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Cervical Cancer Screening at a Crossroads: Learnings from the Past Driving Change for the Future

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Abstract

Cervical cancer screening has been one of the most impactful human interventions in medical history, saving the lives of countless thousands of women since the introduction of organized cytology screening programs. Today, we stand at a crossroads in the fight against cervical cancer, with several countries actively engaged in introducing primary human papillomavirus (HPV) testing and vaccination as more effective means of prevention. This chapter discusses the history of organized screening and how this led to HPV test methods to detect cervical cancer. We go on to examine the technologies used to screen for high-risk HPV types and how they affect clinical performance. We examine the evidence for primary HPV screening and review recent self-collection initiatives to reach underserved women, including the use of urine as novel sample type. In addition, we critically examine the evolution of HPV test methods and make the case for the use of extended genotyping as an improved risk stratification tool for guiding clinical management. Finally, we look to the future of cervical cancer screening and consider options for future management programs.

Keywords: genotyping, HPV screening tests/strategies, Pap, risk stratification, self-sampling

1. Introduction

Cervical cancer screening has advanced considerably since the introduction of the Pap smear in the 1960s: organized cytology screening programs have successfully reduced the burden of disease associated with human papillomavirus (HPV) and sensitive new molecular methods have been developed to detect the virus. Perhaps more importantly, safe and efficacious HPV vaccines are now widely available and offer the prospect of greatly reducing the incidence of cervical cancer if sufficient numbers of the target population can be vaccinated. Vaccines offer the best hope for developing countries, which lack the resources to implement an effective screening program. Despite the introduction of vaccination, there is still a need for improved screening modalities as both screening and vaccination will necessarily coexist for some decades to come. It is against this backdrop that we briefly review the history of the Pap smear and the development of organized cytology screening programs and then go on to discuss the development of molecular methods. We discuss the pros and cons of the different molecular assay design approaches and review the case for primary HPV screening. Despite the effective tools at our disposal, reaching underserved women remains the biggest challenge to controlling cervical disease. However, new self-sampling methods offer the prospect of an effective outreach program to reach women most in need. Finally, we discuss the benefits of extended genotyping and how this might influence future screening algorithms. We conclude that the biggest hurdle to preventing and detecting cervical disease may lie in our inability to adapt to change and effectively implement new strategies. This history of cervical cancer screening suggests that the pace of change is slow but we must respond more quickly to the global threat posed by cervical cancer and make efficient use of the tools at our disposal.

2. The history of organized screening programs

George Nicholas Papanicolaou is almost single-handedly credited with developing cervical cancer screening. He was encouraged to leave his native Greece by the renowned geneticist, Thomas Hunt Morgan, who helped him gain a position in the Department of Pathology and Bacteriology at New York Hospital as an assistant, and later at the Department of Anatomy at Cornell Medical School. Dr. Papanicolaou began to study vaginal secretions in women, beginning with a case study of his wife, Mary [1]. Later work led to the observation of cancer cells while studying smears of women in the New York Hospital, which led him to propose that a systematic study of smears could lead to early cancer detection. His “New Cancer Diagnosis” theory was initially very poorly received by his peers [2]. It was not until over 10 years later that a successful collaboration with Herbert Traut (1894–1963), a renowned gynecologist and pathologist at Cornell University, resulted in a more widely received manuscript “The diagnostic value of vaginal smears in carcinoma of the uterus.” This work was presented to the New York Gynecological Society in 1941 and published in the prestigious *American Journal of Obstetrics and Gynecology* [3]. In 1947, Dr. Papanicolaou and the head of the Anatomy Department, Dr. Joseph Hinsey, began focusing on training other physicians on the new technique. This led to a gradual adoption of the screening method, and by 1960 it was

being adopted across the United States. However, it was not until just before Papanicolaou's death that the impact of his discovery was becoming apparent and he finally began to get the recognition he deserved. Today, he is remembered as "the father of exfoliative cytology" and cervical cytology stands as one of the most successful clinical interventions of the twentieth century. Organized cervical cancer screening has led to an over two-fold reduction in the incidence of cervical cancer in the United States in the period 1975–2012. This success in the United States has been replicated in other countries with organized screening programs such as the United Kingdom, which has seen a similar reduction in mortality from 1985 to 2012. However, over the last decade it has become evident that cytology screening may have reached the limits of its effectiveness in terms of reducing the incidence rate, with both the US and UK trend lines reaching a plateau (**Figure 1**).

Figure 1. (A) US age-standardized incidence and mortality rates per 100,000 women (1975–2012). Source: <http://seer.cancer.gov/statfacts/html/cervix.html> (accessed January 2016). (B) United Kingdom age-standardized incidence rates per 100,000 women (1979–2012). Source: Cancer Research UK, <http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/cervical-cancer/incidence#heading=Two> (accessed January 2016).

3. The development of molecular screening tools

The development of molecular biology techniques in the late 1970s and early 1980s paved the way for the discovery that the HPV was both necessary and responsible for cervical disease. zur Hausen played a pivotal role in establishing the link between papillomaviruses and cervical cancer [4]. As interest intensified, novel HPV types began to be isolated from genital warts (HPV6 [5–7] and laryngeal papillomas (HPV11) [8]). The detection of additional type-specific viral sequences followed: [9, 10], HPV16 [11], HPV18 [12–14], and high-risk types HPV31 and HPV45, which were first described by Lorincz et al. [15] in the United States [16]. These discoveries laid the foundation for the development of cervical cancer diagnostic tests and the development of the first HPV cancer vaccines. Harald zur Hausen went on to win the Nobel Prize in Physiology or Medicine in 2008 for this work on the association of papillomaviruses with human cancers.

While the work of Harald zur Hausen and others set the stage for modern cervical cancer diagnostics, it took another wave of development to advance HPV detection to the point that it could be widely adopted in the clinical laboratory. The early work led to rapid development of basic laboratory tests to detect various HPV types. However, the techniques employed involved the use of ^{32}P -labeled probes that were hybridized using Southern blotting or slot blot methods, which meant they were labor intensive. These early methods (ViraPap and ViraType) were commercialized by Life Technologies Inc. and later sold to the Digene Corporation but they did not achieve widespread adoption, primarily because they did not detect all oncogenic HPV types and also lacked sensitivity. This period also saw the development of *in situ* hybridization (ISH) techniques by Enzo Diagnostics and Life Technologies Inc., and additional methods were later automated by Ventana Medical Systems (now a Roche Company), the Dako Corporation, and others [17]. These tests also suffered from lack of sensitivity [18] and were ultimately not adopted as screening tools. However, ISH methods may prove to be useful in assisting in the diagnosis of Cervical Intraepithelial Neoplasia cases (CIN) [19].

4. The digenehybrid capture 2 assay

The Digene Corporation (now part of QIAGEN) under the scientific leadership of another early HPV molecular pioneer, Dr. Attila Lorincz [15, 16], developed the modern market for HPV cervical cancer screening. Lorincz et al. developed a technology called hybrid capture that fused traditional immunoassay techniques with the newer nucleic acid isolation technology [20]. The core principle leveraged the fact that DNA–RNA hybrids have a distinct shape different than a DNA helix that is detectable using antibodies. By generating *in vitro* RNA transcripts of each of the target HPV types and pooling them into a single probe cocktail, the method was capable of detecting as many as 13 high-risk HPV types at once. The capture antibody is bound to a solid surface that binds cognate target hybrids which in turn are then detected using a secondary antibody coupled with alkaline phosphatase using a chemiluminescent substrate. The first generation hybrid capture assay utilized two mixtures of single-stranded RNA probes: one for low-risk HPV types 6, 11, 42, 43, and 44, and one for high-risk

HPV types 16, 18, 31, 33, 35, 45, 51, 52, and 56. The analytic sensitivity of the first generation test was estimated at 50,000 copies of HPV16 DNA [21, 22], and this was not found to be sensitive enough versus polymerase chain reaction (PCR) and histology [23]. Digene researchers then launched a second generation assay (Hybrid Capture 2) whose analytical sensitivity was increased to 1000 HPV DNA copies by the reformulation of hybridization reagents and by the addition of new probes for four high-risk HPV types, 39, 58, 59, and 68. This assay was launched in Europe in 1998 and was subsequently approved by the US Food and Drug Administration (FDA) in 2003, for the triage of Atypical Squamous Cells of Undetermined Significance (ASC-US) cytology patients and as an adjunctive test with cytology for cervical cancer screening [23]. The assay went on to achieve worldwide acceptance and became the *de facto* clinical standard for all subsequent assay development. It has the largest body of clinical evidence supporting its use, and it has been used in numerous clinical trials across the globe [24, 25].

5. PCR- and other amplification-based technologies

The development of PCR technology [26, 27] meant that small quantities of DNA or RNA could now be amplified and detected either directly in endpoint PCR [28] or through the use of accumulating fluorescence that could be monitored continuously in real-time (RT) PCR [29–31]. As with most new techniques, refinements and improvements overcame some of the early difficulties such as assay robustness and the potential for contamination/false positives [32, 33]. The use of PCR to detect HPVs mirrored that of other methods, with HPV initially being detected using gel analysis of PCR products [34] and subsequently using real-time methods [35, 36]. Type-specific primer amplification methods eventually gave way to consensus PCR approaches where multiple HPV types could be detected in one reaction [37]. Most PCR approaches targeted the L1 gene region, and included the MY09–MY11 (MY09/MY11) and GP51–GP61 (GP51/GP61) primer systems. The MY primers were later redesigned to improve performance (PGMY09/PGMY11 [38]) as were the GP primers, to GP5+/GP6+, which were subsequently widely adopted [39, 40]. PCR-based tests are intrinsically more analytically sensitive than Hybrid Capture 2 technology, detecting as little as 10–100 copies of target. However, the Hybrid Capture 2 assay established that approximately 1 pg/ml or 5000 copies of HPV16 target DNA per reaction equated to an actionable clinical result (CIN2+ histology) [41]. Therefore, assays that are clinically more sensitive than this would have poor specificity, referring low level infections unnecessarily to follow colposcopy and possible biopsy or treatment. This has been the subject of discussion in cervical cancer screening since the introduction of highly sensitive molecular methods. Key opinion leaders have cautioned against the use of analytically validated assays whose clinical performance has not been well established in large longitudinal studies, emphasizing the need to strike the proper balance between sensitivity and specificity [42]. In one case, even an FDA approved test was considered clinically unacceptable due to its high HPV positivity [43]. The issue is compounded by the fact that even large reference laboratories and smaller commercial companies cannot afford to fund large studies with a minimum of 3-year follow up of enrolled patients. This led a group

of international HPV experts to propose acceptance criteria for HPV tests that wish to be used in primary HPV screening [44]. These criteria, commonly known as “Meijer Criteria” after the lead author, were founded on the principle that “candidate high-risk HPV tests to be used for screening should reach an optimal balance between clinical sensitivity and specificity for detection of high-grade CIN and cervical cancer to minimize redundant or excessive follow-up procedures for high-risk HPV positive women without cervical lesions” and set forth the following guidelines for an acceptable HPV screening test:

1. The sensitivity of the candidate test for $\geq$ CIN2 should be at least 90% of the sensitivity of the HC2 (i.e., relative sensitivity of at least 90%) as assessed by a noninferiority score test.
2. The specificity of the candidate test for $\geq$ CIN2 should be at least 98% of the specificity of HC2.
3. The intralaboratory reproducibility in time and interlaboratory agreement should be determined by evaluation of at least 500 samples, 30% of which tested positive in a reference laboratory using a clinically validated assay. This should result in a percentage of agreement with a lower confidence bound not less than 87% (kappa value of at least 0.5 in this series of samples including 30% positives).

Meijer criteria have been well accepted as a critical litmus test in the absence of large-scale longitudinal trial data and have become a clinical benchmark for validation of tests, especially in the European Union. Despite the use of this leaner approach where the disease samples can be sourced retrospectively, only a small number of existing commercially available assays have actually met the criteria. A 2015 review of currently available commercial assays “identified 193 distinct commercial HPV tests” [45]. However, Arbyn et al. [46], also reported in the same year that only five commercially available tests fully met the Meijer criteria and could be considered suitable for use in primary HPV screening: PapilloCheck® HPV-Screening test; Abbott RealTime hrHPV test; cobas® 4800 HPV test; BD Onclarity™ HPV assay; HPV-Risk assay and the Aptima assay, targeting E6/E7 mRNA. The authors stated that the Cervista® assay should also be added to the list, despite one report of a lack of noninferiority for specificity (see also reference [104]). Three other assays met the sensitivity/specificity criteria but did not disclose accuracy and reproducibility data and were thus considered to partially meet the criteria (an in-house quantitative RT-PCR targeting E6/E7 DNA sequences, a GP5+/GP6+ PCR with Luminex identification of high-risk types and a MALDITOF assay). The authors also concluded that the Aptima assay, while fully meeting the criteria, needed further longitudinal validation of its long-term negative predictive value (NPV) because it was an RNA-based assay and to date this had only been established for DNA assays [46].

E6 and E7 gene RNA-based assays have been extensively tested in cervical cancer screening. The impetus for using RNA- versus a DNA-based targets, is likely based on the observation that both the E6 and E7 genes encode oncogenes that are involved in the development of cancer and are upregulated as disease progresses [47]. E6 and E7 viral oncoproteins bind and modulate cellular gene products (p53 and pRb) that play a key role in cell cycle control and DNA repair. The resulting genomic instability caused by E6 and E7 oncoproteins is a necessary condition for cell transformation and immortalization [48, 49]. Thus, it is reasonable to

postulate that an E6/E7 RNA target might offer an advantage over DNA in that it should be overexpressed in high- versus low-grade disease and that it might offer both a sensitivity and specificity advantage. There are two commercially based E6/E7 RNA assays: the Proofer Assay (Norchip, Klokkestua, Norway) is a real-time multiplex nucleic acid sequence-based amplification assay (NASBA) for isothermal amplification and detection of E6/E7 mRNA from five high-risk oncogenic types, HPV16, 18, 31, 33, and 45, using molecular beacon probes, and the Aptima® HPV Assay (Hologic—GenProbe) that is a qualitative nucleic acid amplification test that detects HPV E6/E7 mRNA from 14 high-risk HPV types [50]. The Aptima assay uses target amplification using transcription-mediated amplification (TMA) [51] and detection of the amplification products (amplicon) by the hybridization protection assay (HPA) [52]. HPV mRNA is captured on magnetic particles and then amplified using TMA that is a transcription-based nucleic acid amplification method that utilizes two enzymes, Moloney Murine Leukemia Virus (MMLV) reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy of the target mRNA sequence containing a promoter sequence for T7 RNA polymerase. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template. Detection of the amplicon is achieved by HPA using single-stranded nucleic acid probes with chemiluminescent labels that are complementary to the amplicon. The labeled nucleic acid probes hybridize specifically to the amplicon. The selection reagent differentiates between hybridized and unhybridized probes by inactivating the label on the unhybridized probes. During the detection step, light emitted from the labeled RNA–DNA hybrids is measured as photon signals called relative light units (RLU) in a luminometer. Final assay results are interpreted based on the analyte signal-to-cutoff (S/CO) [53]. The Norchip Proofer assay has been shown to be substantially less sensitive but more specific when compared to other clinically validated assays, including Roche cobas and Hybrid Capture 2 [54–56]. Arbyn et al. [57] performed a meta-analysis of the performance of the Aptima HPV assay versus Hybrid Capture 2 and found that for both triage of ASC-US and Low-Grade Squamous Intraepithelial Lesion (LSIL), Aptima is as sensitive but more specific than HC2 for detecting cervical precancer. In head to head tests, Aptima had a substantially higher sensitivity than Proofer in both a screening and a referral population, but Proofer showed improved specificity [55, 56]. The Aptima test has broader clinical application and relevance because it detects all 14 high-risk types versus just five high-risk types in the Proofer assay. However, as noted above, it needs further confirmation of its long-term NPV over at least a 5-year period, as recommended by Arbyn et al. [46]. A recent report with a 3-year longitudinal follow up found that the NPV is similar to that of Hybrid Capture 2 and again that its specificity was significantly better (96.3% compared with HC2 specificity of 94.8%; $P < 0.001$ [58]. A comprehensive review of the clinical performance of the assay versus HC2 also concluded that the NPV was sufficient to justify a 3-year interval and that the specificity of the assay was consistently higher [59]. The increase in specificity is similar to that previously reported [57] and is relatively modest. It could also result from a slight clinical cut-point bias toward the specificity axis of the receiver operating characteristic (ROC) plot of sensitivity versus 1–specificity for this assay. Whatever the driver, it seems clear that there is no significant difference in sensitivity when comparing RNA- and DNA-based assays and the specificity differences are not as high as one might have predicted from the upregulation of E6/E7 RNA

during oncogenesis. The latter may reflect that one does not see the full potential of upregulation when screening for less severe cellular abnormalities versus cancer. The answer may also lie in the observation that neither assay exclusively targets RNA: both assays have been reported to detect cognate DNA sequences present in endocervical specimens at levels where one might expect the DNA signal alone to be sufficient to record a positive clinical result [60, 61]. Thus, it is conceivable that this could render otherwise RNA-negative specimens positive, reducing the specificity of the assay.

