Eur J Clin Microbiol Infect Dis (2012) 31:627–640
DOI 10.1007/s10096-011-1358-z

REVIEW

Detection of human papillomavirus DNA in urine. A review of the literature

A. Vorsters · I. Micalessi · J. Bilcke · M. Ieven · J. Bogers · P. Van Damme

Received: 6 May 2011 / Accepted: 26 June 2011 / Published online: 5 August 2011
© Springer-Verlag 2011

Abstract The detection of human papillomavirus (HPV) DNA in urine, a specimen easily obtained by a non-invasive self-sampling method, has been the subject of a considerable number of studies. This review provides an overview of 41 published studies; assesses how different methods and settings may contribute to the sometimes contradictory outcomes; and discusses the potential relevance of using urine samples in vaccine trials, disease surveillance, epidemiological studies, and specific settings of cervical cancer screening. Urine sampling, storage conditions, sample preparation, DNA extraction, and DNA amplification may all have an important impact on HPV DNA detection and the form of viral DNA that is detected. Possible trends in HPV DNA prevalence in urine

Authors and contributors Alex Vorsters is responsible for the conception and structure of the review, literature and patent search, data extraction, and interpretation of the data.
Isabel Micalessi provided a substantial contribution to the literature and patent search.
Joke Bilcke investigated and reported on the feasibility to perform a meta-analysis.
Isabel Micalessi, Joke Bilcke, Margareta Ieven, Johannes Bogers, and Pierre Van Damme provided a substantial contribution to the conception and design of the manuscript, interpretation of the data, and critical revision of the paper.

A. Vorsters (✉) · I. Micalessi · P. Van Damme
Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, University of Antwerp, Faculty of Medicine, Campus Drie Eiken,
Universiteitsplein 1,
2610 Antwerpen, Belgium
e-mail: alex.vorsters@ua.ac.be

I. Micalessi
e-mail: mariaisabel.micalessi@ua.ac.be

P. Van Damme
e-mail: pierre.vandamme@ua.ac.be

I. Micalessi · J. Bogers
Applied Molecular Biology Research, Laboratory of Cell Biology and Histology, University of Antwerp,
Groenenborgerlaan 171,
2020 Antwerpen, Belgium

J. Bogers
e-mail: john-paul.bogers@ua.ac.be

J. Bilcke
Centre for Health Economic Evaluations and Modelling Infectious Diseases, Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, University of Antwerp,
Universiteitsplein 1,
2610 Antwerpen, Belgium
e-mail: joke.bilcke@ua.ac.be

M. Ieven
Laboratory for Microbiology, Vaccine and Infectious Disease Institute, University Hospital Antwerp, University of Antwerp,
Wilrijkstraat 10,
2650 Edegem, Belgium
e-mail: greet.ieven@uza.be

could be inferred from the presence of risk factors or the diagnosis of cervical lesions. HPV DNA detection in urine is feasible and may become a useful tool but necessitates further improvement and standardization.

Introduction

The human papillomavirus (HPV) is a non-enveloped, double-stranded DNA virus infecting deeper layers of skin and the inner mucosal lining of organs. Of the over 100 known HPV types, 40 preferentially infect the stratified squamous epithelium of the mucosa and genital skin of the cervix, vagina, vulva, penis, and perianal areas. HPV infection is very common and, in most cases, transient or self-limiting. However, 10–20% of HPV-infected women develop a persistent infection and continue to shed HPV DNA from the genital tract for 24 or more months [1]. In many populations, over 50% (up to 80%) of sexually active women, and probably a similar percentage of men, are infected with HPV at some point in their lives [2, 3].

Cytology-based screening, enabling the early detection of cervical pre-cancerous or cancer lesions, was, for decades, the only tool to reduce the incidence and mortality of cervical cancer. Most women with abnormal cervical cytology results received surgical treatment, an expensive intervention with a potential negative impact on subsequent pregnancy outcome [4]. In the 1980s, the link between HPV and cervical cancer was established [5]. Currently, the use of HPV DNA testing to specifically identify cervical infections with high-risk or carcinogenic types is being evaluated in cervical screening programs.

Two HPV vaccines, a quadrivalent vaccine protecting against HPV types 6/11/16/18 and a bivalent one directed against HPV types 16/18, have become available for the primary prevention of HPV 16/18-related cervical cancer.

Virological endpoints, such as incident and persistent HPV infections, constitute important surrogates to monitor and investigate the impact and implementation of vaccination programs. It is essential to have established efficient methods for HPV detection and genotyping that yield high analytical sensitivities. HPV DNA assays, optimized to detect HPV DNA in cervical cytological samples, are less suitable in vaccine trials because detection thresholds may have been adjusted to identify only women with clinically relevant infections [6, 7].

Using urine as a sample for HPV DNA testing has a number of advantages. Urine can be included in a non-invasive self-sampling method. As such, this approach would permit frequent sampling and the sampling of large populations, e.g., to measure the impact of HPV vaccination programs. Moreover, urine sampling, unlike cervical sampling, is a method that is preferred and better accepted by women [8], which may lead to better population coverage in screening programs. Urine samples are acceptable in clinical practice and are used to screen for chlamydia and gonorrhea. HPV DNA detection in urine does not interfere with the natural history of the infection, whereas scraping cells from the cervix, vagina, or glands may create microlesions or induce an inflammatory reaction. Finally, in certain settings, it might avoid the need for a pelvic examination and/or a visit to the clinic.

However, DNA detection in urine also implies a number of challenges. First, it is a diluted sample and contains both known, such as urea and nitrites, and unknown polymerase chain reaction (PCR) inhibitors [9]. Further, the DNA can be deteriorated by contaminating bacteria and/or endonucleases [10, 11]. If HPV DNA detection in urine is to be used in a cervical cancer screening program, additional disadvantages are that the sample is not collected at the original disease site and only contains spontaneously exfoliated cells. Furthermore, the presence of HPV DNA may also be indicative of an HPV infection of the urinary tract or the lower genital tract.

