The Journal of Molecular Diagnostics, Vol. 20, No. 6, November 2018

ELSEVIER

the Journal of Molecular Diagnostics
jmd.amjpathol.org

A Head-to-Head Analytical Comparison of Cobas 4800 HPV, PapilloCheck HPV Screening, and LMNX Genotyping Kit HPV GP for Detection of Human Papillomavirus DNA in Cervical and Cervicovaginal Swabs

Hana Jaworek,[*†] Vladimira Koudelakova,[*†] Jiri Drabek,[*†] Jana Vrbkova,[*†] Blazena Zborilova,[‡§] Ivana Oborna,[‡¶] Jana Brezinova,[‖] Radim Marek,[¶] Karel Huml,[¶] Peter Vanek,[*†] and Marian Hajduch[*†]

From the Institute of Molecular and Translational Medicine and the Departments of Biology[§] and Obstetrics and Gynecology,[¶] Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc; Cancer Research Czech Republic,[†] Olomouc; Fertimed Ltd.,[‡] Olomouc; and Arleta IVF Ltd.,[‖] Kostelec nad Orlici, Czech Republic*

Accepted for publication July 9, 2018.

Address correspondence to Marian Hajduch, M.D., Ph.D., Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Cancer Research Czech Republic, Hnevotinska 5, 775 15 Olomouc, Czech Republic. E-mail: marian.hajduch@upol.cz.

High-risk human papillomavirus (hrHPV) infection is a cause of cervical cancer development. The addition of hrHPV testing to cervical cancer screening and monitoring of cervical intraepithelial neoplasia treatment improves the efficacy of screening and treatment, respectively. Self-sampling for hrHPV testing seems a promising tool for increasing patient participation in cervical cancer screening. In this project, 1198 cervical swabs obtained by physicians and 176 cervicovaginal swabs obtained by self-sampling (not collected in parallel) were analyzed for the presence of 14 hrHPV genotypes using three commercially available assays in comparison. HPV DNA was detected in 21.2% of all samples (21% of cervical swabs and 22.7% of cervicovaginal swabs). The cobas 4800 HPV Test was the most sensitive (0.983) and specific (0.992) for hrHPV detection overall. The PapilloCheck HPV-Screening and LMNX Genotyping Kit HPV GP had comparable specificity with that of the cobas (0.989 and 0.955, respectively), but lesser sensitivity (0.897 and 0.909, respectively). In physician-obtained cervical swabs, the cobas showed the highest sensitivity and specificity (0.980 and 0.994, respectively) for hrHPV detection, whereas in cervicovaginal swabs, the cobas had the highest sensitivity (1.00), but the PapilloCheck had the highest specificity (0.993). In conclusion, all of the detection methods evaluated were highly sensitive and specific for hrHPV detection from both clinician-collected cervical swabs and self-sampled cervicovaginal swabs. *(J Mol Diagn 2018, 20: 849—858; https://doi.org/10.1016/j.jmoldx.2018.07.004)*

Persistent high-risk human papillomavirus (hrHPV) infection is a cause of cervical cancer and high-grade cervical intraepithelial neoplasia.[1,2] HPV-based cervical cancer screening is more effective than cytology-based screening (Papanicolaou test) for preventing invasive cervical cancer development and cervical cancer mortality.[3] Several European randomized trials have shown that the cumulative incidence of cervical cancer in groups with negative HPV tests was lower after 5 years than the incidence in groups with a normal cytology result after 3 years.[4—7]

Validated HPV tests and HPV tests certified by Conformité Européenne In Vitro Diagnostics usually target 14 hrHPV genotypes.[8] These genotypes were classified by the International Agency for Research on Cancer as follows: carcinogenic (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58,

Supported by Palacky University grant LF_2018_005 (H.J.); Ministry of School, Education and Youth grants LO1304, CZ.02.1.01/0.0/0.0/16_013/0001818, and LM2015064 (M.H.); Technological Agency of the Czech Republic grant TE02000058 (M.H.); and Cancer Research Czech Republic.
Disclosures: None declared.

https://doi.org/10.1016/j.jmoldx.2018.07.004

Table 1 Comparison of the Basic Characteristics for Each of the HPV DNA Tests

Assay characteristics	Cobas 4800 HPV test	PapilloCheck HPV screening	LMNX genotyping kit HPV GP
Manufacturer	Roche	Greiner Bio-One	Diassay
Principle of test	Multiplex real-time PCR, fluorimetric detection	PCR, fluorescent labeling, hybridization on chip	PCR with biotinylated GP5+/6 + primers, RHA
Analyzed gene (size of PCR product)	*L1* (200 bp)	*E1* (350 bp)	*L1* (150 bp)
Internal control	*β-globin*	*ADAT1*	Human DNA fragment located on chromosome 14
Detected genotypes	14 hrHPV (**16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68**)	18 hrHPV (**16, 18, 31, 33, 35, 39, 45, 51, 52,** 53, **56, 58, 59, 66, 68,** 70, 73, 82) + 6 lrHPV (6, 11, 40, 42, 43, 44/55)	14 hrHPV (**16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68**)
Form of results	Partial genotyping (HPV16, HPV18, other HPV)	Full genotyping	Full genotyping
Limit of detection* (HPV16; HPV18)	300—600 copy/mL†; 600 copy/mL†	50 copy/reaction; 300 copy/reaction	10—100 copy/reaction; 10—100 copy/reaction
Volume of DNA input	25 μL	5 μL	10 μL
Automatization (DNA isolation included)	Yes	No	No
Turnaround time/ technician time	4 hours/0.5 hours	4.25 hours/1 hour	4 hours/1.25 hours
Cost per sample	7 Euros	11 Euros	29 Euros
Special equipment required	The cobas x 480 (instrument for automatized sample preparation); cobas z 480 (real-time PCR analyzer)	PCR cycler; CheckScanner; CheckReport software	PCR cycler, Luminex 100/200

HPV genotypes detectable by all tested methods are shown in bold.
*Limit of detection was determined by the manufacturer of each assay.
†Per mL of original sample.
ADAT1, adenosine deaminase 1; HPV, human papillomavirus; hrHPV, high-risk HPV; lrHPV, low-risk HPV; RHA, reverse hybridization assay.