6. How target regions impact assay performance

6.1. Which target region to choose?

HPV is double-stranded DNA virus whose circular genome is approximately 8000 base pairs long. It encodes eight open reading frames (ORFs) that are divided into early and late genes (**Figure 2**) involved in replication (i.e., E1 and E2) and packaging (i.e., L1 and L2) with the remaining genes (E6, E7, E5, and E4), playing roles in driving cell cycle entry, immune evasion, and virus release (reviewed in [49]). Papillomaviruses are ancient in origin, believed to have arisen in reptiles approximately 350 million years ago. They have evolved in their various host lineages (including humans) over the millennia and they have relatively stable genomes given their (redundant) double-stranded DNA structure. The rate of nucleotide substitution for HPV18 has been estimated at $\sim 4.5 \times 10^{-7}$ subs/site/year [62]. This means that assay developers can look across the entire genome for conserved target regions in which to design gene probes.

Figure 2. Physical map of the HPV16 genome.

Given the aforementioned large number of commercial assays available on the market, not surprisingly most ORFs have been targeted by one or more assays, including L1 [63], E1 [64], and E6/E7 [65, 66]. However, most commercial assays to date have been developed using the L1 gene as a target region [45, 63], including the Roche cobas assay that has received FDA approval [67].

The question one must then ask is: does it matter which region of the genome you use—are all genomic regions created equal? While a detailed phylogenetic and evolutionary analysis (beyond the scope of the current work) would be required to fully address this question, the following sections discuss some important topics with respect to target analyte performance in clinical diagnostics and how this can influence clinical results.

6.2. Cross-reactivity with nontarget HPV types

There is general agreement that only high-risk types cause cervical cancer and that low-risk types should not be screened for as a part of routine cervical cancer prevention [68]. Previously, there was consensus that 14 high-risk types caused the majority of cancers. However, more recently the evidence for HPV66 in cervical cancers was considered too weak to keep it in the group of 14 high-risk types and it was recommended to remove it [69]. However, all FDA tests approved to date detect HPV66 as part of their 14 high-risk panel (Roche cobas, Hologic-Genprobe Aptima, and Hologic Cervista) and the Hybrid Capture 2 assay detects it via cross-reactivity [70]. In addition, a number of possible/probably carcinogenic types (including HPV66) have been shown to have oncogenic potential and “are biologically active and affect the same cellular pathways as any of the fully recognized carcinogenic HR-HPV types” [71]. Thus, it is difficult to draw an absolute line between known carcinogenic types and those that have that potential but very infrequently result in cancers [69].

Whether you take the view that there are 14 high-risk types or 13 high-risk types (omitting HPV66), there is little doubt that screening for additional HPV types will not increase sensitivity for cancer detection and will reduce specificity. In addition, it may do harm to patients both psychologically and potentially physically if they are treated for lesions that will not result in cancer [68, 72]. Thus, it is important that clinically validated assays perform inclusivity and exclusivity studies to ensure that the assay detects only the intended HPV types for which they have claims. The Hybrid Capture 2 test has been shown to have excellent clinical sensitivity for CIN2+ endpoints and as described earlier, has been used as a clinical benchmark for the last decade. However, it does have a well-documented cross-reactivity with non-high-risk HPV types [70, 73, 74]. This results from the fact that the assay detects the entire HPV genome (**Figure 2**) and thus there is more potential for closely related nononcogenic sequences to be detected. One study found that “some 20% of HC2-positive samples did not contain the targeted HPV types. About two-thirds of them resulted from cross-hybridization, especially with HPV53, HPV66, and HPV70” [75]. When split sample testing is performed with HC2 and a second assay, one typically sees a larger proportion of HC2+/other assay–results which, if they are resolved by third-party genotyping or sequencing method, shows both low-risk and no HPV present results, confirming the negative result as truth with respect to high-risk HPV [66, 76]. In another recent study ($n = 6172$) comparing Roche cobas assay with Hybrid Capture

2, where discordants were resolved using the Roche Linear Array genotyping assay, the authors reported that “HC2+/COBAS– were less likely to contain hrHPV genotypes (12.3 versus 68.9%; $P < 0.0001$) and more likely to contain only lrHPV genotypes (52.8 versus 12.1%; $P < 0.0001$) than those HC2–/COBAS+” and they found “lower CIN2+ rates among women with HC2+/COBAS– results” [77].

The clinical impact of this cross-reactivity has not been widely discussed in the literature. Castle et al. used a more stringent dual reference assay method to determine the level of HC2 cross-reactivity and concluded that about 8% of all HPV positives were due to cross-reactivity with non-high-risk HPV types in the referred (ALTS Study) population. They concluded that cross-reactivity would likely be further reduced in a screening population where there is less overall infection. However, they cautioned that these non-high-risk HPV “cases of CIN2 might be treated by excisional procedures, which can cause iatrogenic morbidity and adversely affect reproductive outcomes” [70]. The previously cited study of Gillio-Tos et al. [75] concluded “If only the samples containing HC2-targeted types tested positive, the positive predictive value (PPV) would have increased from 7.0% (95% CI, 6.1–8.0%) to 8.4% (95% CI, 7.3–9.6%), although 4.9% (95% CI, 2.4–8.8%) of cervical intraepithelial neoplasia grade 2+ (CIN2+) cases would have been missed”. Regarding the latter point, “missing” CIN2+ disease caused by non-high-risk HPV types should be the goal of future assays since it has an extremely low risk of resulting in cancer. In summary, there is an 8–20% false-positivity associated with the Hybrid Capture 2 assay that unfortunately is correlated with bona fide abnormal cytology and histology (CIN2+). However, it is important to recognize that since these abnormalities are *not* caused by high-risk HPV types, they have a very low cancer risk and should be managed accordingly. While this phenomenon is well documented, it tends to be largely ignored and considered noise in the overall clinical performance of an assay. It does however have implications for benchmarking studies and in our view should also be taken into account in any future revisions of the Meijer criteria, since it has a direct impact on the maximum sensitivity and specificity of any assay which only detects high-risk HPV.

6.3. L1 consensus-based versus gene-specific PCR approaches

All molecular assays require some sort of amplification technology in order for them to have the required sensitivity to detect small quantities of target nucleic acid. This can take the form of signal amplification such as that described for the Hybrid Capture 2 assay (where the signal rather than the target is amplified using a chemiluminescent substrate), or in the case of the Hologic Cervista assay, where signal amplification is achieved through the use of a unique isothermal Invader® chemistry that leverages a reusable universal flap and fluorescence energy transfer (FRET) to amplify the signal [78, 79] or it can use RNA amplification (Proofer and Aptima assays described earlier) or PCR that accounts for the majority of HPV assays on the market [45]. Traditional PCR methods where the amplicon is detected in a secondary process such as on an agarose gel or via hybridization on strips have gradually been replaced by real-time detection methods for clinical use, although the Roche Linear Array and InnoLIPA line blot assays have remained popular where researchers are interested in determining which genotypes are present in clinical samples or for discordant result anal-

ysis [76, 80, 81]. With PCR one can choose to selectively amplify each viral gene target individually (gene-specific PCR) or utilize a consensus (broad spectrum) primer approach. The latter typically uses degenerate primer sequences to detect conserved protein coding regions of multiple HPV types [82]. This has the advantage that multiple (e.g., all high-risk) HPV types can be detected in a single reaction but it means that you can only record a pooled type result, unless you use a secondary method such as a line blot or bead approach to subsequently identify the specific genotypes present [83–85]. Gene-specific detection approaches offer the possibility of direct individual genotype detection but require the use of multiple reactions [86] or the combining of one or more genotypes in different fluorescent channels using real-time PCR technology [66, 87]. **Table 1** lists the major approaches to HPV DNA detection and details some of their advantages and disadvantages. Head to head testing suggests that the top performing assays have broadly similar clinical sensitivity and specificity [55, 56, 88, 89] so clinical laboratories have a choice in the assay they use. The decision on which assay to adopt may be made based on the needs of a particular laboratory. However, it is instructive to consider the following assay characteristics when choosing an HPV test:

Assay name	HPV target region	Nucleic acid type	Consensus or gene-specific	High-risk type coverage	Advantages	Disadvantages	References
QIAGEN Hybrid Capture 2	Entire genome	DNA(signal amplification)	Not applicable	13	Single reaction Clinically validated CE marked/FDA approved	No genotyping or internal control. High level of nontarget cross-reactivity	[41, 70, 75]
PreTect HPV-Proofer	E6/E7	RNA	Gene-specific	5	High specificity CE marked	Low sensitivity Does not detect all cancer-causing types	[55, 56]
Hologic Aptima (screening)	E6/E7	RNA	Gene-specific	14	Single reaction Clinically validated CE marked/FDA approved Fully automated	No genotyping. Sample processing control only	[53, 90]
Hologic Aptima (genotyping)	E6/E7	RNA	Gene-specific	3—HPV16, HPV18+45	Single reaction Clinically validated CE marked/FDA approved Fully automated	Additional test required for limited genotyping Sample processing control only	[91]

Assay name	HPV target region	Nucleic acid type	Consensus or gene-specific	High-risk type coverage	Advantages	Disadvantages	References
Hologic Cervista (screening)	E6/E7/L 1	DNA	Gene-specific	14	Single reaction Clinically validated. Internal control CE marked/FDA approved	No genotyping High positivity rate (clinical specificity has been questioned)	[43, 78]
Hologic Cervista (genotyping)	E6/E7/L 1	DNA	Gene-specific	2—HPV16, HPV18	Single reaction Clinically validated. Internal control CE marked/FDA approved	Additional test required for limited genotyping Limited genotyping	[79]
Abbott Real-time high-risk HPV	L1	DNA	Consensus	14	Single reaction that includes HPV16/18 genotyping. Internal control Semiautomated, CE marked	Limited genotyping	[92, 93]
Greiner Papillocheck	E1	DNA	Consensus (genotypes resolved using array	24— includes 14 high-risk Or 14 high-hybridization) risk only	Full genotyping CE marked	Labor-intensive workflow requiring three separate rooms	[64, 94]
Sonic Laboratories (formerly RIATOL) E6/E7 real-time PCR test	E6/E7	DNA	Gene-specific	14 + 3 moderate risk types	Full genotyping Partially validated per Meijer criteria Semiautomated	17 individual PCR reactions plus controls required Laboratory developed test (LDT)	[86]
BD Onclarity HPV Assay	E6/E7	DNA	Gene-specific	14	Extended genotyping HPV16, 18, 45, 31, 51, 52 Clinically validated Fully automated CE marked	Three-well assay design reduces throughput	[66, 95, 96]
Cepheid Xpert® HPV	E6/E7	DNA	Gene-specific	14	Single reaction that includes HPV16/18+45 genotyping. Internal control	Limited genotyping capability, Meijer criteria not yet established	[87]

Assay name	HPV target region	Nucleic acid type	Consensus or gene-specific	High-risk type coverage	Advantages	Disadvantages	References
Roche cobas assay	L1	DNA	Consensus	14	CE marked Single reaction that includes HPV16/HPV18 genotyping. Internal control. Clinically validated CE marked and FDA approved(cotesting and primary screening) Semiautomated	Limited genotyping capability	[72, 97]

Table 1. HPV detection methods.

1. Is the assay and required equipment cost competitive?
2. What level of automation is involved—manual processing, semiautomated, or a fully integrated workflow?
3. What is the time to result and the hands on time of laboratory personnel to run the assay?
4. Is the assay part of a complete offering or does the user have to procure third party equipment or reagents to complement the workflow?
5. Does the assay target only high-risk HPV types?
6. Does the assay provide the required level of genotyping information to assist with informed patient management and risk stratification?
7. Has the assay demonstrated the required ability to accurately diagnose both single and mixed infections?
8. Has the clinical performance of the assay been demonstrated in the intended target population using the specified collection devices and preservative media (sensitivity/specificity/longitudinal NPV and PPV)?
9. Have the required regulatory approvals and quality requirements for the product been fully met?
10. Has the clinical cutoff of the assay been tested in several different patient populations with different risk profiles to ensure that patients are neither being over-or underreferred for biopsy and treatment?

The first decision one may want to make is whether to choose an assay that targets RNA or DNA. Clinical performance is very similar so it may come down to a practical decision on workflow and laboratory suitability. At the current time, the long-term NPV of DNA-based assays is well established and enshrined in consensus guidelines whereas the evidence for

RNA assays is still accumulating [58, 59, 98]. DNA assays also have the advantage of improved target stability which imposes stricter adherence to laboratory cleaning methods to avoid degrading the more labile RNA targets [53]. As mentioned above, real-time PCR approaches now predominate in the market so one has the choice of either a consensus-based primer design or a gene-specific primer approach (**Table 1**). Consensus approaches yield a pooled high-risk result with HPV16/HPV18 genotype identification from a single well whereas gene-specific approaches can offer more extended genotyping information which can limit throughput. The clinical benefits of extended genotyping will be discussed later in this chapter but here we will focus on the analytical performance of the two assay design approaches.