Objectives

The objectives of this paper are: (1) to provide an overview of all published studies reporting on the detection of HPV DNA in urine; (2) to assess how the different settings and methods contribute to the contradictory outcomes; and (3) to discuss the potential relevance of using urine samples in the HPV field.

Materials and methods

A literature search was performed with Endnote X and X3 software on April 6th 2010 using the databases PubMed and Web of Science. Any field (PubMed) or the title/keywords/abstract fields (Web of Science) were checked for ‘HPV’, ‘detection’, and ‘urine’. All reference lists of identified papers were screened manually.

For each paper, the available data were extracted at the level of the reported cohorts or subgroups, i.e., same gender, similar lesions, or similar risk of being infected. The data were either entered as presented in the paper or calculated based on the provided information. Per identified cohort, the following details were entered in SPSS 16.0: number of subjects, gender, risk of HPV infection (normal=no specific indication; low=pre-adolescent or not sexually active; high=partner of HPV-positive patient, HIV-positive, attending a sexually transmitted infection [STI] clinic, or referred to a colposcopy clinic; mixed; or not relevant=in case of known HPV infection), lesions (not specified, none [none or atypical squamous cells of undetermined significance (ASCUS)], low grade [low-

grade squamous intraepithelial lesion (LSIL) or low-grade cervical intraepithelial neoplasia (CIN1)], high grade [high-grade squamous intraepithelial lesion (HSIL) or high-grade cervical intraepithelial neoplasia (CIN2-3)], cancer, or condylomata), type of DNA detection (conventional PCR, nested PCR, real-time PCR, or Hybrid Capture 2 [HC2, Qiagen (formally known as Digene)]), used primer sets, number of samples which were human DNA-positive, number samples tested for human DNA, number of HPV DNA-positive samples, number of samples tested for HPV DNA, original paper, and publication year.

Studies detecting human DNA in less than 85% of the urine samples or using less sensitive HPV PCR primers (GP5/GP6) were considered as substandard.

The possibility of statistically testing and quantifying relationships between HPV DNA detection in urine and the different subjects' groups and methodologies was investigated. Bubble graphs were created in order to explore the possible relationships between HPV DNA detection rates in urine and the different subjects' groups (Microsoft Office Excel 2007).

Results and discussion

Papers studied

Forty-four papers dealing with HPV DNA detection in urine were initially selected. The papers were published between 1991 and 2009. Specific studies and patent applications on the improvement of DNA detection in urine were also taken into account. Papers providing insufficient details on the methodology, HPV DNA detection rates, or using previously collected and published data were excluded [12–14]. Table 1 gives the details of the 41 papers considered in this review [8, 15–53]. Table 2 summarizes the various methods and settings.

Urine sampling

The majority of the papers did not mention which fraction or type of urine was analyzed. However, human DNA levels in urine are not constant during a void, as reported by Johnson et al. [54]. For most subjects, the first-void fractions contained higher amounts of DNA than the midstream or total void [54]. This study also confirmed that the urine of men, in general, contains less DNA than the urine of women, as reported by Brinkman et al. [18]. Midstream urine—i.e., the standard for the diagnosis of metabolic diseases and urinary tract infections—is preferred by some authors, since it is thought to contain less PCR inhibitors. Three studies using midstream urine did not report on human DNA detection [45, 46, 50].

Due to the high degree of variation across the studies and the lack of relevant information on this topic in the studies, it is difficult to determine the most appropriate urine fraction for analysis. If the goal is to analyze a maximum of exfoliated cells, it seems reasonable to use the first-void fraction.

Storage conditions

Milde et al. showed that human DNA in urine containing 40 mM EDTA stored at room temperature for 8 days was less degraded than the DNA in urine samples without EDTA frozen at −20°C [10]. EDTA, known to be an effective nuclease inhibitor, is a chelating agent of bi-valent cations, cofactors of DNA nucleases. Cell-free DNA is more likely to be exposed to degradation than intracellular DNA. Several patent applications have been submitted to conserve DNA integrity in urine [55–57]. The million-fold inhibition described by Brinkman et al. is most likely due to nuclease activity [18]. Spiking experiments in our laboratory confirmed the quasi-disappearance of free HPV plasmid DNA in urine after Amicon Ultra filtration (Millipore N.V., Brussel, Belgium) and QIAamp DNA mini kit extraction (Qiagen Benelux B.V., Venlo, the Netherlands), as also performed by Brinkman et al. The addition of EDTA to the spiked urine fully eliminated this so-called inhibition (A. Vorsters and I. Micalessi, unpublished data). Spiking urine with cancer cells as described by Daponte et al. and Vossler et al. did not lead to such a dramatic reduction in detection rates [20, 51], as also observed in our laboratory. Although many authors are aware of the possible inhibitors in urine, the presence of nucleases is often not taken into account. Conversely, in studies investigating cell-free transrenal DNA, i.e., DNA from the blood circulation that has passed the kidney barrier, collected urine is immediately mixed with EDTA to a final concentration of 10 mM EDTA in order to inhibit possible nuclease activity [58–60]. The impact of storage on the detection of beta-globin and HPV DNA in urine was demonstrated by Brinkman et al., with overnight storage at 4°C being the most favorable method [18].

Storage conditions of urine have a major impact on DNA detection, and, hence, should be optimized and properly reported in papers. To conserve cell-free DNA, an appropriate storage buffer needs to be applied as soon as possible after collection of the urine sample.

