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Analytic and Clinical Performance of cobas HPV Testing in Anal Specimens from HIV-Positive Men Who Have Sex with Men

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Anal human papillomavirus (HPV) infections are common, and the incidence of anal cancer is high in HIV-infected men who have sex with men (MSM). To evaluate the performance of HPV assays in anal samples, we compared the cobas HPV test (cobas) to the Roche Linear Array HPV genotyping assay (LA) and cytology in HIV-infected MSM. Cytology and cobas and LA HPV testing were conducted for 342 subjects. We calculated agreement between the HPV assays and the clinical performance of HPV testing and HPV genotyping alone and in combination with anal cytology. We observed high agreement between cobas and LA, with cobas more likely than LA to show positive results for HPV16, HPV18, and other carcinogenic types. Specimens testing positive in cobas but not in LA were more likely to be positive for other markers of HPV-related disease compared to those testing negative in both assays, suggesting that at least some of these were true positives for HPV. cobas and LA showed high sensitivities but low specificities for the detection of anal intraepithelial neoplasia grade 2/3 (AIN2/3) in this population (100% sensitivity and 26% specificity for cobas versus 98.4% sensitivity and 28.9% specificity for LA). A combination of anal cytology and HPV genotyping provided the highest accuracy for detecting anal precancer. A higher HPV load was associated with a higher risk of AIN2/3 with HPV16 ($P_{trend} < 0.001$), HPV18 ($P_{trend} = 0.07$), and other carcinogenic types ($P_{trend} < 0.001$). We demonstrate that cobas can be used for HPV detection in anal cytology specimens. Additional tests are necessary to identify men at the highest risk of anal cancer among those infected with high-risk HPV.

The incidence of anal cancer is low in the general population but high among well-defined populations, such as women with a history of cervical precancer and men who have sex with men (MSM), particularly HIV-infected MSM, in whom the incidence is up to 80-fold higher than that in the general population (1, 2). A large proportion of anal cancer is caused by anal infections with carcinogenic human papillomavirus (HPV) (3), with approximately 80% to 85% caused by HPV16 or HPV18 (4, 5). Recent studies suggest that the epidemiology and biology of anal precancer are similar to those of cervical precancers. For example, a history of multiple anal sex partners is associated with a higher risk of anal HPV infection, and smoking is associated with an increased risk of anal precancer (6). Likewise, several well-characterized biomarkers for cervical cancer, such as HPV genotyping, HPV mRNA detection, and $p16^{INK4a}$/Ki-67 immunocytochemistry, show similar clinical performance characteristics for the detection of anal precancer compared to those for cervical precancer (7).

The high prevalence of anal cancer in high-risk populations, particularly HIV-infected MSM, and the similarity in natural histories between anal and cervical disease suggest that, similar to the successful cervical cancer screening model, the detection and treatment of anal precancer may prevent progression to cancer.

Primary HPV testing and cervical cytology have been recommended for primary cervical cancer screening of women between 30 and 65 years of age (8). However, anal specimens have different characteristics than cervical specimens, and they require independent evaluation with HPV DNA assays proven to be effective for cervical cancer screening.

Here, we evaluated the performance of the cobas HPV DNA test (cobas), which was recently approved by the FDA for HPV-cytology cotesting for cervical cancer screening, in a population of HIV-infected MSM.

MATERIALS AND METHODS

Study population. The study was conducted at the Kaiser Permanente Northern California (KPNC) Anal Cancer Screening Clinic in San Francisco, CA. Between August 2009 and June 2010, we enrolled 363 men who were identified as HIV infected through the Kaiser HIV registry, were 18 years or older, were not diagnosed with anal cancer prior to enrollment, and provided informed consent. In this analysis, the 342 subjects (94.2%) who had had both cobas and Roche Linear Array HPV genotyping assay (LA) testing were included. The 21 samples that were excluded were not evaluable for LA (5.8%), while only 3 samples were not evaluable for cobas (0.8%). The study was reviewed and approved by the institutional review boards at KPNC and at the National Cancer Institute.