6.4. The link between analytical and clinical assay performance

Good analytical assay performance is a requirement but not a guarantee of clinical assay performance. The viral load that correlates well with prediction of CIN2+ risk is approximately 5000 copies per reaction as established by the Hybrid Capture 2 test [41]. Thus, it is important to determine the clinical cutoff (a level of positivity in the assay that best correlates with histologically confirmed disease) for each individual assay using standard receiver operating characteristic plots of sensitivity versus 1-specificity so as to provide the highest possible sensitivity and an optimal specificity [55, 56, 88]. Assays that are analytically too sensitive will refer an unacceptably high number of women to colposcopy for possible biopsy and treatment [43]. **Table 2** describes some of the unique diagnostic challenges associated with cervical cancer screening that make this task a complicated one. This is reflected in the reports in the literature on viral load measurements, which are mixed at best. While HPV16 viral load has been shown to correlate with disease progression [99, 100], non-HPV16 types have been reported to show less correlation [101]. A recent well controlled study that focused exclusively on single infections found that HPV16/HPV18/HPV31/HPV45 viral load was correlated with abnormal cytology. This study is likely to have benefited by excluding the complication of mixed infections and the use of a high quality E6 gene-specific real-time PCR assay [102]. A positive correlation for these types (and HPV33) with disease severity was also confirmed in an independent study with histological endpoints [103]. These apparent discrepancies may be explained by analytical performance differences in the methods used to measure viral load. As mentioned above, most commercial assays use consensus L1 primers because of the ease of use offered by a single reaction that can detect 14 high-risk viruses. However, consensus primer designs exhibit poor detection of mixed infections due to HPV-type suppression or restriction, where the overabundance of one HPV type in a mixed infection can lead to failure to detect lower levels of a coinfecting virus. Van Doorn et al. were one of the first to demonstrate this phenomenon using spiking experiments where detectable levels of HPV18 were eliminated using an increasing concentration of a competing HPV16 target [104]. This result was later confirmed in cervical smear and biopsy specimens where the L1 SPF10 consensus primer assay was compared to a gene-specific E6 assay where significantly more genotypes ($P < 0.0001$) were identified by the E6 assay, especially for HPV types 16, 35, 39, 45, 58, and 59 and the authors concluded “that broad-spectrum PCRs are hampered by type competition when multiple HPV genotypes are present in the same sample” [105]. Similar results have been

reported by Mori et al. who found that “three consensus primers frequently caused incorrect genotyping in the selected clinical specimens containing HPV16 and one or two of HPV18, 31, 51, 52, and 58” and went on to conclude “that PCR with consensus primers is not suitable for genotyping HPV in specimens containing multiple HPV types” [106]. Given that approximately one-third or more of clinical specimens can harbor more than one HPV type [107], this should be considered carefully when designing vaccine monitoring or other studies requiring genotyping. This has recently been underlined by a *post-hoc* analysis of the PATRICIA vaccine trial where Struyf et al. found that an E6-based multiplex type-specific PCR and reverse hybridization assay showed improved sensitivity versus the L1-based SPF10 PCR-DNA enzyme immunoassay (DEIA)/line probe assay (LiPA25) used in the original trial, resulting in higher vaccine efficacy estimates for nonvaccine oncogenic HPV types [108]. The E6-assay was developed by Van Doorn et al. and had previously been shown to increase genotype detection by 14.3% [105]. Another negative impact of HPV-type suppression was reported by Cornall et al. who hypothesized that the sensitivity of consensus-based PCR approaches could be altered in highly vaccinated populations such as Australia. They confirmed their hypothesis using the Roche HPV linear array genotype assay by simulating samples containing 1000 copies of one or two high-risk HPV DNA genomes in the presence and the absence of 10,000 copies of the HPV16 genome. HPV16 alone did not affect detection of other high-risk genotypes; however, when HPV16 and an additional genotype were present, detection of HPV31, 33, 51, or 59 was impeded, indicating potential for misrepresentation of population-based prevalence of these genotypes and false evidence for type replacement following vaccination [109]. A next-generation sequencing (NGS) method also found that consensus MY09/MY11 primers had “lower sensitivity for some HPV types than LiPA, conceivably due to the poor sensitivity of the MY09/MY11-based primers” [110]. The results from the WHO LabNet genotyping panel broadly support these findings. The panel consists of approximately 43 DNA standardized DNA samples with single and mixed infections of the 14 high-risk HPV types (and low-risk types HPV6 and HPV11) and three cell line extraction controls. Candidate assays are considered proficient if they can detect 50 international units (IU) of HPV type 16 (HPV16) and HPV18 DNA and 500 genome equivalents (GE) for the other 14 HPV types. The 2010 panel results reported the data from 98 laboratories who submitted 132 datasets, only ~20% of which were deemed proficient for all HPV types. In addition, approximately 35% of the test panels had multiple false positive results and were considered nonproficient. Virtually all of the assays that submitted test results were L1 consensus-primer based [63]. The results from the 2014 panel from 119 participating global laboratories (146 datasets) were recently reported and the overall results showed an improvement, with 59% of the test results deemed 100% proficient and 20% nonproficient [111]. Nevertheless, there is little room for complacency when one considers that this implies that 40% of current assays do not accurately detect HPV types. In summary, assay design has a direct impact on clinical performance and the ability to accurately genotype both single and mixed infections will play an ever-increasing role, especially in a postvaccination era.

Diagnostic challenge	Impact	Mitigation	Current status	Comments
Most infections do no result in cancer	Cancer incidence can be as low as 5–8 per 100,000 women screened [112]	Organized screening programs need to reach as many women as possible	50% of cancers still occur in women who have not been screened	Need to continue to expand programs to reach underserved women (self-sampling)
Younger women are more likely to be infected with HPV	Screening of women < 25–30 may lead to overdiagnosis [113]	Current guidelines recommend not screening women <25 or <30	Primary screening approved in US for >25, with >30 more common for cotesting with cytology	Ongoing countrywide programs will shed more light on the correct age to start screening (which could be population specific)
Older women are more likely to develop cancer	Overtesting may cause unnecessary anxiety in younger patients	Adhere to expert guidelines and screen appropriately		
Analytical positive ≠ clinical positive [42]	Over- and underreferral possible	Establish appropriate clinical cutoff in target population	Meijer guidelines and FDA approval provide reassurance but in-country real-world validation is also informative	Need more research on the impact of cross-reactivity and molecular mimics as well as mixed infections
Results impacted by quality of specimen collection	Poorly collected or expressed samples increases unsatisfactory rate and can lead to false negative results if no cellular control present	Include cellular versus PCR processing control in the assay	Performance of different collection devices should be considered [114, 115]	Physicians and laboratory personnel need to adhere to recommended procedures for collection and processing of specimens
Large number of normal versus abnormal cells present in sample leads to sampling variation	Sampling errors can occur and lead to false negative results [116]	Assay needs to be robust to differences in pipetting volumes around recommended test volume	Most of the newer assays have a cellular processing control which helps mitigate this risk	Most manufacturers have adapted their methods for the two common liquid-based-cytology media on the market
Most endocervical samples are collected in preservative which lead to clumping of exfoliated cells		Samples need to be vortexed adequately and aliquots removed promptly prior to robust extraction and	Semiautomated or fully automated methods improve reliability (Table 1)	

Diagnostic challenge	Impact	Mitigation	Current status	Comments
		homogenization of the specimen [67, 117]		
Samples can be inhibited with a variety of exogenous and endogenous interfering substances [118]	False negative or indeterminate results obtained	Product needs to undergo rigorous analytical validation with a variety of interfering substances	Most assays are robust to interfering substances	Less of an issue than sampling variation
Infections are dynamic and can produce a large dynamic range of viral titers	Infections may be regressing or proliferating leading to false results	Correlate with cytology or other biomarker results and repeat test as needed	Clinically validated assays have good correlation with disease endpoints. One assay reported predictive ability of viral load measurements taken sequentially [119]	Viral load measurements are difficult to perform and may not be clinically practical if sequential. Genotyping provides information on persistence which may aid in patient management
The same pathology can be caused by non-high-risk HPV or other “mimics”	Non-high-risk HPV pathology can lead to overtreatment	Assays should not detect nontarget HPV types. Histology results should be considered in the context of high-risk HPV results	Newer assays have no cross-reactivity to nontarget types. Pathology review and biomarker qualification improve diagnostic cytopathology accuracy	The belief that “gold standard” pathology is truth needs to be critically challenged [120]
Viral clearance may precede resolution of cellular abnormalities	Lingering pathology complicates patient management	Adhere to screening guidelines and retest as needed	p16 biomarker is increasingly being used to augment diagnosis and guidelines have been proposed [121]	Extended genotyping offers improved risk stratification which may aid in patient management [96]

Table 2. Diagnostic challenges associated with cervical cancer screening.

6.5. HPV integration-induced deletions: impact on screening programs?

It is now well established that the HPV viral genome can integrate into the host genome during disease progression. The ability to integrate varies by high-risk HPV type with approximately 70% of HPV16-associated cervical cancers containing integrated HPV16 sequences, rising to almost 100% for HPV18 [122, 123]. Next-generation sequencing methods are now shedding new light on this phenomenon and the emerging picture suggests the following:

1. Viral integration appears to be a random (nontargeted) event with disruption of a wide variety of host genes across the human genome [124–126].
2. Integration is associated with fragile sites in human DNA and is likely opportunistic, with the HPV virus taking advantage of exposed DNA to integrate into the host genome. Regions of microhomology between the viral and human sequences are enriched near integration sites suggesting that integration may be driven by the host DNA repair machinery [124, 125].
3. Viral sequences are frequently deleted during the integration process [124, 125].
4. Contrary to what was previously believed E6 and E7 oncogenes can also be deleted during integration. However, this occurs less frequently than in other viral genes such as L1, L2, E1, and E2 and there appears to be significant enrichment of (intact) viral gene E7 > E4 > E5 > E6 reads among the cervical tumor samples [124, 125, 127]. (It is possible that this may simply be due to the random nature of the integration process and reflect the fact that L1, L2, E1, and E2 are the largest ORFs in the HPV genome, **Figure 2**).
5. Viral integration appears to occur at a higher frequency than perhaps previously understood and can also be detected in Pap smears, one study reporting integration in 53% of cytology samples with histology grades CIN1–3 [124].

Thus, it appears that viral integration HPV integration is an unintended consequence of HPV replication and that following integration, the virus life cycle is aborted. Nevertheless, virus integration is a routine consequence of infection and the observation that it can occur earlier in the disease progression prompts the question whether it has a measureable effect on the ability of HPV assays to detect the virus. Several authors have expressed concern that L1-based assays can fail to detect late-stage cancers due to target deletion [128–130] and there is no doubt that L1-deleted cancers occur as evidenced by case studies [129, 131]. Several studies have reported that the E6/E7 assays have increased sensitivity for cancer versus L1-based assays [132–134]. Some have dismissed this as due to earlier methodological differences due to the difficulty of detecting longer L1 amplicons in formalin fixed paraffin embedded (FFPE) tissue. However, this is not supported by more recent literature where even using gold standard FFPE processing methods, one group reported only a 91% detection rate in cancer specimens with two different L1 primer assays [135]. Another recent study of cervical adenocarcinomas reported that PCR using a very sensitive L1-based SPF10-PCR resulted in 482 cases (67%) positive for HPV DNA, but that testing using type-specific E6 PCR added 53 HPV-positive cases (a 7% increase in detection). The study design also accounted for DNA adequacy in the samples [136]. Adenocarcinomas represent about 25% of all cervical cancers [137, 138] and are known to be prone to HPV DNA integration, so this increase in sensitivity for detection of adenocarcinomas could potentially translate to the detection of another 1–2% of total cancer cases (assuming an increase in just adenocarcinoma cases). Large-scale studies will be needed to address the question of whether or not the above reported difference in assay performance (due to increased maintenance of intact target regions postintegration) has a measureable impact on clinical sensitivity in cervical cancer screening. This warrants further study, especially given the reported ability of the virus to integrate in approximately 50% of cervical

smears from patients with CIN [124]. Finally, the Roche cobas assay has recently been reported to have a 94% detection rate in the smears from women with histologically confirmed invasive cervical cancer [139].

7. The evolution of HPV screening

7.1. Cotesting with cytology versus primary HPV screening

It has been known for 10 years that HPV testing is substantially more sensitive in detecting CIN2+ than cytology (96.1 versus 53.0%) but less specific (90.7 versus 96.3%) [140]. In the intervening period, large randomized controlled trials (RCTs) have continued to reaffirm this point. Pileggi et al. [141] recently performed a meta-analysis of global RCTs which included trials from Italy (NTCC trials I+II [142, 143]), the UK (ARTISTIC Trial [144]), Finland [145], India [146], Canada [147], and the Netherlands (POBASCAM [148]). The analysis from this larger more comprehensive dataset showed a significantly higher detection of both CIN2+ and CIN3+ by HPV testing versus cytology and that the relative specificity of cytology was higher. However, in women greater than 30 years of age, the specificity was not statistically different ("almost overlapping"). In addition, the pooled relative PPV was not significantly lower for HPV compared with cytology. These results suggest that the difference in specificity is actually less than previously thought, at least in women over 30. A recent US *post-hoc* analysis of a large group of women from multiple practices reported that positive Pap + HPV (cotest) was more sensitive than either a positive HPV alone or Pap alone for detection of CIN3+ and suggested that HPV primary screening might miss cancers if not combined with the Pap [149]. However, the study design has come under criticism from an independent expert, because the short follow up time of 1 year biases results in favor of cytology and all women were not followed equally [150]. Thus, large global studies support the use of primary HPV testing as a means to improve cervical cancer detection and it explains why many countries are now in the process of implementing either pilot or countrywide programs to replace cytology and/or cotesting [151, 152]. In the United States, one HPV test has received FDA approval for primary screening in women >25 years of age [153] and interim guidelines for its use have been issued [113]. The reaction to this decision has been mixed with some arguing that cotesting detects more disease [154]. However, data continue to accumulate both in the United States and in Europe that supports the long-term NPV of a primary HPV negative result, thus permitting safe interval extension [155, 156]. A study comparing primary HPV versus cotesting versus primary cytology concluded that "primary HPV testing every 3 years might provide as much, if not more, reassurance against precancer and cancer, compared to primary Pap testing every 3 years and cotesting every 5 years" [156]. Thus, the choice of which screening paradigm to adopt will likely be influenced by the resources available together with the desired screening interval and risk tolerance of the medical community and the patients they serve. Despite the slow pace of change, it seems clear that HPV primary screening will gradually be adopted worldwide, especially in countries which do not have current cytology infrastructure. HPV testing is considerably more reproducible than cytology [157] and with the advent of automation, highly skilled personnel are no longer required to implement IVD-qualified tests. It should be noted,

however, that primary HPV screening cannot be used in isolation to refer women to colposcopy. Biomarkers such as p16 [121] or cytology or HPV16/HPV18 genotyping need to be considered to increase specificity and reduce the number of unnecessary colposcopies [72, 153, 158].