Detection of human DNA

Lack of the detection of human DNA in urine by a validated amplification and detection method could be a good indicator of poor storage, and/or mediocre extraction conditions. Table 1 shows that some authors systematically found all urine samples to be positive for human DNA,

Table 1 Details of the papers in the literature describing the detection of human papillomavirus (HPV) DNA in urine

Author, year, reference	Subjects/patients	Country, city	Mean age and range (years)	DNA extraction methods tested/used	Storage	Amplification/primers	hDNA-positive, % (no. positive/no. tested)	HPV DNA-positive, % (no. positive/no. tested)
Alameda et al., 2007 [15]	50 women referred to a gynecology clinic	Spain, Barcelona	36 (28 to 55)	Pellet extracted with Genomica kit (Genomica, Madrid, Spain)	Urine was collected in 30 ml PreservCyt (Cytyc Corporation)	PCR MY09/MY11	Not determined	22% (11/50) overall; 0% (0/20) with ASCUS; 6.2% (1/16) LSIL; 81.8% (11/14) in case of HSIL
Astori et al., 1995 [16]	70 asymptomatic male partners of HPV DNA-positive women	Italy, Udine	Not reported	Proteinase K phenol chloroform extraction	Pellet suspended in 0.1 NaCl, 10 mM Tris-HCL, 1 mM EDTA, and stored at −20°C	PCR L1 consensus primers MY09/MY11. Beta-globin was tested with PC04 GH20 primers	79% (55/70)	33% (18/55) of adequate samples
Brinkman et al., 2002 [17]	101 HIV-positive women attending an HIV out patient clinic	US, New Orleans	20 to 40+ (50% between 30 and 39)	Amicon concentration filter (Millipore), followed by Qiagen DNA mini kit	At −20°C at least 24 h before extraction. DNA extractions stored at −20°C	L1 PGMY09–PGMY11 consensus primers labeled with biotin and GH20-PC04 beta-biotin primer	73% (74/101)	65% (48/74)
Brinkman et al., 2004 [18]	Different patient populations (HIV-positive women, male/female students, college-age women, and women enrolled at a local family planning clinic) and urine spiked with cloned HPV DNA	US, New Orleans	Different groups studied, age not reported	Different techniques tested. Phenol chloroform; QIAamp DNA mini kit, QIAamp RNA mini kit; phenol chloroform and QIAamp DNA Mini Kit extractor; and benzyl alcohol-guanidine HCL DNA extraction)	Overnight refrigeration is better than immediate processing or freezing at −20°C for 24 h.	L1 PGMY09–PGMY11 consensus primers labeled with biotin and GH20-PC04 beta-biotin primer	Qiagen DNA mini kit 15/30 (50%), AM+ QIAgen 10/30 (33%), 1/3 PBS AM QIAgen 19/30 (63%), cohort A; storage conditions: direct processing 46% (24/52), overnight at 4°C 62% (32/52), and overnight at −20°C 46% (24/52) for the urine of cohort B and using PBS dilution, AM, and Qiagen	HPV was found respectively in 13%, 20%, and 30% of the different extractions for urine in cohort A; and 13% (7/52), 17% (9/52), and 10% (5/52) for the different storage conditions in the urine of cohort B
Costa et al., 2009 [19]	Asymptomatic men infected with HIV-1 attending an HIV referral centre and outpatient clinic for HIV	Brazil, Sao Paulo	7 (18–29); 49 (30–39); 167 (40+)	Genomic Blood DNA Purification Kit (Amersham Pharmacia Biotech, Inc.)	−70°C	PGMY9 and PGMY11 (450 bp of L1) adapted to real-time PCR using SYBR green. Positive samples were amplified with type-specific PCR for 6, 11, 16, and 18…	Not determined	30.9% (69/223)
Daponte et al., 2006 [20]	Women with abnormal cervical cytology (ASCUS or worse) referred to a colposcopy clinic. Women were HIV-negative	Greece, Larissa	Not reported	Diluted urine pellet extracted with QIAamp Viral RNA Mini Kit Extraction	24–48 h at 4°C	In-house PCR for HPV 16 and 18 or commercial PCR kits. Also, specific E6 genes of HPV types 6, 11, 16, 18, and 33 were tested with a commercial multiplex assay (Maxim Biotech). Beta-globin was detected using in-house PCR (509-bp PCR product)	Not all samples tested	88% (8/9) in patients with cancer; 44.8% (13/29) in patients with high-grade lesions; 12.8% (5/39) in patients with low-grade lesions
Daponte et al., 2008 [21]	100 patients referred for colposcopy and positive for HPV-16 (15 cancerous, 36 high-grade and 49 low-grade lesions) were recruited	Greece, Larissa	Not reported	Diluted urine pellet extracted with QIAamp Viral RNA Mini Kit Extraction	Not disclosed	HPV 16 primers specific for E1 and E6 genes classic PCR and QRT-PCR were performed. Viral load is expressed as copies/µg DNA but how µg DNA is determined is not disclosed	Not all samples tested	Overall 63% (63/100); cancer 93.3% (14/15); high-grade 83.3% (30/36); low-grade 38.8% (19/49)
D'Hauwers et al., 2007 [22]	HPV-positive women and male partners	Belgium, Antwerpen	Not reported	Roche Amplicor protocol	Not disclosed	Real-time PCR for beta-globin detection (in-house primers, PCR product is 167-bp long). If positive, then MY9/11 consensus primer. Ifpositive, then type-specific PCR	Females 71.0% (22/31); males 66.7% (20/30)	Females 3.2% (1/31); males 0% (0/30)

Table 1 (continued)

