Human Papillomavirus (HPV) Detection in Cytologic Specimens: Similarities and Differences of Available Methodology

Michel P. Bihl, PhD,* Luigi Tornillo, MD,* André B. Kind, MD,† Ellen Obermann, MD,*
Christoph Noppen, PhD,‡ Rosemarie Chaffard,* Patricia Wynne,* Bruno Grilli,*
Anja Foerster,* Luigi M. Terracciano, MD,* and Sylvia Hoeller, MD*

Abstract: Accumulating evidence regarding the causative role of human papillomavirus (HPV) in a wide range of malignant and nonmalignant diseases highlights the importance of HPV testing. This study describes and discusses the efficacy and characteristics of 4 well-established and commercially available tests. Here, 181 cytologic specimens from cervical smears were analyzed using the HPV SIGN PQ (Diatech) and the Linear Array (Roche) method. Discrepant results were further studied with the Real Time High-Risk HPV (Abbott) method and the INNO-LiPA (Fujirebio) method. Of 181 cytologic specimens, 61 (34%) showed discrepant results. High-risk HPV was not detected in 9 cases by HPV SIGN PQ, in 16 cases by Linear Array, in 10 cases by Real Time High-Risk HPV, and in 6 cases by INNO-LiPA, respectively. Lack of DNA detection or problems in interpreting the result were seen in 9 cases with HPV SIGN PQ, 8 cases with Linear Array, 3 cases with Real Time High-Risk HPV, and 3 cases with INNO-LiPA, respectively. This study indicates that the choice of HPV detection method has a substantial influence on the HPV risk classification of tested PAP smears and clinical follow-up decisions.

Key Words: human papillomavirus, genotyping, HPV testing

(*Appl Immunohistochem Mol Morphol* 2017;25:184–189)

Human papillomavirus (HPV) is a family of over 200 different double-stranded DNA viruses about 8 kbp¹ in size. HPV primarily infects basal epithelial cells of the

skin or inner lining of mucosal tissues such as the mouth, the respiratory tract, or the anogenital epithelium.² Persistent infection with HPV is necessary for the transformation from normal epithelial cells of the cervix uteri to invasive squamous cell carcinoma of the cervix. Because of the high prevalence of this disease, HPV has been extensively studied.³ Depending on the risk of inducing transformation to cervical cancer, HPV types were classified into different risk groups: low risk, probable high risk, and high risk.⁴ However, this classification has not yet been definitively established, and to date there is no generally accepted risk assessment scheme for HPV subtypes. One of the most common classification system is that proposed by Muñoz et al,⁴ although there are also others that classify HPV with slight differences⁵; 3 HPV subtypes (69, 71, and 74) remain unclassified. The HPV genome can be functionally divided into 3 regions by which the DNA sequence is differentially conserved between the HPV types. The noncoding, so-called long-control region, shows the highest degree of variation. The second early region is involved in viral replication, and the third late region encodes for 2 structural proteins for the capsid, which are more conserved.² This sequence homology is important for the design of primers used in diagnostic testing. If the homology is too high, the risk of cross-reaction with other HPV types increases, but if the homology of the targeted region is low, it is not possible to use consensus primers due to the unacceptably high number of primers required.

As primer design is the primary factor in establishing a specific HPV test, and HPV classification is not definitively established, it is evident that the diagnosis, and especially the genotyping, of HPV patients is critical. Our study focused on the genotyping capacity of 4 well-established and commercially available tests, namely, HPV SIGN, Linear Array, High-Risk HPV, and INNO-LiPA.

MATERIALS AND METHODS

Samples

We collected from our daily routine practice 181 cytology specimens from cervical smears in which HPV genotyping was to be performed following internal guidelines. The majority of samples were taken from

Received for publication May 12, 2015; accepted September 5, 2015.

From the *Institute of Pathology; †Department of Gynaecology and Obstetrics, University Hospital Basel; and ‡Viollier AG, Genetics and Molecular Biology, Basel, Switzerland.

The authors declare no conflict of interest.

Reprints: Michel P. Bihl, PhD, Institute of Pathology, University Hospital Basel, Schoenbeinstrasse 40, 4031 Basel, Switzerland (e-mail: michel.bihl@usb.ch).

Copyright © 2015 The Author(s). Published by Wolters Kluwer Health, Inc. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

women with atypical squamous cells of uncertain significance (ASCUS) or low-grade squamous intraepithelial lesion (LSIL). This comparative study was done using the same DNA extraction for each sample.

