Patient Safety and the Next Generation of HPV DNA Tests

DOI: 10.1309/AJCP96UDYH4OJJU

To the Editor

The special commentary, “Patient Safety and the Next Generation of HPV DNA Tests,” by Kinney et al1 asserted that the analytic and clinical sensitivity of the US Food and Drug Administration (FDA) newly approved Cervista human papillomavirus (HPV) test (Hologic, Bedford, MA) is too high for the test kit to be used clinically because it generates 2 to 4 times more positive results than the other FDA-approved Hybrid Capture 2 (HC2) HPV DNA test (Qiagen, Gaithersburg, MD). The authors further stated “any new test must demonstrate acceptable clinical performance based on the standards of reliable clinical sensitivity and specificity set by the community of experts,” designating Drs Stoler, Castle, Solomon, and Schiffman as the experts in a reference.2 The first author of the Special Commentary, Dr Kinney, should have known that neither of the 2 FDA-approved HPV DNA tests has been validated according to the experts’ set standards of “accounting for the majority (>90%) of CIN3+ [cervical intraepithelial neoplasia, grade 3 or more].” As a consultant to Digene in 2002, he presented data to the FDA in support of approval of the HC2 HPV assay, and stated on public record “because invasive cancer is not an option for an endpoint, CIN2+ was used as the clinically relevant endpoint instead.”3 At this FDA premarketing approval open session advisory meeting, the HC2 assay was proposed “for use as a general population screening test in conjunction with the Papanicolaou (Pap) smear for women age 30 and older, as an aid to determining the absence of high-grade cervical disease or cancer.”4 How to use HPV test results to determine the presence of high-grade cervical disease or cancer was not on the agenda for discussion. Now, postmarketing surveys have shown that about 95% of the referrals to colposcopic biopsies based on cotesting by cytology and HC2 assay in the United States have been found to be unnecessary, regardless of the patients’ age,4 since a combined HC2+ and atypical squamous cells of undetermined significance cytology result only has a 3.2% positive predictive value for CIN2/3 detection.5 For patient safety, health care providers must accept the truth that the HPV test is a virology test, not a test for predicting cancer.6

The authors compared the results of HPV DNA detection by the Cervista HPV HR assay and those by a reference standard consisting of “combined detection of HPV DNA by the Hybrid Capture 2 (HC2) assay and DNA sequencing of the polymerase chain reaction (PCR)-amplified human papillomavirus (HPV) DNA,” summarized in Table 1 of the Special Commentary. The discordant data in the table clearly showed that 10 (37%) of the 27 reference standard samples classified as HPV+ by HC2 were negative for HPV by PCR DNA sequencing and that the HC2 assay failed to detect HPV DNA in 9 (90%) of 10 reference standard samples that were proven to be positive for HPV by PCR DNA sequencing. With this inherently high false-positive rate (37%) and high false-negative rate (90%) in the HC2 testing system, comparing HC2 with another test with similar analytic performance easily generates a 2 to 4 times difference in positive results between the 2 tests. The FDA has now recommended PCR DNA sequencing to be used as the standard for validating any new HPV genotyping test.7

Sin Hang Lee, MD
Pathology
Milford Hospital
Milford, CT

References

The Authors’ Reply

We thank Dr Lee for his dogged responses to our various commentaries on the criteria for a clinically useful HPV DNA test.1,2 For the record, we point out that Dr Lee has a significant conflict of interest as the president and a shareholder of HiFi DNA Tech (http://www.hifidna.com), a company that “specializes in transferring the Sanger DNA sequencing technology to community hospital laboratories to increase specificity of DNA tests.” This is a technology that HiFi DNA Tech is currently offering as an in-house-developed (homebrew) laboratory HPV genotyping test for cervical cancer screening, without evidence of rigorous and proper validation against clinically relevant end points of cervical precancer and cancer. Although HiFi DNA Tech is within its rights to offer a homebrew test (nb, homebrews cannot be regulated by the US Food and Drug Administration [FDA]), the use of poorly performing or unvalidated HPV tests for clinical decision making is exactly the main focus of this and our prior commentary. Such tests have the potential for significant physical and psychosocial harm due to false-positive and false-negative results.3,4 False-positive results may result in unnecessary referral of women to colposcopy, potentially resulting in treatment, which has important negative reproductive consequences such as preterm delivery and perinatal mortality.3 It may also unnecessarily stigmatize some women by labeling them as having HPV infections although they are at very low risk of cervical precancer and cancer. False-negative results can put women with a precancerous lesion at risk for invasive cervical cancer.