Cytology, anoscopy, and histology. Two cytology specimens were collected from each patient during the clinical examination by inserting a wetted swab into the anal canal up to the distal rectal vault and withdrawing with rotation and lateral pressure. Both specimens were transferred to PreservCyt medium (Hologic, Bedford, MA, USA). After specimen collection, participants received a digital anorectal exam followed by high-resolution anoscopy (HRA). One to two suspicious-appearing lesions

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TABLE 1 Single and paired results for cobas and LA for the detection of any carcinogenic HPV, HPV16, HPV18, and 12 other (non-HPV16/18) carcinogenic HPV genotypes in 342 anal specimens[a]

HPV status	Testing results (no. [%])						% agreement	% positive agreement	Kappa	*P*
	cobas$^+$	LA$^+$	cobas$^-$, LA$^-$	cobas$^-$, LA$^+$	cobas$^+$, LA$^-$	cobas$^+$, LA$^+$				
Any carcinogenic type	271 (79.2)	254 (74.3)	64 (18.7)	7 (2.0)	24 (7.0)	247 (72.2)	90.9	88.8	0.75	0.003
HPV16	106 (31.0)	96 (28.1)	236 (69.0)	0 (0.0)	10 (2.9)	96 (28.1)	97.1	90.6	0.93	0.002
HPV18	42 (12.3)	36 (10.5)	298 (87.1)	2 (0.6)	8 (2.3)	34 (9.9)	97.1	77.3	0.86	0.1
Other carcinogenic type	246 (71.9)	229 (67.0)	84 (24.6)	12 (3.5)	29 (8.5)	217 (63.5)	88.0	84.1	0.72	0.01

[a] An exact version of McNemar's chi-square was used to test for statistical differences in testing positive.

identified by HRA were biopsied and sent for routine histopathological review. From the first specimen, a ThinPrep slide was prepared for routine Pap staining; cytology results were reported analogous to the Bethesda classification for cervical cytology (9) using the categories negative for intraepithelial lesion or malignancy (NILM), atypical squamous cell of undetermined significance (ASC-US), atypical squamous cells-cannot exclude high-grade lesion (ASC-H), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesions-anal intraepithelial neoplasia grade 2 (HSIL-AIN2), and HSIL-AIN3. We previously observed moderate agreement between two independent expert cytology reviews (10); in this analysis, primary cytology results from one pathologist (T.M.D.) were used. The histology results were reported as negative, condyloma acuminata, and AIN grades 1 to 3. In contrast to our previous analyses from the same study population, to allow for the evaluation of anal cytology as an independent test, we do not present combined cytology-histology endpoints in this study (7).

HPV DNA testing. The two HPV DNA tests, cobas and Linear Array, were conducted on the specimen in the second container by Roche (Pleasanton, CA, USA), which was blinded to all study data as previously described (7). In brief, 500 μl of the PreservCyt specimen was pipetted into a secondary tube. The tube was vortexed, uncapped, and loaded onto the x-480 sample extraction module of the cobas 4800 system. The x-480 extraction module inputs 400 μl of this material into the specimen preparation process. cobas 4800 and Linear Array were performed as previously described (7, 11). So far, neither cobas HPV nor Linear Array has been approved for anal cancer screening by the Food and Drug Administration.

RNAproofer HPV mRNA testing. HPV mRNA testing was conducted on the specimen in the second container by NorChip (Klokkarstua, Norway), which was blinded to all study data as previously described (7). In brief, DNA/RNA was isolated from 5-ml PreservCyt specimens by using the NucliSENS easyMAG system (bioMérieux, Marcy l'Etoile, France). Detection of HPV E6 and E7 mRNA from HPV16, -18, -31, -33, and -45 was conducted by real-time multiplex nucleic acid sequence-based amplification (NASBA) using the PreTect HPV-Proofer assay (NorChip AS) according to the manufacturer's instructions (7).

p16/Ki-67 testing. p16/Ki-67 dual-staining (CINtec PLUS) was performed on the sample in the first container by Roche mtm Laboratories (Roche Ventana, Mannheim, Germany), which was blinded to all study data as previously described (7). In brief, a second cytology slide was prepared from the residual PreservCyt material using a T2000 slide processor (Hologic, Bedford, MA, USA). Immunostaining of anal cytology slides for p16/Ki-67 was performed using the CINtec PLUS kit (Roche mtm Laboratories) according to the manufacturer's instructions. A trained cytotechnologist reviewed all cases for the presence of cells staining positively with both markers. A case was considered positive if one or more squamous epithelial cells stained with a brown cytoplasmic stain (p16) and a red nuclear (Ki-67) stain, irrespective of the interpretation of morphological abnormalities.