The 4 analytical methods of this study are broadly used in routine diagnostics and have well-established experimental procedures, thus we will simply summarize the principle of each method and provide the Internet link for the detailed protocols.

The study was approved by the local ethics committee EKNZ (Ethikkommission Nordwest und Zentralschweiz).

HPV SIGN PQ Genotyping (Diatech) test is based on 2 consecutive analyses. The aim of the first analysis is to detect the presence or absence of HPV DNA using a multiplex real-time polymerase chain reaction (PCR) containing primers targeting a hypervariable region of L1 ORF and the human β -globin gene. The melting curve analysis obtained with the EVA Green chemistry provides a semiquantitative signal of the presence of β -globin by a peak at around 87°C, and HPV amplicons by 1 or more peaks in the range of 78 to 81°C. Only positive samples were further analyzed by 4 different sequencing primers' mixtures and processed on the Pyromark Q24 (Qiagen). The sequences obtained were compared by multialignment on the Identifier software (Biotage, Uppsala Sweden), and its genotyping was scored by percentage of identity to references sequences. A list of the major HPV genotypes identifiable through HPV SIGN PQ is provided, and includes 16 HPV high risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 54, 56, 58, 59, 68, 73, 82), 12 HPV low risk (6, 11, 34, 40, 42, 43, 44, 61, 70, 72, 81, 89), and 7 probable high risk (in the instruction manual called HPV intermediate risk) (26, 53, 66, 67, 84, 90, 91). However, further typing HPV by sequencing should be possible as the results are generated from a HPV library containing all variants available in public databases (HPV1 to HPV117 including JEB2, RTRX7, SIBX3, SIBX9) for a 45 bp hypervariable region of a highly conserved HPV gene. This method also distinguishes between the sequence variations within the same HPV genotype, for example, HPV16, HPV16 African 1, and HPV16 African 2.

Linear Array HPV Genotyping (Roche) is an adaptation of the MY09/11 system by Gravitt et al⁶ called PGMY09/11. HPV sequences of about 450 bp are amplified from the L1 ORF region by multiplex PCR. Probes for the following 37 HPV types are fixed on a membrane strip: 13 HPV high-risk genotypes (13, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and 24 low-risk genotypes (6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, and CP6108). The PCR product containing the biotin-labeled primer is hybridized to the strip. Streptavidin-horseradish peroxidase conjugate is linked to biotin and the presence of HPV is detected visually by addition of tetramethylbenzidine as substrate.

Real-Time High-Risk HPV (Abbott)

The Abbott Real-Time High-Risk (HR) HPV assay is a qualitative in vitro PCR assay that utilizes homoge-

nous target amplification and detection technology for the detection of high-risk HPV DNA in cervical cells collected in liquid cytology media. The Abbott Real-Time HR HPV assay is intended to detect 14 high-risk HPV genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and to partially genotype 16, 18 from other 12 high-risk genotypes.

Inno-Lipa HPV Genotyping Extra (Fujirebio) is a line probe assay using the SPF10 primer system. Short HPV sequences of about 50 to 65 bp are amplified from the L1 ORF region by multiplex PCR. In addition, a set of primers for amplification of the human HLA-DPB1 gene was added to monitor sample quality and extraction. Probes for the following 28 HPV types are fixed on a membrane strip: 15 high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82), 3 probable high-risk (26, 53, 66), 7 low-risk (6, 11, 40, 43, 44, 54, 70), and 3 unclassified (69, 71, and 74) genotypes. The PCR product containing the biotin-labeled primer is hybridized to the strip. Streptavidin-horseradish peroxidase conjugate is linked to biotin and the presence of HPV is detected visually by addition of 5-bromo-4-chloro-3-indolylphosphate (BCIP) and nitroblue tetrazolium chloride (NBT) as substrate. Interpretation of the result can be done by direct visualization or using the software LIRAS for LiPA HPV.

HPV Risk Classification Used for This Study

We used the classification of HPV types associated with cervical cancer proposed by Muñoz et al,⁴ Varnai et al,⁵ or HPV SIGN PQ instruction manual:

From Muñoz et al

High-risk HPV (HR): 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82.

Probable high risk (pHR): 26, 53, 66.

Low risk (LR): 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 73, 81, CP6108.

From Varnai et al

Probable high risk (pHR): 9, 30, 67, 34.

Low risk (LR): 6, 11, 32, 40, 42, 43, 54, 61, 62, 70, 72, 74, 81, 83, 84, 86, 87, 91, and CP6108.