and 59), probably carcinogenic (HPV66), and possibly carcinogenic (HPV68).[1]

Many HPV detection tests are commercially available and typically detect clusters of hrHPV genotypes or provide partial genotyping. Only a few tests provide full genotype-specific information.[8] Partial genotyping for HPV16 and HPV18 could be beneficial because these genotypes pose a greater risk of causing cervical cancer than the other hrHPV genotypes.[9,10] Genotyping hrHPVs other than HPV16 and HPV18 is valuable for the identification of type-specific persistent infection, follow-up evaluation of women who screen positive, and an indication of residual or recurrent disease in women treated for high-grade cervical lesions.[11,12]

Three Conformité Européenne In Vitro Diagnostics—certified methods were compared in this study: the cobas 4800 HPV Test (Roche Diagnostics, Mannheim, Germany; referred to as the cobas 4800), the PapilloCheck HPV-Screening (Greiner Bio-One, Frickenhausen, Germany; referred to as PapilloCheck), and the LMNX Genotyping Kit HPV GP (Diassay, Rijswijk, the Netherlands; referred to as LMNX). The cobas 4800[13,14] and PapilloCheck[15] were fully validated according to the Meijer protocol.[16] The Meijer protocol was assembled by an expert committee in 2009 and proposes that new hrHPV DNA detection methods should be highly reproducible (sensitivity, ≥0.90; specificity, ≥0.98) to detect high-grade cervical intraepithelial neoplasia (CIN2/3) or cancer.[16]

Hybrid Capture 2 (HC2; Qiagen, Hilden, Germany) and the GP5+/6+ PCR enzyme immunoassay were used as standard comparator assays and are considered fully clinically and epidemiologically validated.[16] LMNX fulfills the criteria for clinical accuracy based on comparisons with standard comparator assays; however, no publication exists showing its reproducibility according to the Meijer protocol. Thus, the LMNX assay may be considered partially validated according to the Meijer protocol.[17]

Self-sampling seems a promising method for improving patient participation in cervical cancer screening.[18,19] Thus, we tested the performance of selected diagnostic systems as well for women self-collected samples, even if the paired self-collected and physician-collected samples were not available.

The aim of this study was to directly compare the detection of HPV16, 18, and a pool of 12 other hrHPV genotypes using the cobas 4800, PapilloCheck, and LMNX.

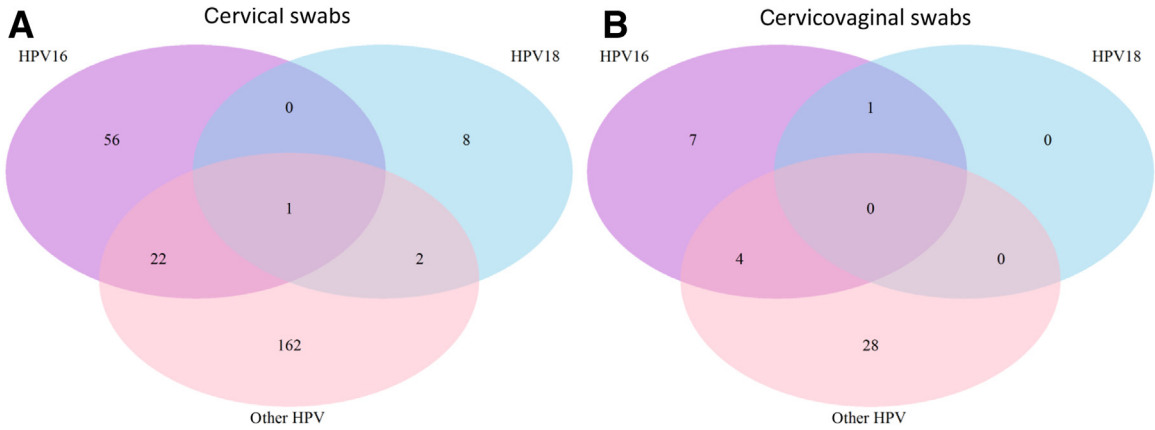

Figure 1 **A** and **B:** Venn diagrams showing the distribution of positive detection of human papillomavirus (HPV)16, HPV18, and the other 12 high-risk HPV (hrHPV) genotypes (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) in cervical (**A**) and cervicovaginal (**B**) swabs.

Materials and Methods

Ethical Considerations

This study was performed in compliance with the Helsinki Declaration according to the study ethics proposal approved by the Ethics Committee of the Faculty of Medicine and Dentistry at Palacky University and the Faculty Hospital in Olomouc. Written informed consent for the use of collected samples for research was obtained from all study participants.

Clinical Specimen Collection

For this study, 1374 samples were collected from February 2013 to August 2015 from Czech women ages 17 to 72 years (median age, 33.7 years), regardless of histopathology or cytomorphology findings. Physicians collected 1198 cervical swabs for this study in cervical screening centers (302 cases) or *in vitro* fertilization clinics (896 cases). After sampling, each cervical brush was rinsed in cobas PCR Cell Collection Media (Roche Diagnostics). All samples were stored and transported at room temperature.

A total of 176 cervicovaginal swabs were obtained by self-sampling using the Evalyn Brush device (Rovers Medical Devices B.V., Oss, the Netherlands) via a cervical cancer prevention program organized by the Cancer Research Czech Republic (*http://www.vyzkumrakoviny.cz/lets-combat-cancer-together*, last accessed May 9, 2018). After sampling, each specimen was sealed in its original dry state inside its case with a cap and sent by mail for HPV testing. Each Evalyn Brush received was rinsed in cobas PCR Cell Collection Media. The median time between sampling and sample receipt was 3 days. All samples were stored at room temperature. Cervical and cervicovaginal swabs were not collected in parallel.