7.2. Reaching underserved women: self-sampling methods

It is well established that the effectiveness of cervical cancer screening programs is limited by the number of women who do not participate—with 50% or more of disease being detected in women who have not been screened [159–162]. While there may be many reasons (cultural, socioeconomic, and religious) why women choose not to participate in screening programs available to them, it is clear that self-collection offers an effective outreach tool with which to increase participation. For example, in Finland the participation rate was <70% but was increased to 72.6–79.9% by sending reminder letters to nonattendees in 22 municipalities, and to 83.4% by sending self-collection kits to those who did not attend after receiving the initial invitation letter [163]. Similar finding on outreach were reported in Sweden where telephone invitations to long-term nonattendees increased the participation rate within the following 12 months to 18.0% versus 10.6% in a control group [164]. In Canada, reminder letters were compared head to head with sending a self-sampling kit and standard screening and women receiving the self-collected HPV kit were 3.7 times more likely to undergo screening compared with the standard of care [165]. Finally, a recent Danish outreach program where 5000 women were invited to “opt-in” and receive a self-collection kit resulted in the detection of nine cancers. This translates to yield of 1.8 per 1000, a dramatic enrichment for disease detection versus the Danish population rate, estimated at 12.9 per 100,000 women [166, 167]. Thus, self-sampling is emerging as a very important tool in national screening programs and one that continues to generate a high degree of interest [168]. Two principal self-collection methods that have been described in the literature: cervico-vaginal collection and urine collection.

7.2.1. *Cervico-vaginal collection methods*

A number of different cervico-vaginal methods have been described in the literature (reviewed in [169, 170]) and range from simple brush devices currently used by physicians to custom designed self-collection devices such as the Rovers® Evalyn® brush (Rovers Medical Devices BV, the Netherlands) and HerSwab™ (Eve Medical, Canada). Earlier literature reported mixed results versus physician collected endocervical samples in terms of sensitivity and specificity. However, more recent studies have confirmed that self-collected specimens can have the same sensitivity for CIN2+ as physician-collected specimens [171]. A recent meta-analysis concluded that signal amplification methods were not sensitive enough for self-collection use but that certain PCR methods had similar sensitivity to physician-collected samples [172]. This has been confirmed in a number of small studies using clinically validated HPV tests where sensitivities for CIN2+ were similar to physician-collected samples [173–176]. It is likely that both the quality of the instructions used and the sample workflow/assay play a role in the ability to accurately detect infections. Further large-scale studies are needed to further demonstrate the utility of these methods in screening populations.

7.2.2. HPV testing from urine

HPV testing from urine has been performed for over 20 years and similar to cervico-vaginal methods, early studies were somewhat discouraging with decreased sensitivity versus physician-collected endocervical samples being reported [177]. This was compounded by the demonstration that male urine samples were a poor sample type for HPV detection [178], which has been confirmed in more recent studies and likely reflects a true biological difference between men and women [179, 180]. The general interest in self-sampling and the introduction of HPV vaccines, which can benefit from noninvasive population-based monitoring, has resulted in a renewed interest in urine as an alternative sample type for cervical cancer screening [181]. There is now a growing body of evidence that urine, in particular first void urine, has a lot of potential for detecting HPV infections [182, 183]. The basic premise of HPV urine testing is founded on the hypothesis that “at the start of the void, urine gets contaminated by debris and impurities lining the urethra opening, including mucus and debris of exfoliated cells from the vagina, cervix and uterus” [181]. Recent research has also demonstrated that optimal sample workflows and sensitive detection methods improve clinical performance:

1. Urine contains substantial amounts of free-DNA and virus, so processing methods that utilize recovered cell pellets may underestimate the amount of virus present. Processing of neat urine is preferable [184, 185]. A recent study found similar performance using 0.5 ml of neat urine versus up to 80-fold concentrated cell pellets [186]. Another study found HPV DNA sequences in both urinary tract infection positive and negative patient urine using cesium chloride density gradient-purified virus particles, suggesting that intact virions are present [187]. (Urine also contains transrenal DNA [short DNA fragments from the blood circulation that has passed the kidney barrier [188], but this is not believed to be the main source of HPV target DNA as evidenced by the observation that first void urine collected during menses is contaminated with blood and cells.)
2. Urine from HPV positive women appears to be consistently positive even when sampled multiple times from the same patient. However, first void urine samples are analytically more positive, especially if the initial catchment is in a smaller volume [181, 182, 186, 189].
3. Virus appears to be shed continuously from infected women and is simply flushed out of the vaginal canal during urination. Therefore, it is not advisable to clean the local area with alcohol wipes prior to collection in order to maximize recovery of cells, virus, and free-DNA [181].
4. Urine samples are labile at room temperature (due to the action of proteases and nucleases) so urine samples should be mixed promptly with an appropriate preservative such as EDTA to ensure sample integrity. Addition of the preservative to the urine cup or collection tube is optimal since degradation can occur immediately post-collection prior to the arrival of the sample in the testing laboratory [184, 188, 190–192].
5. An assay cellularity control targeting a human gene is recommended to ensure sample adequacy. Routine urine samples should be positive for human DNA and confirms that an adequate catchment has been obtained. Analytically sensitive molecular methods such

as real-time PCR should be used to ensure optimal results and clinical cutoff adjustments may be required versus endocervical specimens [193].

8. “Safety in numbers”: time for extended genotyping in cervical cancer screening

The majority of (clinically validated) HPV screening tests have the ability to provide genotyping information on only two types, HPV16 and HPV18 [46]. There is now a growing body of evidence that extended genotyping (beyond types 16 and 18) better stratifies a woman’s risk for subsequent disease: A recent Danish study looked at the long-term absolute risk for CIN3+ in women following a baseline HPV genotyping result and found that HPV31 and HPV33 had the same or greater longitudinal risk as HPV18 over a 10-year period [194]; Schiffman et al. [195] confirmed this finding for HPV31 in a US cohort over a 15-year follow up period; Cuzick et al. [196] looked at the positive predictive value of genotyping in a referred UK population and found that HPV33 had a higher PPV than HPV16 (59.8 versus 57.8) and that HPV31 was higher than HPV18 (39.5 versus 29.3). Similar trends were reported in a much larger New Mexico study of 21,297 women of whom 77% had biopsies. Among women with CIN3+ ($n = 1880$), 14.9% were attributable to HPV31, 5.2% to HPV33 and 4.9% to HPV18, with HPV16 responsible for 54.1% [197]. Roche Linear Array genotyping analysis of patients from the ATHENA trial also support these findings: among 40,901 women aged ≥ 25 years HPV16 conferred the greatest absolute risk of $\geq$ CIN3 both in women aged 25–29 and ≥ 30 years (14.2% and 15.1%, respectively) followed by HPV31 (8.0% and 7.9%), HPV52 (6.7% and 4.4%), and HPV18 (2.7% and 9.0%) [198].

While the majority of cancers are caused by HPV16 and HPV18, presumably due to the increased ability of these genotypes to persist and induce oncogenic changes, one must equally concede that HPV31, 33, 45, 52, and 58 are the next most potent carcinogens of the high-risk HPV types [199]. This is supported by the introduction of the nine-valent HPV vaccine, where the addition of these types is estimated to provide protection against an additional ~20% of cancers [200]. The clinical paradigm of equal management of equal risk [201] strongly suggests that knowledge of HPV31 and HPV33 infections has clinical value and can aid in patient management. In addition, the widespread introduction of the new nine-valent vaccine is expected to have a profound effect on the incidence of CIN3+ disease, further underlining the need for a more comprehensive genotype profile of pre- and postvaccinated women [202]. Finally, the use of gene-specific versus consensus PCR genotyping approaches can alleviate the issue of apparent type replacement in vaccinated women where HPV types such as HPV31 and HPV33 may appear to be increasing in prevalence but are simply being unmasked due to the reduced incidence of HPV16 and HPV18 in the postvaccination era [109].

Extended genotyping also offers a potential solution to key emerging issues in cervical cancer screening:

- I. Accurate risk stratification—absolute risk calculations *a priori* are underestimated when two or more HPV types with different CIN3 risks are pooled together (e.g., the 10-year longitudinal study of Kahn et al. attributes a very low risk to pooled non-16/18

types [203] which has been consistently found *not* to be the case in subsequent studies where individual genotypes have been detected [194, 196–198]). Precise risk stratification is essential for consistent and informed patient management. This is especially true in cytology-negative HPV-positive women where genotyping can provide clinicians with a much more informed assessment of future risk versus a pooled HPV positive result.

- II. On the other hand, extended genotyping allows one to group HPV types with similar risk, thereby simplifying patient management algorithms. Cuzick et al. [196] proposed a three tiered risk group approach to patient stratification based on the positive predictive value of different genotypes for CIN3+ disease in a referred population. Schiffman et al. recently published the largest longitudinal ASC-US population analysis to date (Persistence and Progression cohort with 13,890 women aged 21+ with HC2 (QIAGEN)-positive ASC-US at enrollment and median follow-up of 3.0 years). The authors used the concept of equal management of equal risk approach and calculated the 3-year CIN3+ risk for all HC2-positive women with ASC-US (5.2%), using this as the “benchmark” risk for colposcopic referral. They concluded that the 3-year risk for developing CIN3+ associated with high-risk HPV types 35, 39, 51, 56, 59, 66, and 68 (2.7% for HPV51, 1.6% for HPV39/HPV68/HPV35, and 1.3% for HPV59/HPV56/HPV66) “might be low enough to recommend 1-year retesting, permitting viral clearance. This strategy would defer colposcopy for 40% of women with HPV-positive ASC-US, half of whom would be cotest-negative at 1-year return” [96].

Thus, extended genotyping offers the potential of improved risk stratification and simpler patient management, helping to improve patient outcomes with reduced intervention.

9. Future perspectives

While we have made great strides in both detecting and preventing cervical cancer over the last 20 years, it still represents a significant challenge in both developed and developing settings. In countries with a high disease burden such as Mexico, Ecuador, Samoa, and Colombia, it is the number one cancer in young women and the number two cancer in women of all ages, whereas in several areas in Africa and in Cambodia, it is primary cause of cancer and death [112]. With 50% of cancers occurring in underserved women, it is clear that the biggest remaining challenges are in implementation, not technology development. Self-collection methods offer new hope of reaching women who are currently not captured in traditional screening programs. Ongoing studies should shed further light on the ability of these methods to equal those used in the physician’s office or at least provide a means to target these women for follow up in the medical system (analogous to current home pregnancy test kits). HPV vaccines have been shown to be safe and efficacious but here again the biggest hurdle is achieving the required vaccine coverage in the target population. It is hoped that the successful implementation

models such as those in the UK and Australia will be adopted elsewhere to boost global vaccine coverage. It should also not go unmentioned that the other barrier to improved disease prevention and detection is resistance to change. While medicine can be understandably conservative, waiting for substantial bodies of evidence to accumulate prior to changing clinical management, we should also constantly challenge ourselves on this point, given the severity of the disease burden imposed by HPV. With the benefit of hindsight, one can justifiably argue that even the Pap smear itself took 30 years too long to be implemented, HPV primary screening could have been adopted 10 years ago based on the available evidence, and vaccine coverage is suffering today due to the emergence of vaccine opposition which is not supported by scientific evidence [204]. We should also continue to challenge the *status quo* on cervical cytology and histopathology – while they have an important role to play, their future role should be evaluated in the context of comprehensive screening strategies [205, 206]. There is an urgent need to eradicate cervical cancer for the sake of both the current generation of women and the men and women who follow them. At the same time, there is an ever increasing pressure on global healthcare systems to do more with less. Ironically, this will likely serve as a catalyst for change as health economic studies identify which screening and vaccination strategies are the most cost-effective and offer the highest adoption rate. Ongoing and future research should focus on answering key questions in cervical cancer program implementation such as the effect of vaccination on HPV prevalence, and the ability of extended genotyping to better stratify a patient's risk. Optimal triage strategies should also be investigated together with new studies on the long-term effects on the physical and psychological health of patients who are overtested and overtreated. As we settle into the twenty-first century of cervical cancer screening, one thing is clear, we have effective means to detect and prevent disease. The real question is: do we have the resolve to deploy these tools in an effective manner? We join others in the hope that we will learn from the past and quicken the pace of change in response to the global threat posed by cervical disease [207].

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References

- [1] Diamantis A, Magiorkinis E, Koutselini H. 50 years after the death of George Nicholas Papanicolaou (1883–1962): evaluation of his scientific work. *Acta Med Hist Adriat.* 2014;12(1):181–8.
- [2] Classes in oncology: George Nicholas Papanicolaou's new cancer diagnosis presented at the Third Race Betterment Conference, Battle Creek, Michigan, January 2–6, 1928, and published in the Proceedings of the Conference. *CA Cancer J Clin.* 1973;23(3):174–9.
- [3] Papanicolaou GN, Traut HF. The diagnostic value of vaginal smears in carcinoma of the uterus. 1941. *Arch Pathol Lab Med.* 1997;121(3):211–24.
- [4] zur Hausen H. Oncogenic Herpes viruses. *Biochim Biophys Acta.* 1975;417(1):25–53.
- [5] Gissmann L, zur Hausen H. Partial characterization of viral DNA from human genital warts (*Condylomata acuminata*). *Int J Cancer.* 1980;25(5):605–9.
- [6] de Villiers EM, Gissmann L, zur Hausen H. Molecular cloning of viral DNA from human genital warts. *J Virol.* 1981;40(3):932–5.
- [7] Gissmann L, deVilliers EM, zur Hausen H. Analysis of human genital warts (*Condylomata acuminata*) and other genital tumors for human papillomavirus type 6 DNA. *Int J Cancer.* 1982;29(2):143–6.
- [8] Gissmann L, Wolnik L, Ikenberg H, Koldovsky U, Schnurch HG, zur Hausen H. Human papillomavirus types 6 and 11 DNA sequences in genital and laryngeal papillomas and in some cervical cancers. *Proc Natl Acad Sci USA.* 1983;80(2):560–3.
- [9] Zachow KR, Ostrow RS, Bender M, Watts S, Okagaki T, Pass F, Faras AJ. Detection of human papillomavirus DNA in anogenital neoplasias. *Nature.* 1982;300(5894):771–3.
- [10] Green M, Brackmann KH, Sanders PR, Loewenstein PM, Freel JH, Eisinger M, Switlyk SA. Isolation of a human papillomavirus from a patient with epidermodysplasia verruciformis: presence of related viral DNA genomes in human urogenital tumors. *Proc Natl Acad Sci USA.* 1982;79(14):4437–41.
- [11] Durst M, Gissmann L, Ikenberg H, zur Hausen H. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. *Proc Natl Acad Sci USA.* 1983;80(12):3812–5.
- [12] Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlen W, zur Hausen H. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. *EMBO J.* 1984;3(5):1151–7.
- [13] Ikenberg H, Gissmann L, Gross G, Grussendorf-Conen EI, zur Hausen H. Human papillomavirus type-16-related DNA in genital Bowen's disease and in Bowenoid papulosis. *Int J Cancer.* 1983;32(5):563–5.