Statistical analysis. To evaluate the agreement between cobas and Linear Array HPV testing, we calculated the percent agreement, the percent positive agreement, the kappa values, and McNemar's chi-square values for any carcinogenic type and for strata of HPV genotypes (HPV16, HPV18, and other carcinogenic types). In addition, we evaluated the percent agreement in HPV categories grouped hierarchically according to cancer risk (HPV16, else HPV18, else other carcinogenic HPV, else carcinogenic HPV negative). We compared two markers of anal precancer, HPV E6/E7 mRNA and p16/Ki-67, in categories of cobas negativity and LA positivity for HPV16, HPV18, and any carcinogenic HPV type (cobas negative/LA negative, cobas negative/LA positive, cobas positive/LA negative, cobas positive/LA positive). To evaluate whether the additional HPV detection by cobas was associated with other disease markers, we compared biomarker positivity in the cobas-positive/LA-negative group to the cobas-negative/LA-negative group using the chi-square test. To evaluate the clinical performance of cobas and LA, we calculated the sensitivities, specificities, Youden's indices (sensitivity + specificity − 1), and positive predictive values (PPVs) for the two assays to detect AIN2 or AIN3 and AIN3 alone. In addition, we evaluated the combination of HPV DNA detection with cytology using the logical operator "or," indicating that a test was considered positive if either HPV testing or cytology was positive. Using logistic regression, we evaluated the association of viral load quartiles (as estimated by threshold cycle [C_T] values provided by the cobas assay) with risk of AIN2 or greater. Analyses were conducted for any carcinogenic type (HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, or -68), HPV16, HPV18, or HPV16 and HPV18 combined. All *P* values are two sided, and *P* values of <0.05 were considered statistically significant. All analyses were run in Stata 10.1 (Stata Corp., College Station, TX).

RESULTS

Agreement between cobas and LA HPV testing. cobas was more likely than LA to show a positive result for any carcinogenic HPV (79.2% versus 74.3%, respectively; $P = 0.003$), HPV16 (31.0% versus 28.1%, $P = 0.002$), HPV18 (12.3% versus 10.5%, respectively; $P = 0.1$), and other carcinogenic HPV types (71.9% versus 67.0%, respectively; $P = 0.01$) (Table 1). The percent agreement, percent positive agreement, and kappa value for the detection of any carcinogenic HPV type by cobas and LA were 90.9%, 88.8%, and 0.75, respectively.

Four of 10 (40.0%) specimens that were HPV16 positive by cobas and negative by LA also tested positive for HPV16 E6/E7 mRNA, and 7 tested positive for p16/Ki-67. By comparison, only 5 of 233 (2.1%) that were HPV16 negative by both cobas and LA tested positive for HPV16 E6/E7 mRNA ($P = 0.0002$). Two of 7 (28.6%) specimens that were HPV18 positive by cobas and negative by LA also tested positive for HPV18 E6/E7 mRNA. By comparison, only 2 of 296 (0.7%) that were HPV18 negative by both cobas and LA tested positive for HPV18 E6/E7 mRNA ($P = 0.003$). Thirteen of 23 (56.5%) specimens that were carcinogenic HPV positive by cobas and negative by LA also tested p16/Ki-67 positive. By comparison, only 17 of 52 (32.7%) that were carcinogenic HPV negative by cobas and LA tested p16/Ki-67 positive ($P =$

TABLE 2 Paired results for cobas and LA for the detection of HPV, categorized hierarchically according to cancer risk

HPV type with Cobas	Results (no. [cell %]) for indicated HPV type with LA[a]				
	HPV16	HPV18	Other carcinogenic type	Carcinogenic negative	Total
HPV16	**96 (28.1)**	1 (0.3)	5 (1.5)	4 (1.2)	106 (31.0)
HPV18	0 (0.0)	**20 (5.8)**	1 (0.3)	4 (1.2)	25 (7.3)
Other carcinogenic type	0 (0.0)	0 (0.0)	**124 (36.3)**	16 (4.7)	140 (40.9)
Carcinogenic negative	0 (0.0)	0 (0.0)	7 (2.0%	**64 (18.7)**	71 (20.8)
Total	96 (28.1)	21 (6.1)	137 (40.1)	88 (25.7)	342 (100.0)

[a] Hierarchical grouping of HPV genotype results: HPV16, else HPV16 negative and HPV18 positive, else HPV16 and HPV18 negative but positive for a pool of 12 other carcinogenic HPV types. The exact agreement for each HPV category is in bold type. The overall exact agreement was 88.9%, and the kappa value was 0.8392. cobas was more likely than LA to categorize the HPV results in the riskier HPV group ($P = 0.0007$, exact McNemar's chi-square).

