clinic site and their HIV infection in general was well managed, as indicated by the high percentage of MSM that had CD4 counts 350 per μL or higher and HIV viral loads of less than 75 copies per ml. While our results may not be generalizable to a general population, there are no efforts to implement anal cancer screening in the general population, because the prevalence is so low. Thus, our participants are representative for the growing population of HIV-infected MSM, where anal cancer is an increasing problem and where screening and early detection options need to be evaluated. Furthermore, our results may apply to other populations with high HPV prevalence, where distinguishing between benign HPV infections and those infections that have caused precancer is important.

In conclusion, once we accounted for the differences in the targeted HPV genotypes between RNA and DNA tests, we found only minor differences between detection of these two biomarkers of HPV infection in anal specimens from HIV-infected MSM for identify those anal precancer. E6/E7 expression is not exclusive to transforming infections as E6/E7 must be expressed for genome maintenance in productive HPV infections [36]. The E6/E7 mRNA test, which amplifies its target, may not effectively differentiate between E6/E7 expression detected in productive viral infections versus its over-expression detected in precancer. The clinical relevance of the incremental improvement in specificity of RNA over DNA detection will depend on the population in which the test is applied. In the high-risk population analyzed in this study, the improved specificity of RNA testing resulted only in a marginal increase of positive predictive value. An algorithm similar as that used for cervical cancer screening [37], which may use HPV16/18 DNA detection, could serve as a model for anal cancer screening: rule out disease with carcinogenic HPV DNA detection and use DNA or RNA detection for a subset of highly carcinogenic HPV genotypes, perhaps in combination with cytology to determine which carcinogenic HPV-positive people need immediate HRA and directed biopsies. The choice between DNA and RNA detection may depend on laboratory ease of use and costs given the similarities in clinical performance characteristics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The Intramural Research Program of NIH/National Cancer Institute supported this study.

Reference List

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin*. 2012 Jan-Feb;62(1): 10–29. [PubMed: 22237781]
2. Silverberg MJ, Lau B, Justice AC, Engels E, Gill MJ, Goedert JJ, et al. Risk of anal cancer in HIV-infected and HIV-uninfected individuals in North America. *Clin Infect Dis*. 2012 Apr; 54(7):1026–34. [PubMed: 22291097]
3. Shiels MS, Pfeiffer RM, Gail MH, Hall HI, Li J, Chaturvedi AK, et al. Cancer burden in the HIV-infected population in the United States. *J Natl Cancer Inst*. 2011 May 4; 103(9):753–62. [PubMed: 21483021]

