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Editorial
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Quality assessment for human papillomavirus testing

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Abstract. There are over 30 commercial, as well as numerous in-house assays, available for human papillomavirus testing. Laboratories performing such assays would need to assess accuracy and reproducibility of their results by incorporating ongoing internal control as well as participating in external quality-assurance schemes (EQAS) as part of their quality assurance program. Several EQAS are available and participation in which is a requirement for laboratories engaged in HPV testing. It is important that laboratories select the appropriate panels for detection of targeted types covered by assay used. Failure to do so can possibly alter patient management and increase the cost of treatment.

Additional keywords: assays, external quality-assurance schemes.

Detection of human papillomavirus (HPV) is becoming increasingly utilised in various clinical protocols. Proposed applications include: (i) monitoring women for evidence of persistent disease and/or test of cure; (ii) triage of women with minimally abnormal (borderline, equivocal, inconclusive) Pap smears to discriminate those truly HPV related and requiring follow up; and (iii) primary screening using HPV DNA alone in particular in women more than 30 years old. With increase clinical demand for HPV testing, more laboratories are requested to perform HPV detection as part of their routine diagnostic regiment.

The lack of ability to utilise conventional culture methods, has directed all HPV detection, based on molecular biological techniques. Furthermore, assignment of HPV genotypes is done by comparison of HPV genomes, particularly the L1 gene. Overall HPV has been shown to be highly heterogenous with over 100 genotypes fully sequenced and identified to date, with many more likely in the near future.[1] Biologically, HPV types are divided into two groups: cutaneous and mucosal. A subset of ~40 genotypes appears to regularly infect the genital and mucosal epithelium, with the remaining infecting cutaneous areas causing skin warts and lesions. Genital mucosal HPV types such as 6, 11, 42, 43, 44, 53, 54 and 55 are mainly found in low-grade cervical lesions with type 6 and 11 being the primary cause of genital warts. Genotypes such as 16, 18, 31, 33, 35, 38, 45, 51, 52, 56, 58, 59 and 68 are found regularly in high-grade dysplasias or high-grade squamous intraepithelial lesions (HSIL) and cervical cancers, and are designated high-risk (HR) HPV types. Among these HR types, risk of cancer may be an order of magnitude higher for some genotypes, like HPV16 than for other HR HPV types.[2]

HPV detection methods include a wide array of commercial and in-house assays using either signal or target amplification methods. However, performance of these assays is subject to limitations. In-house assays requires, the combination of various processes such as initial sample processing, nucleic acid extraction, amplification and detection methodology as well as utilising different reagents and concentrations, which would virtually make all such assays performed in laboratories different. Commercial assays have better standardisation with superior inter and intra assay performance when compared with in-house assays. However, commercial assays can also be subject to variation mainly due to differences in performance of individual assay steps, in particular when manufacturers have not specified utilisation of specific equipment or pre-analytic step (i.e. sample processing or nucleic acid extraction). There are currently over 30 different commercial assays (Table 1) available with many more in development. Each of these assays has different sensitivity and specificity and would need to be utilised appropriately for the particular clinical algorithm. Several assays also have ability to identify a particular genotype present (Table 1). Although type-specific assays are mainly used in research and epidemiological projects, there are data suggesting detection of type specific 16 and 18 may have important clinical relevance in management of abnormal smears. Therefore, in addition to detection of HR genotypes, identification of these particular two genotypes has been incorporated in several assays (Table 1). Currently only two assays have Food and Drug Administration approval with others having limited accreditation such as Certified European *In-Vitro* Diagnostic marking for use in European countries.