Sample Preparation

All samples were collected in cobas PCR Cell Collection media, which is recommended for the cobas 4800 HPV Test. PreservCyt transport medium (Hologic, Inc., Marlborough, MA) is recommended for PapilloCheck HPV-Screening and the LMNX Genotyping Kit HPV GP. Because the chemical composition of the PreservCyt and the cobas PCR Cell Collection media is not available from manufacturers and thus not comparable, we have performed extensive chemical analysis to compare both preservation medias. Of interest, both compositions are based on 55.4% or 57.5% buffered methanol without further significant differences as evidenced

Table 2 Summary of HPV Positivity Rates in Overall, Cervical, and Cervicovaginal Swabs

	All samples	%	Cervical swabs	%	Cervicovaginal swabs	%
Total number	1372		1196		176	
HPV negative	1081	78.8*	945	79.0*	136	77.3*
HPV positive	291	21.2*	251	21.0*	40	22.7*
HPV16	63	21.6†	56	22.3†	7	17.5†
HPV16 and 18	1	0.34†	0	0	1	2.50†
HPV16, 18, and other HPV	1	0.34†	1	0.40†	0	0
HPV16 and other HPV	26	8.93†	22	8.76†	4	10.0†
HPV18	8	2.75†	8	3.19†	0	0
HPV18 and other HPV	2	0.69†	2	0.80†	0	0
Other HPV	190	65.3†	162	64.5†	28	70.0†

*Percentage from a total number of samples.
†Percentage from a number of HPV-positive samples.
HPV, human papillomavirus.

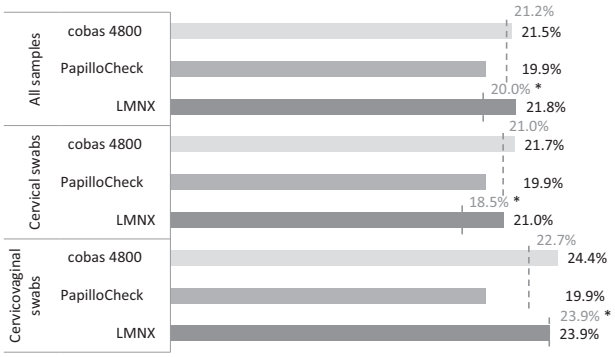

Figure 2 Comparison of method-specific human papilloma virus (HPV)-positive rates with consensus results. The **dashed lines** represent the HPV-positive detection rate values of consensus results in 1372 cervical ($n = 1196$) and cervicovaginal ($n = 176$) samples analyzed by the cobas 4800 and PapilloCheck. Because only part of the sample collection was examined by the LMNX method, the **asterisks** mark the HPV-positive detection rate values of consensus results in 330 (238 cervical and 92 cervicovaginal) samples analyzed by the cobas 4800, PapilloCheck, and the LMNX in parallel. The consensus HPV result for a given sample was obtained when at least two methods were concordant.

by elementary analysis and nuclear magnetic spectroscopy (Supplemental Table S1; Supplemental Figure S1).

DNA was extracted from all of the samples using the cobas x 480 for use with all three HPV detection methods, although the PapilloCheck HPV-Screening and the LMNX Genotyping Kit HPV GP recommend using different DNA extraction methods. The oCheck DNA extraction kit (Greiner Bio-One) is recommended for PapilloCheck and the QIAamp DNA Micro kit (Qiagen, Hilden, Germany) for LMNX. For this reason, the validation of the cobas x 480 DNA extraction for these two HPV detection assays was performed according to International Organization for Standardization ISO 15189. For this validation study, a set of 193 cervical swabs (collected during 2012) was selected and tested by the cobas 4800. The results from using the cobas x 480 DNA extraction were compared with those using the oCheck DNA extraction kit on 49 HPV-positive and 50 HPV-negative cervical swabs and compared with those using the QIAamp DNA Micro kit on 46 HPV-positive and 48 HPV-negative cervical swabs (Supplemental Table S2).

HPV DNA Detection

All samples were tested for HPV DNA using the cobas 4800 HPV Test according to the manufacturer's recommendations (Table 1). PapilloCheck was used to test all samples according to the manufacturer's instructions, except for the nucleic acid preparation. LMNX was used for parallel testing of the first 337 samples according to the manufacturer's instructions, except for the nucleic acid preparation. Unfortunately, the concentration and quality (as measured by a 260/280 ratio) of the DNA isolated using the cobas x 480 (*HPV Detection Failures*) was lower than that obtained using the QIAamp DNA Micro kit and contributed to an unacceptable failure rate of both the PapilloCheck and LMNX assays. DNA extraction using the recommended methods reduced the failure rate from 0.44% to 0% and from 11.1% to 1.59%, respectively. Because of this, the LMNX assay was used only for result confirmation in the remaining 42 samples, in which the results from cobas 4800 and PapilloCheck were not concordant (Supplemental Table S3).

Although each detection method tests a slightly different spectrum of genotypes, only 14 high-risk HPV types detected by all three methods were analyzed for comparison: HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. HPV16 and HPV18 results were analyzed individually, and the remaining 12 high-risk HPV types were pooled for analysis. Of the 1374 samples collected, 1 produced inconclusive results and 1 failed in all tested assays, most probably because of inappropriate sample collection. These two samples were excluded from our study. In total, 1372 cervical and cervicovaginal samples were analyzed. The consensus HPV result for a given sample was obtained when at least two detection methods were in agreement.

Analyses of samples deemed invalid by PapilloCheck (6 cases) or LMNX (42 cases) were repeated after DNA extraction according to the assay manufacturer's recommendations to clarify the role of the DNA extraction method in assay robustness. DNA concentration was measured using the fluorescence-based Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA).

Statistical Analysis

The statistical software R version 3.2.1 (R Core Team, R Foundation for Statistical Computing, *http://www.r-project.org*, last accessed February 26, 2018) was used for data evaluation. Measures of agreement, such as sensitivity, specificity, and Cohen's κ coefficients with 95% CIs, were calculated for each method and compared with the HPV consensus result using functions from the epibasix R package, version 1.3. The McNemar test was used to evaluate the symmetry of positive results. HPV consensus results (concordance of at least two methods) were considered the gold standard for sensitivity, specificity, and κ coefficient calculations. The sensitivity, specificity, Cohen's κ coefficient, and concordance of results obtained by the LMNX assay were calculated only for 337 samples tested in parallel with the cobas 4800 and PapilloCheck. Concordance between the results of the HPV detection methods was analyzed only when both methods being compared produced valid results. In a validation study, the cobas 4800 was considered the gold standard for sensitivity, specificity, and accuracy assessment (Supplemental Table S2).

