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Human Papillomavirus (HPV) Genotyping: Automation and Application in Routine Laboratory Testing

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Abstract: A large number of assays designed for genotyping human papillomaviruses (HPV) have been developed in the last years. They perform within a wide range of analytical sensitivity and specificity values for the different viral types, and are used either for diagnosis, epidemiological studies, evaluation of vaccines and implementing and monitoring of vaccination programs. Methods for specific genotyping of HPV-16 and HPV-18 are also useful for the prevention of cervical cancer in screening programs. Some commercial tests are, in addition, fully or partially automated. Automation of HPV genotyping presents advantages such as the simplicity of the testing procedure for the operator, the ability to process a large number of samples in a short time, and the reduction of human errors from manual operations, allowing a better quality assurance and a reduction of cost. The present review collects information about the current HPV genotyping tests, with special attention to practical aspects influencing their use in clinical laboratories.

Keywords: Automation, Human Papillomavirus, genotyping.

INTRODUCTION

The establishment of a persistent infection by some of the several high-risk human papillomavirus (HR-HPV) is a necessary step in the development of cervical carcinoma [1-3]. HPV infection is frequent in sexually active people, but the persistent infection develops only in a small proportion of cases. HPV persistency involves a risk of developing precancerous cervix lesions and, eventually, cervical cancer [4-6]. Among the HPVs from the genus alpha (those infecting the mucosal epithelium), 10 to 15 genotypes have been qualified as HR-HPV on the basis of their association with cancer [7-10]. The World Health Organization (WHO) International Agency for Research on Cancer (IARC) include, however, at present only twelve of them (HPV-16, -18, -31, -33, -45, -51, -52, -56, -58 and -59) into such consideration [7], the types 66 and 68 being excluded from their last classifications. However, these two types were present in the widespread classifications previously published, and most commercial tests for HR-HPV detection have included both into the design [8-10]. HR-HPV genotypes 16 and 18 are the most clinically relevant, since they are involved in about 70% of cases of cervical cancer [8, 11, 12].

HPVs cannot be cultured *in vitro* by conventional methods, and the wide natural variation of the humoral immune response after the infection impairs the use of HPV-specific antibody testing in diagnosis [13]. Diagnosis of the HPV infection is, therefore, achieved by molecular testing, mainly by detection of genomic HPV DNA.

Several practice guidelines for cervical screening have proposed in the last years the use of HR-HPV detection adjunctively with cervical cytology to screen women aged 30 and older and to determine the need for referral to colposcopy of women with ASCUS (atypical squamous cells of undetermined significance) cervical cytology results [14, 15]. Also, the genotype specific identification of HPV-16 and HPV-18 in women with a positive HR-HPV test and negative cytological results has been recommended [16].

Two HR-HPV DNA-based screening tests have the US Food and Drug Administration (FDA) approval: in April 2003, Hybrid Capture 2 (HC2) High-Risk DNA Test (Qiagen, Gaithersburg, MD, USA) and in April 2009, Cervista™ HPV HR, (Hologic, Inc, Marlborough, MA, USA). Both techniques detect concurrently 13 HPV genotypes (HPV-16, -18, -31, -33, -45, -51, -52, -56, -58, -59 and -68); Cervista™ HPV HR test further includes HPV-66. HC2 is an *in vitro* nucleic acid hybridization assay with signal-amplification and Cervista is based on the Invader Chemistry®, which detects specific nucleic acid sequences using two isothermal reactions simultaneously. Other commercially available tests for pooled detection of HR-HPV genotypes are Amplicor HPV test (Roche Molecular Systems, Inc, Pleasanton, CA, USA), based on PCR amplification and Care HPV Test (Qiagen) based on hybrid capture technology detecting the same 13 HPV types included in HC2 plus HPV-66.

Given the clinical relevance of HPV-16 and HPV-18, some HPV screening assays include the specific identification of these types, and are collectively referred to

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as HR-HPV DNA-based screening assays with concurrent or reflex HPV-16 and HPV-18 genotyping.