4. Machalek DA, Poynten M, Jin F, Fairley CK, Farnsworth A, Garland SM, et al. Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic review and meta-analysis. *Lancet Oncol.* 2012 May; 13(5):487–500. [PubMed: 22445259]
5. Smith JS, Melendy A, Rana RK, Pimenta JM. Age-specific prevalence of infection with human papillomavirus in females: a global review. *J Adolesc Health.* 2008 Oct; 43(4 Suppl):S5–25. S e1–41. [PubMed: 18809145]
6. Molden T, Kraus I, Karlsen F, Skomedal H, Nygard JF, Hagmar B. Comparison of human papillomavirus messenger RNA and DNA detection: a cross-sectional study of 4,136 women >30 years of age with a 2-year follow-up of high-grade squamous intraepithelial lesion. *Cancer Epidemiol Biomarkers Prev.* 2005 Feb; 14(2):367–72. [PubMed: 15734959]
7. Molden T, Nygard JF, Kraus I, Karlsen F, Nygard M, Skare GB, et al. Predicting CIN2+ when detecting HPV mRNA and DNA by PreTect HPV-proofer and consensus PCR: A 2-year follow-up of women with ASCUS or LSIL Pap smear. *Int J Cancer.* 2005 May 10; 114(6):973–6. [PubMed: 15645423]
8. Kraus I, Molden T, Holm R, Lie AK, Karlsen F, Kristensen GB, et al. Presence of E6 and E7 mRNA from human papillomavirus types 16, 18, 31, 33, and 45 in the majority of cervical carcinomas. *J Clin Microbiol.* 2006 Apr; 44(4):1310–7. [PubMed: 16597856]
9. Molden T, Kraus I, Karlsen F, Skomedal H, Hagmar B. Human papillomavirus E6/E7 mRNA expression in women younger than 30 years of age. *Gynecol Oncol.* 2006 Jan; 100(1):95–100. [PubMed: 16153696]
10. Molden T, Kraus I, Skomedal H, Nordstrom T, Karlsen F. PreTect HPV-Proofer: real-time detection and typing of E6/E7 mRNA from carcinogenic human papillomaviruses. *J Virol Methods.* 2007 Jun; 142(1–2):204–12. [PubMed: 17379322]
11. Sorbye SW, Fismen S, Gutteberg T, Mortensen ES. Triage of women with minor cervical lesions: data suggesting a “test and treat” approach for HPV E6/E7 mRNA testing. *PLoS One.* 2010; 5(9):e12724. [PubMed: 20856930]
12. Valari O, Koliopoulos G, Karakitsos P, Valasoulis G, Founta C, Godevenos D, et al. Human papillomavirus DNA and mRNA positivity of the anal canal in women with lower genital tract HPV lesions: predictors and clinical implications. *Gynecol Oncol.* 2011 Sep; 122(3):505–8. [PubMed: 21665253]
13. Silling S, Kreuter A, Hellmich M, Swoboda J, Pfister H, Wieland U. Human papillomavirus oncogene mRNA testing for the detection of anal dysplasia in HIV-positive men who have sex with men. *J Clin Virol.* 2012 Apr; 53(4):325–31. [PubMed: 22261122]
14. Szarewski A, Mesher D, Cadman L, Austin J, Ashdown-Barr L, Ho L, et al. Comparison of seven tests for high-grade cervical intraepithelial neoplasia in women with abnormal smears: the Predictors 2 study. *J Clin Microbiol.* 2012 Jun; 50(6):1867–73. [PubMed: 22422852]
15. Cuschieri K, Wentzensen N. Human papillomavirus mRNA and p16 detection as biomarkers for the improved diagnosis of cervical neoplasia. *Cancer Epidemiol Biomarkers Prev.* 2008 Oct; 17(10):2536–45. [PubMed: 18842994]
16. 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. [PubMed: 20808869]
17. Chiao EY, Giordano TP, Palefsky JM, Tying S, El Serag H. Screening HIV-infected individuals for anal cancer precursor lesions: a systematic review. *Clin Infect Dis.* 2006 Jul 15; 43(2):223–33. [PubMed: 16779751]
18. 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. [PubMed: 21791907]
19. Darragh TM, Tokugawa D, Castle PE, Follansbee S, Borgonovo S, Lamere BJ, et al. Interrater agreement of anal cytology. *Cancer Cytopathol.* 2012 Jul 18.
20. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA.* 2002 Apr 24; 287(16):2114–9. [PubMed: 11966386]