Results

HPV Positivity Rates

Three HPV DNA detection methods, the cobas 4800 HPV Test, PapilloCheck HPV-Screening, and the LMNX

Table 3 Pairwise Concordance between HPV DNA Detection Methods and Consensus Results

	cobas 4800		PapilloCheck		LMNX		Consensus HPV result	
	HPV+	HPV−	HPV+	HPV−	HPV+	HPV−	HPV+	HPV−
cobas 4800								
HPV+			0.876 (0.844 to 0.907)*		0.767 (0.681–0.852)*		0.970 (0.954–0.985)*	
HPV−			0.005†		0.860†		0.423†	
PapilloCheck								
HPV+	256	17			0.705 (0.609–0.802)*		0.906 (0.878–0.934)*	
HPV−	39	1060			0.031†		0.009†	
LMNX								
HPV+	58	14	50	22			0.835 (0.762–0.909)*	
HPV−	12	246	9	249			0.239†	
Consensus HPV result								
HPV+	286	5	261	30	60	6		
HPV−	9	1072	12	1069	12	252		

Only samples with valid results from each method were included in the analyses. Every intersection of method row and method column corresponds to a 2 × 2 contingency table for those two methods.

*κ (95% CI) concordance metrics.

†*P* value was calculated using the McNemar test.

HPV, human papillomavirus.

Genotyping Kit HPV GP, were used to identify HPV infection in cervical and cervicovaginal samples. In total, 1196 of 1198 cervical samples and all 176 cervicovaginal samples were included in the study. For one cervical specimen, all three methods showed an invalid result (low DNA content); the other cervical specimen had an inconclusive result, probably owing to hemorrhage. These two samples were not analyzed further (Supplemental Table S3).

HPV DNA was detected in 291 of 1372 samples (21.2%) (Figure 1, Table 2). HPV16 alone was identified positively in 63 of the 291 samples (21.6%). HPV16 co-infection with other HPVs was found in 26 cases (8.93%). One (0.34%) of the 291 samples showed co-infection with HPV16 and HPV18, and co-infection with HPV16, HPV18, and the other HPVs was found in 1 case also. HPV18 alone was detected positively in 8 of the 291 samples (2.75%), whereas co-infection with HPV18 and the other HPVs was detected in 2 samples (0.69%). The other 12 HPV types were found in the majority of positive cases (190 of 291; 65.3%).

Age information was available for 94.5% (1297 of 1372) of the women in the study. Of these, 10.6% (137 of 1297) were younger than age 25 years, and 1.3% (17 of 1297) were older than age 60 years. The median age of all women examined was 32.7 years. A majority of the women (88.1%) were within the recommended age range for cytologic screening (age range, 25 to 60 years).[20] HPV-positive women were significantly younger than HPV-negative women (median age, 30.6 versus 33.2 years; $P < 0.001$). This association of positive HPV detection with younger age was observed both in women sampled by physicians (median age, 30.1 versus 32.7 years; $P < 0.001$), as well as in self-sampled women (median age, 34.3 versus 38.0 years; $P = 0.012$).

Comparison of HPV Positivity Rates in Cervical and Cervicovaginal Swabs

Twenty-one percent of cervical samples (251 of 1196) collected by physicians were HPV positive. Similarly, 22.7% (40 of 176) of cervicovaginal self-samples were HPV positive. The distribution of HPV-positive results between cervical and cervicovaginal swabs was comparable within individual HPV subgroups (Figure 1, Table 2).

Comparison of HPV Results from the cobas 4800 HPV Test, PapilloCheck HPV-Screening, and LMNX Genotyping Kit HPV GP Methods

HPV was detected in 295 of 1372 samples (21.5%) using the cobas 4800, and in 273 of 1372 samples (19.9%) using PapilloCheck (Figure 2). LMNX produced valid results in 330 of 337 samples tested. Of the 330 samples with valid results, 72 (21.8%) were HPV positive.

Irrespective of HPV genotype, the three HPV detection assays used produced concordant results in 291 of 330 cases (88.2%). Genotyping results of all three methods coincided in 288 (87.3%) cases. The cobas 4800 HPV detection results were concordant with PapilloCheck results in 95.9% (1316 of 1372) of samples, and genotyping results were concordant between the two methods in 95.3% (1307 of 1372) of cases. The cobas 4800 and LMNX methods produced concordant HPV identification results in 92.1% (304 of 330) of cases, and concordant genotyping results in 90.6% (299 of 330) of cases.

The lowest concordance was observed between the PapilloCheck and LMNX methods: 90.6% (299 of 330) concordant for HPV detection and 90.0% (297 of 330)

Table 4 Sensitivity, Specificity, and Cohen's κ Coefficient of the Different Methods Calculated Using Consensus HPV Status (Defined by Concordance of at Least Two Methods) as a Reference

		All samples					Cervical swabs	
HPV type	Method	N	SE	SP	κ (95% CI)	*P* value*	N	SE
hrHPV	cobas 4800	1372	0.983	0.992	0.970 (0.954–0.985)	0.423	1196	0.98
	PapilloCheck	1372	0.897	0.989	0.906 (0.878–0.934)	0.009	1196	0.904
	LMNX	330	0.909	0.955	0.835 (0.762–0.909)	0.239	238	0.886
HPV16	cobas 4800	1372	0.989	0.995	0.954 (0.923–0.986)	0.077	1196	0.987
	PapilloCheck	1372	0.956	1	0.976 (0.952–0.999)	0.134	1196	0.949
	LMNX	330	0.950	0.990	0.898 (0.800–0.997)	0.617	238	0.923
HPV18	cobas 4800	1372	1	0.997	0.856 (0.716–0.995)	0.134	1196	1.000
	PapilloCheck	1372	0.667	1	0.799 (0.606–0.992)	0.134	1196	0.636
	LMNX	330	0.500	0.991	0.328 (−0.161 to 0.817)	0.617	238	1.000
Other HPV	cobas 4800	1372	0.977	0.994	0.968 (0.949–0.986)	0.773	1196	0.973
	PapilloCheck	1372	0.886	0.989	0.894 (0.861–0.927)	0.074	1196	0.898
	LMNX	330	0.902	0.975	0.863 (0.787–0.939)	0.773	238	0.882

(*table continues*)

Other HPV includes HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 genotypes.
*The *P* value was calculated using the McNemar test.
HPV, human papillomavirus; hrHPV, high-risk human papillomavirus; κ, Cohen's κ coefficient; NA, not available; SE, sensitivity; SP, specificity.

concordant for HPV genotyping (Table 3, Supplemental Table S3).