From the HPV SIGN Instruction Manual

High-risk HPV (HR): 16, 18, 31, 33, 35, 39, 45, 51, 52, 54, 56, 58, 59, 68, 73, and 82.

Probable high risk (pHR, also described as IR): 26, 53, 66, 67, 84, and 90.

Low risk (LR): 6, 11, 34, 40, 42, 43, 44, 61, 70, 72, 81, and 89.

As these classification schemes are arbitrary, the risk stratification of a given HPV was chosen corresponding to the study with the highest number of studied cases (higher probability of accuracy). In cases of doubt, the higher risk class was chosen for a given HPV subtype. With 1 exception, HPV subtype 54 was indicated as high-risk HPV by the HPV SIGN instruction manual, but was classified as low risk according to the 2 other hallmark

papers of HPV risk classification, and was therefore always classified as low-risk HPV in our study.

As no “gold standard” exists for HPV typing, to compare the capacity of the different methods to correctly evaluate detection accuracy of HPV status, we developed a scoring system (Table 1) based on the same score used in external quality control for HR HPV. Basically, the scoring system “rewards” the absence of clinical consequences; for example, 2 points are given for the detection of confirmed HR HPV as HR and –1 point if the method failed to detect a confirmed HPV HR. We did not differentiate between negative and low-risk (LR) HPV, both of which belong to the same category for sample scoring. One point was given in the case of misclassification between high-risk and potential high-risk samples, misclassification of any sample status with an unknown risk (?R) result. Correct identification of a negative/low-risk sample status as negative/low-risk result was scored 2 points, invalid results 0 points.

RESULTS

Of 181 samples initially analyzed by 2 methods (HPV SIGN and Linear Array), 120 showed concordant results, among which 42 were positive for HPV and the following genotypes were detected: HPV16 ($n = 16$), HPV42 ($n = 5$), HPV18 ($n = 4$), HPV81 ($n = 3$), HPV6, 31, 56 ($n = 2$), and only once HPV39, 43, 45, 54, 58, 62, 67, and 73, respectively.

Sixty-one samples showed unclear HPV status and were therefore considered discordant. These 61 cases were further analyzed with both High-Risk HPV (Abbott) and INNO-LiPA (Fujirebio) methods (Table 2).

A wide range of discrepancies was observed, from undetected HPV to detection of other HPV types, including a shift from high-risk HPV to low-risk HPV or vice versa (Table 3). Undetected low-risk HPV and negative results were noted separately in our results, but due to external quality control standards they were considered as equal for the scoring system (Table 1).

On the basis of the scoring system described above, every method was assessed with a total score comprising the results of all 61 samples (Table 3). The highest ranking was achieved by the INNO-LiPA method with 102 points (of 122 possible points), followed by Real Time HR with 72 points, HPV SIGN with 66 points, and Linear Array with 60 points, respectively. However, with the Real Time HR method, the distinction between pHR and HR was not possible, therefore the result Other was always adjusted to the corresponding sample status (pHR or HR) leading to the maximum possible points.

As the number of possible detectable HPV subtypes is rather high, we looked for representations of different types in the results of each method among the 61 cases (Table 2). We found imbalances in HPV16 detection 10 times with HPV SIGN, once with Linear Array, 5 times

with Real Time HR, and 7 times with INNO-LiPA, respectively.

Moreover, we observed an absence of detection for HPV51, HPV52, and HPV53 by the method HPV SIGN, whereas these types were detected 4, 4, and 8 times by Linear Array, respectively, and 6, 13, and 8 times by INNO-LiPA method, respectively (Table 2).

The HPV SIGN method detected only a few double infections, and no triple or quadruple infections, whereas Real Time HR, Linear Array, and INNO-LiPA detected multiple infections more frequently (Table 4).

Samples that were not applicable due to failure of HPV detection or that did not allow detection of a pHR or HR HPV were analyzed in correlation with their DNA concentrations (Fig. 1). In all methods, the majority of inapplicable results appeared in samples with low DNA content.

DISCUSSION

The detection and genotyping of HPV is important because there is a strong link between high-risk HPV infection and the development of squamous cell carcinoma of the cervix,³ as well as anogenital and head and neck SCC.⁷ Assessment of HPV status in cervical cytology classified as ASCUS or LSIL helps to refine clinical decision making on follow-up examinations and further investigation.^{8–11} For this reason, we compared the benefits and limitations of 4 commercially available PCR-based detection tests.

