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Molecular testing and cervical screening: will one test fit all?

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Introduction Cervical screening has undergone significant changes in recent years, with molecular human papillomavirus (HPV) testing for HPV 16 and 18 at the forefront of clinical practice. But is molecular testing more effective than morphologic testing for cervical screening? Does current information on HPV hold true across all populations? As a public health laboratory serving high-risk, underserved populations, these remain important considerations for our practice.

Materials and methods The subject population largely consisted of young women within 200% or less of the poverty line. Correlation of Papanicolaou and HPV results was performed via retrospective review, focusing on Papanicolaou cases with high-grade diagnoses and an associated HPV test using the cobas 4800 HPV test. Secondary HPV testing and typing was performed via PCR at an outside laboratory for 205 cases with sufficient residual material and negative for HPV 16/18 by cobas.

Results Of 20,211 cytology tests reviewed from July 2013 to May 2015, 521 were diagnosed as high-grade; 387 had concurrent HPV tests. Of those with concurrent HPV tests, 58% (225 of 387) of the high-grade Papanicolaou cases were not HPV 16/18 positive; furthermore, no HPV was detected in 14% (55 of 387) of these cases. Secondary testing revealed the presence of 25 unique genotypes.

Conclusions With recent emphasis on molecular HPV testing, the results of this review are concerning. As we move forward with evolution of cervical screening practices, it will be important to explore these questions for the continued quality and integrity of women's health services.

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Introduction

Over time, cervical screening has evolved from a simple glass-slide smear to a sophisticated test involving liquid-based processing, automated screening, and human papillomavirus (HPV) testing. Recent emphasis on molecular testing seeks to identify HPV strains considered "high-risk" for carcinogenesis, with HPV 16 and HPV 18 being shown to be the viral types most frequently associated with squamous neoplasia. Is the new wave of molecular testing more effective and efficient than classical morphologic testing for cervical screening, however? Do current data on HPV hold true across all populations? As a public health laboratory serving higher-risk, underserved populations, these remain important considerations for our practice.

Current data indicates that HPV 16 and HPV 18 account for approximately 70% of all cervical cancers worldwide.[1-3] There is evidence to suggest that prevalence of these and other HPV genotypes can vary significantly across populations. Recent studies indicate that prevalence of HPV 16 in cervical cancer cases range from as low as 47% in Africa to 68% in Central Asia.[4] HPV 16 prevalence in North America falls in the middle, around 55%. It is plausible that similar variations exist within subsets of any given population, despite overall national and regional trends. This begs the question of which genotypes are accountable for the remaining cervical cancer cases, and whether focusing on HPV 16 and 18 is adequate for preventative screening in all populations.

Further complicating the matter is the fact that HPV testing lacks the specificity of morphologic diagnoses rendered from a quality cytology laboratory. A positive HPV test simply indicates exposure to the virus. It cannot discern between recent exposure, transient infection, persistent infection, and progression of dysplastic lesions. This can create uncertainty of how to handle clinical follow-up for HPV+ patients, especially those with limited resources and disproportionate burden of disease. To examine these issues further, we investigated HPV prevalence within our public health-based population, focusing on our highest-risk patients; those with current high-grade Papanicolaou tests.

Materials and methods

The subject population consisted of Wisconsin women with current liquid-based cytology tests submitted to the Wisconsin State Laboratory of Hygiene between July 2013 and May 2015. Tests were submitted from 79 clinics across the state of Wisconsin, including both rural and urban settings. Ages ranged from 17 to 62 years, with an average age of 28 years. Although the age range does not strictly adhere to current ASCCP guidelines,[5,6] one must understand that guidelines may be tempered by clinical judgment, especially in a public health population that is underserved, frequently transient, and at general high risk.

Correlation of Papanicolaou and HPV results was performed via retrospective review for liquid-based Papanicolaou tests with a current high-grade squamous intraepithelial lesion (HSIL) diagnosis and an associated HPV test using the cobas 4800 HPV platform from Roche Diagnostics (Indianapolis, Ind.). All samples for cytologic and HPV analysis were obtained by means of ThinPrep liquid-based Papanicolaou test methodology (Hologic Corp, Marlborough, Mass.). For the sake of this study, HSIL Papanicolaou test diagnoses included: atypical squamous cells—cannot exclude high grade squamous intraepithelial lesion (ASC-H); those interpretations encompassed under the Bethesda System HSIL category (morphologic moderate dysplasia; severe dysplasia; carcinoma in situ [CIS]); plus squamous cell carcinoma. The cobas HPV test detects 14 high-risk genotypes (16, 18, and other high-risk cocktail encompassing 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). Results included not detected (negative), detected-HPV 16, detected-HPV 18, detected-HPV other high-risk, and detected-with a combination of two or more genotypes. The proportion of HSIL cases within each HPV diagnostic category was assessed.

Following correlation with initial HPV testing using the cobas HPV test, additional molecular analysis was performed on a subset of cases at a CLIA- and CAP-accredited reference laboratory (Access Genetics, Eden Prairie, Minn.) to provide secondary HPV detection and genotyping. Secondary testing was performed on cases with negative or other high-risk HPV results on initial testing, omitting those cases with positive HPV 16 or 18 results by the cobas method. The Access Genetics HPV test detects and identifies 50 HPV genotypes (6, 11, 16, 18, 26, 30, 31, 32, 33, 34, 35, 39, 40, 41, 42, 43, 44, 45, 49, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 66, 67, 68, 69, 70, 72, 73, 74, 75, 77, 80, 81, 82, 83, 84, CP6108, CP8061, and LVX160) using two consensus oligonucleotide primers designed to detect and type a broad range of HPV sequences by polymerase chain reaction (PCR) methodology as previously described.[7,8] The secondary HPV results were correlated

Table 1 Initial HPV results using cobas 4800 HPV test on 387 liquid-based cytology specimens with concurrent HSIL diagnosis.

HPV result	Number	Number biopsy-confirmed	Percent HPV type	Percent biopsy-confirmed
16	72	14	18.6	13.5
18	6	2	1.6	1.9
Other high-risk	170	59	43.9	56.7
Multiple	84	19	21.7	18.3
Negative	55	10	14.2	9.6
Total	387	104	100	100

The 104 cases with biopsy confirmed CIN 2+ are shown in the second column. Number and percentage of cases are broken down by HPV result. HPV results designated as Multiple indicate that more than one genotype was present, including HPV 16 and/or HPV 18.