- [14] Crum CP, Ikenberg H, Richart RM, Gissman L. Human papillomavirus type 16 and early cervical neoplasia. *N Engl J Med*. 1984;310(14):880–3. DOI:10.1056/nejm198404053101403.
- [15] Lorincz AT, Lancaster WD, Temple GF. Cloning and characterization of the DNA of a new human papillomavirus from a woman with dysplasia of the uterine cervix. *J Virol*. 1986;58(1):225–9.
- [16] Naghashfar ZS, Rosenshein NB, Lorincz AT, Buscema J, Shah KV. Characterization of human papillomavirus type 45, a new type 18-related virus of the genital tract. *J Gen Virol*. 1987;68 (Pt 12):3073–9. DOI:10.1099/0022-1317-68-12-3073.
- [17] Hubbard RA. Human papillomavirus testing methods. *Arch Pathol Lab Med*. 2003 Aug;127(8):940–5. DOI:10.1043/1543-2165(2003)127<940:hptm>2.0.co;2.
- [18] Guo M, Gong Y, Deavers M, Silva EG, Jan YJ, Cogdell DE, Luthra R, Lin E, Lai HC, Zhang W, Sneige N. Evaluation of a commercialized *in situ* hybridization assay for detecting human papillomavirus DNA in tissue specimens from patients with cervical intraepithelial neoplasia and cervical carcinoma. *J Clin Microbiol*. 2008;46(1):274–80. DOI:10.1128/jcm.01299-07.
- [19] Zhang W, Kapadia M, Sugarman M, Free H, Upchurch C, Gniewek R, White K, Miller M, Vladich F, Ferenczy A, Wright TC, Stoler MH, Pestic-Dragovich L. Adjunctive HPV in-situ hybridization (ISH) assay as an aid in the diagnosis of cervical intraepithelial neoplasia in cervical tissue specimens: an analytical and functional characterization. *Int J Gynecol Pathol*. 2012;31(6):588–95. DOI:10.1097/PGP.0b013e318254349a.
- [20] Lorincz AT. Hybrid Capture method for detection of human papillomavirus DNA in clinical specimens: a tool for clinical management of equivocal Pap smears and for population screening. *J Obstet Gynaecol Res*. 1996;22(6):629–36.
- [21] Cavuslu S, Mant C, Starkey WG, Bible JM, Biswas C, Kell B, Rice P, Best JM, Cason J. Analytic sensitivities of hybrid-capture, consensus and type-specific polymerase chain reactions for the detection of human papillomavirus type 16 DNA. *J Med Virol*. 1996;49(4):319–24. DOI:10.1002/(sici)1096-9071(199608)49:4<319::aid-jmv10>3.0.co;2-5.
- [22] Reid R, Lorincz AT. Human papillomavirus tests. *Baillieres Clin Obstet Gynaecol*. 1995;9(1):65–103.
- [23] Poljak M, Brencic A, Seme K, Vince A, Marin IJ. Comparative evaluation of first- and second-generation digene hybrid capture assays for detection of human papillomaviruses associated with high or intermediate risk for cervical cancer. *J Clin Microbiol*. 1999;37(3):796–7.
- [24] 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(12):1789–99. DOI:10.1086/657321.
- [25] Patanwala IY, Bauer HM, Miyamoto J, Park IU, Huchko MJ, Smith-McCune KK. A systematic review of randomized trials assessing human papillomavirus testing in

cervical cancer screening. *Am J Obstet Gynecol.* 2013;208(5):343–53. DOI:10.1016/j.ajog.2012.11.013.

- [26] Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific enzymatic amplification of DNA *in vitro*: the polymerase chain reaction. *Cold Spring Harb Symp Quant Biol.* 1986;51(Pt 1):263–73.
- [27] Saiki RK, Bugawan TL, Horn GT, Mullis KB, Erlich HA. Analysis of enzymatically amplified beta-globin and HLA-DQ alpha DNA with allele-specific oligonucleotide probes. *Nature.* 1986;324(6093):163–6. DOI:10.1038/324163a0.
- [28] Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific enzymatic amplification of DNA *in vitro*: the polymerase chain reaction. 1986. *Biotechnology.* 1992;24:17–27.
- [29] Higuchi R, Fockler C, Dollinger G, Watson R. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. *Biotechnology (N Y).* 1993;11(9):1026–30.
- [30] Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. *Genome Res.* 1996;6(10):986–94.
- [31] Gibson UE, Heid CA, Williams PM. A novel method for real time quantitative RT-PCR. *Genome Res.* 1996;6(10):995–1001.
- [32] Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene.* 1990;93(1):125–8.
- [33] Kwok S, Higuchi R. Avoiding false positives with PCR. *Nature.* 1989;339(6221):237–8. DOI:10.1038/339237a0.
- [34] Dallas PB, Flanagan JL, Nightingale BN, Morris BJ. Polymerase chain reaction for fast, nonradioactive detection of high- and low-risk papillomavirus types in routine cervical specimens and in biopsies. *J Med Virol.* 1989;27(2):105–11.
- [35] Josefsson A, Livak K, Gyllensten U. Detection and quantitation of human papillomavirus by using the fluorescent 5' exonuclease assay. *J Clin Microbiol.* 1999;37(3):490–6.
- [36] Cuschieri KS, Cubie HA, Whitley MW, Seagar AL, Arends MJ, Moore C, Gilkisson G, McGoogan E. Multiple high-risk HPV infections are common in cervical neoplasia and young women in a cervical screening population. *J Clin Pathol.* 2004;57(1):68–72.
- [37] Snijders PJ, van den Brule AJ, Schrijnemakers HF, Snow G, Meijer CJ, Walboomers JM. The use of general primers in the polymerase chain reaction permits the detection of a broad spectrum of human papillomavirus genotypes. *J Gen Virol.* 1990;71(Pt 1):173–81. DOI:10.1099/0022-1317-71-1-173.
- [38] Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Coutlee F, Hildesheim A, Schiffman MH, Scott DR, Apple RJ. Improved amplification of genital human papillomaviruses. *J Clin Microbiol.* 2000;38(1):357–61.

- [39] de Roda Husman AM, Walboomers JM, van den Brule AJ, Meijer CJ, Snijders PJ. The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. *J Gen Virol*. 1995;76(Pt 4):1057–62. DOI:10.1099/0022-1317-76-4-1057.
- [40] Bulkman NW, Rozendaal L, Snijders PJ, Voorhorst FJ, Boeke AJ, Zandwijken GR, van Kemenade FJ, Verheijen RH, v Groningen K, Boon ME, Keuning HJ, van Ballegooijen M, van den Brule AJ, Meijer CJ. POBASCAM, a population-based randomized controlled trial for implementation of high-risk HPV testing in cervical screening: design, methods and baseline data of 44,102 women. *Int J Cancer*. 2004;110(1):94–101. DOI: 10.1002/ijc.20076.
- [41] QIAGEN. Hybrid Capture 2 High-Risk HPV DNA Test Package Insert. 2007;L00665 Rev. 2.
- [42] Stoler MH, Castle PE, Solomon D, Schiffman M. The expanded use of HPV testing in gynecologic practice per ASCCP-guided management requires the use of well-validated assays. *Am J Clin Pathol*. 2007;127(3):335–7. DOI:10.1309/rnf3c01jkadqclkp.
- [43] Kinney W, Stoler MH, Castle PE. Special commentary: patient safety and the next generation of HPV DNA tests. *Am J Clin Pathol*. 2010;134(2):193–9. DOI:10.1309/ajcpri8xpqueaa3k.
- [44] 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(3):516–20. DOI:10.1002/ijc.24010.
- [45] Poljak M, Kocjan BJ, Ostrbenk A, Seme K. Commercially available molecular tests for human papillomaviruses (HPV): 2015 update. *J Clin Virol*. 2016;76 Suppl 1:S3–S13. DOI: 10.1016/j.jcv.2015.10.023.
- [46] 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(9):817–26. DOI:10.1016/j.cmi.2015.04.015.
- [47] Bosch FX, Broker TR, Forman D, Moscicki AB, Gillison ML, Doorbar J, Stern PL, Stanley M, Arbyn M, Poljak M, Cuzick J, Castle PE, Schiller JT, Markowitz LE, Fisher WA, Canfell K, Denny LA, Franco EL, Steben M, Kane MA, Schiffman M, Meijer CJ, Sankaranarayanan R, Castellsague X, Kim JJ, Brotons M, Alemany L, Albero G, Diaz M, de Sanjose S. Comprehensive control of human papillomavirus infections and related diseases. *Vaccine*. 2013;31(Suppl 7):H1–31. DOI:10.1016/j.vaccine.2013.10.003.
- [48] Munger K, Howley PM. Human papillomavirus immortalization and transformation functions. *Virus Res*. 2002;89(2):213–28.

- [49] Doorbar J, Egawa N, Griffin H, Kranjec C, Murakami I. Human papillomavirus molecular biology and disease association. *Rev Med Virol.* 2015;25(Suppl 1):2–23. DOI: 10.1002/rmv.1822.
- [50] Dockter J, Schroder A, Hill C, Guzinski L, Monsonego J, Giachetti C. Clinical performance of the APTIMA HPV Assay for the detection of high-risk HPV and high-grade cervical lesions. *J Clin Virol.* 2009;45(Suppl 1):S55–61. DOI:10.1016/s1386-6532(09)70009-5.
- [51] Kacian, D.L. and T.J. Fultz. 1995. Nucleic acid sequence amplification methods. U. S. Patent 5,399,491.
- [52] Arnold LJ, Jr., Hammond PW, Wiese WA, Nelson NC. Assay formats involving acridinium-ester-labeled DNA probes. *Clin Chem.* 1989;35(8):1588–94.
- [53] GenProbe. Aptima HPV Assay. 2011;502170 Rev. A.
- [54] Alaghehbandan R, Fontaine D, Bentley J, Escott N, Ghatage P, Lear A, Coutlee F, Ratnam S. Performance of ProEx C and PreTect HPV-Proofer E6/E7 mRNA tests in comparison with the hybrid capture 2 HPV DNA test for triaging ASCUS and LSIL cytology. *Diagn Cytopathol.* 2013;41(9):767–75. DOI:10.1002/dc.22944.
- [55] Szarewski A, Mesher D, Cadman L, Austin J, Ashdown-Barr L, Ho L, Terry G, Liddle S, Young M, Stoler M, McCarthy J, Wright C, Bergeron C, Soutter WP, Lyons D, Cuzick J. Comparison of seven tests for high-grade cervical intraepithelial neoplasia in women with abnormal smears: the Predictors 2 study. *J Clin Microbiol.* 2012;50(6):1867–73. DOI:10.1128/JCM.00181-12.
- [56] 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(4):908–13. DOI:10.1038/bjc.2013.22.
- [57] Arbyn M, Roelens J, Cuschieri K, Cuzick J, Szarewski A, Ratnam S, Reuschenbach M, Belinson S, Belinson JL, Monsonego J. The APTIMA HPV assay versus the Hybrid Capture 2 test in triage of women with ASC-US or LSIL cervical cytology: a meta-analysis of the diagnostic accuracy. *Int J Cancer.* 2013;132(1):101–8. DOI:10.1002/ijc.27636.
- [58] Reid JL, Wright TC, Jr., Stoler MH, Cuzick J, Castle PE, Dockter J, Getman D, Giachetti C. Human papillomavirus oncogenic mRNA testing for cervical cancer screening: baseline and longitudinal results from the CLEAR study. *Am J Clin Pathol.* 2015;144(3):473–83. DOI:10.1309/ajcphvd7mip3fyvv.
- [59] Haedicke J, Iftner T. A review of the clinical performance of the Aptima HPV assay. *J Clin Virol.* 2016;76 Suppl 1:S40-8. DOI:10.1016/j.jcv.2015.10.027

- [60] Getman D, Aiyer A, Dockter J, Giachetti C, Zhang F, Ginocchio CC. Efficiency of the APTIMA HPV Assay for detection of HPV RNA and DNA targets. *J Clin Virol*. 2009;45(Suppl 1):S49–54. DOI:10.1016/s1386-6532(09)70008-3.
- [61] Boulet GA, Micalessi IM, Horvath CA, Benoy IH, Depuydt CE, Bogers JJ. Nucleic acid sequence-based amplification assay for human papillomavirus mRNA detection and typing: evidence for DNA amplification. *J Clin Microbiol*. 2010;48(7):2524–9. DOI:10.1128/jcm.00173-10.
- [62] Ong CK, Chan SY, Campo MS, Fujinaga K, Mavromara-Nazos P, Labropoulou V, Pfister H, Tay SK, ter Meulen J, Villa LL, et al. Evolution of human papillomavirus type 18: an ancient phylogenetic root in Africa and intratype diversity reflect coevolution with human ethnic groups. *J Virol*. 1993;67(11):6424–31.
- [63] Eklund C, Zhou T, Dillner J. Global proficiency study of human papillomavirus genotyping. *J Clin Microbiol*. 2010;48(11):4147–55. DOI:10.1128/jcm.00918-10.
- [64] Didelot MN, Boulle N, Damay A, Costes V, Segondy M. Comparison of the Papillo-Check(R) assay with the digene HC2 HPV DNA assay for the detection of 13 high-risk human papillomaviruses in cervical and anal scrapes. *J Med Virol*. 2011;83(8):1377–82. DOI:10.1002/jmv.22148.
- [65] Stoler MH, Wright TC, Jr., Cuzick J, Dockter J, Reid JL, Getman D, Giachetti C. APTIMA HPV assay performance in women with atypical squamous cells of undetermined significance cytology results. *Am J Obstet Gynecol*. 2013;208(2):144 e1–8. DOI:10.1016/j.ajog.2012.12.003.
- [66] Wright TC, Jr., Stoler MH, Agreda PM, Beitman GH, Gutierrez EC, Harris JM, Koch KR, Kuebler M, LaViers WD, Legendre BL, Jr., Leitch SV, Maus CE, McMillian RA, Nussbaumer WA, Palmer ML, Porter MJ, Richart GA, Schwab RJ, Vaughan LM. Clinical performance of the BD onclarity HPV assay using an adjudicated cohort of BD SurePath liquid-based cytology specimens. *Am J Clin Pathol*. 2014;142(1):43–50. DOI:10.1309/ajcp53kmhnrldicbl.
- [67] Roche. cobas 4800 HPV Test, Instructions For Use. 2013;Reference 05641225001-10EN(Doc. Rev, 8.0).
- [68] Castle PE, Hunt WC, Langsfeld E, Wheeler CM. Three-year risk of cervical precancer and cancer after the detection of low-risk human papillomavirus genotypes targeted by a commercial test. *Obstet Gynecol*. 2014;123(1):49–56. DOI:10.1097/aog.0000000000000013.
- [69] Schiffman M, Clifford G, Buonaguro FM. Classification of weakly carcinogenic human papillomavirus types: addressing the limits of epidemiology at the borderline. *Infect Agent Cancer*. 2009;4:8. DOI:10.1186/1750-9378-4-8.