CHARACTERISTICS OF COMMERCIAL TESTS FOR HR-HPV GENOTYPING

HPV genotyping tests are used in studies of HPV-type specific prevalence among the population, in the evaluation of vaccines, and in the implementation and monitoring of vaccination programs. Specific HPV-16 and HPV-18 identification is, in addition, recommended for screening programs, as mentioned above. Since a proper quality assurance is required in all cases, the WHO HPV Laboratory Network (LabNet) agreed and implemented international proficiency studies for monitoring the performance of assays for HPV DNA detection and typing [17, 18]. Approval of any of these assays for clinical use is achieved only after careful consideration of the results obtained in validation studies, which can eventually record an acceptable performance in terms of sensitivity and specificity for detecting clinically relevant disease (CIN 2) [19].

A large number of assays for HPV genotyping have been developed in the last years, and some of them have been commercialised and introduced in clinical and research laboratories. Full or partial automation is offered by some assays. The analytical sensitivities of theses assays for each HPV type have been shown very diverse [20-22]. Several studies have been published comparing HR-HPV-DNA based screening with concurrent and reflex HPV-16 and HPV-18 genotyping and DNA based genotyping assays in the last years [23-26]. Relevant issues such as the sample size, the population studied, the procedure used for specimen collection, or the method used for DNA extraction must be taken into account for an appropriate comparison of the results obtained in these studies.

The performance of automated methods for HPV genotyping is an issue of special interest, since automation simplifies the testing procedure, increases the sample processing capability, minimises the human errors, facilitates the quality assurance and reduces the cost. In addition, screening tests with reflex or concurrent HPV-16 and HPV-18 identification are also of consideration.

Table **1** shows a list of the main assays for HPV genotyping or HPV-16 and HPV-18 identification currently available. The technologies used by these tests include hybrid capture, Invader® chemistry, real time PCR, PCR-reverse hybridisation and PCR-microarray.

HR-HPV Screening Methods with Reflex or Concurrent HPV-16 and HPV-18 Identification

Four HR-HPV screening methods with reflex or concurrent HPV-16 and HPV-18 identification are commerc-ialised at present, two of them under the US FDA approval: Cobas HPV (Roche Molecular Systems) and Cervista HPV-16/18 (Hologic). The other two are the RealTime High Risk HPV test (Abbott Molecular, Des Plaines, IL, USA) and the HR-HPV 16/18/45 Probe Set Test (Qiagen).

Cobas 4800 HPV Test (Roche Molecular Systems)

Cobas 4800 HPV is a multiplex assay based on the real time PCR technology, intended to identify HPV-16 and HPV-18 with concurrent detection of twelve other HPV types (HPV-31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68). It achieved the US FDA approval in April 2011 for use in women aged 30 years and over, or women aged 21 years and older with borderline cellular cytology results, in order to evaluate the need of performing additional diagnostic procedures or follow-up. The PCR amplification is achieved with specific primers and probes for the 14 HPV types and for the endogenous human gene for *beta globin*, used for assessing sample celularity, DNA extraction and absence of PCR inhibitors (internal control). Four different fluorescent dyes are used for the detection of the PCR products: one for the *beta globin* gene, one for HPV-16, one for HPV-18 and one for the remaining twelve HPV types. Samples collected in Cobas® PCR Cell Collection Media (Roche Molecular Systems), ThinPrep®PreservCyt® Solution (Hologic) and SurePath® Preservative Fluid (not approved in the US) (BD Diagnostics, Burlington, NC, USA) are valid for use in this assay. The analytical sensitivity reported by the manufacturer is genotype dependent and ranges from 80 to 2,400 copies of the target per ml of sample.

The test is fully automated by the Cobas system, which is composed of two different instruments: the Cobas x 480 instrument, for the extraction of DNA and the preparation of the PCR mixture; and the Cobas z 480 analyzer, which performs the PCR reaction. Recording and interpretation of results is done by the Cobas 4800 System software.

The Cobas 4800 system can process up to 280 samples in one day and can be connected to a laboratory network system. The only manual step that the operator should perform is the loading of the PCR microwell plate in the Cobas z 480 analyzer.

Cervista HPV 16/18 Test (Hologic)

This test uses the patented Invader® chemistry and the Invader Reporter™ software to detect HPV-16 and HPV-18 DNA, and enjoys the US FDA approval since 2009 with two indications: Identification of HPV-16 and HPV-18 in women aged 30 and over (adjunctively with Cervista™ HPV HR and in combination with cervical cytology) and in women with ASCUS result in screening. Cervical samples collected in ThinPrep®PreservCyt® Solution (Hologic) are valid for this assay. The analytical sensitivity reported by the manufacturer ranges from 625 to 1,250 copies per reaction for both types.