Author, year, reference	Subjects/patients	Country, city	Mean age and range (years)	DNA extraction methods tested/used	Storage	Amplification/primers	hDNA-positive, % (no. positive/no. tested)	HPV DNA-positive, % (no. positive/no. tested)
Fambrini et al.,2008 [23]	Women with HR-CIN before and after treatment (laser CO_2 conization). Excluded: patients with diabetes, HIV-positive, chronic steroidal therapy	Italy, Florence	37.9 (18–59)	EZ1 DNA Tissue Kit QIAGEN; automated DNA purification Bio Robot EZ1, QIAGEN	Urine stored overnight at 4°C	PCR kit Bioline first L1 consensus primers	Not determined	80.8% (42/52)
Feng et al., 2007 [24]	Women with biopsy-proven cervical neoplasia (9 CIN1, 29 CIN2 –3, 72 ICC) and 19 without cervical neoplasia on biopsy	Senegal, Dakar	46.8 (±10.9)	Cell pellet resuspended in STM digene, proteinase K, Qiagen Amp blood microcolumn	At 4°C; processed within 24 h; cellpellets resuspended in STM (Digene) and frozen, shipped to Seattle; remained at −80°C there until processing	PCR MY09, MY11, HMB01, and PC04/GH20 beta-globin	99.2% (128/129)	69% overall; in 5/19 (26%) negative and a typical;5/9 (56%) CIN-1; 22/29 (76%) CIN-2 and CIS; and 56/71 (79%) ICC.
Fife et al., 2003 [25]	Men attending STD clinic. Three groups were established. GI visible external genital warts at the time of visit and no history of treatment. GII history of warts with successful treatment. GIII no clinical history of warts	US, Indianapolis	Between 18 and 50	Qiagen quick DNA purification method	Not reported	PCR HPV type-specific primers HPV L1, E6 andE7 detection by dot-blot hybridization. Beta-globin was used to determine sampleadequacy	93% (56/60)	Not relevant, only HPV 6 and 11 tested
Forslund et al., 1993 [26]	Group I: military conscripts 143. Group II: attending an adolescent clinic, 12 females and 8 males. Group III: 343 women referred to a colposcopy clinic. Patients were seen more than once; in total, 489 paired urine and cervix samples were taken	Sweden, Malmö	GI: 21 (20–23); GII 19 (17–22); GIII: 37(17–79)	Pellets were treated with proteinase K. If samples were beta-globin PCR-negative, phenol-free DNA extraction was performed	Stored in refrigerator before further processing on the same day, after centrifugation pellet frozen at−20°C	PCR used TS primers for 6, 11, 33, and 16–18(run separately). Third tube E6 primersselected from published sequences and beta-globin (PC03, PC04). Fourth tube used consensus primers. Bovine serum albumin (0.2%) was included in PCR mix, has been shown to overcome inhibitory activities of unknown origin in archaeological samples	GI military conscripts, after phenol extraction of the 18 negative samples, 96.5% (138/143) were positive; GIII samples of 343 women referred to a colposcopy clinic, after extraction of the 56 negative samples, 95.5% (489/512) were positive	GI: HPV DNA was detected in 5.8% (8/138); GIII: 38.2% (187/489)
Geddy et al., 1993 [27]	73 male patients attending a genitourinary clinic	UK, Leeds	Not reported	Sediment washed, digestion buffer and proteinase K, phenol chloroform extraction and DNA precipitation	4°C up to 4 h	PCR (consensus primers HPV and beta-globin for hDNA)	55% (40/73)	0% (0/73)
Giuliano et al., 2007 [28]	Men (recruited via advertisements in city and university newspapers, etc.) 18-40 yearsold	US, Tucson	Between 18 and 40	Not disclosed	At 4°C before storage at −80°C.	ND for urine	51% (n=226)	0.4% (1/226)
Golijow et al., 2005 [29]	Male patients attending the urological department with HPV-positive sexual partner (n=112) or HPV-associated penile lesions (n=73)	Argentina, La Plata	GI: 31 (17–71); GII: 27(20–56)	Pellet was washed, proteinaseK digestion, and boiled	Samples stored at 4°C and processed on the same day. Celldigests were stored at –20°C	DNA quality by amplifying thymidine kinase gene. HPV DNA was analyzed using nested PCR, first round MY09/11 (450 bp) second round GP5+/6+(150 bp)	100% (n=185)	73% (135/185); 68.8% 77/112 for men with HPV-positive partners and 79.5% (58/73) for men with penile lesions
Gupta et al., 2006 [30]	Women with histologically confirmed invasive cervical cancer and their husbands, and a control group of 30	India, New Delhi	Women 42, men 46	Pellets washed, proteinase digestion, if required, phenol chloroform extraction	Not disclosed	HPV-L1 consensus primer and in-house beta-globin primers	In total, 98.3% (118/120); in urine samples of cases, 93.3%(28/30)	82% (23/28) for women with CC; 66.7% (20/30) partners of women with CC; 26.7% (8/30) healthy

Table 1 (continued)

Author, year, reference	Subjects/patients	Country, city	Mean age and range (years)	DNA extraction methods tested/used	Storage	Amplification/primers	hDNA-positive, % (no. positive/no. tested)	HPV DNA-positive, % (no. positive/no. tested)
	age-matched women with normal or inflammatory or negative cervical cytology and their husbands							control women; 26.7% (8/30) partners of healthy control women
Hernandez et al., 2008 [31]	Male undergraduate and graduate students (University of Hawaii)	US, Hawaii	29	Not disclosed, commercial extraction kits (Qiagen) were used	Not disclosed	All specimens were tested using GH20 and PC04 primers. PCR for HPV DNA was done using the PGMY9 and PGMY11 primers	Not reported	10% (20/200)
Hillman et al., 1993 [32]	Men attending a clinic for the surgical removal of anogenital warts	UK, London	28.6 (16.1–61.6)	Sediment resuspended in TRIS-HCl, NaCl, EDTA buffer. Pronase E digestion, phenolchloro form extraction	Not disclosed	PCR GP5/GP6	Not determined	6.7% (1/15)
Hillman et al., 1993 [33]	100 men with gonorrhea, 32 men with penile warts, and 37 men with genital dermatoses	UK, London	GI: 26.7(17.0–55.6); other groups similar features	Sediment resuspended in lysisbuffer, pronase E digestion, phenol chloroform DNA extraction	Not disclosed	PCR GP5/GP6	Not determined	12.5% (11/88) men with gonorrhea; 0/5 men with penial warts; 13.5% (5/37) of men with genital dermatoses
Hillman et al., 1993 [34]	Men undergoing genital biopsy at agenital dermatosis clinic	UK, London	35.9 (18.6–68.6)	Sediment resuspended in lysisbuffer, pronase E digestion, phenol chloroform DNA extraction	Not disclosed	PCR GP5/GP6	Not determined	10.2% (6/59)
Iwasawa et al., 1997 [35]	47 male patients, 29 with urethralcondylomata, three with penile condylomata, 15 controls without any symptoms or signs	Finland, Helsinki	25.2 (20–47)	Sediment resuspended in Tris-HCl, EDTA buffer, sonicated, proteinase K digestion, phenol chloro form extraction	Collected in sterile plastic containers and stored at −70°C	PCR MY09/MY11	Not determined	75.9% (22/29)
Jacobson et al., 2000 [36]	Adolescents recruited from 37 women attending an STI clinic and 63 women attending an adolescent clinic for pelvic examination	US, Baltimore	17.5 (11–20)	Digestion with proteinase K3 µl used in PCR. For HCII as recommended by vendor	Time between getting the urine sample and processing not revealed (immediate?); pellet resuspended in 0.5 ml Digene transport medium stored at −20°C	PCR: MY09/MY11/HMB01 HPV L1 consensus primers and beta-globin PC04 and GH20. Detection by Hybrid Capture Probe B (13 cancer-associated types)	Not reported	HCII: 46.8% (37/80); PCR: 75% (55/80); no lesion (32/49); ASCUS 86.7% (13/15); SIL 100%(15/15)
Jong et al., 2008 [37]	HIV-infected women recruited from different clinical settings, varying from out patients of an HIV clinic to patients referred to colposcopy because of cervical dysplasia	The Netherlands, Amsterdam	(20–40+) (more than half were older than 40)	DNA was isolated using Roche viral nucleic acid isolation kits	Not clear at −20°C in Sure Path?	PCR SPF10 (Kleter et al., 1999) and part of the beta-globin gene. The use of PhHV-1 (sealherpes virus) was used to check inhibition	Not reported	81.5% (22/27)
Jong et al., 2009 [38]	HIV-infected men and a control group of 231 men without known risk factors for HIV attending a urology outpatient clinic	The Netherlands, Amsterdam	45 (24–74)	Not disclosed	At −20°C prior to processing	PCR SPF10 and beta-globin	Not reported	27.5% (67/243) HIV-positive men and 12.6%(29/231) HIV-negative men
Lazcano-Ponce et al., 2001 [39]	43 male college students and 77 male industry workers from an automobile factory. None of the males had evident external	Mexico, Cuernavaca	14–55	Presumably, PCR was initially done on crude cell extraction after freeze thawing. Some	Pellet suspended in PBS	Beta-globin (209-bp amplicon) and GP5+/GP6+	14% (17/120)	11.8% (of the initial 17 beta-globin-positive urine samples, two were HPV-positive). After