0.08). Among the 10 samples that were positive for HPV16 by cobas but negative for HPV16 by LA, we did not observe a predominance of other types detected by LA that were closely related to HPV16, arguing against cross-reactivity.

When HPV genotypes were grouped hierarchically by clinically relevant risk categories (Table 2) (HPV16, else HPV18, else other carcinogenic types, else negative for carcinogenic types), cobas was more likely than LA to categorize HPV results in higher-risk categories ($P = 0.0007$). The exact agreement across the four categories was 89%, and the kappa value was 0.84.

Detection of AIN2 and AIN3. The sensitivity, specificity, and PPV of carcinogenic HPV DNA detection by cobas for AIN2/3 ($n = 68$) were 100%, 26.0%, and 24.1%, respectively, very similar to the clinical performance of LA (Table 3). By comparison, the sensitivity, specificity, and PPV of anal liquid-based cytology (LBC) with an atypical squamous cell of undetermined significance (ASC-US) positive cut point were 83.6%, 51.5%, and 28.9%, respectively. At the positive cut point of high-grade cytology, the sensitivity, specificity, and PPV were 40.3%, 87.2%, and 42.9%, respectively. Similar results were observed for an AIN3 ($n = 22$) endpoint.

We also looked at combinations of HPV16, HPV18, and LBC to improve AIN2/3 and AIN3 detection. We observed that the highest Youden's index for the detection of AIN2/3 was HPV16/18 detection or high-grade cytology LBC results, which had 74.6% sensitivity, 64.2% specificity, and 33.3% PPV. The highest Youden's index for detection of AIN3 was HPV16 detection or high-grade cytology LBC results, which had 85.7% sensitivity, 65.5% specificity, and 13.8% PPV.

Finally, we examined the association of HPV load and AIN2/3.

TABLE 3 The clinical performance of LA, cobas, LBC, or combinations of HPV genotypes detected by cobas and LBC for detection of AIN2 or AIN3 or AIN3 alone[a]

Test and HPV status[b]	Performance (%) of indicated test to detect[c]:									
	AIN2 or AIN3 ($n = 68$)					AIN3 ($n = 22$)				
	Se	Sp	YI	PPV	NPV	Se	Sp	YI	PPV	NPV
LA										
HPV16	55.6	78.0	33.5	36.5	88.5	63.6	74.2	37.9	14.6	96.7
HPV18	19.0	91.3	10.4	33.3	83.2	13.6	89.6	3.3	8.3	93.8
HPV16 and HPV18	65.1	72.6	37.6	35.0	90.1	68.2	67.9	36.1	12.8	96.9
Any carcinogenic type	98.4	28.9	27.3	23.9	98.8	95.5	25.2	20.6	8.1	98.8
cobas										
HPV16	57.4	74.4	31.7	34.5	88.1	63.6	70.4	34.1	12.39	96.7
HPV18	20.6	90.0	10.6	32.6	82.8	13.6	88.1	1.7	6.98	93.9
HPV16 and HPV18	66.2	67.8	34.0	32.6	89.5	68.2	63.3	31.5	10.87	96.8
Any carcinogenic type	100.0	26.0	26.0	24.1	100.0	100.0	22.4	22.4	7.80	100.0
LBC (T.M.D.)										
ASC-US	83.6	51.1	34.6	28.9	92.9	90.5	46.6	37.1	9.8	98.7
HG	40.3	87.2	27.5	42.9	86.0	61.9	84.8	46.7	20.6	97.2
Genotypes and LBC (T.M.D.)										
≥ASC-US or HPV16	92.5	42.7	35.2	27.9	96.0	95.2	37.8	33.1	9.0	99.2
≥ASC-US or HPV16/18	95.5	40.1	35.7	27.7	97.4	95.2	35.1	30.3	8.7	99.1
HG or HPV16	68.7	69.9	38.5	35.4	90.3	85.7	65.5	51.3	13.8	98.6
HG or HPV16/18	74.6	64.2	38.8	33.3	91.3	85.7	59.4	45.1	12.0	98.5

[a] LBC, liquid-based cytology; AIN2, anal intraepithelial neoplasia grade 2; AIN3, anal intraepithelial neoplasia grade 3.
[b] ASC-US, atypical squamous cells of undetermined significance; ≥ASC-US, ASC-US or more severe cytology; HG, high-grade cytology (high-grade squamous intraepithelial lesion [HSIL] or atypical squamous cells cannot rule out HSIL [ASC-H] cytology).
[c] Se, sensitivity; Sp, specificity; YI, Youden's index; PPV, positive predictive value.