In our diagnostic HPV testing routine, only 66% (120 of 181) of results from Linear Array and HPV

TABLE 1. Scoring System Proposed to Evaluate the Performance of Each Method in the Style of External Quality Control Examinations

Sample Status*	Result	Score	Comment
HR pHR Neg/LR	HR pHR Neg/LR	2	Absolute concordance
HR HR pHR pHR Neg/LR	pHR ?R HR ?R ?R	1	Minor discordance (no consequences on treatment)
pHR Neg/LR Neg/LR	Neg/LR HR pHR	0	Moderate discordance (may lead to overtreatment or undertreatment)
Invalid	Invalid	0	Repetition
HR	Neg/LR	–1	Severe discordance (leads to undertreatment)

Scoring is adjusted with consideration of clinical consequences.

*Sample status is defined by the most probable result in consideration of the results of all 4 methods.

?R indicates unknown risk; HR, high risk; Neg/LR, negative/low risk; pHR, probable high risk.

TABLE 2. Results Obtained From DNA Analysis of 61 Selected Discrepant Samples Analyzed for HPV Using 4 Methods

ID	HPV SIGN	Linear Array	Real Time HR	INNO-LiPA	DNA Concentration (ng/ μ L)	Status
1	Neg	51	neg	51	17.5	HR
3	16	54	NA, no hDNA	NA, no hDNA	0	HR
7	16, 45	45, 59, 61	Other	66, 11	58.1	HR
8	Neg	52	Other	52	4.6	HR
9	NA	52, 84	Other	52	49.9	HR
10	NA	39, 59	Other	39	5.8	HR
11	Neg	52, 84	Other	52	14	HR
12	Neg	82	Neg	82	4.4	HR
13	45	45, 62	Other	45	19.5	HR
14	16	83	Neg	Neg	6.8	HR
16	66	51, 66	Other	51, 52, 66	7.1	HR
17	NA	16, 39, 58	16 + Other	16, 39, 58, 74	9.9	HR
18	16	Neg	Neg	74	1.3	HR
20	58, 81	58, 61, 81	Other	39, 58, 52	0	HR
21	58	58	Other	58	200	HR
24	33	Neg	Neg	Neg	6.3	HR
26	56 HR	56	Other	56	4.5	HR
28	73	Neg	Neg	Neg	0.9	HR
29	Neg	NA, no hDNA	Other	39, 51	11.7	HR
33	58	58, 53	Other	51, 52	8.5	HR
35	86	84	Other	16	43.8	HR
36	16	Neg	16	16	11.1	HR
38	66	66	Other	39, 66	42.2	HR
39	33	Neg	NA, no hDNA	33	30.8	HR
41	16	NA, no hDNA	16 + Other	16, 31	39.4	HR
42	58	55	Neg	52	72.4	HR
43	Neg	51	Other	51	2.6	HR
45	16	Neg	Other	16, 31, 52	2.5	HR
46	NA	51	Other	51	4.8	HR
47	67	Neg	Neg	44, 52	92.7	HR
48	16	NA, no hDNA	16	16	12.3	HR
49	16	53	16 + Other	31, 53	24.2	HR
50	90	52	Other	52	88.9	HR
51	59	NA, no hDNA	Other	52	8.7	HR
52	73	NA, no hDNA	Neg	X	9.5	HR
53	16	NA, no hDNA	16	16, 52	11.6	HR
56	58	Neg	Other	58	24.4	HR
57	73	73	Neg	73	14.4	HR
59	81	Neg	Other	51, 52	8.2	HR
61	56	Neg	Other	56	1.8	HR
4	Neg	53	Neg	53	10.5	pHR
6	42 LR	53	Other	53	29.2	pHR
19	NA	53, 54	Neg	53, 54	1.3	pHR
22	NA	53	Neg	53	7.1	pHR
23	Neg	53	Neg	53	3.9	pHR
32	Neg	53	Neg	53	11.3	pHR
40	87	Neg	Neg	53	66.3	pHR
44	66	66	Other	66	3.7	pHR
55	NA	26	Neg	26	3.1	pHR
60	66	Neg	Other	66	10.7	pHR
2	42	Neg	Neg	Neg	5.8	LR
5	87	Neg	NA, no hDNA	NA, no hDNA	24.5	LR
25	42	Neg	Neg	X	17.4	LR
27	87	Neg	Neg	44	16.2	LR
37	70	NA, no hDNA	Neg	NA, no hDNA	28.6	LR
54	91	Neg	Neg	Neg	13.8	LR
15	Neg	55	Neg	X	5.7	?/LR
30	NA	62	Neg	X	26.4	?/LR
31	Neg	Neg	Neg	Neg	34	Neg
34	Neg	NA, no hDNA	Neg	Neg	10.4	Neg
58	NA	Neg	Neg	Neg	40.4	Neg

HPV types detected and risk classifications are indicated.