Sensitivity and Specificity of Detection Methods Tested in Comparison with Consensus Result

Overall, the cobas 4800 had the highest sensitivity (0.983) and specificity (0.992) of the three detection methods evaluated. The LMNX had lower sensitivity (0.909) and comparable specificity (0.955). Similarly, PapilloCheck had comparable specificity (0.989), but lesser sensitivity (0.897) (Table 4). PapilloCheck showed higher false negativity compared with the cobas 4800 and LMNX (2.33% versus 0.44% and 2.12%, respectively), whereas higher false positivity was observed in the LMNX (3.94% versus 1.31% for cobas 4800 and 0.95% for PapilloCheck) (Supplemental Table S3).

In addition, all tests showed comparably high specificity for HPV16 (0.995 to 1.0), HPV18 (0.991 to 1.0), and the other 12 hrHPV (0.975 to 0.994) genotype detection.

Comparison of Sensitivity and Specificity in Cervical Versus Cervicovaginal Swabs

In cervical swabs, the cobas 4800 showed the highest sensitivity (0.980) and specificity (0.994) for hrHPV detection. Similarly, in cervicovaginal swabs, the cobas 4800 was the most sensitive (1.0); however, PapilloCheck was the most specific (0.993) (Table 4).

The cobas 4800 was similarly sensitive and specific for both cervical and cervicovaginal swabs tested for HPV16 (sensitivity, 0.987 versus 1.0; specificity, 0.997 versus 0.976), HPV18 (sensitivity, 1.0 versus 1.0; specificity, 0.997 versus 0.994), and for the other 12 hrHPV genotypes (sensitivity, 0.973 versus 1.0; specificity, 0.994 versus 0.993). PapilloCheck showed comparable specificity for cervical and cervicovaginal swabs tested for HPV16 (specificity, 1.0 versus 1.0), HPV18 (specificity, 1.0 versus 1.0), and the other hrHPV genotypes (specificity, 0.988 versus 0.993), but was less sensitive to HPV16 and HPV18 in cervical swabs than in cervicovaginal swabs (sensitivity, 0.949 versus 1.0; sensitivity, 0.636 versus 1.0, respectively). In contrast, PapilloCheck showed greater sensitivity to the other 12 hrHPV genotypes in cervical swabs than in cervicovaginal swabs (sensitivity, 0.898 versus 0.813).

The LMNX showed comparable specificity for HPV16 (0.991 versus 0.988) and HPV18 (0.987 versus 1.0) in cervical and cervicovaginal swabs, but lesser sensitivity to HPV16 in cervical swabs than in cervicovaginal swabs (0.923 versus 1.0). In contrast to the absolute sensitivity of LMNX to HPV18 in cervical swabs, LMNX had zero sensitivity to HPV18 in cervicovaginal swabs because of the false-negative result of only one HPV18-positive case. The LMNX showed comparable sensitivity (0.95 versus 0.926) but higher specificity (1.0 versus 0.968) for the other hrHPV genotypes in cervicovaginal swabs than in cervical swabs (Table 4).

HPV Detection Failures

One or more of the HPV DNA detection methods repeatedly failed to detect HPV DNA in 50 of 1374 samples (3.64%). DNA isolated using the cobas x 480 showed lower concentration and purity compared with DNA isolated by the QIAamp DNA Micro kit and oCheck DNA extraction kit. In the invalid samples, DNA therefore was re-extracted according to the assay manufacturer's recommendations and the detection method was repeated. Two samples were excluded because of poor quality (see the *Materials and*

Table 4 (*continued*)

Cervical swabs			Cervicovaginal swabs				
SP	κ (95% CI)	*P* value*	N	SE	SP	κ (95% CI)	*P* value*
0.994	0.972 (0.956–0.989)	1.000	176	1.000	0.978	0.953 (0.900–1.0060)	0.248
0.988	0.91 (0.881–0.939)	0.043	176	0.85	0.993	0.882 (0.796–0.967)	0.131
0.943	0.788 (0.689–0.887)	0.211	92	0.955	0.986	0.940 (0.858–1.022)	1.000
0.997	0.973 (0.947–0.999)	0.617	176	1.000	0.976	0.845 (0.697–0.993)	0.134
1.000	0.972 (0.945–0.999)	0.134	176	1.000	1.000	1.000 (−1.100)	NA
0.991	0.882 (0.751–1.01)	1.000	92	1.000	0.988	0.927 (0.786–1.069)	1.000
0.997	0.879 (0.743–1.015)	0.248	176	1.000	0.994	0.664 (0.046–1.283)	1.000
1.000	0.776 (0.563–0.990)	0.134	176	1.000	1.000	1.000 (−1.10)	NA
0.987	0.396 (−0.147 to 0.939)	0.248	92	0	1.000	0 (−0.203 to 0.203)	1.000
0.994	0.965 (0.945–0.986)	1.000	176	1.000	0.993	0.981 (0.944–1.018)	1.000
0.988	0.900 (0.866–0.935)	0.281	176	0.813	0.993	0.858 (0.755–0.960)	0.131
0.966	0.818 (0.714–0.922)	0.546	92	0.941	1.000	0.963 (0.891–1.035)	1.000

Methods section). After repeated testing of 6 of 1372 (0.44%) samples in which the PapilloCheck assay failed, 1 sample was found to be "other HPV positive" and 5 samples had HPV-negative results. Of the 42 of 377 cases (11.1%) initially determined invalid by the LMNX assay, 1 was found HPV16 positive, 4 had the "other HPV positive" result, 31 were HPV negative, and 6 remained invalid after repeated testing. The median DNA concentration of the LMNX-invalid samples was 0.222 μg/mL (0.0608 to 0.504 μg/mL) compared with 11.61 μg/mL (6.42 to 125 μg/mL) in 40 randomly selected LMNX-valid samples. The DNA concentration of LMNX-invalid samples was significantly lower ($P < 0.001$) compared with other tested samples, even after using the recommended isolation method (Supplemental Table S4).