Table 1 (continued)

Author, year, reference	Subjects/patients	Country, city	Mean age and range (years)	DNA extraction methods tested/used	Storage	Amplification/primers	hDNA-positive, % (no. positive/no. tested)	HPV DNA-positive, % (no. positive/no. tested)
	genital lesions upon clinical examination			beta-globin-negative urine samples were tested using the high pure PCR template from Boehringer				DNA extraction, 12 additional urine samples became beta-globin-positive; in none was HPV DNA detected: 6.9% (2/29)
Manhart et al., 2006 [40]	Sexually active women 18–25	US	18–25	Cell pellet resuspended in 600 µl STM (Digene) digested with protease K and DNA isolated using QIAamp blood DNA microcolumn	At reception by the laboratory, 5 ml of urine was aliquoted and frozen at −70°C. Shipment was on dry ice	PCR MY09/MY11/HMB01. Beta-globinPC04/GH20	95.8% (3,585/3,741)	28.6% (934/3,262)
Melchers et al., 1989 [41]	Male patients attending an STI clinic for condylomata acuminata in the urethrae. Control group 14 male volunteers from the department of pathology at the Diagnostic Centre SSDZ Delft.	The Netherlands, Rotterdam	Not reported	Sediment resuspended in PBS, SDS added, Pronase digestion, phenol chloroform extraction.	No info, urine sediments were suspended in 0.5 ml PBS.	Type-specific primers HPV 6 and HPV 11 wereused	Not determined	88% (15/17)
Nakazawa et al., 1991 [42]	Male sexual partners of HPV 16- or 18-positive patients with cervical cancer	Japan, Osaka	Not reported	Proteinase K digestion, phenol chloroform extraction	Sediment suspended in 2 ml PBS and stored at −20°C	HPV 16 18 E6 PCR detection by the Southern blot method	Not determined	22% (2/9)
Payan et al., 2007 [43]	Cervical scrape samples and first-streamurine samples 5–10 ml from women consulting a gynecologist	France, Angerand Brest	Not reported	Qiagen RNA mini kit	Urine stored at −80°C before extraction	Real-time PCR. Mx4000 (Stratagene Europe) and Light Cycler (Roche Diagnostics). SYBR green using modified SPF10 primers and GAPDH primers	85% (150/177)	37% (66/177)
Payan et al., 2009 [44]	Women refusing a pap smear	France, Morlaix	25–65	EasyMag extractor (bioMérieux)		Real-time PCR	Not reported	19% (222/1,169)
Powell et al., 2003 [45]	Children with vulval (lichen sclerosus) LS and with non-LS vulval disease and ascontrol, girls with no recorded vulval disease	UK, Oxford	2–11	Guanidiniumisothiocyanate/silica (Boommethod)	Not disclosed	Nested PCR MY09/MY11 and GP5+/GP6+negative samples were checked for quality by beta-globulin DNA PCR	Not determined	25% (8/32) with LS; 6.4% (2/31) other vulvar diseases; 24% (7/29) healthy controls
Prusty et al., 2005 [46]	Sexually inexperienced, unmarried college girls and 55 normal healthy (negative pap smear) sexually active married women	India, New Delhi	18–25	Non-organic method. Urine smears DNA extracted by the boiling method	At 4° for 2–3 h before processing. Cell sediment was washed twice with sterile PBS and smeared on paper, stored at RT	Type-specific primers HPV (sequences shown) and consensus L1 primer in-house PCR procedure	Not determined	6% (6/100) of sexually inexperienced girls and 9% (5/55) of sexually active women
Rintala et al., 2002 [47]	Healthy men undergoing vasectomy. Mean age 40.3	Finland, Turku	40.3 (33–49)	Lysing using proteinase K at 37°C overnight proteins precipitated with saturate 6M NaCl	Pellet stored at −20°C	Nested PCR MY09/MY11 and GP5+/GP6+. Samples were checked for quality by beta-globulin DNA PCR	Not reported	22.2% (4/18)
Sellors et al., 2000 [8]	Women referred to a colposcopy clinic at a teaching hospital because of abnormalities upon cervical cytology screening	Canada, Hamilton	31.5 (SD9.4)	Procedure of HC II assay	Urine stored at 4°C for a maximum of two weeks, shipped at RT before processing. 20 ml of urine was mixed with 20 ml of urine preparation buffer solution Digene Corp.	Hybrid capture II; results for PCR with consensus HPV L1 primers and beta-globin are not provided	Not reported	35% (69/200); HSIL: 44,8% (26/58); LSIL: 54.2%(13/24); normal 25.4% (30/118)

