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Detection and genotyping of human papillomavirus by five assays according to cytologic results

Jonghyeon Choi[a,b], Yongjung Park[a], Eun Hee Lee[c], Sinyoung Kim[d], Jeong-Ho Kim[a], Hyon-Suk Kim[a,*]

[a] Department of Laboratory Medicine, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-752, South Korea
[b] Neodin Medical Institute, 2-3 Yongdap-dong Sungdong-gu, Seoul, South Korea
[c] Green Cross Reference Laboratory, 314 Bojeong-dong, Kiheung-gu, Yongin, Gyeonggi-do, South Korea
[d] Department of Laboratory Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, 211 Eonju-ro, Gangnam-gu, Seoul, South Korea

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A B S T R A C T

Five assays for the detection of human papillomavirus (HPV) with different assay principles were evaluated. A total of 230 cervical swab specimens were collected from subjects according to the cytologic results. All specimens were tested by the following assays: hybrid capture 2 (HC2), two real-time PCR assays (Abbott RealTime HR and AdvanSure RealTime), liquid beads microarray (GeneFinder) and peptide nucleic acid-based array (PANArray). The HPV DNA of 99 samples was sequenced to identify genotypes. Concordance rates between the results for the identification of 14 high risk HPV genotypes by any two of the evaluated assays, except for AdvanSure RealTime, ranged from 83.0% to 88.3%, and those for the identification of genotypes 16 and 18, except for HC2, were 93.0% and 96.1%, respectively. The results for the evaluation of high risk HPV genotypes by HC2 agreed with those of the other assays in 76.5–86.5% of cases. Identification of HPV genotype by GeneFinder and PANArray corresponded with that by direct sequencing in 88.9% and 84.8% of sequenced samples. This study demonstrated that HC2 and the two real-time PCR assays could be used for routine HPV screening, and the other genotyping assays can be applied for epidemiologic surveillance.

1. Introduction

According to recent reports by the World Health Organization, cervical cancer is the second most common malignancy in women worldwide, causing almost 250,000 deaths each year (Department of Reproductive Health and Research, 2008; Report of the GAVI-UNFPA-WHO meeting, 2009). Cervical cancer occurs at the epithelial transformation zone of the cervical canal, where the squamous epithelium of the vagina replaces the glandular epithelium of the cervix. Persistent infection with human papillomavirus (HPV) is an important risk factor for cervical cancer; moreover, an etiologic link between persistent infections of high-risk (HR) HPV, cervical cancer and its precancerous lesions (i.e., intraepithelial neoplasia grade 3, cervical intraepithelial neoplasia 3) has been shown (Carozzi et al., 2011). HPV causes cancers when it infects epithelial cells of the cervical mucosa and its DNA integrates into the cellular genome (Schiffman et al., 2011). After the discovery of HPV DNA in patients with cervical cancer, various genotypes of HPV have been identified. More than 150 HPV genotypes have been defined, and approximately 50 genotypes can infect cervical epithelia. Recently, the International Agency for Research on Cancer (IARC) classified 12 HR-HPV types as group 1 carcinogens: HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 (Bouvard et al., 2009). Particularly, HPV 16 is considered the most carcinogenic in respect to the number of infected cases among cervical intraepithelial neoplasia 3 and cervical cancer patients (Bosch et al., 2008; Li et al., 2011). HPV 16 also causes most HPV-related cancers in other genital and oropharynx epithelia (Fakhry and Gillison, 2006; Parkin and Bray, 2006). In terms of etiologic significance, HPV 18 is known as the second most common cause of HPV-related cancers (Li et al., 2011).

For the prevention of cervical cancer, a cervix screening test based on morphological cytology, originally described by Papanicolaou, has been performed in pathological laboratories over the past 50 years (Gustafsson et al., 1997a,b). A major target of the cervical screening test is treatable cervical intraepithelial neoplasia 3. However, this cytology could be limited in predicting cervical cancer. According to a report by Kovacic et al. (2006) abnormal findings in cervical cytology were concurrently present in only about one quarter to one-third of the patients who were detected with carcinogenic HR HPV types, and most of these abnormal findings were equivocal or minor. Utilizing the current U.S. cytology classification, the Bethesda system was devised to reveal further crucial information on the history of HPV infection in squamous lesions (Nayar and

Abbreviations: HC2, hybrid capture 2 HPV test; HR, high risk.

* Corresponding author. Tel.: +82 2 2228 2443; fax: +82 2 364 1583.
E-mail address: kimhs54@yuhs.ac (H.-S. Kim).

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Table 1
Detectable HPV genotypes according to assays.

Assays (*n* of detectable genotypes)	HPV genotypes[a]			Genotype reporting
	High risk	Low risk	Uncertain	
HC2 (13)[b]	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	–	–	None
RealTime HR (14)[b]	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	–	–	16, 18, and other high risk HPVs
AdvanSure RealTime (41)	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 61, 62, 66, 67, 68, 72	3, 6, 10, 11, 27, 32, 34, 40, 42, 43, 44, 53, 55, 69, 73	26, 54, 57, 70, 71, 74, 81, 84	16, 18, and other HPVs
GeneFinder (43)	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 61, 62, 66, 67, 68, 72	6, 10, 11, 30, 32, 34, 40, 42, 43, 44, 53, 55, 69, 73	26, 54, 57, 70, 71, 81, 83, 84, 86, 90, 97	Each specified genotype
PANArray (32)	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	6, 11, 32, 34, 40, 42, 43, 44, 53, 54, 55, 62, 69, 73	26, 70, 81, 83, other	Each specified genotype

Abbreviations: HC2, hybrid capture 2; HR, high risk.
[a] The criteria were categorized by manually classified HPV types (Eom et al., 2004).
[b] HC2 and RealTime HR can only detect high risk genotypes of HPV.