The assay can be performed either manually or fully automated using the Sample Tranfer System (STS) (Thinprep®5000) and the Medium Throughput Automation (MTA) (Cervista). The STS automatically transfers the samples to the 96 well microplate and allows the processing of 96 samples in two hours. The MTA platform performs automatically DNA extraction, preparation of the Cervista test, incubation, detection, and analysis of results. The processing capability of the automated procedure is 24, 48 or 96 samples per run.

RealTime High Risk HPV Test (Abbott Molecular)

The Abbott RealTime High Risk (HR) HPV test is an assay based on the real time PCR technology that achieves pooled detection of twelve HR-HPV genotypes (HPV-31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68) with concurrent HPV-16 and HPV-18 detection. Amplification is

Table 1. Characteristics and Analytical Sensitivity of the Main HR-HPV Screening Assays with Reflex or Concurrent HPV-16 and HPV-18 Genotyping and HPV Genotyping Assays

HPV Assay	Technology	Automation	Analytical Sensitivity
HR-HPV screening assays with HPV-16/18 genotyping			
Cobas 4800 HPV Test (Roche Molecular Systems) */**	Real time PCR	Fully automated high-throughput **(COBAS 4800)**	80-2,400 copies/ml
Cervista HPV 16/18 Test (Hologic) */**	Invader® chemistry	Automation available **(Cervista Medium throughput automation - MTA)**	625-1,250 copies/reaction
RealTime High Risk HPV test (Abbott Molecular)*	Real time PCR	Fully automated high-throughput **(m2000sp + m2000rt)**	500-5,000 copies/assay
HR-HPV 16/18/45 Probe Set Test (Qiagen)*	Hybrid Capture	Automation available **(Rapid Capture System-RCS)**	5,000 copies/assay
HPV genotyping assays			
INNO-LiPA HPV Genotyping Extra (Innogenetics) *	Reverse hybridisation	Partially automated ▪ DNA extraction ▪ Hybridization/Detection ▪ Interpretation	20-70 copies/assay
Linear Array HPV Genotyping Test (Roche) *	Reverse hybridisation	Partially automated ▪ DNA extraction ▪ Hybridization/Detection	50-8,000 copies/ml
PapilloCheck HPV-Screening Test (Greiner Bio-One) *	PCR-microarray	Partially automated ▪ DNA extraction ▪ Interpretation	30-750 copies/reaction
Clart HPV 2 (Genomica) *	PCR-microarray	Partially automated ▪ DNA extraction: ▪ Detection/Interpretation	10-100 copies/reaction
Infiniti HPV Genotyping assay (AutoGenomics) *	PCR-microarray	Fully automated: Infiniti analyzer Partially automated ▪ Detection/Interpretation	300-3,000/assay

*Conformité Européenne (CE marked); **U.S Food and Drug Administration approval.

performed using five different primers (designed in the *L1* HPV genomic region) and single stranded DNA probes. Endogenous human *beta globin* gene fragment detection is used as internal control. Cervical samples collected in ThinPrep®PreservCyt® Solution (Hologic), Surephath (BD Diagnostics) or Abbott Cervi-Collect (Abbott Molecular) can be tested by this assay. The analytical sensitivity reported by the manufacturer is 500 copies per assay for HPV types -16, -18, -35, -39, -45, -51, -59, -66 and -68; 2,000 copies per assay for HPV types -31, -33, -52 and -56; and 5,000 copies per assay for HPV-58.

Testing is performed automatically by the Abbott Systems m24sp or m2000sp for DNA extraction, and by the Abbott m2000rt device for amplification, detection and analysis of results, with hands-on time less than 15 minutes. The processing capability is 24, 48 or 96 samples per run. The total processing time for each run depends on the number of samples tested, because of the variable time consumed by DNA extraction and reagent preparation. Ninety-six samples can be tested in less than six hours using m2000sp in combination with m2000rt.

HR-HPV 16/18/45 Probe Set Test (Qiagen)

HR-HPV 16/18/45 Probe Set Test is a signal amplification assay based on the hybrid capture technology using a probe mix comprising short sequence-specific synthetic probes designed for HPV-16, -18 and -45 detection. The analytical sensitivity of the assay is 5,000 copies per reaction.