Table 1 (continued)

Author, year, reference	Subjects/patients	Country, city	Mean age and range (years)	DNA extraction methods tested/used	Storage	Amplification/primers	hDNA-positive, % (no. positive/no. tested)	HPV DNA-positive, % (no. positive/no. tested)
Smits et al., 2005 [48]	Male HIV patients with CD4 count below 100 per mm^3 taken during routine visit. 115 HIV-negative males served as controls (provided anonymously)	The Netherlands, Amsterdam	Not reported	Guanidinium thiocyanate lyses binding to silica particles and washing and elution as described by Boom et al.	Urine stored at −20°C	SPF10 and geno typing (LiPA) followed by qPCR with SPF10. Beta-globin gene was amplified to assess the presence of human cells. Beta-actinwas used to determine the number of human cells	100%	39.4% (41/104) for HIV-positive men; 9.6% (11/115) for HIV-negative men
Stanczuk et al., 2003 [49]	Women with invasive cervical cancer were enrolled from gynecological clinics at the university hospital	Zimbabwe, Harare	44 (24–70)	Qiagen DNA extraction kit	Urine samples were stored at −20°C before processing	Conventional nested PCR, reagents fromRoche, degenerated nested primers as described by Williamson and Rybicki 1991. PCRfor human cytokine gene was used to assess the presence of DNA (Perrey et al. 1999).	81% (35/43)	72% (31/43)
Song et al., 2007 [66]	Women visiting the Department of Obstetrics and Gynecology; 100 with adequate cervical swabs (beta-globin-positive) and biopsy-proven histological diagnosis were selected for this study. 23 chronic cervicitis, 48 CIN, and 29 ICC, including three adenocarcinomas	Korea, Incheon	45.2 (26–77)	Wizard genomic DNA purification kit (Promega). Using a detergent lysis buffer and protease digestion, ribonuclease treatment	No info on storage or immediate processing	HPV DNA chip PCR-based DNA microarray system (using HPV and beta-globin primers)	90% (90/100)	52.2% (47/90); cervicitis 13% (3/23); CIN:62.8% (27/43); carcinoma 70.8% (17/24)
Strauss et al., 1999 [50]	Women (aged 16–57, mean age 26) attending a genitourinary hospital, selected at random without considering clinical history or current signs of HPV infection	UK, Cambridge	26 (16–57)	Several methods tested, including proteinase K 60 min at 55°C and boiling at 95°C for 10 min	No info on storage	Nested PCR: first round MY09 and MY11, second round GP5+ and GP6+. HPV DNA-negative samples were tested for beta-globin	Not reported	15% (20/136) first round; 65% (89/136) with nested PCR
Vossler et al., 1995 [51]	Female patients (19–82) seen in the gynecological offices. 15 patients showed evidence of condylomata, dysplasia or invasive carcinoma	US, New York	19–82	Sediment resuspended in and washed with Tris-EDTA buffer. Proteinase K digestion. Phenol chloroformextraction. Samples negative for beta-globin further treated with GeneClean	Stored at 4°C for up to 24 h prior to processing	PCR MY11 and MY09 beta-globin was amplified using GH20 and PC04 primers	45% (10/22) initially after using Gene Clean 95% (21/22)	76% (16/21 beta-globin-positive samples); 87% (13/15) of patients showing evidence of condylomata, dysplasia, or invasive carcinoma; 50% (3/6) of patients with negative or benign lesions
Weaver et al., 2004 [52]	Heterosexual men attending a sexually transmitted disease clinic foroptimization; and male universitystudents	US, Seattle	18–25 (both groups)	Not reported	Some left at RT before processing (max. 6 h) after centri fugation pellet first at −20°C or immediately at −70°C	MY09, MY11, and HMB01 and beta-globin PC04 and GH20. If positive for HPV, then amplified by PGMY09 PGMY11 and beta-globin PC04 and GH20 primers	86.6% (26/30); 99.7% (313/314) students	3.8% (1/26); 5.7% (18/313)
Zambrano et al., 2002 [53]	Males undergoing surgical treatment for prostate cancer	US, Long Beach	62 (52–69)	Not reported	Urine samples were stored at 4°C; before centrifugation, pellet was suspended in PBS and transferred to micro centrifuge tube and kept at −70°C until further processing	Nested PCR MY09/MY11 and GP5+/GP6+	Not reported	50% (6/12)

Table 2 Divergence of the methods, settings, and tested subjects among 41 papers analyzed