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Table 1. List of commercial assay for human papillomavirus testing currently available
HR, high-risk; LR, low-risk

Assay	Company	Detects	Can identify human papillomavirus 16 and 18
Abbott RealTime High Risk HPV	Abbott	14 genotypes (HR)	Yes
ProDect Chip HPV Typing	Bcs Biotech S.P.A.	24 genotypes (HR and LR)	Yes
NucliSENS EasyQ HPV	Biomerieux	5 genotypes (HR)- mRNA	Yes
HPV-DNA Qualitative Assay	Catch by Gene	15 genotypes (HR)	No
REBA HPV-ID	M&D, Inc.	25 genotypes (HR and LR)	Yes
LCD Array HPV 3.5	Chipron	32 genotypes (HR and LR)	Yes
Genpoint Tm HPV Test	Dako-Oxoid	13 genotypes (HR)	Yes
PapType	Genera Biosystems	14 genotypes (HR)	Yes
AID HPV Screening Kit	GenoID	49 genotypes (HR and LR)	No
AID HPV Typing Kit	GenoID	14 genotypes (HR)	Yes
AID STD Assay	GenoID	14 genotypes (HR)	No
Reveal HPV Real-Time HPV Detection Kit	GenooID	19 genotypes (HR and LR)	No
Array Papillomavirus	Genomica	35 genotypes (HR and LR)	Yes
Aptima	Gen-Probe	14 genotypes (HR)- mRNA	Yes
Papillocheck	Greiner BioOne	24 genotypes (HR and LR)	Yes
Cervista HPV HR Test[A]	Hologic	14 genotypes (HR)	Yes
Linear ArrayExtra HPV Genotyping Kit	Innogenetics	28 genotypes (HR and LR)	Yes
HPV OncoTect	Invirion Diagnostics	HR E6 and E7 mRNA expression in cells	No
Genesquare- HPV	Kurabo Industries	23 genotypes (HR and LR)	Yes
BIOPAP QTS HPV Kit	Loxo	6 genotypes (HR)	Yes
fHPV typing	molGENTIX	15 genotypes (HR and LR)	Yes
Luminex HPV Genotyping	Multimetrix/Progen	24 genotypes (HR and LR)	Yes
Care HPV	Qiagen	14 genotypes (HR)	No
Consensus HPV Typing Kit	Qiagen	18 genotypes (HR and LR)	Yes
Hybrid Capture 2[A]	Qiagen	13 genotypes (HR)	No
Luminex HPV Assay	Qiagen	17 genotypes (HR and LR)	Yes
Amplicor HPV Test	Roche	13 genotypes (HR)	No
COBAS 4800 HPV Test	Roche	14 genotypes (HR)	Yes
Linear Array HPV Genotyping Test	Roche	37 genotypes (HR and LR)	Yes
Seeplex HPV Genotyping	Seegene	20 genotypes (HR and LR)	Yes
PCR Human Papillomavirus Detection Set	Takara Mirus Bio	7 genotypes (HR and LR)	Yes
Viroactiv	Virofem	8 genotypes (HR)	No

[A]Food and Drug Administration approved.

Assessment of the operating characteristics of the test utilised by each laboratory is a regulatory guideline and requirement for conducting laboratory testing across many countries. Quality assurance program is essential to ensure quality of test results is maintained. Such programs incorporate several elements, including regular audits, equipment monitoring, incorporation of quality control (QC) (internal QC and kit controls) as well as quality assessment. An important mechanism for quality assessment is verification of assay performance by participation in external quality assessment schemes. This will ensure accurate reporting of patient results, and would allow laboratories to compare their performance to other laboratories. These panels are generally distributed by reference laboratories and comprise of clinical or spiked samples to assess proficiency of a wide range of techniques and assays performed. Most available panels would measure proficiency of detection of HR genotypes and may not cover measurement of accuracy of HPV genotyping. Programs such as those established in USA through College of American Pathologist (CAP), Czech Republic,[3] UK[4] and recently in Australia through Royal College of Pathologist of Australia measure proficiency of detection of HR genotypes and can be utilised by laboratories involved with assays which HPV testing.

Recently, WHO HPV Laboratory Network, have distributed proficiency panels for primarily assessment of accuracy of HPV genotyping to reference laboratories with further panels becoming available to all laboratories in the near future. Furthermore, WHO have also established the first international standards for HPV, in particular HPV 16 and 18 DNA for use in the amplification and detection steps of the nucleic acid-based assays.[5]

As the demand for HPV testing increases and laboratories are utilising varied assays, participation in quality assessment programs is of outmost importance in order to improve the overall quality and reliability of HPV testing in the respective laboratories and result in enhanced roles of laboratory in better patient management.

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