Finally, the cobas 4800 and PapilloCheck assays did not fail to detect HPV in any sample included in the analysis whereas the LMNX assay failed in 1.59% of samples (6 of 377; $P < 0.001$), even though the analysis was repeated with the recommended DNA extraction method (Supplemental Table S4).

Discussion

This study compared the performance of three Conformité Européenne In Vitro Diagnostics hrHPV detection methods: the cobas 4800 HPV Test, PapilloCheck HPV-Screening, and the LMNX Genotyping Kit HPV GP in 94 cervicovaginal self-samples and 243 cervical clinician-collected samples and the cobas 4800 HPV Test and PapilloCheck HPV-Screening in 176 cervicovaginal self-samples and 1198 cervical clinician-collected samples.

Of the three assays evaluated, the cobas 4800 was the most sensitive and specific for detecting the 14 hrHPV genotypes overall, and in particular from the cervical swabs. In the cervicovaginal swabs, the cobas 4800 was the most sensitive, but PapilloCheck was the most specific. The sensitivity and specificity of the LMNX method may have been influenced by the lower number of samples tested using this method compared with the other two methods. The sensitivity and specificity of the cobas 4800 and PapilloCheck was calculated from 1372 cervical/cervicovaginal samples including 42 samples with discordant cobas 4800 and PapilloCheck results verified by the LMNX. The sensitivity and specificity of the cobas 4800 and PapilloCheck therefore could be slightly affected by the bias. Clinical validation of all methods tested was confirmed in several studies.[13–15,17] The Cobas 4800 and PapilloCheck yielded concordant results, with consensus in more than 97% of samples, and the LMNX aligned with the consensus results in 94.5% of samples (Table 3).

Few publications exist on the analytical sensitivity and specificity of the cobas 4800, PapilloCheck, and the LMNX for patient specimens, in contrast with the high number of clinical validation studies mentioned in the previous paragraph.[21–25] Only two studies[23,25] comparing the analytical sensitivity and specificity of the cobas 4800 with a consensus HPV result (a true positive/negative result) have been published. No study comparing the analytical sensitivity and specificity of PapilloCheck or the LMNX with a consensus HPV result has been published to our best knowledge.

Lindemann et al[25] performed an analytical comparison of the cobas 4800 and the HC2. In a set of 1360 cervical samples, the cobas 4800 was comparable with the HC2, with concordance of both methods in 86.6% of samples. However, only 82.4% (140 of 170) of the inconclusive results from the cobas 4800 and the HC2 were analyzed by the Linear Array HPV Genotyping test.[25] Park et al[23] published the only study ($n = 356$) comparing the analytical sensitivity and specificity of the cobas 4800, RealTime HR HPV assay, and the HC2 with consensus HPV results. Samples with discrepant results were analyzed both by sequencing

and by the GeneFinder HPV liquid beads microarray (Innomeditech Inc., Seoul, South Korea). Compared with the findings of Park et al[23], it was found that the cobas 4800 showed a higher sensitivity for the 14 hrHPV genotypes (0.98 versus 0.917), and for HPV16 individually (0.987 versus 0.885). HPV18 sensitivity (1 versus 1) and specificity for the 14 hrHPV genotypes (0.994 versus 0.97), HPV16 (0.997 versus 0.991), and HPV18 (0.997 versus 0.994) were comparable.

PapilloCheck and the LMNX were compared in the VALidation of HPV GENotyping Tests study, but these assays were not compared with the gold standard. The concordance of PapilloCheck with the LMNX was 0.947 ($\kappa = 0.875$) for all 14 hrHPV genotypes, 0.99 ($\kappa = 0.936$) for HPV16, and 0.99 ($\kappa = 0.801$) for HPV18.[24] This study showed a concordance of PapilloCheck with the LMNX of only 0.906 ($\kappa = 0.705$) for all 14 hrHPVs, 0.991 ($\kappa = 0.898$) for HPV 16, and 0.988 ($\kappa = -0.005$) for HPV18. Several false-positive/false-negative results were probably a reflection of different analytical sensitivities (limits of detection) of each assay. The highest limit of detection of almost all tested genotypes was indicated by the manufacturer in PapilloCheck, which showed the highest number of false-negative samples. The highest number of false-positive results was produced by the LMNX assay, which described the lowest limit of detection.

The accuracy of HPV detection and genotyping in all laboratories using the cobas 4800 and PapilloCheck has been proved by the HPV Laboratory Network's international proficiency study. No laboratory using LMNX participated in that study.[26]

The main disadvantage of the LMNX was its high detection failure rate (42 of 377; 11.1%; $P < 0.001$) when using DNA extracted using the cobas x 480. As performed in other studies, the DNA extraction method was unified to reduce cost, use limited sample quantity, and reduce technician hands-on time requirements.[23] However, DNA quantity and quality were an issue with the cobas x 480 and required validation of the other recommended DNA extraction methods. DNA extracted using the cobas x 480 was of sufficiently good quality in almost all cases for the internal control to be amplified by the cobas 4800 and by the PapilloCheck assays. The concentration and purity of the DNA isolated by the cobas x 480, however, was lower compared with the DNA isolated by the QIAamp DNA Micro kit and likely contributed to the failure rates of the PapilloCheck and the LMNX assays because the detection rates improved from 0.44% to 0% and from 11.1% to 1.59%, respectively, when the recommended DNA extraction methods were used. Despite the use of the recommended isolation method, a significantly lower DNA concentration was measured in the remaining six LMNX-invalid samples (0.222 μg/mL compared with 11.61 μg/mL in the LMNX-valid samples). This clearly shows that the LMNX has higher demands for DNA content compared with the cobas 4800 and PapilloCheck. Nevertheless, using two different DNA extraction methods for HPV detection and genotyping is extremely inconvenient in clinical practice.

hrHPV infection was observed in 21.2% of samples, with the highest prevalence of hrHPV-positive results found in the 26- to 30-year−old age category. These observations correspond with another study performed on the Czech population. Tachezy et al[27] showed a 22.3% (310 of 1393) prevalence of hrHPV infection using a PCR-based HPV detection method but, in their study, hrHPV infection was most prevalent in the 21- to 25-year−old age group.