Dr Lee fails to recognize that the purpose of screening tests is to reliably rule out disease in a mostly healthy population, rather than to diagnose the small percentage with disease, which is accomplished in the clinical follow-up of the screen-positive population. HPV DNA testing does this very well: a negative HPV test connotes excellent safety against cervical precancer, invasive cervical cancer, and even cervical cancer–related death for many years.5,6 Dr Lee is correct that a positive HPV test does not indicate the presence of a precancerous lesion, which is why triage of the HPV+ test result using a more specific biomarker of risk is necessary. The current method of triage of an HPV+ result is to use cervical cytology, which would refer the same population of women to colposcopy as cytology-only screening but recommends increased surveillance of HPV+ women with normal cytology (HPV+Cyo−) – who are at an elevated risk of cervical precancer and cancer compared with the general populace. Validated HPV genotyping for the most carcinogenic HPV genotypes, HPV-16 and HPV-18, may be useful for referring HPV+Cyo− women at higher risk immediately to colposcopy.8 In the future, to increase specificity for cervical precancer and cancer, screening programs may incorporate p16INK4a immunocytochemical studies9 and 1-year HPV persistence,10 although HPV genotyping beyond perhaps HPV-16 and HPV-18 may not provide additional risk stratification above what can be achieved by the pool of other HPV genotypes.10,11

Whether HPV DNA tests like the Hybrid Capture 2 (HC2; Qiagen, Gaithersburg, MD) are “virology” tests is a semantic point. The goal of HPV DNA testing in cervical cancer screening is the detection of the clinically relevant HPV infections, which are those associated with precancerous lesions, not the detection of oncogenic HPV at any viral load or the detection of HPV genotypes that do not cause cancer.1,2,12 HC2 uses a positive cutpoint of 1.0 relative light units per positive control (rlu/pc), based on a receiver operating characteristic curve analysis vs cervical intraepithelial neoplasia grade 2 or more severe (CIN 2+) to minimize the detection of lower viral load HPV infections that are mostly benign.13 Retrospective analyses have shown that raising the cutpoint to 2.0 rlu/pc may increase the accuracy of HC2,14 perhaps in part by reducing the cross-reactivity with some noncarcinogenic HPV genotypes.15 Yet, HC2 or any other HPV DNA test does not perfectly distinguish between benign and clinically important HPV infections. That is why not all HPV+ women are referred immediately to colposcopy.8

In our most recent commentary,1 we contrasted the performance of HC2 with the next HPV DNA test that was recently approved by the FDA, Cervista (Hologic, Bedford, MA), as an example of the potential dangers of excessive analytic sensitivity. The evidence presented in the manufacturer’s package insert suggested that the use of Cervista would result in excessive positivity in routine screening. Given that HC2 has consistently demonstrated sensitivity of more than 90%,4 it seems unlikely that increasing the analytic sensitivity for HPV DNA will do much to increase the clinical sensitivity for cervical precancer but will drastically hurt the clinical specificity of a test. By using the data from the manufacturer’s package insert, we illustrated that the use of Cervista in cervical cancer screening might label 2- to 4-fold more women as HPV+ without any evidence of benefit, ie, improved clinical sensitivity. Such an increase in HPV+ test results will only result in stigmatizing more women and, ultimately, referring more women to colposcopy and probably leading to more unnecessary treatments. It is also noteworthy that the age-group prevalence of Cervista HPV+ results did not sharply decline with age, as expected in the United States, suggesting that Cervista positivity may not be specific for HPV DNA.

Dr Lee cites a single article16 on the positive predictive value (PPV) of HPV testing among women 50 years and older with atypical squamous cell of undetermined significance (ASC-US) as evidence of the nonspecificity and lack of usefulness of HPV DNA testing. There is a wealth of evidence, including data from several clinical trials, that the PPV of HPV testing among women with ASC-US is
approximately 20% for CIN 2+ and 10% for CIN 3+.4,17,18 HPV DNA testing is now well proven and accepted as a triage test for ASC-US cytology,8 distinguishing women at risk of CIN 2+ that is comparable to women with cytologic findings of low-grade squamous intraepithelial lesion who need colposcopy from women who have very low risk of CIN 2+ and do not need colposcopy. The low PPV in the cited article is easily understood based on our current understanding of HPV natural history and cervical carcinogenesis19: women 50 years and older are approximately 20 to 25 years older than the peak age of CIN 2/CIN 3 in the United States.20

Finally, Dr Lee misrepresents our presentation of the paired results by Cervista and a combination of HC2 and PCR sequencing testing on a subset of specimens from the Cervista premarketing approval trial. A fraction of Cervista positives (10/94 [11%]) were judged as indeterminate by the combination of HC2 and PCR sequencing because the results were discordant (positive/negative or negative/positive). We were not privy to distribution of discordant results so cannot speculate as to which test was negative. However, even if we maximized the analytic sensitivity of the combination of HC2 and PCR sequencing (which will have a greater analytic sensitivity than PCR sensitivity alone) by treating either or both positive as positive (and, therefore, reclassifying the indeterminates as positive), Cervista was still much more likely to test positive than the combined testing (odds ratio, 6.7; 95% confidence interval, 3.4-15). Without evidence of patient benefit, such excessive test positivity by Cervista cannot be justified in clinical practice. It is only the detection of the subset of HPV infections strongly associated with CIN 2/CIN 3 that has clinical usefulness.1,2,12

Philip E. Castle, PhD, MPH
American Society for Clinical Pathology
Institute
Washington, DC

Mark Stoler, MD
Department of Pathology
University of Virginia
Charlottesville

Walter Kinney, MD
Division of Gynecologic Oncology
Kaiser Permanente Medical Care Program
Oakland, CA

References