21. Castle PE, Gravitt PE, Solomon D, Wheeler CM, Schiffman M. Comparison of linear array and line blot assay for detection of human papillomavirus and diagnosis of cervical precancer and cancer in the atypical squamous cell of undetermined significance and low-grade squamous intraepithelial lesion triage study. *J Clin Microbiol.* 2008 Jan; 46(1):109–17. [PubMed: 17989194]
22. Gravitt PE, Schiffman M, Solomon D, Wheeler CM, Castle PE. A comparison of linear array and hybrid capture 2 for detection of carcinogenic human papillomavirus and cervical precancer in ASCUS-LSIL triage study. *Cancer Epidemiol Biomarkers Prev.* 2008 May; 17(5):1248–54. [PubMed: 18483347]
23. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens--Part B: biological agents. *Lancet Oncol.* 2009 Apr; 10(4):321–2. [PubMed: 19350698]
24. Cuzick J. A Wilcoxon-type test for trend. *Stat Med.* 1985 Jan-Mar;4(1):87–90. [PubMed: 3992076]
25. Wentzensen N, Follansbee S, Borgonovo S, Tokugawa D, Schwartz L, Lorey TS, Sahasrabudhe VV, Lamere B, Gage JC, Fetterman B, Darragh TM, Castle PE. HPV genotyping, HPV mRNA expression, and p16/Ki-67 cytology to detect anal cancer precursors in HIV-infected men who have sex with men. *AIDS.* 2012 Sep 26. (E-pub).
26. Abramowitz L, Jacquard AC, Jaroud F, Haesebaert J, Siproudhis L, Pradat P, et al. Human papillomavirus genotype distribution in anal cancer in France: the EDiTH V study. *Int J Cancer.* 2011 Jul 15; 129(2):433–9. [PubMed: 20839262]
27. De Vuyst H, Clifford GM, Nascimento MC, Madeleine MM, Franceschi S. Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: a meta-analysis. *Int J Cancer.* 2009 Apr 1; 124(7):1626–36. [PubMed: 19115209]
28. Gohy L, Gorska I, Rouleau D, Ghattas G, Pokomandy A, Vezina S, et al. Genotyping of human papillomavirus DNA in anal biopsies and anal swabs collected from HIV-seropositive men with anal dysplasia. *J Acquir Immune Defic Syndr.* 2008 Sep 1; 49(1):32–9. [PubMed: 18667921]
29. Salit IE, Tinmouth J, Chong S, Raboud J, Diong C, Su D, et al. Screening for HIV-associated anal cancer: correlation of HPV genotypes, p16, and E6 transcripts with anal pathology. *Cancer Epidemiol Biomarkers Prev.* 2009 Jul; 18(7):1986–92. [PubMed: 19567510]
30. Wentzensen N, Schiffman M, Dunn T, Zuna RE, Gold MA, Allen RA, et al. Multiple human papillomavirus genotype infections in cervical cancer progression in the study to understand cervical cancer early endpoints and determinants. *Int J Cancer.* 2009 Nov 1; 125(9):2151–8. [PubMed: 19585494]
31. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol.* 2010 Nov; 11(11):1048–56. [PubMed: 20952254]
32. Schiffman M, Clifford G, Buonaguro FM. Classification of weakly carcinogenic human papillomavirus types: addressing the limits of epidemiology at the borderline. *Infect Agent Cancer.* 2009; 4:8. [PubMed: 19486508]
33. Castle PE, Dockter J, Giachetti C, Garcia FA, McCormick MK, Mitchell AL, et al. A cross-sectional study of a prototype carcinogenic human papillomavirus E6/E7 messenger RNA assay for detection of cervical precancer and cancer. *Clin Cancer Res.* 2007 May 1; 13(9):2599–605. [PubMed: 17473189]
34. Monsonego J, Hudgens MG, Zerat L, Zerat JC, Syrjanen K, Halfon P, et al. Evaluation of oncogenic human papillomavirus RNA and DNA tests with liquid-based cytology in primary cervical cancer screening: the FASE study. *Int J Cancer.* 2011 Aug 1; 129(3):691–701. [PubMed: 20941740]
35. Darragh TM, Colgan TJ, Cox JT, Heller DS, Henry MR, Luff RD, et al. The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: Background and Consensus Recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. *Arch Pathol Lab Med.* 2012 Jun 28.
36. Doorbar J. Molecular biology of human papillomavirus infection and cervical cancer. *Clin Sci (Lond).* 2006 May; 110(5):525–41. [PubMed: 16597322]
37. Saslow D, Solomon D, Lawson HW, Killackey M, Kulasingam SL, Cain J, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for

Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer.
CA Cancer J Clin. 2012 May-Jun;62(3):147–72. [PubMed: 22422631]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

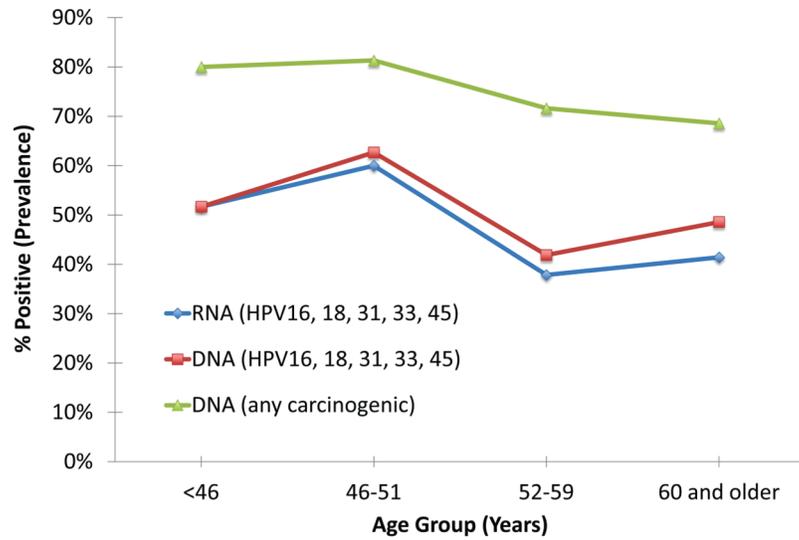


Figure 1. The age-group specific prevalence (percent positive) of HPV16, 18, 31, 33, and 45 in aggregate by DNA and E6/E7 mRNA detection and of all 13 carcinogenic HPV genotypes by DNA detection.