TABLE 4 The relationships of quartiles of viral load, as measured by C_T values from cobas, for HPV16, HPV18, and other carcinogenic HPV types with histologically confirmed AIN2 or AIN3 among HPV-positive men[a]

Quartile of viral load	HPV16					HPV18					Other carcinogenic type				
	<AIN2[b]	AIN2/3[b]	Total[b]	OR[c]	95% CI[d]	<AIN2[b]	AIN2/3[b]	Total[b]	OR	95% CI	<AIN2[b]	AIN2/3[b]	Total[b]	OR	95% CI
4th	13 (45)	16 (55)	29 (100)	11	2.3–64	7 (64)	4 (36)	11 (100)	5.7	0.41–310	44 (66)	23 (34)	67 (100)	35	5.2–1400
3rd	23 (79)	6 (21)	29 (100)	2.3	0.42–15	8 (67)	4 (33)	12 (100)	5.0	0.36–270	60 (83)	12 (17)	72 (100)	13	1.9–580
2nd	25 (83)	5 (17)	30 (100)	1.7	0.3–13	10 (91)	1 (9)	11 (100)	1.0	0.012–86	62 (89)	8 (11)	70 (100)	8.6	1.1–390
1st	26 (90)	3 (10)	29 (100)	1.0	Ref	10 (91)	1 (9)	11 (100)	1.0	Ref	67 (99)	1 (1)	68 (100)	1.0	Ref
P_{trend}[e]	<0.001					0.07					<0.001				

[a] Logistic regression was used to calculate the odds ratio and 95% confidence interval as measures of association of the higher viral load (versus 1st quartile) with anal intraepithelial neoplasia grade 2/3 (AIN2/3).
[b] Values shown are no. (row %).
[c] OR, odds ratio.
[d] CI, confidence interval; Ref, reference category.
[e] As a test of trend, quartiles of viral load were also treated as continuous variables in the logistic regression model.

Viral loads (as estimated by C_T values) for HPV16, HPV18, and other carcinogenic HPV types were categorized into quartiles. As shown in Table 4, higher viral load quartiles of HPV16 ($P_{trend} < 0.001$), HPV18 ($P_{trend} = 0.07$), and other carcinogenic HPV genotypes ($P_{trend} < 0.001$) were more strongly associated with AIN2/3. The risks of AIN2/3 for the highest viral load quartiles for HPV16, HPV18, and other carcinogenic HPV types were 55%, 36%, and 34%, respectively.

DISCUSSION

We present here the first report of cobas compared to LA in the testing of anal specimens for the detection of clinically relevant carcinogenic HPV and AIN2/3. We made the following observations. First, cobas detection of HPV16, HPV18, other carcinogenic HPV types, and any carcinogenic HPV type agreed well with detection by LA, a well-validated standard for HPV detection and HPV genotyping. For all HPV categories, cobas was more likely than LA to show positive results. Men with cobas-positive LA-negative results were more likely to be positive for other measures of HPV (e.g., HPV E6/E7 mRNA and p16 immunocytochemistry) compared to those who tested negative in both tests, suggesting that some of the cobas-positive LA-negative results represent true HPV infections that may have clinical importance. It was notable that the patients who were cobas positive and LA negative for HPV16, HPV18, and other carcinogenic HPV types had a lower viral load than those who tested positive in both assays (data not shown).

In a previous comparison of cobas and LA in cervical specimens, a very similar agreement for the detection of carcinogenic HPV types was observed (91.0% versus 90.9% in this study), but in contrast to our findings, LA detected more infections than cobas in that study (12). In agreement with the previous study, we observed that cobas tended to classify individuals in higher HPV risk groups. There were several differences between the two studies. The previous study evaluated cervical specimens collected in specimen transport medium (STM), while the current study evaluated anal specimens collected in PreservCyt solution. The different specimen types and collection media may differentially affect the performance of the assays. The larger number of invalid samples for LA and the slightly lower HPV detection rate compared to those of cobas may suggest that LA performance is specifically more affected by biochemical components (i.e., PCR inhibitors) in anal samples than in cervical specimens. It is also worth noting that while cobas testing was done in the same laboratory, LA was done in two different laboratories, which may have further contributed to the variability in analytic performances.