For HPV SIGN, NA indicates that the melting curve analysis revealed a peak of HPV presence, but pyrosequencing failed to assign it to a special genotype. ?R indicates unknown risk; HPV, indicates human papillomavirus; HR, high risk; NA, not applicable; Neg, negative; LR, low risk; pHR, probable high risk.

TABLE 3. Total Score (See Also Table 1) and Detection Failures of the 61 Samples Analyzed by All 4 Methods

Method	Score	Failures	N
HPV Sign	66	Fail HR	9
		Fail pHR	5
		NA	9
Linear Array	60	Fail HR	16
		Fail pHR	2
		NA	8
Real Time HR	72	Fail HR	10
		Fail pHR	7
		NA	3
INNO-Lipa	102	Fail HR	6
		Fail pHR	0
		NA	3

HPV indicates human papillomavirus; HR, high risk; NA, not applicable.

SIGN were identical. This is quite a low concordance rate and resulted mainly from misses of HPV (21 cases) or differently detected HPV subtypes, representing a clinically important shift in risk stratification in a subfraction of 13 cases.

In the absence of a gold standard method for assessing the HPV status of cytologic specimens, it is difficult to be certain of the actual HPV status/subtype of a given sample. To shed more light on the real HPV status of a given sample, about which nobody knows the fact, we decided to perform a discrepancy analysis with 2 additional methods, also in the knowledge of possibly overestimating the tests that were added after the first analytic run.¹² We are aware that there might be false-positive (eg, clinically irrelevant HPV presence or methodical bias) and false-negative (HPV not detected despite presence) results in this group. However, all LSIL were in 1 or more method positive for HPV, meaning that in this subgroup negative results are most probably false-negative results. For the whole group there are only 2 cases that were positive for HPV only, with 1 of the 4 methods suggesting that false-positive results (eg, due to methodical bias) are quite infrequent. However, the goal of our work was not to compare 4 different test methods, but to show significant differences in the results obtained from the same DNA extraction of ambiguous cases and to discuss possible reasons for that.

We used a scoring system similar to that used in external quality schemes to estimate the performance of the different methods in the defined discrepant cases. INNO-LiPA showed a high efficiency in detecting high-

FIGURE 1. Correlation between sample DNA concentration and failure of HPV detection. NA corresponds to a technical failure. pHR and HR to failure of detection of probable high risk and high risk HPV, respectively.

risk HPV and multiple infections with a low rate of nonevaluable specimens (8 cases). This performance is likely due to the short size of the HPV amplicons generated (65 bp), which also allows detection of HPV with low DNA content and suboptimally preserved DNA. For instance, HPV type 52 was detected in 13/61 cases by the INNO-LiPA methodology, whereas all other methods detected this type in only 4 cases or not at all (Table 2). Of course, the possibility of an overestimation of this type, for example, due to cross-reactions with other HPV types cannot be excluded.

Linear Array showed the highest number of possible detectable genotypes, and the lowest rate of HPV16 detection (only 1 of the discrepant cases), whereas the other methods detected this genotype 10, 7, and 5 times, respectively (HPV SIGN, INNO-LiPA, and Real time HPV, respectively). In all these cases, a low DNA concentration was detected, meaning that the quality was probably insufficient for the Linear Array technology, which works with a rather high amplicon length of 450 bp, requiring sufficient DNA of good quality. In addition, the high number of unevaluable cases (8, due to lack of human DNA) is probably the result of the requirement for good quality and large amount of DNA to reach an amplicon length of 450 bp. This would also be problematic in formalin-fixed and paraffin-embedded

TABLE 4. Summary of the Number of Negative or Nonapplicable (NA), Single Infections, or Multiple Infections Detected by Each Method

No. Viral Subtypes Found in Each Case	NA/Negative	Single Infection	2 Subtypes	3 Subtypes	4 Subtypes
HPV SIGN	22	38	2	0	0
Linear Array	27	25	6	3	0
Real Time HR	31	26	4	0	0
INNO-LiPA	13	34	10	3	1

tissues where the length of achievable DNA amplicon is normally below 300 bp.

