HPV Laboratory Methods:

Impact on studies of epidemiology and vaccine preparedness

Elizabeth R. Unger

Centers for Disease Control and Prevention, USA

Sampling and testing for HPV are increasingly important because results are being used to guide clinical decisions and prophylactic vaccines are in development. HPV testing is complicated because of the nature of the virus. It cannot be cultured and antibody methods are relatively insensitive; therefore HPV detection requires some form of nucleic acid test. The viral life cycle is restricted to differentiating epithelium; therefore a cellular sample collected from the site of infection is required. Further, HPV is not a single virus, but a family of more than 100 closely related viral types. The types are distinguished based on differences in their DNA sequence. Assays must be designed to handle the complexity introduced by the large number of HPV types.

HPV testing has been used to understand the epidemiology and natural history of the virus. The nature of the sample and the assay will frame the view of infection. Definitions of occult, persistent or recurrent infection are complicated because of these issues. Based on association with disease, HPV types found in the anogenital tract are grouped into high risk (HR) and low risk (LR) types. HR types are detected frequently in cancers, yet these are the most prevalent types in the general population. HPV is the most common sexually transmitted infection. In the US it is estimated that 80% of the sexually active population will be exposed during their lifetime. The vast majority of HPV infections are transient and asymptomatic.

HPV testing is used clinically to detect HPV-associated disease. HPV infection is not treated; only HPV-associated disease is treated. HPV testing is evaluated in terms of clinical sensitivity and specificity (ability to detect disease) or analytic sensitivity and specificity (ability to detect HPV). This talk will focus on analytic characteristics of HPV tests.

The only HPV test approved by the US FDA for clinical use is Digene's Hybrid Capture® 2 HPV DNA test (HC2). This test can be applied to cervical cells collected with the Digene Cervical Sampler™ (includes brush and media), cervical cells collected for ThinPrep® cytology in PreservCyt® solution or with cervical biopsies collected in Digene Specimen Transport Medium™ . The current assay format uses liquid hybridization in a microarray platform. Samples are lysed to release nucleic acids and combined with the RNA probe mixture. The high risk probe mix includes 13 types (HPV16, 18, 31, 33, 35, 39,

45, 51, 52, 56, 58, 59, 68). The RNA probes hybridize to the DNA targets in liquid phase and are bound to the well (i.e. captured) by antibodies specific for DNA-RNA hybrids. The same antibody linked to alkaline phosphatase is used to generate a signal after addition of chemiluminescent substrate. Cut-off for a positive result is determined by comparison of the intensity of sample to that of the 1 pg/ml control. The test has good inter-laboratory comparisons and has been widely used in trials determining the clinical utility of HPV testing. The test does not control for sample cellularity and does not give type-specific results. Because the RNA probes are genetically complex, including the majority of the HPV genes, cross-hybridization with types not included as probes occurs. This could be advantageous for detection of other closely related HR types. Occasionally large numbers of copies of LR types will yield a positive result.

Type-specific PCR assays for HPV are available and can be particularly useful in quantitative assessments of HPV. However, the large number of types that are of interest limit their usefulness in most studies. PCR assays that target sequences that are highly conserved are called consensus assays. These assays will generate a product for nearly all HPV types. Type-specific identification requires analysis using sequencing, restriction-fragment length polymorphism assays or hybridization. The three most widely used consensus PCR assays are PGMY09/11, GP5+/6+ and SPF (10). All target the L1 region. They differ in the primer mix, the size of the amplified product and the method for type identification. Results differ slightly in terms of type-specific sensitivity and specificity. Typing assays using linear arrays or strips allow for efficient detection of multiple HPV types within a sample.

Other HPV assays include serology and in situ hybridization. Serologic assays use ELISA-based detection of type-specific antibodies against L1-VLPs (viral-like particles). A positive reaction indicates past or current infection. Less than 70% of HPV positive subjects develop detectable antibodies and these develop after a lag-time of several months. There is currently no gold standard for setting the threshold for positive results and few inter-laboratory comparisons are available. Competitive ELISA formats that allow titer determination are being used to follow response to HPV vaccination. The WHO is developing one or more standard sera that will assist in assay standardization. In situ hybridization assays are useful for detecting virus within a morphologic context and can give information about integration status. Requiring virus to be localized in abnormal cells may increase the specificity of test results, but sensitivity may be affected. The utility of this format is under investigation.

References

Davies P, Kornegay J, and Iftner T: Current methods of testing for human papillomavirus. Best Pract Res Clin Obstet Gynaecol 2001, 15: 677-700

Gravitt PE, Viscidi RP: Measurement of exposure to human papillomaviruses. In: Rohan TE, Shah KV (Ed), Cervical Cancer from Etiology to Prevention, Kluwer Academic

Publishers, Boston, 2004, p. 119-141

Kornegay JR, Roger M, Davies PO, Shepard AP, Guerrero NA, Lloveras B, Evans D, and Coutlee F: International proficiency study of a consensus L1 PCR assay for the detection and typing of human papillomavirus DNA: evaluation of accuracy and intralaboratory and interlaboratory agreement. J Clin Microbiol 2003, 41: 1080-1086

Unger ER: In situ diagnosis of human papillomaviruses. (In) Carr J (ed) Clinics in Laboratory Medicine, Vol. 20, WB Saunders, Philadelphia, 2000, pp. 289-301