- [70] Castle PE, Solomon D, Wheeler CM, Gravitt PE, Wacholder S, Schiffman M. Human papillomavirus genotype specificity of hybrid capture 2. *J Clin Microbiol.* 2008;46(8): 2595–604. DOI:10.1128/jcm.00824-08.
- [71] Halec G, Alemany L, Lloveras B, Schmitt M, Alejo M, Bosch FX, Tous S, Klaustermeier JE, Guimera N, Grabe N, Lahrmann B, Gissmann L, Quint W, Bosch FX, de Sanjose S, Pawlita M. Pathogenic role of the eight probably/possibly carcinogenic HPV types 26, 53, 66, 67, 68, 70, 73 and 82 in cervical cancer. *J Pathol.* 2014;234(4):441–51. DOI:10.1002/path.4405.
- [72] Cox JT, Castle PE, Behrens CM, Sharma A, Wright TC, Jr., Cuzick J, hena HPVSG. Comparison of cervical cancer screening strategies incorporating different combinations of cytology, HPV testing, and genotyping for HPV 16/18: results from the ATHENA HPV study. *Am J Obstet Gynecol.* 2013;208(3):184 e1–11. DOI:10.1016/j.ajog.2012.11.020.
- [73] 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. JMD.* 2012;14(1):65–70. DOI:10.1016/j.jmoldx.2011.09.005.
- [74] Poljak M, Kocjan BJ, Kovanda A, Lunar MM, Lepej SZ, Planinic A, Seme K, Vince A. Human papillomavirus genotype specificity of hybrid capture 2 low-risk probe cocktail. *J Clin Microbiol.* 2009;47(8):2611–5. DOI:10.1128/jcm.00278-09.
- [75] Gillio-Tos A, De Marco L, Carozzi FM, Del Mistro A, Girlando S, Burrioni E, Frayle-Salamanca H, Giorgi Rossi P, Pierotti P, Ronco G. Clinical impact of the analytical specificity of the hybrid capture 2 test: data from the New Technologies for Cervical Cancer (NTCC) study. *J Clin Microbiol.* 2013;51(9):2901–7. DOI:10.1128/jcm.01047-13.
- [76] Bottari F, Sideri M, Gulmini C, Igidbashian S, Tricca A, Casadio C, Carinelli S, Boveri S, Ejegod D, Bonde J, Sandri MT. Comparison of the performance of the BD Onclarity HPV Assay with Hybrid Capture II in detection of CIN2+ lesions. *J Clin Microbiol.* 2015;53(7):2109-14. DOI:10.1128/jcm.00246-15.
- [77] Cook DA, Mei W, Smith LW, van Niekerk DJ, Ceballos K, Franco EL, Coldman AJ, Ogilvie GS, Krajden M. Comparison of the Roche cobas(R) 4800 and Digene Hybrid Capture(R) 2 HPV tests for primary cervical cancer screening in the HPV FOCAL trial. *BMC Cancer.* 2015;15(1):968. DOI:10.1186/s12885-015-1959-5.
- [78] Hologic. Cervista HPV HR, Instructions For Use. 2011;Ref. 92-011 PRD-015620 (Part number 15-3053 Revision 103).
- [79] Guo M, Khanna A, Feng J, Patel S, Zhang W, Gong Y, Huo L, Staerckel G. Analytical performance of cervista HPV 16/18 in SurePath pap specimens. *Diagn Cytopathol.* 2015;43(4):301–6. DOI:10.1002/dc.23221.

- [80] Ejegod DM, Rebolj M, Bonde J. Comparison of analytical and clinical performance of CLART HPV2 genotyping assay to Linear Array and Hybrid Capture 2: a split-sample study. *BMC Cancer*. 2015;15:216. DOI:10.1186/s12885-015-1223-z.
- [81] Orlando G, Fasolo M, Mazza F, Ricci E, Esposito S, Frati E, Zuccotti GV, Cetin I, Gramegna M, Rizzardini G, Tanzi E. Risk of cervical HPV infection and prevalence of vaccine-type and other high-risk HPV types among sexually active teens and young women (13–26 years) enrolled in the VALHIDATE study. *Hum Vaccin Immunother*. 2014;10(4):986–94.
- [82] Snijders PJ, Meijer CJ, Walboomers JM. Degenerate primers based on highly conserved regions of amino acid sequence in papillomaviruses can be used in a generalized polymerase chain reaction to detect productive human papillomavirus infection. *J Gen Virol*. 1991;72(Pt 11):2781–6. DOI:10.1099/0022-1317-72-11-2781.
- [83] Ozaki S, Kato K, Abe Y, Hara H, Kubota H, Kubushiro K, Kawahara E, Inoue M. Analytical performance of newly developed multiplex human papillomavirus genotyping assay using Luminex xMAP technology (Mebgen HPV Kit). *J Virol Methods*. 2014;204:73–80. DOI:10.1016/j.jviromet.2014.04.010.
- [84] Schalasta G, Rosenthal T, Grothe M. Roche AMPLICOR human papilloma virus (HPV) and LINEAR ARRAY HPV tests will profit from automated DNA extraction. *Clin Lab*. 2007;53(3–4):131–3.
- [85] van Hamont D, van Ham MA, Bakkers JM, Massuger LF, Melchers WJ. Evaluation of the SPF10-INNO LiPA human papillomavirus (HPV) genotyping test and the roche linear array HPV genotyping test. *J Clin Microbiol*. 2006;44(9):3122–9. DOI:10.1128/JCM.00517-06.
- [86] Depuydt CE, Benoy IH, Beert JF, Criel AM, Bogers JJ, Arbyn M. Clinical validation of a type-specific real-time quantitative human papillomavirus PCR against the performance of hybrid capture 2 for the purpose of cervical cancer screening. *J Clin Microbiol*. 2012;50(12):4073–7. DOI:10.1128/JCM.01231-12.
- [87] Castle PE, Smith KM, Davis TE, Schmeler KM, Ferris DG, Savage AH, Gray JE, Stoler MH, Wright TC, Jr., Ferenczy A, Einstein MH. Reliability of the Xpert HPV assay to detect high-risk human papillomavirus DNA in a colposcopy referral population. *Am J Clin Pathol*. 2015;143(1):126–33. DOI:10.1309/ajcp4q0nsdhwizgu.
- [88] Mesher D, Szarewski A, Cadman L, Austin J, Ashdown-Barr L, Ho L, Terry G, Young M, Stoler M, Bergeron C, McCarthy J, Wright C, Liddle S, Soutter WP, Lyons D, Cuzick J. Comparison of human papillomavirus testing strategies for triage of women referred with low-grade cytological abnormalities. *Eur J Cancer*. 2013;49:2179–86. DOI:10.1016/j.ejca.2013.01.018.
- [89] Rebolj M, Lynge E, Ejegod D, Preisler S, Rygaard C, Bonde J. Comparison of three human papillomavirus DNA assays and one mRNA assay in women with abnormal cytology. *Gynecol Oncol*. 2014;135(3):474–80. DOI:10.1016/j.ygyno.2014.10.014.

- [90] Heideman DA, Hesselink AT, van Kemenade FJ, Iftner T, Berkhof J, Topal F, Agard D, Meijer CJ, Snijders PJ. The Aptima HPV assay fulfills the cross-sectional clinical and reproducibility criteria of international guidelines for human papillomavirus test requirements for cervical screening. *J Clin Microbiol.* 2013;51(11):3653–7. DOI:10.1128/jcm.01517-13.
- [91] Castle PE, Eaton B, Reid J, Getman D, Dockter J. Comparison of human papillomavirus detection by Aptima HPV and cobas HPV tests in a population of women referred for colposcopy following detection of atypical squamous cells of undetermined significance by Pap cytology. *J Clin Microbiol.* 2015;53(4):1277–81. DOI:10.1128/jcm.03558-14.
- [92] Hesselink AT, Meijer CJ, Poljak M, Berkhof J, van Kemenade FJ, van der Salm ML, Bogaarts M, Snijders PJ, Heideman DA. Clinical validation of the Abbott RealTime High-Risk HPV assay according to the guidelines for human papillomavirus DNA test requirements for cervical screening. *J Clin Microbiol.* 2013;51(7):2409–10. DOI:10.1128/jcm.00633-13.
- [93] Abbott. Abbott RealTime High-Risk HPV, Instructions For Use. 2010;Ref. 2N09 49-2028/R3 B2N090.
- [94] Greiner-Bio-One. PapilloCheck® high-risk –Instructions For Use. Revision: BQ-065-01;2011.
- [95] Castle PE, Gutierrez EC, Leitch SV, Maus CE, McMillian RA, Nussbaumer WA, Vaughan LM, Wheeler CM, Gravitt PE, Schiffman M. Evaluation of a new DNA test for detection of carcinogenic human papillomavirus. *J Clin Microbiol.* 2011;49(8):3029–32. DOI:10.1128/JCM.00422-11.
- [96] Schiffman M, Vaughan LM, Raine-Bennett TR, Castle PE, Katki HA, Gage JC, Fetterman B, Befano B, Wentzensen N. A study of HPV typing for the management of HPV-positive ASC-US cervical cytologic results. *Gynecol Oncol.* 2015;138(3):573–8. DOI: 10.1016/j.ygyno.2015.06.040.
- [97] Wright TC, Jr., Stoler MH, Behrens CM, Apple R, Derion T, Wright TL. The ATHENA human papillomavirus study: design, methods, and baseline results. *Am J Obstet Gynecol.* 2012;206(1):46 e1–11. DOI:10.1016/j.ajog.2011.07.024.
- [98] Saslow D, Solomon D, Lawson HW, Killackey M, Kulasingam SL, Cain J, Garcia FA, Moriarty AT, Waxman AG, Wilbur DC, Wentzensen N, Downs LS, Jr., Spitzer M, Moscicki AB, Franco EL, Stoler MH, Schiffman M, Castle PE, Myers ER, American Cancer S, American Society for C, Cervical P, American Society for Clinical P. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. *Am J Clin Pathol.* 2012;137(4):516–42. DOI:10.1309/AJCPTGD94EVR SJCG.

- [99] Carcopino X, Henry M, Mancini J, Giusiano S, Boubli L, Olive D, Tamalet C. Significance of HPV 16 and 18 viral load quantitation in women referred for colposcopy. *J Med Virol*. 2012;84(2):306–13. DOI:10.1002/jmv.23190.
- [100] Sun Z, Zhang R, Liu Z, Liu C, Li X, Zhou W, Yang L, Ruan Q, Zhang X. Development of a fluorescence-based multiplex genotyping method for simultaneous determination of human papillomavirus infections and viral loads. *BMC Cancer*. 2015;15:860. DOI: 10.1186/s12885-015-1874-9.
- [101] Wentzensen N, Gravitt PE, Long R, Schiffman M, Dunn ST, Carreon JD, Allen RA, Gunja M, Zuna RE, Sherman ME, Gold MA, Walker JL, Wang SS. Human papilloma-virus load measured by Linear Array correlates with quantitative PCR in cervical cytology specimens. *J Clin Microbiol*. 2012;50(5):1564–70. DOI:10.1128/jcm.06240-11.
- [102] Marongiu L, Godi A, Parry JV, Beddows S. Human Papillomavirus 16, 18, 31 and 45 viral load, integration and methylation status stratified by cervical disease stage. *BMC Cancer*. 2014;14:384. DOI:10.1186/1471-2407-14-384.
- [103] Baron C, Henry M, Tamalet C, Villeret J, Richet H, Carcopino X. Relationship between HPV 16, 18, 31, 33, 45 DNA detection and quantitation and E6/E7 mRNA detection among a series of cervical specimens with various degrees of histological lesions. *J Med Virol*. 2015;87(8):1389–96. DOI:10.1002/jmv.24157.
- [104] van Doorn LJ, Molijn A, Kleter B, Quint W, Colau B. Highly effective detection of human papillomavirus 16 and 18 DNA by a testing algorithm combining broad-spectrum and type-specific PCR. *J Clin Microbiol*. 2006;44(9):3292–8. DOI:10.1128/jcm.00539-06.
- [105] van Alewijk D, Kleter B, Vent M, Delroisse JM, de Koning M, van Doorn LJ, Quint W, Colau B. A human papilloma virus testing algorithm comprising a combination of the L1 broad-spectrum SPF10 PCR assay and a novel E6 high-risk multiplex type-specific genotyping PCR assay. *J Clin Microbiol*. 2013;51(4):1171–8. DOI:10.1128/jcm.02831-12.
- [106] Mori S, Nakao S, Kukimoto I, Kusumoto-Matsuo R, Kondo K, Kanda T. Biased amplification of human papillomavirus DNA in specimens containing multiple human papillomavirus types by PCR with consensus primers. *Cancer Sci*. 2011;102(6):1223–7. DOI:10.1111/j.1349-7006.2011.01922.x.
- [107] Vaccarella S, Franceschi S, Snijders PJ, Herrero R, Meijer CJ, Plummer M. Concurrent infection with multiple human papillomavirus types: pooled analysis of the IARC HPV Prevalence Surveys. *Cancer Epidemiol Biomarkers Prev*. 2010;19(2):503–10. DOI: 10.1158/1055-9965.epi-09-0983.
- [108] Struyf F, Colau B, Wheeler CM, Naud P, Garland S, Quint W, Chow SN, Salmeron J, Lehtinen M, Del Rosario-Raymundo MR, Paavonen J, Teixeira JC, Germar MJ, Peters K, Skinner SR, Limson G, Castellsague X, Poppe WA, Ramjattan B, Klein TD, Schwarz TF, Chatterjee A, Tjalma WA, Diaz-Mitoma F, Lewis DJ, Harper DM, Molijn A, van Doorn LJ, David MP, Dubin G. Post hoc analysis of the PATRICIA randomized trial of the efficacy of human papillomavirus type 16 (HPV-16)/HPV-18 AS04-adjuvanted

vaccine against incident and persistent infection with nonvaccine oncogenic HPV types using an alternative multiplex type-specific PCR assay for HPV DNA. *Clin Vaccin Immunol.* 2015;22(2):235–44. DOI:10.1128/cvi.00457-14.