Agreement for DNA and E6/E7 mRNA detection of 5 human papillomavirus (HPV) genotypes (HPV16, 18, 31, 33, and 45) individually and in aggregate (“any”) in anal cytologic specimens

Table 1

	DNA+	DNA+		DNA-		DNA- RNA-	% Agreement	% Positive Agreement	Kappa	p	
		RNA+	RNA-	RNA+	RNA-						
HPV16	n	92	88	79	13	9	233	93.4%	78.2%	0.83	0.5
	row%	28%	26%	24%	4%	3%	70%				
HPV18	n	35	31	27	8	4	295	96.4%	69.2%	0.80	0.4
	row%	10%	9%	8%	2%	1%	88%				
HPV31	n	50	48	37	13	11	273	92.8%	60.7%	0.71	0.8
	row%	15%	14%	11%	4%	3%	82%				
HPV33	n	38	36	31	7	5	291	96.4%	72.1%	0.82	0.8
	row%	11%	11%	9%	2%	1%	87%				
HPV45	n	40	39	30	10	9	285	94.3%	61.2%	0.73	1
	row%	12%	12%	9%	3%	3%	85%				
Any	n	178	161	146	32	15	141	85.9%	75.6%	0.72	0.02
	row%	53%	48%	44%	10%	4%	42%				

Table 2
 Number of HPV16, 18, 31, 33, and 45 infections detected by human papillomavirus (HPV) DNA and E6/E7 mRNA

		No. of HPV16, 18, 31, 33, 45 Genotypes (RNA)						
		0	1	2	3	4	Total	
No. of HPV16, 18, 31, 33, 45 Genotypes (DNA)	0	n	141	12	2	1	0	156
		cell%	42%	4%	1%	0%	0%	47%
1	n	29	82	8	1	1	1	121
	cell%	9%	25%	2%	0%	0%	0%	36%
2	n	2	6	28	4	0	0	40
	cell%	1%	2%	8%	1%	0%	0%	12%
3	n	1	1	2	11	1	1	16
	cell%	0%	0%	1%	3%	0%	0%	5%
4	n	0	0	0	0	1	1	1
	cell%	0%	0%	0%	0%	0%	0%	0%
Total		n	173	101	40	17	3	334
		cell%	52%	30%	12%	5%	1%	

Table 3

Percent positive for DNA and E6/E7 mRNA detection of 5 human papillomavirus (HPV) genotypes (HPV16, 18, 31, 33, and 45) stratified by cytology and histology results

	N	HPV16/18/31/33/45		p
		%DNA+	%RNA+	
Cytology				
Pathologist 1				
Missing/Unsatisfactory	19	37%	37%	1
Negative	103	37%	30%	0.1
ASC-US/LSIL	127	50%	46%	0.2
High-Grade Cytology (ASC-H or HSIL)	85	82%	76%	0.1
Pathologist 2				
Missing/Unsatisfactory	9	44%	44%	1
Negative	143	35%	27%	0.03
ASC-US/LSIL	107	57%	53%	0.3
High-Grade Cytology (ASC-H or HSIL)	75	84%	81%	0.4
Both Pathologists				
No High-Grade Cytology*	233	41%	36%	0.1
One High-Grade Cytology	42	79%	62%	0.02
Both High-Grade Cytology	59	85%	85%	1
Histology				
<AIN2	272	46%	40%	0.01
AIN2	40	88%	85%	1
AIN3	22	82%	86%	1
Histology and Cytology				
No High-Grade Cytology* or Histology	206	36%	31%	0.1
Any High-Grade Cytology or Histology	128	81%	76%	0.1
AIN2/3 & Any High-Grade Cytology	35	91%	91%	1
AIN3 & Any High-Grade Cytology	14	93%	93%	1
AIN2/3 & Both High-Grade Cytology	25	100%	100%	1
AIN3 & Both High-Grade Cytology	11	100%	100%	1

Clinical performance of DNA and E6/E7 mRNA detection of different combinations of carcinogenic HPV genotypes for identification of anal precancer

Table 4

	Se	Sp	LR+	LR-	PPV	NPV	OR	95%CI
HPV16/18 RNA	62%	81%	3.3	0.48	57%	84%	6.8	4.1–12
HPV16/18 DNA	62%	77%	2.8	0.50	53%	83%	5.6	3.3–9.3
HPV16/18/31/33/45 RNA	81%	65%	2.3	0.29	48%	90%	8.1	4.5–14
HPV16/18/31/33/45 DNA	81%	58%	1.9	0.32	44%	89%	6.0	3.4–11
Carcinogenic* HPV DNA	96%	33%	1.4	0.13	37%	95%	11	4.0–32

* HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68