The worldwide HPV prevalence in women with normal cytologic findings ranged from 10.4% to 12%, and prevalence varied between continents and regions.[28–30] Overall, the HPV prevalence in Europe was 8.1% to 14.2%,[28–30] with the highest prevalence (21.4%) in Eastern Europe.[29,30] The high prevalence of HPV in the Czech Republic is comparable with that in the Eastern European countries, and has been confirmed by our data. HPV16 and HPV18 are the most common HPV genotypes in the Czech Republic and also worldwide. The HPV16 and HPV18 genotypes have a frequency of 20.4% to 24% and 7.4% to 9.8% respectively, worldwide, and a frequency of 24.2% to 55% and 7.7% to 10.3%, respectively, in the Czech Republic.[27,31] Similarly, HPV16 was the most frequent genotype in this study, reflected in the finding that 31.3% of HPV-positive samples were HPV16 positive. On the other hand, HPV18 was detected in only 4.12% cases.

Although parallel cervical and cervicovaginal samples were not compared, the frequency of hrHPV-positive samples was comparable for both clinician-collected samples and self-samples, with a difference of only 1.7%. Similarly, the distribution of HPV-positive samples between cervical and cervicovaginal swabs was comparable within individual HPV subgroups.

This finding was consistent with the results of several large meta-analyses.[18,32,33] Petignat et al[33] reported a comparable average frequency of hrHPV-positive samples between self-sample and physician-collected sample groups (24.1% versus 24.8%), with a difference ranging between 0.3% and 22.2% (median, 4.9%). These differences could have been caused by the use of different types of self-sampling devices, which affects the sensitivity and specificity of the examination. The PCR-based HPV testing of self-samples collected by brush or lavage has reached the highest relative sensitivity and specificity.[32] In this study, the analytical sensitivity and specificity of all detection methods evaluated also were comparable for self- and clinician-sampling. Only the sensitivity of the PapilloCheck assay was 5.4% higher for cervical swabs than for cervicovaginal swabs ($P = 0.441$). The marginal differences between the sensitivity (1.7% to 5.4%) and specificity values (0.5% to 4.0%) of HPV detection methods analyzed using self- and clinician-samples could be caused by the varied sizes of the sample groups analyzed.

In conclusion, this study showed a high concordance between all tested methods. The concordance was the

highest between the cobas 4800 HPV Test and the PapilloCheck HPV-Screening assay, and the lowest between the PapilloCheck HPV-Screening assay and the LMNX Genotyping Kit HPV GP. The analytical parameters of all methods tested were comparable; however, the cobas 4800 HPV Test showed the highest analytical sensitivity and specificity. The LMNX Genotyping Kit HPV GP showed the highest detection failure rate and the highest demands for DNA content, which may be limiting in clinical practice.

Acknowledgments

We thank Rastislav Slavkovsky (Palacky University Olomouc, Olomouc, the Czech Republic) for writing and editing assistance, and Miroslav Soural (Palacky University Olomouc, Olomouc, the Czech Republic), Jiri Lukes (University Hospital Olomouc, Olomouc, the Czech Republic), and Vaclav Ranc (Palacky University Olomouc, Olomouc, the Czech Republic) for analysis of transport media composition.

Supplemental Data

Supplemental material for this article can be found at *https://doi.org/10.1016/j.jmoldx.2018.07.004*.