Urine collection	- 17 papers report using first-void or first-catch urine, three use midstream, 21 didnot disclose which fraction was analyzed
	- volumes analyzed range from 200 μl to 400–600 ml
	- urine is collected at the clinic, at home, or sent by mail to the laboratory
	- one study asked not to wash genitals, another study washed genitals with ethanol before urination [28, 66]
Storage	- urine was processed immediately, stored at RT, at 4°C, −20°C, or −70°C
	- storage times varied from a few hours to several weeks
	- urine was stored undiluted, diluted, or after centrifugation and removal of the supernatant. Pellets were frozen as such or suspended in phosphate-buffered saline, an in-house or commercial buffer
Centrifugation	- all but one paper indicate centrifugation as one of the initial processing steps
	- centrifugation speeds range from 500g to 100,000g
	- centrifugation times from 5 min to 1 h
	- if reported, centrifugation was done at RT or at 4°C
	- a number of papers uses Amicon Ultrafiltration filters (Millipore) to concentrate urine and remove potential PCR inhibitors
	- 20 papers did not mention the centrifugation conditions
DNA extraction	- applied extraction methods ranged from crude proteinase K digestion,ethanol-chloroform extraction, to guanidium isothiocyanate/silica-based extraction (Boom extraction), commercially available kits, and automated extraction methods
	- a number of authors washed the pellet several times to remove potential PCRinhibitors
Detection of HPV DNA	- 27 publications used conventional PCR, six nested PCR, six real-time PCR, and two HC2
	- due to the large time frame in which these studies were performed (1993–2009), a wide range of primers was used: GP5/GP6, GP5+/GP6+, MY09/MY11,MY09/MY11/HMB01, PGMY09/PGMY11, SPF10, SPF10 modified, in-house consensus primers, as well as combinations of type-specific primers
	- detection of amplified product ranged from gel electrophoresis to real-time PCR
Detection of human DNA	- testing the urine for the presence of human DNA was performed in 25 studies
	- in 22 papers, beta-globin gene was targeted, although different primers were used to generate amplicons from 506 bp to 110 bp; in 11 papers, the GH20/PC04 primers, generating a 268-bp-long amplicon were used; in the other studies, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), humancytokine, beta-actin, or thymidine kinase genes were targeted
	- only 19 of the 25 studies reported the detection rates
	- a number of studies only tested the presence of human DNA if a sample was negative for HPV DNA; the majority of the studies only performed HPV DNA testing if human DNA was detectable
Subjects studied	- various groups of subjects were tested: pre-adolescent children, students, military recruits, healthy controls, women attending a genito-urinary clinic, HIV-infected persons, persons with genital warts, males attending an STI clinic, male partners from women with HPV cervical infection, women with HPVcervical cancer, and partners of women with HPV cancers

even from male subjects, while others detected human DNA in less than 50% of the samples. Interestingly, poor human DNA detection is also linked to very low HPV DNA detection rates [22, 27, 28], indicating that the ability to detect human DNA in urine could have been affected by poor sample storage and/or extraction. The majority of the studies used human DNA as an internal control for sample validity. In a number of public health STD screening programs, the manipulation of urine specimens, e.g., providing water instead of urine, has been reported [61]. Human DNA detection could help to identify falsified samples. However, the detection of cell-free HPV viral particles, even in the absence of human DNA, is equally relevant in certain settings.

It is unclear to what extent the shedding and exfoliation of cells are influenced by the viral type and stage of HPV infection. Payan et al. did not find any correlation between HPV viral loads and cell levels in urine or cervical samples [43].

Extraction, amplification, and detection of HPV DNA

The analytical sensitivity of HPV DNA detection in urine depends on a cascade of factors that all influence the outcome: type of urine, storage conditions, quality of DNA extraction, amplification method, and the detection of amplified DNA. A poorly performed step can, to a certain extent, be compensated for by a well-performed step downstream, e.g., a very sensitive DNA amplification (short PCR fragments in a real-time PCR setting) may overcome mediocre extraction conditions or the fact that DNA has been partly degraded due to poor storage conditions.

The possible impact of different steps can be inferred from the results of the different studies. Vossler et al. described that further processing the ethanol-chloroform-extracted samples using GeneClean (BIO 101, Inc., La Jolla, CA, USA) increased the beta-globin positivity from 45 to 95% [51]. Forslund et al. reported that crude sample preparation, i.e., simple proteinase K treatment, resulted in 18 (male) and 56 (female) inadequate urine samples, of which 13 and 23, respectively, became beta-globin-positive after simple phenol-free DNA extraction [26]. Also, Lazcano-Ponce et al. initially detected beta-globin positivity in only 14.1% of the male urine samples, whereas all 12 initially beta-globin-negative samples became positive after extraction with a High Pure PCR template (Boehringer, Mannheim, Germany) assay; using nested PCR, Strauss et al. improved the detection of HPV DNA in urine from 15 to 65% [50]. Daponte et al., finally, showed that HPV detection in urine was more sensitive by real-time PCR than by conventional PCR [21].

Methods in which the cellular fraction of urine is concentrated do not necessarily improve sensitivity. Brinkman et al. compared three extraction and concentration methods (QIAmp DNA Mini kit, Amicon ultrafiltration followed by QIAamp DNA Mini Kit and 1/3 PBS dilution, Amicon ultrafiltration followed by QIAamp DNA Mini Kit), and detected 50, 33, and 63% human DNA, respectively, in 30 previously frozen urine samples [18]. The analyzed volumes were 200 μl, 15 ml, and 5 ml respectively. Strauss et al. detected HPV DNA only in the supernatant and not in the pellet/sediment in 7.8% of 89 HPV DNA-positive urine samples. This is an unexpected finding as the pellet was resuspended in 500 μl of the urine supernatant [50]. Both studies show that concentrating the urine can even increase inhibition and/or DNA degradation by the possible concentration of PCR inhibitors and/or DNA-degrading proteins.

Further research is required in order to determine the optimal extraction method.

Different forms of viral DNA can be detected in urine

The amount of viral DNA in infected cells correlates to the life cycle and stage of infection. The basal layer contains only low-copy numbers of viral episomes. In a productive cycle, viral episomes are multiplied and eventually packaged in viral particles, while the cells differentiate and migrate to the epithelial surface. Cells and infectious virions are shed from the epithelial surface during desquamation. In cancer cells, viral DNA is nearly always integrated and the productive cycle is arrested. Middleton et al. showed with surrogate markers that LSIL or CIN1 lesions are linked to the productive life cycle of the virus, while HSIL or CIN2 and CIN3 are indicative of abortive infection, during which very limited viral DNA and almost no L1, the major viral particle protein, are produced [62].

Considering the different stages of infection and possible cell disintegration, HPV DNA can appear in urine as being integrated in the cellular genome (cell-associated or cell-free), as intracellular episomal DNA, as cell-associated viral DNA-containing particles, as free viral DNA-containing particles, or as free viral DNA.