The sample processing is the same as that for the HC2 test, where DNA-RNA hybrids are captured in a solid phase (microtiter well) and are detected by specific antibodies conjugated with alkaline phosphatase, which generate quimioluminiscence once the substrate is added. The test performs like the HC2 test for the three HPV types targeted [27], and the manual steps are kept to a minimum using the Rapid Capture® System (RCS), since hybridisation, capture and signal amplification steps are achieved automatically. The processing capability of the automated procedure is 352 samples per run.

HPV Genotyping Tests

Several methods for HPV genotyping are at present available for testing clinical samples and five of the most

frequently used can be partially automated. Table **2** shows the HPV genotypes identified by them:

INNO-LiPA HPV Genotyping Extra (Innogenetics, Gent, Belgium)

This test is based on reverse line blot hybridisation, and was designed for the identification of 28 different HPV genotypes: 17 HR and probably carcinogenic HPV types (HPV-16, -18, -26, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68 and -82); seven low risk (LR)-HPV types (HPV-6, -11, -40, -43, -44, -54 and -70); and four HPV types of unknown oncogenic risk (HPV-69, -71, -73 and -74) [7]. Amplification of the human *major histocompatibility complex, class II, DP beta 1* (*HLA-DPB1)* gene is used to monitor the sample quality and the efficiency of the DNA extraction.

The assay has been validated for samples collected in Surepath® medium (BD Diagnostics). The DNA extraction is not part of the INNO-LiPA HPV Genotyping Extra, and the manufacturer recommends proteinase K treatment as the method of choice. Standard protocols for cervical cell sampling in collection media as ThinPrep®PreservCyt® Solution (Hologic), combined with HPV DNA extraction by commercially available kits, can be used, but require in-house validation. Automatic DNA extractors as Magnapure (Roche Molecular Systems), BioRobot M48 or EZ1 workstation (Qiagen), or m2000sp (Abbott Molecular) allow partial automation.

The limit of detection ranges between 20 and 70 copies per reaction depending on the genotype tested. Genotype inclusivity testing showed that all genotypes were detected with a threshold limit of 1,000 copies per PCR reaction except for HPV-59, which required 10,000 copies.

Hybridisation of the amplified PCR products with HPV-specific probes immobilized in the strip can be performed automatically by the Auto-LIPA 48 device (Innogenetics), which achieves also the detection step. The interpretation of results can also be automated using LIRAS for LIPA HPV software.

Linear Array HPV Genotyping Test (Roche Molecular Systems)

The Linear Array HPV Genotyping Test is an assay based on reverse line blot hybridisation registered in the European Union for the detection of 37 HPV genotypes, including HPV-6, -11, -16, -18, -26, -31, -33, -35, -39, -40, -42, -45, -51, -52, -53, -54, -55, -56, -58, -59, -61, -62, -64, -66, -67, -68, -69, -70, -71, -72, -73 (MM9), -81, -82 (MM4), -83 (MM7), -84 (MM8), IS39, and CP6108.

A pool of primers designed in the HPV *L1* genomic region are used for PCR amplification. Amplification of an endogenous human *beta globin* gene sequence is used as internal control. The test has been validated for use in cervical samples collected in Cobas® PCR Cell Collection Media (Roche Molecular Systems) or ThinPrep® PreservCyt® Solution (Hologic).

The limit of detection reported by the manufacturer for 18 of the 37 HPV genotypes detected (HPV-6, -16, -18, -26, -31, -33, -35, -39, -45, -51, -53, -56, -58, -59, -66, -68, -73 and -82) ranges from 53 to 8,089 copies per ml of sample.

The method recommended by the manufactured for DNA extraction, Amplilute Liquid Media Extraction Kit (Roche Molecular Systems), is a manual method, but the step can be performed by other methods after in-house validation. Automatic DNA extractors as Magnapure (Roche Molecular Systems), BioRobot M48 or EZ1 workstation (Qiagen), m2000sp (Abbott Molecular) allow partial automation. As for the INNO-LiPA HPV Genotyping Extra, the hybridisation and detection steps can be automated using Auto-LIPA 48 (Innogenetics) and ProfiBlot™ T48 (Tecan Group, Mannedorf, Switzerland).