Carcinogenic HPV type detection by cobas was very sensitive but nonspecific for the detection of AIN2/3 and AIN3 alone. By comparison, anal LBC read by an expert pathologist was less sensitive but significantly more specific, resulting in an overall more accurate test. The highest Youden's index for detection of AIN2/3 was observed for a combination of HSIL cytology and HPV16/18 positivity. However, test accuracy is context dependent and needs to be defined for each specific application. For example, to rule out disease in a screening population, high sensitivity and a high negative predictive value are required (13).

We observed an association of higher HPV load measured in the cobas assay with a higher risk of high-grade AIN for HPV16, HPV18, and the other carcinogenic types combined, with a higher magnitude compared to that of previous findings from studies of cervical cancer and precancer (14). These data may implicate high viral load as a triage for HPV-positive MSM to decide who needs anoscopy immediately and who may be deferred from anoscopy. Those who may be deferred can be followed for 6 to 12 months to allow benign HPV infections to resolve and to identify a subset of MSM with evidence of persistent HPV infection, which presumably carries an elevated risk of anal precancer as it does in the cervix (15). It may also suggest that a higher cut point, especially for the detection of other carcinogenic HPV types (1 AIN2/3 out of 68 at the lowest viral load), might improve the overall specificity while having a minimal impact on sensitivity. The higher agreement between the two assays for HPV16 and HPV18 may be related to the higher viral loads with these infections compared to those with other carcinogenic types.

The cobas assay has been evaluated for several cervical cancer screening applications (16–18); it has been approved for ASC-US triage and for HPV-cytology cotesting in primary screening, and it was recently approved by the Food and Drug Administration for primary screening without cytology cotesting. Our study demonstrates the feasibility of using the cobas HPV DNA assay with anal

cytology specimens. The good agreement with LA, an assay previously evaluated in anal specimens, suggests that the cobas assay could be used to identify individuals at increased risk of anal cancer by anal HPV DNA testing. Almost 80% of the men enrolled in this study were positive for carcinogenic HPV DNA, and about 20% of them had AIN2 or AIN3. These figures are similar to those reported in a large meta-analysis of HPV infections and HPV-related disease in MSM, demonstrating the high HPV burden in HIV-positive MSM (3). Due to the high HPV prevalence among HIV-infected MSM, the specificity of any HPV DNA-based assay is expected to be low. We previously evaluated the performance of HPV mRNA testing for the detection of precancers, since the expression of HPV oncogenes is increased in precancerous lesions over that in productive infections. However, we found only a slight increase in the specificity of mRNA testing over that of HPV DNA detection of the same types (19). Other tests, such as Pap cytology and p16/Ki-67 cytology, have also shown increased specificities for anal precancers and may help to decide who among the HPV-positive HIV-positive population of MSM should be referred for further diagnostic evaluation (7). Prospective studies are needed to evaluate whether the reassurance of not having anal precancer or cancer following a negative anal HPV test is comparable to that from a negative HPV test in cervical cancer screening.

The strengths of this study include the large homogeneous population of HIV-infected MSM who had highly standardized anal cytology samples collected. All the molecular assays evaluated in this study were conducted in reference laboratories. All the men enrolled in the study had thorough disease ascertainment based on anal cytology and high-resolution anoscopy. Due to the lack of a true gold standard for cervical or anal HPV DNA status, studies evaluating new HPV assays usually rely on comparisons with established assays and evaluation of associations with disease endpoints (20). Since cobas does not provide genotyping information beyond HPV16 and HPV18, a more extensive comparison of individual genotyping results with LA was not possible.

In summary, we demonstrate that the cobas HPV DNA assay has a high agreement with LA in anal cytology specimens. Similar to cervical cancer screening using HPV DNA tests, anal HPV detection has a limited specificity for AIN2/3. Further studies are required to determine whether anal HPV DNA testing can be used efficiently for risk stratification in populations at increased risk of anal cancer.

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