References

1. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, Cogliano V: A review of human carcinogens—part B: biological agents. Lancet Oncol 2009, 10:321—322
2. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJ, Meijer CJ: Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003, 348:518—527
3. Arbyn M, Ronco G, Anttila A, Meijer CJ, Poljak M, Ogilvie G, Koliopoulos G, Naucler P, Sankaranarayanan R, Peto J: Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. Vaccine 2012, 30 Suppl 5:F88—F99
4. Naucler P, Ryd W, Tornberg S, Strand A, Wadell G, Elfgren K, Radberg T, Strander B, Johansson B, Forslund O, Hansson BG, Rylander E, Dillner J: Human papillomavirus and Papanicolaou tests to screen for cervical cancer. N Engl J Med 2007, 357:1589—1597
5. Ronco G, Giorgi-Rossi P, Carozzi F, Confortini M, Dalla Palma P, Del Mistro A, Ghiringhello B, Girlando S, Gillio-Tos A, De Marco L, Naldoni C, Pierotti P, Rizzolo R, Schincaglia P, Zorzi M, Zappa M, Segnan N, Cuzick J; New Technologies for Cervical Cancer screening (NTCC) Working Group: Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. Lancet Oncol 2010, 11: 249—257
6. Bulkmans NW, Berkhof J, Rozendaal L, van Kemenade FJ, Boeke AJ, Bulk S, Voorhorst FJ, Verheijen RH, van Gronongen K, Boon ME, Ruitinga W, van Ballegooijen M, Snijders PJ, Meijer CJ: Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. Lancet 2007, 370: 1764—1772
7. Kitchener HC, Almonte M, Thomson C, Wheeler P, Sargent A, Stoykova B, Gilham C, Baysson H, Roberts C, Dowie R, Desai M, Mather J, Bailey A, Turner A, Moss S, Peto J: HPV testing in combination with liquid-based cytology in primary cervical screening (ARTISTIC): a randomised controlled trial. Lancet Oncol 2009, 10:672—682
8. Arbyn M, Snijders PJ, Meijer CJ, Berkhof J, Cuschieri K, Kocjan BJ, Poljak M: Which high-risk HPV assays fulfil criteria for use in primary cervical cancer screening? Clin Microbiol Infect 2015, 21:817—826
9. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al: Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 2010, 11:1048—1056
10. Khan MJ, Castle PE, Lorincz AT, Wacholder S, Sherman M, Scott DR, Rush BB, Glass AG, Schiffman M: The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. J Natl Cancer Inst 2005, 97:1072—1079
11. Meijer CJ, Snijders PJ, Castle PE: Clinical utility of HPV genotyping. Gynecol Oncol 2006, 103:12—17
12. Soderlund-Strand A, Kjellberg L, Dillner J: Human papillomavirus type-specific persistence and recurrence after treatment for cervical dysplasia. J Med Virol 2014, 86:634—641
13. Heideman DA, Hesselink AT, Berkhof J, van Kemenade F, WJ Melchers WJ, Daalmeijer NF, Verkuijten M, Meijer CJ, Snijders PJ: Clinical validation of the cobas 4800 HPV test for cervical screening purposes. J Clin Microbiol 2011, 49:3983—3985
14. Lloveras B, Gomez S, Alameda F, Bellosillo B, Mojal S, Muset M, Parra M, Palomares JC, Serrano S: HPV testing by cobas HPV test in a population from Catalonia. PLoS One 2013, 8:e58153
15. Hesselink AT, Heideman DA, Berkhof J, Topal F, Pol RP, Meijer CJ, Snijders PJ: Comparison of the clinical performance of PapilloCheck human papillomavirus detection with that of the GP5+/6+-PCR-enzyme immunoassay in population-based cervical screening. J Clin Microbiol 2010, 48:797—801
16. Meijer CJ, Berkhof J, Castle PE, Hesselink AT, Franco EL, Ronco G, Arbyn M, Bosch FX, Cuzick J, Dillner J, Heideman DA, Snijders PJ: Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. Int J Cancer 2009, 124:516—520
17. Geraets DT, Cuschieri K, de Koning MN, van Doorn LJ, Snijders PJ, Meijer CJ, Quint WG, Arbyn M: Clinical evaluation of a GP5+/6+-based luminex assay having full high-risk human papillomavirus genotyping capability and an internal control. J Clin Microbiol 2014, 52:3996—4002
18. Arbyn M, Castle PE: Offering self-sampling kits for HPV testing to reach women who do not attend in the regular cervical cancer screening program. Cancer Epidemiol Biomarkers Prev 2015, 24:769—772
19. Gok M, Heideman DA, van Kemenade FJ, Berkhof J, Rozendaal L, Spruyt JW, Voorhorst F, Belien JA, Babovic M, Snijders PJ, Meijer CJ: HPV testing on self collected cervicovaginal lavage specimens as screening method for women who do not attend cervical screening: cohort study. BMJ 2010, 340:c1040
20. Duskova J, Bekova A, Dvorak V, Majek O, Dusek L: Results of the Czech National cervical cancer screening programme. Klin Onkol 2014, 27 Suppl 2:79—86
21. Dalstein V, Merlin S, Bali C, Saunier M, Dachez R, Ronsin C: Analytical evaluation of the PapilloCheck test, a new commercial DNA chip for detection and genotyping of human papillomavirus. J Virol Methods 2009, 156:77—83
22. Cuzick J, Cadman L, Mesher D, Austin J, Ashdown-Barr L, Ho L, Terry G, Liddle S, Wright C, Lyons D, Szarewski A: Comparing the performance of six human papillomavirus tests in a screening population. Br J Cancer 2013, 108:908—913
23. Park Y, Lee E, Choi J, Jeong S, Kim HS: Comparison of the Abbott RealTime high-risk human papillomavirus (HPV), Roche Cobas HPV, and Hybrid Capture 2 assays to direct sequencing and genotyping of HPV DNA. J Clin Microbiol 2012, 50:2359—2365

24. Arbyn M, Depuydt C, Benoy I, Bogers J, Cuschieri K, Schmitt M, Pawlita M, Geraets D, Heard I, Gheit T, Tommasino M, Poljak M, Bonde J, Quint W: VALGENT: a protocol for clinical validation of human papillomavirus assays. J Clin Virol 2016, 76 Suppl 1: S14−S21
25. Lindemann ML, Dominguez MJ, de Antonio JC, Sandri MT, Tricca A, Sideri M, Khiri H, Ravet S, Boyle S, Aldrich C, Halfon P: Analytical comparison of the cobas HPV test with Hybrid Capture 2 for the detection of high-risk HPV genotypes. J Mol Diagn 2012, 14:65−70
26. Eklund C, Forslund O, Wallin KL, Dillner J: Global improvement in genotyping of human papillomavirus DNA: the 2011 HPV LabNet International Proficiency Study. J Clin Microbiol 2014, 52:449−459
27. Tachezy R, Smahelova J, Kaspirkova J, Salakova M: Human papillomavirus type-specific prevalence in the cervical cancer screening population of Czech women. PLoS One 2013, 8:e79156
28. Guan P, Howell-Jones R, Li N, Bruni L, de Sanjose S, Franceschi S, Clifford GM: Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. Int J Cancer 2012, 131:2349−2359
29. Bruni L, Diaz M, Castellsague X, Ferrer E, Bosch FX, de Sanjose S: Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis 2010, 202:1789−1799
30. de Sanjose S, Diaz M, Castellsague X, Clifford G, Bruni L, Munoz N, Bosch FX: Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis 2007, 7:453−459
31. Tachezy R, Hamsikova E, Hajek T, Mikyskova I, Smahel M, Van Ranst M, Kanka J, Havrankova A, Rob L, Guttner V, Slavik V, Anton M, Kratochvil B, Kotrsova L, Vonka V: Human papillomavirus genotype spectrum in Czech women: correlation of HPV DNA presence with antibodies against HPV-16, 18, and 33 virus-like particles. J Med Virol 1999, 58:378−386
32. Arbyn M, Verdoodt F, Snijders PJ, Verhoef VM, Suonio E, Dillner L, Minozzi S, Bellisario C, Banzi R, Zhao FH, Hillemanns P, Anttila A: Accuracy of human papillomavirus testing on self-collected versus clinician-collected samples: a meta-analysis. Lancet Oncol 2014, 15: 172−183
33. Petignat P, Faltin DL, Bruchim I, Tramér MR, Franco EL, Coutlée F: Are self-collected samples comparable to physician-collected cervical specimens for human papillomavirus DNA testing? A systematic review and meta-analysis. Gynecol Oncol 2007, 105:530−535