- [109] Cornall AM, Phillips S, Cummins E, Garland SM, Tabrizi SN. *In vitro* assessment of the effect of vaccine-targeted human papillomavirus (HPV) depletion on detection of non-vaccine HPV types: implications for post-vaccine surveillance studies. *J Virol Methods.* 2015;214:10–4. DOI:10.1016/j.jviromet.2014.12.007.
- [110] Barzon L, Militello V, Lavezzo E, Franchin E, Peta E, Squarzon L, Trevisan M, Pagni S, Dal Bello F, Toppo S, Palu G. Human papillomavirus genotyping by 454 next generation sequencing technology. *J Clin Virol.* 2011;52(2):93–7. DOI:10.1016/j.jcv.2011.07.006.
- [111] Eklund C, Wallin K-L, Forslund O, Dillner J. Continued global improvement in HPV DNA genotyping: The 2014 HPV LabNet International Proficiency Study. International Papillomavirus Conference, Lisbon, Portugal, 17–21 September 2015.
- [112] ICO. Information Centre on HPV and Cancer (Institut Català d'Oncologia). 2016; Available from: <http://www.hpvcentre.net/> (accessed: 2016-01-10).
- [113] Huh WK, Ault KA, Chelmow D, Davey DD, Goulart RA, Garcia FA, Kinney WK, Massad LS, Mayeaux EJ, Saslow D, Schiffman M, Wentzensen N, Lawson HW, Einstein MH. Use of primary high-risk human papillomavirus testing for cervical cancer screening: Interim clinical guidance. *Gynecol Oncol.* 2015;136(2):178–82. DOI:10.1016/j.ygyno.2014.12.022.
- [114] Fontaine D, Narine N, Naugler C. Unsatisfactory rates vary between cervical cytology samples prepared using ThinPrep and SurePath platforms: a review and meta-analysis. *BMJ Open.* 2012;2(2):e000847. DOI:10.1136/bmjopen-2012-000847.
- [115] Kenyon S, Sweeney BJ, Happel J, Marchilli GE, Weinstein B, Schneider D. Comparison of BD Surepath and ThinPrep Pap systems in the processing of mucus-rich specimens. *Cancer Cytopathol.* 2010;118(5):244–9. DOI:10.1002/cncy.20096.
- [116] Chen G, Kobayashi L, Nazarenko I. Effect of sample aliquot size on the limit of detection and reproducibility of clinical assays. *Clin Chem.* 2007;53(11):1962–5. DOI:10.1373/clinchem.2007.089854.
- [117] Becton-Dickinson. BD Onclarity™ HPV Assay, CE Mark Instructions for use, Ref. 442946, 8089899(02). 2014.
- [118] Power Y, Bowes C. Reprocessing bloodstained ThinPrep Papanicolaou cervical cytology samples using glacial acetic acid increases the satisfactory rate in previously unsatisfactory smears. *Br J Biomed Sci.* 2013;70(3):130.
- [119] Depuydt CE, Jonckheere J, Berth M, Salembier GM, Vereecken AJ, Bogers JJ. Serial type-specific human papillomavirus (HPV) load measurement allows differentiation between regressing cervical lesions and serial virion productive transient infections. *Cancer Med.* 2015;4(8):1294–302. DOI:10.1002/cam4.473.

- [120] Stoler MH, Ronnett BM, Joste NE, Hunt WC, Cuzick J, Wheeler CM. The interpretive variability of cervical biopsies and its relationship to HPV status. *Am J Surg Pathol*. 2015;39(6):729–36. DOI:10.1097/pas.0000000000000381.
- [121] Clinton LK, Miyazaki K, Ayabe A, Davis J, Tauchi-Nishi P, Shimizu D. The LAST guidelines in clinical practice: implementing recommendations for p16 use. *Am J Clin Pathol*. 2015;144(6):844–9. DOI:10.1309/ajcpuxlp7xd8oqyy.
- [122] Badaracco G, Venuti A, Sedati A, Marcante ML. HPV16 and HPV18 in genital tumors: significantly different levels of viral integration and correlation to tumor invasiveness. *J Med Virol*. 2002;67(4):574–82. DOI:10.1002/jmv.10141.
- [123] Cullen AP, Reid R, Campion M, Lorincz AT. Analysis of the physical state of different human papillomavirus DNAs in intraepithelial and invasive cervical neoplasm. *J Virol*. 1991;65(2):606–12.
- [124] Hu Z, Zhu D, Wang W, Li W, Jia W, Zeng X, Ding W, Yu L, Wang X, Wang L, Shen H, Zhang C, Liu H, Liu X, Zhao Y, Fang X, Li S, Chen W, Tang T, Fu A, Wang Z, Chen G, Gao Q, Li S, Xi L, Wang C, Liao S, Ma X, Wu P, Li K, Wang S, Zhou J, Wang J, Xu X, Wang H, Ma D. Genome-wide profiling of HPV integration in cervical cancer identifies clustered genomic hot spots and a potential microhomology-mediated integration mechanism. *Nat Genet*. 2015;47(2):158–63. DOI:10.1038/ng.3178.
- [125] Liu Y, Lu Z, Xu R, Ke Y. Comprehensive mapping of the human papillomavirus (HPV) DNA integration sites in cervical carcinomas by HPV capture technology. *Oncotarget*. 2016;2;7(5):5852–64. DOI:10.18632/oncotarget.6809.
- [126] Cullen M, Boland JF, Schiffman M, Zhang X, Wentzensen N, Yang Q, Chen Z, Yu K, Mitchell J, Roberson D, Bass S, Burdette L, Machado M, Ravichandran S, Luke B, Machiela MJ, Andersen M, Osentoski M, Laptewicz M, Wacholder S, Feldman A, Raine-Bennett T, Lorey T, Castle PE, Yeager M, Burk RD, Mirabello L. Deep sequencing of HPV16 genomes: A new high-throughput tool for exploring the carcinogenicity and natural history of HPV16 infection. *Papillomavirus Res*. 2015;1:3–11. DOI:10.1016/j.pvr.2015.05.004.
- [127] Chandrani P, Kulkarni V, Iyer P, Upadhyay P, Chaubal R, Das P, Mulherkar R, Singh R, Dutt A. NGS-based approach to determine the presence of HPV and their sites of integration in human cancer genome. *Br J Cancer*. 2015;112(12):1958–65. DOI:10.1038/bjc.2015.121.
- [128] Tjalma WA, Depuydt CE. Cervical cancer screening: which HPV test should be used- L1 or E6/E7? *Eur J Obstet Gynecol Reprod Biol*. 2013;170(1):45–6. DOI:10.1016/j.ejogrb.2013.06.027.
- [129] Tjalma WA, Depuydt CE. Cervical atypical glandular cells and false negative HPV testing: a dramatic reality of the wrong test at the right place. *Eur J Gynaecol Oncol*. 2014;35(2):117–20.

- [130] Morris BJ. Cervical human papillomavirus screening by PCR: advantages of targeting the E6/E7 region. *Clin Chem Lab Med.* 2005;43(11):1171–7. DOI:10.1515/CCLM.2005.203.
- [131] Einstein MH, Cruz Y, El-Awady MK, Popescu NC, DiPaolo JA, van Ranst M, Kadish AS, Romney S, Runowicz CD, Burk RD. Utilization of the human genome sequence localizes human papillomavirus type 16 DNA integrated into the TNFAIP2 gene in a fatal cervical cancer from a 39-year-old woman. *Clin Cancer Res.* 2002;8(2):549–54.
- [132] Karlsen F, Kalantari M, Jenkins A, Pettersen E, Kristensen G, Holm R, Johansson B, Hagmar B. Use of multiple PCR primer sets for optimal detection of human papillomavirus. *J Clin Microbiol.* 1996;34(9):2095–100.
- [133] Hagmar B, Johansson B, Kalantari M, Petersson Z, Skyldberg B, Walaas L. The incidence of HPV in a Swedish series of invasive cervical carcinoma. *Med Oncol Tumor Pharmacother.* 1992;9(3):113–7.
- [134] Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Munoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol.* 1999;189(1):12–9. DOI:10.1002/(SICI)1096-9896(199909)189:1<12::AID-PATH431>3.0.CO;2-F.
- [135] Hopenhayn C, Christian A, Christian WJ, Watson M, Unger ER, Lynch CF, Peters ES, Wilkinson EJ, Huang Y, Copeland G, Cozen W, Saber MS, Goodman MT, Hernandez BY, Steinau M, Lyu C, Tucker TT, Saraiya M. Prevalence of human papillomavirus types in invasive cervical cancers from 7 US cancer registries before vaccine introduction. *J Low Genit Tract Dis.* 2014;18(2):182–9. DOI:10.1097/LGT.0b013e3182a577c7.
- [136] Molijn A, Jenkins D, Chen W, Zhang X, Pirog E, Enqi W, Liu B, Schmidt J, Cui J, Qiao Y, Quint W. The complex relationship between human papillomavirus and cervical adenocarcinoma. *Int J Cancer.* 2015;15;138(2):409-16. DOI:10.1002/ijc.29722.
- [137] Holl K, Nowakowski AM, Powell N, McCluggage WG, Pirog EC, Collas De Souza S, Tjalma WA, Rosenlund M, Fiander A, Castro Sanchez M, Damaskou V, Joura EA, Kirschner B, Koiss R, O'Leary J, Quint W, Reich O, Torne A, Wells M, Rob L, Kolomiets L, Molijn A, Savicheva A, Shipitsyna E, Rosillon D, Jenkins D. Human papillomavirus prevalence and type-distribution in cervical glandular neoplasias: results from a European multinational epidemiological study. *Int J Cancer.* 2015;137(12):2858–68. DOI:10.1002/ijc.29651.
- [138] Adegoke O, Kulasingam S, Virnig B. Cervical cancer trends in the United States: a 35-year population-based analysis. *J.Women's Health (2002).* 2012;21(10):1031–7. DOI: 10.1089/jwh.2011.3385.
- [139] Mbatani N, Adams T, Wijk LV, Behrens C, Tam T, Wright T, Jr., Stoler M, Denny L. Performance of an Human Papillomavirus Test in Samples From Women With Histopathologically Confirmed Invasive Cervical Cancer. *J Low Genit Tract Dis.* 2016;20(2):151-3. DOI:10.1097/lgt.0000000000000183.

- [140] Cuzick J, Clavel C, Petry KU, Meijer CJ, Hoyer H, Ratnam S, Szarewski A, Birembaut P, Kulasingam S, Sasieni P, Iftner T. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int J Cancer*. 2006;119(5):1095-101. DOI:10.1002/ijc.21955.
- [141] Pileggi C, Flotta D, Bianco A, Nobile CG, Pavia M. Is HPV DNA testing specificity comparable to that of cytological testing in primary cervical cancer screening? Results of a meta-analysis of randomized controlled trials. *Int J Cancer*. 2014;135(1):166–77. DOI:10.1002/ijc.28640.
- [142] Ronco G, Segnan N, Giorgi-Rossi P, Zappa M, Casadei GP, Carozzi F, Dalla Palma P, Del Mistro A, Folicaldi S, Gillio-Tos A, Nardo G, Naldoni C, Schincaglia P, Zorzi M, Confortini M, Cuzick J. Human papillomavirus testing and liquid-based cytology: results at recruitment from the new technologies for cervical cancer randomized controlled trial. *J Natl Cancer Inst*. 2006;98(11):765–74. DOI:10.1093/jnci/djj209.
- [143] 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. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. *Lancet Oncol*. 2010;11(3):249–57. DOI:10.1016/s1470-2045(09)70360-2.
- [144] Kitchener HC, Almonte M, Gilham C, Dowie R, Stoykova B, Sargent A, Roberts C, Desai M, Peto J. ARTISTIC: a randomised trial of human papillomavirus (HPV) testing in primary cervical screening. *Health Technol Assess*. 2009;13(51):1–150, iii–iv. DOI:10.3310/hta13510.
- [145] Leinonen M, Nieminen P, Kotaniemi-Talonen L, Malila N, Tarkkanen J, Laurila P, Anttila A. Age-specific evaluation of primary human papillomavirus screening vs conventional cytology in a randomized setting. *J Natl Cancer Inst*. 2009;101(23):1612–23. DOI:10.1093/jnci/djp367.
- [146] Sankaranarayanan R, Nene BM, Shastri SS, Jayant K, Muwonge R, Budukh AM, Hingmire S, Malvi SG, Thorat R, Kothari A, Chinoy R, Kelkar R, Kane S, Desai S, Keskar VR, Rajeshwarkar R, Panse N, Dinshaw KA. HPV screening for cervical cancer in rural India. *N Engl J Med*. 2009;360(14):1385–94. DOI:10.1056/NEJMoa0808516.
- [147] Mayrand MH, Franco EL. Integrating novel primary- and secondary-prevention strategies: the next challenge for cervical cancer control. *Future Oncol*. 2010;6(11):1725–33. DOI:10.2217/fon.10.141.
- [148] Rijkaart DC, Berkhof J, Rozendaal L, van Kemenade FJ, Bulkman NW, Heideman DA, Kenter GG, Cuzick J, Snijders PJ, Meijer CJ. Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial. *Lancet Oncol*. 2012;13(1):78–88. DOI:10.1016/s1470-2045(11)70296-0.

- [149] Blatt AJ, Kennedy R, Luff RD, Austin RM, Rabin DS. Comparison of cervical cancer screening results among 256,648 women in multiple clinical practices. *Cancer Cytopathol.* 2015;123(5):282–8. DOI:10.1002/cncy.21544.
- [150] Castle PE. Comparison of cervical cancer screening results among 256,648 women in multiple clinical practices. *Cancer Cytopathol.* 2015;123(9):566. DOI:10.1002/cncy.21572.
- [151] Carozzi FM, Del Mistro A, Cuschieri K, Frayle H, Sani C, Burroni E. HPV testing for primary cervical screening: Laboratory issues and evolving requirements for robust quality assurance. *J Clin Virol.* 2016;76 Suppl 1:S228. DOI:10.1016/j.jcv.2015.10.025.
- [152] El-Zein M, Richardson L, Franco EL. Cervical cancer screening of HPV vaccinated populations: cytology, molecular testing, both or none. *J Clin Virol.* 2016;76 Suppl 1:S62-8. DOI:10.1016/j.jcv.2015.11.020.
- [153] Wright TC, Stoler MH, Behrens CM, Sharma A, Zhang G, Wright TL. Primary cervical cancer screening with human papillomavirus: end of study results from the ATHENA study using HPV as the first-line screening test. *Gynecol Oncol.* 2015;136(2):189–97. DOI:10.1016/j.ygyno.2014.11.076.
- [154] Stoler MH, Austin RM, Zhao C. Point-counterpoint: cervical cancer screening should be done by primary human papillomavirus testing with genotyping and reflex cytology for women over the age of 25 years. *J Clin Microbiol.* 2015;53(9):2798–804. DOI:10.1128/jcm.01087-15.
- [155] Uijterwaal MH, Polman NJ, Van Kemenade FJ, Van Den Haselkamp S, Witte BI, Rijkaart D, Berkhof J, Snijders PJ, Meijer CJ. Five-year cervical (pre)cancer risk of women screened by HPV and cytology testing. *Cancer Prev Res (Phila).* 2015;8(6):502–8. DOI: 10.1158/1940-6207.capr-14-0409.
- [156] Gage JC, Schiffman M, Katki HA, Castle PE, Fetterman B, Wentzensen N, Poitras NE, Lorey T, Cheung LC, Kinney WK. Reassurance against future risk of precancer and cancer conferred by a negative human papillomavirus test. *J Natl Cancer Inst.* 2014;106(8):1-4. DOI:10.1093/jnci/dju153.
- [157] Wright TC, Jr., Stoler MH, Behrens CM, Sharma A, Sharma K, Apple R. Interlaboratory variation in the performance of liquid-based cytology: insights from the ATHENA trial. *Int J Cancer.* 2014;134(8):1835–43. DOI:10.1002/ijc.28514.
- [158] Rijkaart DC, Berkhof J, van Kemenade FJ, Coupe VM, Hesselink AT, Rozendaal L, Heideman DA, Verheijen RH, Bulk S, Verweij WM, Snijders PJ, Meijer CJ. Evaluation of 14 triage strategies for HPV DNA-positive women in population-based cervical screening. *Int J Cancer.* 2012;130(3):602–10. DOI:10.1002/ijc.26056.
- [159] van Ballegooijen M, van den Akker-van Marle E, Patnick J, Lynge E, Arbyn M, Anttila A, Ronco G, Dik J, Habbema F. Overview of important cervical cancer screening process

