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Clinical Performance of Roche Cobas 4800 HPV Test

Miao Cui, Nicholas Chan, Momo Liu, Khanh Thai, Joanna Malaczynska, Ila Singh, David Zhang, Fei Ye

Department of Pathology, Mount Sinai School of Medicine, New York, New York, USA

Evaluation of the Cobas 4800 test demonstrated that Cobas had a low rate of cross-reactivity with low-risk human papillomavirus (lrHPV), a 3.74% disconcordance rate between prealiquots and postaliquots, and failure rates of 4.57% and 1.16%, respectively, after vortexing and swirling. This study demonstrated that the Cobas test has good sensitivity, accuracy, and reproducibility for detecting 14 high-risk HPV (hrHPV) genotypes.

Human papillomavirus (HPV) is one of the most common sexually transmitted viruses worldwide and a major contributor to cancer, causing almost 60,000 new cancer cases per year in Europe (1) and leading to the development of cervical cancer in women. HPV16 and HPV18 are the most carcinogenic (2, 3), as approximately 70% of all cervical cancers present with HPV16 or HPV18 infections (4). Almost 30% of cervical cancers are missed by initial cervical cytology screening (5), so accurate and objective tests for HPV-associated cervical cancer that use more sensitive molecular techniques are necessary to provide effective prevention and treatment to reduce future risks and incidence of cervical cancer.

The Roche Cobas 4800 HPV test (Cobas) is a novel molecular method based on real-time PCR (RT-PCR) (6, 7), with a fully automated system allowing quick and efficient sample processing. Cobas can detect HPV16, HPV18, 12 other high-risk HPVs (hrHPVs) (HPV31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68, as a pooled result), and the β-globin control independently in the same PCR. The primary purpose of our study was to evaluate the technical and clinical performance characteristics of Cobas. Cervical samples from 6,056 women referred to the Department of Pathology at the Icahn School of Medicine at Mount Sinai for routine examination of cervical lesions between November 2011 and February 2012 were collected in a liquid-based cytology medium (PreservCyt; Hologic, Marlborough, MA) and stored at 4°C until use.

One hundred eighty specimens were tested for hrHPV genotypes by both the Roche Cobas 4800 HPV test and the Digene Hybrid Capture 2 (HC2) hrHPV DNA test (Table 1), with any discrepancies being resolved using the Linear Array (LA) HPV genotyping test (Roche Molecular Systems, Pleasanton, CA) (8). According to Pearson's chi-square test, there was a significant difference in the number of positive and negative samples identified by the two HPV tests ($P < 0.001$). However, the concordance between the two tests was statistically strong, with an agreement level of 88.33% ($P = 0.0218$) and Cohen's kappa coefficient of 0.767 (95% confidence interval [95% CI], 0.674 to 0.860; $P < 0.001$), where 95% confidence intervals of the kappa values were deduced from a binomial distribution.

In the discordant samples, the LA HPV test detected low-risk HPV (lrHPV) genotypes in 50% (2/4) of the samples that were HC2 positive and Cobas negative and in 13.33% (2/15) of samples that were HC2 negative and Cobas positive. HPV53 was found in two samples that were HC2 positive and Cobas negative, suggesting that the probes in the Digene kit cross-reacted with this HPV genotype. This observation is unsurprising, since the cross-reactivity of the HC2 test with lrHPV genotypes, specifically HPV53, was observed previously (9). The consequence of lrHPV genotype cross-reactivity in an HPV test is an increased number of false-positive results. This could lead to overinvestigation and overtreatment through follow-up testing of women who possess only lrHPV genotypes and are actually at low risk of developing cervical cancer. In two HC2-negative/Cobas-positive samples, HPV62 and HPV89 (CP6108) were detected in both samples and HPV42, HPV54, HPV55, HPV61, HPV70, and HPV84 were detected in one of the samples, suggesting that mixed HPV infection leads to false-positive results by the Cobas HPV test.

TABLE 1 Comparison between HC2 and Cobas HPV tests

Cobas HPV test result	No. of samples with result in HC2 HPV test			
	HPV+	HPV−	Indeterminate	Total
HPV+	72	15	7	94
HPV−	4	80	2	86
Total	76	95	9	180

The LA HPV test detected hrHPV genotypes in none of the samples that were HC2 positive and Cobas negative (0/4) and in 80% (12/15) of the samples that were HC2 negative and Cobas positive. The HC2 test failed to detect hrHPV in these samples, which indicates that the HC2 test missed more hrHPV genotypes than the Cobas test, implying that Cobas is a potentially more effective screening assay due to better sensitivity and accuracy. In contrast, HPV was not detected by the LA HPV test in 6.67% (1/15) of the HC2-negative/Cobas-positive samples. Overall, however, the difference in detecting hrHPV between HC2 and Cobas tests was still statistically insignificant ($\chi^2 = 2.901$, $P = 0.0885$).

Sixty-eight samples were then used to determine the reproducibility of the Cobas HPV test. The reproducibility between the initial and repeat tests, as calculated with a standard 2-by-2 contingency table, was 93.55%; the kappa coefficient between results of the first and second rounds of Cobas testing was 0.869 (95% CI,

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Address correspondence to Fei Ye, fei.ye@mssm.edu.

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0.744 to 0.993; $P < 0.001$). The strength of this agreement is considered very good, suggesting that the assay is highly reliable.

One hundred eighty-seven samples were tested by the Cobas HPV test to determine the difference in HPV detection between prealiquoted and postaliquoted samples. Only 7 samples were mismatched, for a rate of 3.74% (95% CI, 1.02% to 6.46%). Statistical analysis revealed that the difference between prealiquoted and postaliquoted samples was not significant ($\chi^2 = 0.233$, $P = 0.36290$). Since no increase in the HPV-positive rate was observed before and after the cytology process (prealiquot versus postaliquot), cross-contamination of specimens during cytology processing is extremely rare. HPV testing after cytology can significantly improve workflow and reduce unnecessary aliquoting when a reflex HPV test for abnormal cytology is intended.

Finally, 5,621 samples were tested using the Cobas HPV test to determine the effects of vortexing and swirling (before Cobas testing) on failure rates. Among 1,380 samples processed by vortexing, the failure rate was 4.57% (95% CI, 3.65% to 5.91%). In contrast, among 4,241 samples processed by swirling, the failure rate was 1.16% (95% CI, 0.84% to 1.48%). Pearson's chi-square test revealed that the differences of the failure rates between vortexed and gently swirled samples were extremely significant ($\chi^2 = 59.983$, $P < 0.0001$), with gentle swirling being recommended to decrease Cobas' failure rate.

In conclusion, the Cobas 4800 HPV test demonstrated a greater degree of sensitivity and specificity in detecting hrHPV genotypes than the Digene HC2 hrHPV test. The Cobas test also had a lower level of cross-reactivity with lrHPV genotypes than the HC2 test, as evidenced by the fewer false-positive cases. However, the difference in overall performance between these two tests is statistically not significant.

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