PapilloCheck® Test Kit (Greiner Bio-One GmbH, Frickenhausen, Germany)

PapilloCheck® Test Kit is a PCR-based microarray assay intended to use for the qualitative detection and identification of 24 HPV genotypes in DNA preparations from cervical smears. The method detects 16 HR or probably carcinogenic HPV types (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68 and -82), seven LR-HPV types (HPV-6, -11, -40, -42, -43, -44 and -70), and two types of uncertain oncogenic risk (HPV-55 and HPV-73) [7]. HPV types -44 and -55 are detected simultaneously and cannot be discriminated.

Amplification of a fragment of the *E1* HPV gene with specific primers is followed by hybridisation with specific DNA probes fixed on Papillocheck® DNA chip. Scanning of the chip is performed by the CheckScanner™ device. Amplification and detection of a human *Adenosine deaminase tRNA-specific 1 (ADAT1)* gene sequence is included as internal control. DNA extracted from cervical smears collected in PapilloCheck® collection medium, (Greiner Bio-One) or ThinPrep® PreservCyt® Solution (Hologic) is suitable for testing.

The limit of detection of the assay ranges from 30 to 750 copies per reaction and depends on the genotype.

Only DNA extraction and interpretation of results can be automated. BioRobot M48 or EZ1 workstation (Qiagen), using MagAttractDNAMini M48 Kit and EZ1 DNA Tissue Kit, respectively, or NucliSENS easyMAG (bioMérieux Marcy l'Etoile, France) can be used for DNA extraction, as declared by the manufacturer. The CheckReport™ software records the scanning of the chip and performs the evaluation and analysis of results.

Clart® Papillomavirus Humano 2 (Genomica, Madrid, Spain)

Clart® Papillomavirus Humano 2 is a PCR-based microarray assay which detects 35 HPV types, 17 HR or probably carcinogenic HPV types (HPV-16, -18, -26, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68 and -82), twelve LR-HPV types (HPV-6, -11, -40, -42, -43, -44, -54, -61, -70, -72, -81 and -89), and six of undetermined oncogenic risk HPV types (HPV-62, -71, -73, -83, -84 and -85) [7]. The amplification of a 450 pb fragment of the *L1* HPV gene is followed by the detection using the low density microarray technology CLART® (Clinical Array Technology). Amplification of a sequence from the *cystic fibrosis transmembrane conductance regulator* (*CFRT)* human gene is used as internal control. Testing can be performed on samples collected with swabs and ThinPrep®

Table 2. HPV Genotypes Detected by HR-HPV Screening Tests with Reflex or Concurrent HPV-16 and HPV-18 Genotyping and by Tests Intended for HPV Genotyping

Oncogenic Risk[a]	HPV TYPES																
HR/Probably Carcinogenic	**16**	**18**	**26**	**31**	**33**	**35**	**39**	**45**	**51**	**52**	**53**	**56**	**58**	**59**	**66**	**68**	**82**
Cobas 4800 HPV Test	X	X		X	X	X	X	X	X	X		X	X	X	X	X	
Cervista HPV 16/18 Test	X	X															
RealTime High Risk HPV test	X	X		X	X	X	X	X	X	X		X	X	X	X	X	
HR-HPV 16/18/45 Probe Set Test	X	X						X									
INNO-LiPA HPV Genotyping Extra	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Linear Array HPV Genotyping Test	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PapilloCheck® Test Kit	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clart® Papillomavirus Humano 2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Infiniti HPV QUAD	X	X		X	X	X	X	X	X	X		X	X	X		X	
Infiniti HR-HPV QUAD	X	X		X	X	X	X	X	X	X		X	X	X	X	X	
Infiniti HPV genotyping	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
LR	**6**	**11**	**13**	**40**	**42**	**43**	**44**	**54**	**61**	**70**	**72**	**81**	**89**				
Cobas 4800 HPV Test																	
Cervista HPV 16/18 Test																	
RealTime High Risk HPV test																	
HR-HPV 16/18/45 Probe Set Test																	
INNO-LiPA HPV Genotyping Extra	X	X		X		X	X	X		X							
Linear Array HPV Genotyping Test	X	X		X	X			X	X	X	X	X					
PapilloCheck® Test Kit	X	X		X	X	X	X			X							
Clart® Papillomavirus Humano 2	X	X		X	X	X	X	X	X	X	X	X	X				
Infiniti HPV QUAD	X	X															
Infiniti HR-HPV QUAD																	
Infiniti HPV genotyping	X	X								X							
Undetermined Risk	**30**	**34**	**55**	**62**	**64**	**67**	**69**	**71**	**73**	**74**	**83**	**84**	**85**				
Cobas 4800 HPV Test																	
Cervista HPV 16/18 Test																	
RealTime High Risk HPV test																	
HR-HPV 16/18/45 Probe Set Test																	
INNO-LiPA HPV Genotyping Extra							X	X	X	X							
Linear Array HPV Genotyping Test			X	X	X	X	X	X	X		X	X					
PapilloCheck® Test Kit			X						X								
Clart® Papillomavirus Humano 2				X				X	X		X	X	X				
Infiniti HPV QUAD																	
Infiniti HR-HPV QUAD																	
Infiniti HPV genotyping	X	X				X	X		X				X				