Solomon, 2004). For instance, based on microscopic findings, a low-grade squamous intraepithelial lesion implies an acute infection with HPV, whereas a high-grade squamous intraepithelial lesion entails the possibility of current cervical intraepithelial neoplasia 3 (or an uncertain precancerous lesion, cervical intraepithelial neoplasia 2). When cytologic findings were classified according to the Bethesda system, approximately two-thirds of low-grade squamous intraepithelial lesions, as well as the majority of high-grade squamous intraepithelial lesions, were associated with carcinogenic HPV types (Clifford et al., 2005; Smith et al., 2007), while roughly half of atypical squamous cells of undetermined significance were positive for HR HPV genotypes (Schiffman et al., 2011). The atypical squamous cells of undetermined significance/low-grade squamous intraepithelial lesions (Triage Study Group, 2000). In general, HPV DNA can be detected earlier and is identifiable for a longer time, compared with cytological changes in the cervix (Schiffman et al., 2002). In this context, additional HPV genotyping can be useful for interpreting cytologic findings. Evidence in previous randomized trials supports the incorporation of HPV-based screening tests with cervical screening programs (Franceschi et al., 2009; Schiffman et al., 2007).

Along with advances in methodological techniques, various assays for detecting HPV have been introduced such as hybridization with RNA probes, polymerase chain reaction (PCR), as well as DNA chip and sequencing. Among them, hybrid capture 2 HPV test (HC2) was approved by the U.S. Food and Drug Administration (FDA) and has been used widely for screening of HPV infections. However, HC2 can only detect 13 HR HPV genotypes and cannot discriminate among the 13 genotypes. Recently, assays including real-time PCR tests and others, which can distinguish among HPV genotypes, have been developed with increasing interest for identifying HPV genotypes. Thus, this study was aimed to compare these newly developed HPV detection assays, which utilize different assay principles, with HC2 and another widely used HPV assay, Abbott RealTime HR. The following five assays were compared: (1) HC2 (Digene, Gaithersburg, MD, USA), (2) RealTime HR PCR HPV test (Abbott, Wiesbaden, Germany), (3) AdvanSure RealTime PCR HPV test (LG Life Science, Seoul, Korea), (4) GeneFinder HPV Liquid Beads MicroArray (Innomeditech, Seoul, Korea) and (5) Peptide Nucleic Acid-based Array (PANArray, Panagene Incorporated, Daejeon, Korea).

2. Materials and methods

2.1. Study subjects and cervical sample collection

Between January and April 2011, a total of 230 cervical swab specimens, which were requested for cervical cytologic examination, were collected from women who visited Severance Hospital or the Green Cross Reference Laboratory for screening of cervical HPV infections. These specimens were divided into four groups according to cytologic results: normal (group 1, $n = 78$), atypical squamous cells of undetermined significance (group 2, $n = 51$), low-grade squamous intraepithelial lesion (group 3, $n = 46$) and high-grade squamous intraepithelial lesion (group 4, $n = 55$). All specimens were placed into ThinPrep® preservcyt solution (Hologic Inc., Marlborough, MA, USA) and were split into several aliquots. Then, HPV DNA from these aliquots was extracted using *m2000sp* (Abbott Molecular Inc. Abbott Park, IL, USA), a fully automated instrument for nucleic acid preparation.

2.2. Assays for HPV detection

2.2.1. HC2 HPV test

HC2 is a sandwich capture hybridization assay comprising chemiluminescence using unlabeled single-stranded RNA probes (Lorincz, 1996; Poljak et al., 1999). After denaturation of HPV DNA, the single-stranded DNA of each specimen was hybridized with mixed RNA probes specific for 13 HR HPV genotypes. RNA–DNA hybrids were captured on the surface of an antibody-coated microplate. These hybrids were coupled with chemiluminescent substrates to produce light. The light emission was measured as a relative light unit to cutoff ratio (RLU/CO). The samples with an RLU/CO over 1.0 were considered positive.

2.2.2. Real-time HPV PCR tests

For the two real-time PCR assays, an m2000rt automated PCR instrument (Abbott Molecular Inc. Abbott Park, IL, USA) was used in the detection of amplified HPV DNA, following the manufacturers' instructions. PCR amplification utilized a mixture of modified GP5+/GP6+ primers and probes, and the assay threshold cycle cutoff of 32.0 cycles and internal control target cutoff of 35.0 for RealTime HR and 32.0 for AdvanSure RealTime were used to interpret the results according to the manufacturers' instructions. The RealTime HR can discriminate HPV genotypes 16, 18 and the group consisted of other 12 HR HPVs, while the AdvanSure RealTime separately reports HPV genotypes 16, 18 and the group of other 39 genotypes regardless of high or low risk (Table 1).

2.2.3. Liquid beads microarray

The GeneFinder HPV Liquid beads microarray uses 5.6 μm polystyrene microspheres (beads), which are internally dyed with red and infrared fluorophores. Each microsphere comprises an oligonucleotide specific to respective genotypes of HPV. Using different amounts of the two dyes for batches of microspheres, up to 100 different microsphere sets can be formed. Microspheres that