The detection of multiple infections continues to be challenging for detection methods (Table 4). Only 2 cases showed multiple infections of the 61 selected cases per the HPV SIGN method. Linear Array and INNO-LiPA showed 9 and 14 multiple infections, respectively. Real Time HR is unable to detect multiple infections other than combinations of 16, 18, or Other. Combinations of high-risk genotypes other than 16 and 18 would be reported as other (of note: not all high-risk HPV genotypes are represented in this assay), and low-risk HPV is not detectable as the method is designed to detect high-risk DNA. This lack of detection of multiple infections with the HPV SIGN method is a result of the sequencing approach with comparison with the BLAST database not being considered in its design, as multiple HPV infections would create overlapping peaks in the chromatogram leading to noninterpretable results. In addition, the HPV SIGN method did not reveal any cases with detected HPV51, 52, or 53 genotypes in our study cohort, whereas Linear Array or INNO-LiPA detected these genotypes at least 4 times each. These cases were not only multiple infection, but primarily single infections that went undetected. A possible explanation might be a detection weakness due to primer design problems.

The multiplex amplification of HPV DNA in 4 certified methods showed significant differences in the results obtained from the same sample/DNA extraction, confirming the need for a gold standard, as indicated in other studies.^{13–17} The weaknesses observed and discussed in this study might encourage companies to improve their tests, and prompt users to be cognizant of possible pitfalls in result interpretation.

REFERENCES

1. Van Doorslaer K. Evolution of the papillomaviridae. *Virology*. 2013;445:11–20.
2. Burd EM. Human papillomavirus and cervical cancer. *Clin Microbiol Rev*. 2003;16:1–17.
3. zur Hausen H. Papillomaviruses—to vaccination and beyond. *Biochemistry (Mosc)*. 2008;73:498–503.
4. Muñoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*. 2003;348:518–527.
5. Varnai AD, Bollmann M, Bankfalvi A, et al. The spectrum of cervical diseases induced by low-risk and undefined-risk HPVS: implications for patient management. *Anticancer Res*. 2007;27(1B):563–570.
6. Gravitt PE, Peyton CL, Apple RJ, et al. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. *J Clin Microbiol*. 1998;36:3020–3027.
7. zur Hausen H. Papillomaviruses in the causation of human cancers - a brief historical account. *Virology*. 2009;384:260–265.
8. Solomon D, Schiffman M, Tarone R. Group AS. Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. *J Natl Cancer Inst*. 2001;93:293–299.
9. Cuzick J, Clavel C, Petry KU, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int J Cancer*. 2006;119:1095–1101.
10. Naucier P, Ryd W, Tornberg S, et al. Efficacy of HPV DNA testing with cytology triage and/or repeat HPV DNA testing in primary cervical cancer screening. *J Natl Cancer Inst*. 2009;101:88–99.
11. Stoler MH, Schiffman M. Atypical Squamous Cells of Undetermined Significance-Low-grade Squamous Intraepithelial Lesion Triage Study Group. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. *JAMA*. 2001;285:1500–1505.
12. Hadgu A. The discrepancy in discrepant analysis. *Lancet*. 1996;348:592–593.
13. Ovestad IT, Vennestrom U, Andersen L, et al. Comparison of different commercial methods for HPV detection in follow-up cytology after ASCUS/LSIL, prediction of CIN2-3 in follow up biopsies and spontaneous regression of CIN2-3. *Gynecol Oncol*. 2011;123:278–283.
14. Keegan H, Mc Inerney J, Pilkington L, et al. Comparison of HPV detection technologies: Hybrid capture 2, PreTect HPV-Proofer and analysis of HPV DNA viral load in HPV16, HPV18 and HPV33 E6/E7 mRNA positive specimens. *J Virol Methods*. 2009;155:61–66.
15. Barbieri D, Nocera M, Gallinella G, et al. Comparison of HPV sign genotyping test with INNO-LiPA HPV genotyping extra assay on histologic and cytologic cervical specimens. *Diagn Microbiol Infect Dis*. 2012;74:43–48.
16. Hwang Y, Lee M. Comparison of the AdvanSure human papillomavirus screening real-time PCR, the Abbott RealTime High Risk human papillomavirus test, and the Hybrid Capture human papillomavirus DNA test for the detection of human papillomavirus. *Ann Lab Med*. 2012;32:201–205.
17. van Ham MA, Bakkers JM, Harbers GK, et al. Comparison of two commercial assays for detection of human papillomavirus (HPV) in cervical scrape specimens: validation of the Roche AMPLICOR HPV test as a means to screen for HPV genotypes associated with a higher risk of cervical disorders. *J Clin Microbiol*. 2005;43:2662–2667.