[a]Oncogenic risk classification of HPV genotypes was establish according to 2009 IARC Monograph Working Group [7].

PreservCyt® Solution (Hologic). Analytical sensitivity ranges from 10 to 100 copies, depending of the genotype.

DNA extraction can be performed automatically by NucliSENS easyMAG (bioMérieux) and BioSprint 96 (Qiagen), which were both validated for use in this test. Recording and interpretation of results can also be automated by the Clinical Array Reader (CAR) and the Clinical Array Processor (CAP) (Genomica).

Infiniti HPV Genotyping Assay (AutoGenomics Inc, California, USA)

There are three different Infiniti HPV genotyping assays for research use, all of them based in PCR–microarray. Automatic hybridization and detection of a 300 bp fragment amplified from the HPV *E1* gene is achieved on fill-based microarrays (BioFilmChip). Infiniti HPV QUAD identifies 13 HPV types individually (HPV-16, -18, -31, -33, -45) or in

combination (-35/-68, -39/-56, -58/-52, -59/-51), as well as two LR-HPV types pooled (HPV-6/-11). Infiniti HR-HPV QUAD detects 14 HR or probably carcinogenic HPV types (HPV-16, -18, -31, -33, -39, -45, -51, -52, -56, -58, -59, -66 and -68). Finally, Infiniti HPV Genotyping identifies 26 HPV types: 17 HR or probably carcinogenic HPV types (HPV-16, -18, -26, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68 and -82), three LR-HPV types (HPV-6, -11 and -70), and six of undetermined oncogenic risk (HPV-30, -34, -67, -69, -73 and -85). Endogenous human *beta globin* gene fragment amplification is used as internal control. Analytical sensitivity reported for Infiniti tests ranges from 300 copies per reaction for HPV-16 to 3,000 copies for HPV-18 [28].

Samples collected in Surepath® medium (BD Diagnostics) and ThinPrep® PreservCyt® Solution (Hologic) have been validated for use in these tests. Assays perform fully automated using the Infiniti™ Analyser, a multiplexing microarray platform.

AUTHORS' RECOMMENDATIONS

- Currently, a wide variety of commercial HPV genotyping methods exist. Selection of the more appropriate one depends on the intended use, i.e.: epidemiology, vaccine evaluation, or clinical studies.
  - For epidemiological studies, HPV genotyping methods allow drawing of type specific prevalence.
  - For vaccine evaluation, these assays provide data in regard to changes in prevalence for HPV types not included in the current vaccines, and facilitate the follow up of persistent infections.
  - For clinical studies, the current international guidelines recommend the use of HPV genotyping tests among women 30 years and older with negative cytology and a HR HPV positive results, in special HPV-16 and HPV-18. Identification of less-carcinogenic HPV types would be of minor interest.
- HPV genotyping methods displaying high analytical sensitivity are especially suitable for epidemiological studies, for evaluating vaccine efficacy and for detecting multiple HPV infections. Methods of lesser analytical sensitivity increase the positive predictive value in screening programs for cervical cancer, and are suitable in follow-up of treated patients.
- Even commercial methods already validated by the manufacturers must be performed with strict standardisation and with observation of quality assurance criteria. Clinical validation is an absolute requirement for using any test in clinical studies.
- Fully automated methods detecting HR HPV types pooled with concurrent or reflex HPV-16 and HPV-18 genotyping may present advantages for routine clinical practice.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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Declared none.

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