- values in European Union (EU) countries, and tentative predictions of the corresponding effectiveness and cost-effectiveness. *Eur J Cancer*. 2000;36(17):2177–88.
- [160] Peto J, Gilham C, Fletcher O, Matthews FE. The cervical cancer epidemic that screening has prevented in the UK. *Lancet*. 2004;364(9430):249–56. DOI:10.1016/s0140-6736(04)16674-9.
- [161] Kinney W, Sung HY, Kearney KA, Miller M, Sawaya G, Hiatt RA. Missed opportunities for cervical cancer screening of HMO members developing invasive cervical cancer (ICC). *Gynecol Oncol*. 1998;71(3):428–30. DOI:10.1006/gyno.1998.5135.
- [162] Dugue PA, Lynge E, Rebolj M. Mortality of non-participants in cervical screening: register-based cohort study. *Int J Cancer*. 2014;134(11):2674–82. DOI:10.1002/ijc.28586.
- [163] Virtanen A, Nieminen P, Malila N, Luostarinen T, Anttila A. Self-sampling and reminder letters increase participation in the Finnish cervical cancer screening programme. *Duodecim*. 2013;129(16):1709–17.
- [164] Broberg G, Jonasson JM, Ellis J, Gyrd-Hansen D, Anjemark B, Glantz A, Soderberg L, Ryd ML, Holtenman M, Milsom I, Strander B. Increasing participation in cervical cancer screening: telephone contact with long-term non-attendees in Sweden. Results from RACOMIP, a randomized controlled trial. *Int J Cancer*. 2013;133(1):164–71. DOI: 10.1002/ijc.27985.
- [165] Racey CS, Gesink DC, Burchell AN, Trivers S, Wong T, Rebbapragada A. Randomized intervention of self-collected sampling for human papillomavirus testing in underscreened rural women: uptake of screening and acceptability. *Journal of Women's Health*. November 2015, ahead of print. doi:10.1089/jwh.2015.5348.
- [166] Rychla L. New self-sampling device for cervical cancer screening tested in Denmark. *Copenhagen Post*(2015) December 8. Available from CPH POST ONLINE: <http://cphpost.dk/news/new-self-sampling-device-for-cervical-cancer-screening-tested-in-denmark.html>
- [167] ICO. HPV Information Center (Institut Català d'Oncologia): Human Papillomavirus and Related Diseases Report –Denmark. Available from: <http://www.hpvcentre.net/statistics/reports/DNKpdf> (accessed: 2015-12-30. 2015).
- [168] Verdoodt F, Jentschke M, Hillemanns P, Racey CS, Snijders PJ, Arbyn M. Reaching women who do not participate in the regular cervical cancer screening programme by offering self-sampling kits: a systematic review and meta-analysis of randomised trials. *Eur J Cancer*. 2015;51(16):2375–85. DOI:10.1016/j.ejca.2015.07.006.
- [169] Othman NH, Mohamad Zaki FH. Self-collection tools for routine cervical cancer screening: a review. *Asian Pac J Cancer Prev*. 2014;15(20):8563–9.
- [170] Snijders PJ, Verhoef VM, Arbyn M, Ogilvie G, Minozzi S, Banzi R, van Kemenade FJ, Heideman DA, Meijer CJ. High-risk HPV testing on self-sampled versus clinician-collected specimens: a review on the clinical accuracy and impact on population

attendance in cervical cancer screening. *Int J Cancer*. 2013;132(10):2223–36. DOI: 10.1002/ijc.27790.

- [171] Petignat P, Faltin DL, Bruchim I, Tramer MR, Franco EL, Coutlee F. Are self-collected samples comparable to physician-collected cervical specimens for human papilloma-virus DNA testing? A systematic review and meta-analysis. *Gynecol Oncol*. 2007;105(2):530–5. DOI:10.1016/j.ygyno.2007.01.023.
- [172] 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 papillo-mavirus testing on self-collected versus clinician-collected samples: a meta-analysis. *Lancet Oncol*. 2014;15(2):172–83. DOI:10.1016/s1470-2045(13)70570-9.
- [173] Chernesky M, Jang D, Gilchrist J, Elit L, Lytwyn A, Smieja M, Dockter J, Getman D, Reid J, Hill C. Evaluation of a new APTIMA specimen collection and transportation kit for high-risk human papillomavirus E6/E7 messenger RNA in cervical and vaginal samples. *Sex Transm Dis*. 2014;41(6):365–8. DOI:10.1097/olq.0000000000000125.
- [174] Eperon I, Vassilakos P, Navarria I, Menoud PA, Gauthier A, Pache JC, Boulvain M, Untiet S, Petignat P. Randomized comparison of vaginal self-sampling by standard vs. dry swabs for human papillomavirus testing. *BMC Cancer*. 2013;13:353. DOI: 10.1186/1471-2407-13-353.
- [175] Lorenzi AT, Fregnani JH, Possati-Resende JC, Neto CS, Villa LL, Longatto-Filho A. Self-collection for high-risk HPV detection in Brazilian women using the careHPV test. *Gynecol Oncol*. 2013;131(1):131–4. DOI:10.1016/j.ygyno.2013.07.092.
- [176] Forslund O, Hansson BG, Rymark P, Bjerre B. Human papillomavirus DNA in urine samples compared with that in simultaneously collected urethra and cervix samples. *J Clin Microbiol*. 1993;31(8):1975–9.
- [177] Geddy PM, Wells M, Lacey CJ. Lack of detection of human papillomavirus DNA in male urine samples. *Genitourin Med*. 1993;69(4):276–9.
- [178] Bianchi S, Frati ER, Panatto D, Martinelli M, Amicizia D, Zotti CM, Martinese M, Bonanni P, Boccacini S, Coppola RC, Masia G, Meloni A, Castiglia P, Piana A, Gasparini R, Tanzi E. Detection and genotyping of human papillomavirus in urine samples from unvaccinated male and female adolescents in Italy. *PLoS One*. 2013;8(11):e79719. DOI: 10.1371/journal.pone.0079719.
- [179] Enerly E, Olofsson C, Nygard M. Monitoring human papillomavirus prevalence in urine samples: a review. *Clin Epidemiol*. 2013;5:67–79. DOI:10.2147/clep.s39799.
- [180] Vorsters A, Van Keer S, Van Damme P. The use of urine in the follow-up of HPV vaccine trials. *Hum Vaccin Immunother*. 2015;11(2):350–2. DOI:10.4161/21645515.2014.995058.
- [181] Vorsters A, Van Damme P, Clifford G. Urine testing for HPV: rationale for using first void. *BMJ*. 2014;349:g6252. DOI:10.1136/bmj.g6252.

- [182] Pathak N, Dodds J, Zamora J, Khan K. Accuracy of urinary human papillomavirus testing for presence of cervical HPV: systematic review and meta-analysis. *BMJ*. 2014;349:g5264. DOI:10.1136/bmj.g5264.
- [183] Vorsters A, Van den Bergh J, Micalessi I, Biesmans S, Bogers J, Hens A, De Coster I, Ieven M, Van Damme P. Optimization of HPV DNA detection in urine by improving collection, storage, and extraction. *Eur J Clin Microbiol Infect Dis*. 2014;33(11):2005–14. DOI:10.1007/s10096-014-2147-2.
- [184] Vorsters A, Micalessi I, Bilcke J, Ieven M, Bogers J, Van Damme P. Detection of human papillomavirus DNA in urine. A review of the literature. *Eur J Clin Microbiol Infect Dis*. 2012;31(5):627–40. DOI:10.1007/s10096-011-1358-z.
- [185] Senkomago V, Des Marais AC, Rahangdale L, Vibat CR, Erlander MG, Smith JS. Comparison of urine specimen collection times and testing fractions for the detection of high-risk human papillomavirus and high-grade cervical precancer. *J Clin Virol*. 2016;74:26–31. DOI:10.1016/j.jcv.2015.11.005.
- [186] Santiago-Rodriguez TM, Ly M, Bonilla N, Pride DT. The human urine virome in association with urinary tract infections. *FrontMicrobiol*. 2015;6:14. DOI:10.3389/fmicb.2015.00014.
- [187] Bryzgunova OE, Laktionov PP. Extracellular nucleic acids in urine: sources, structure, diagnostic potential. *Acta Naturae*. 2015;7(3):48–54.
- [188] Vorsters A, Vankerckhoven V, Beyers K, Van Damme P, Long D, Schwab R, Gutierrez G, Nussbaumer W, Harris J, Vaughan L. Performance of an automated HPV genotyping assay using first void urine specimens. International Papillomavirus Conference, Lisbon, Portugal, 17–21 September 2015.
- [189] Zhang SH, Zhao SM, Zhao ZM, Li CT. Genotyping of urinary samples stored with EDTA for forensic applications. *Genetics Mol Research*. 2012;11(3):3007–12. DOI: 10.4238/2012.May.10.5.
- [190] Musante L, Tataruch D, Gu D, Liu X, Forsblom C, Groop PH, Holthofer H. Proteases and protease inhibitors of urinary extracellular vesicles in diabetic nephropathy. *J Diabetes Res*. 2015;2015:289734. DOI:10.1155/2015/289734.
- [191] Milde A, Haas-Rochholz H, Kaatsch HJ. Improved DNA typing of human urine by adding EDTA. *Int J Legal Med*. 1999;112(3):209–10.
- [192] Ducancelle A, Reiser J, Pivert A, Le Guillou-Guillemette H, Le Duc-Banaszuk AS, Lunel-Fabiani F. Home-based urinary HPV DNA testing in women who do not attend cervical cancer screening clinics. *J Infect*. 2015;71(3):377–84. DOI:10.1016/j.jinf.2015.05.001.
- [193] Thomsen LT, Frederiksen K, Munk C, Junge J, Iftner T, Kjaer SK. Long-term risk of cervical intraepithelial neoplasia grade 3 or worse according to high-risk human

papillomavirus genotype and semi-quantitative viral load among 33,288 women with normal cervical cytology. *Int J Cancer*. 2015;137(1):193–203. DOI:10.1002/ijc.29374.

- [194] Schiffman M, Wentzensen N, Wacholder S, Kinney W, Gage JC, Castle PE. Human papillomavirus testing in the prevention of cervical cancer. *J Natl Cancer Inst*. 2011;103(5):368–83. DOI:10.1093/jnci/djq562.
- [195] Cuzick J, Ho L, Terry G, Kleeman M, Giddings M, Austin J, Cadman L, Ashdown-Barr L, Costa MJ, Szarewski A. Individual detection of 14 high risk human papilloma virus genotypes by the PapType test for the prediction of high grade cervical lesions. *J Clin Virol*. 2014;60(1):44–9. DOI:10.1016/j.jcv.2014.02.002.
- [196] Joste NE, Ronnett BM, Hunt WC, Pearce A, Langsfeld E, Leete T, Jaramillo M, Stoler MH, Castle PE, Wheeler CM. Human papillomavirus genotype-specific prevalence across the continuum of cervical neoplasia and cancer. *Cancer Epidemiol Biomarkers Prev*. 2015;24(1):230–40. DOI:10.1158/1055-9965.epi-14-0775.
- [197] Monsonego J, Cox JT, Behrens C, Sandri M, Franco EL, Yap PS, Huh W. Prevalence of high-risk human papilloma virus genotypes and associated risk of cervical precancerous lesions in a large U.S. screening population: data from the ATHENA trial. *Gynecol Oncol*. 2015;137(1):47–54. DOI:10.1016/j.ygyno.2015.01.551.
- [198] de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, Tous S, Felix A, Bravo LE, Shin HR, Vallejos CS, de Ruiz PA, Lima MA, Guimera N, Clavero O, Alejo M, Llombart-Bosch A, Cheng-Yang C, Tatti SA, Kasamatsu E, Iljazovic E, Odida M, Prado R, Seoud M, Grce M, Usubutun A, Jain A, Suarez GA, Lombardi LE, Banjo A, Menendez C, Domingo EJ, Velasco J, Nessa A, Chichareon SC, Qiao YL, Lerma E, Garland SM, Sasagawa T, Ferrera A, Hammouda D, Mariani L, Pelayo A, Steiner I, Oliva E, Meijer CJ, Al-Jassar WF, Cruz E, Wright TC, Puras A, Llave CL, Tzardi M, Agorastos T, Garcia-Barriola V, Clavel C, Ordi J, Andujar M, Castellsague X, Sanchez GI, Nowakowski AM, Bornstein J, Munoz N, Bosch FX. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol*. 2010;11(11):1048–56. DOI:10.1016/S1470-2045(10)70230-8.
- [199] Joura EA, Ault KA, Bosch FX, Brown D, Cuzick J, Ferris D, Garland SM, Giuliano AR, Hernandez-Avila M, Huh W, Iversen OE, Kjaer SK, Luna J, Miller D, Monsonego J, Munoz N, Myers E, Paavonen J, Pitisuttithum P, Steben M, Wheeler CM, Perez G, Saah A, Luxembourg A, Sings HL, Velicer C. Attribution of 12 high-risk human papilloma-virus genotypes to infection and cervical disease. *Cancer Epidemiol Biomarkers Prev*. 2014;23(10):1997–2008. DOI:10.1158/1055-9965.epi-14-0410.
- [200] Katki HA, Schiffman M, Castle PE, Fetterman B, Poitras NE, Lorey T, Cheung LC, Raine-Bennett T, Gage JC, Kinney WK. Benchmarking CIN3+ risk as the basis for incorporating HPV and Pap cotesting into cervical screening and management guidelines. *J Low Genit Tract Dis*. 2013;17(5 Suppl 1):S28–35. DOI:10.1097/LGT.0b013e318285423c.

- [201] Joura EA, Giuliano AR, Iversen OE, Bouchard C, Mao C, Mehlsen J, Moreira ED, Jr., Ngan Y, Petersen LK, Lazcano-Ponce E, Pitisuttithum P, Restrepo JA, Stuart G, Woelber L, Yang YC, Cuzick J, Garland SM, Huh W, Kjaer SK, Bautista OM, Chan IS, Chen J, Gesser R, Moeller E, Ritter M, Vuocolo S, Luxembourg A. A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. *N Engl J Med*. 2015;372(8): 711–23. DOI:10.1056/NEJMoa1405044.
- [202] 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(14):1072–9. DOI:10.1093/jnci/dji187.
- [203] Larson H. The world must accept that the HPV vaccine is safe. *Nature*. 2015;528(7580): 9. DOI:10.1038/528009a.
- [204] Schiffman M, Wentzensen N. A Suggested Approach to Simplify and Improve Cervical Screening in the United States. *J Low Genit Tract Dis*. 2016;20(1):1–7. DOI:10.1097/lgt.0000000000000170.
- [205] Wentzensen N, Schiffman M, Palmer T, Arbyn M. Triage of HPV positive women in cervical cancer screening. *J Clin Virol*. 2015. DOI:10.1016/j.jcv.2015.11.015.
- [206] Wentzensen N, Schiffman M, Palmer T, Arbyn M. Triage of HPV positive women in cervical cancer screening. *J Clin Virol*. 2016;76 Suppl 1:S49-55. DOI:10.1016/j.jcv.2015.11.015.