Some authors justified the use of low-speed centrifugation, which theoretically only concentrates the cellular fraction of the urine, by arguing that the virus is largely cell-associated. One study combined high-speed and low-speed centrifugation to investigate the urine of men with condylomata acuminata in the urethrae. No additional positive samples were found by high-speed centrifugation, indicating that all samples did contain cell-associated HPV [41]. Strauss et al. were the only group investigating the presence of HPV DNA in the supernatant. Of 89 HPV DNA-positive urine samples, 58.4% had detectable HPV DNA in the supernatant and sediment, 34.8% only in the pellet, and 7.8%, as discussed earlier, only in the supernatant [50]. These results demonstrate inhibition or DNA degradation in the sediment, but also clearly indicate that non-cell-associated HPV DNA can be found in urine.

Then again, it may be that sensitive methods do pick up cell-free HPV DNA or cell-free HPV virions. Smits et al., who did not concentrate the cellular fraction by centrifugation, analyzed 200 μl of urine, a volume that easily remains on the pellet when removing the supernatant after centrifugation. They found that all samples were adequate, hence, positive for human DNA, and detected 39.4 and 9.6% HPV DNA in 104 HIV-positive men and 115 male controls, respectively [48].

Washing the pellet several times with PBS to remove potential inhibitors, as reported in a number of studies, might reduce the possible inhibitors and DNA degradation, but, equally so, the amount of detectable cell-free HPV DNA.

Finally, the possibility of detecting transrenal cell-free DNA also needs further attention. Transrenal tumor DNA has been reported in urine from patients with colorectal cancers and fetal DNA was detected in maternal urine [58–60, 63]. Transrenal DNA molecules are relatively short, i.e., less than 250 nucleotides, and unstable in urine. With improved storage conditions and the use of PCR primer sets generating short amplicons, the detection of transrenal human DNA and HPV DNA cannot be excluded.

The methods applied in the studies may have detected different or additional forms of viral DNA, which should be taken into account when optimizing and standardizing the methodology.

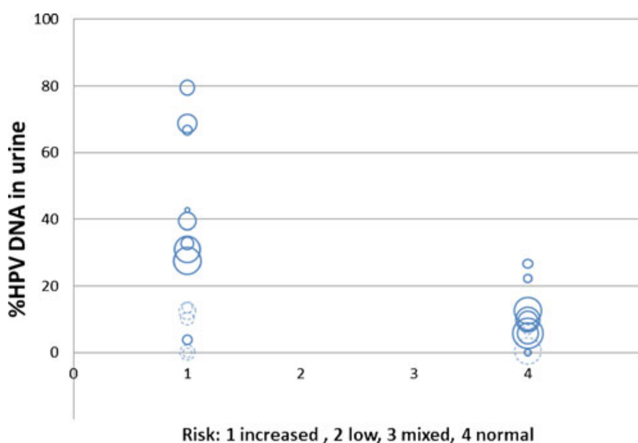
Relevance of HPV DNA detection in urine

The studies are highly heterogeneous with respect to the methods used, specific settings, and tested subjects, which may impact on the prevalence of HPV DNA in urine. It is not possible to statistically correct for this diversity, for instance, by using a random effects meta-regression analysis, because the number of factors that may explain the differences is too high compared to the number of studies. A meta-analysis of the collected data, without correcting for these factors, is not justifiable either, because the diversity may have a non-random impact on variations between studies.

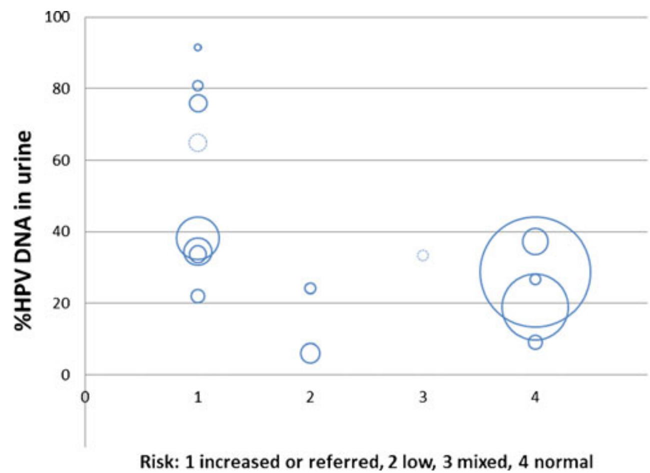
However, the following trends could be observed from the bubble plots. Graph 1 shows the HPV DNA prevalence of the male cohorts classified according to either predefined increased (n=15) or regular risk (n=11) of being infected. There is a clear trend towards a higher prevalence of HPV DNA in the urine of men with risky behavior, increased exposure, or increased susceptibility as compared to men without reported risk factors. Eight studies considered as substandard also reported an HPV DNA prevalence of less than 20% [27, 28, 32–34, 39, 41, 42, 64].

Graph 2 shows the female cohorts according to predefined risk of being HPV DNA-positive, increased (n=8), normal (n=5), mixed (n=1), and low (n=2). Although limited data points are available, there is a trend that HPV DNA prevalence in urine is correlated with increased exposure or risk of having an HPV infection. Two studies were considered as substandard [17, 18].

Graph 3 shows the HPV DNA prevalence in 35 cohorts categorized according to the reported cervical lesions.



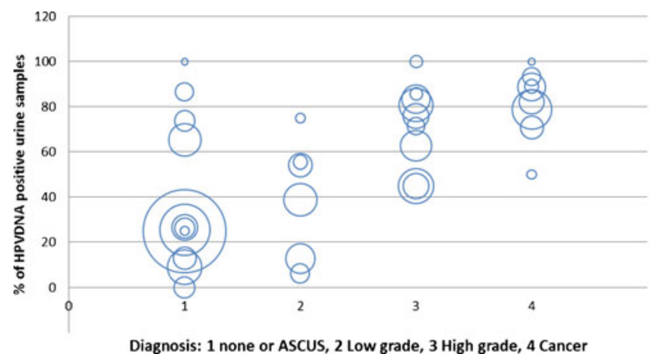
Graph 1 HPV in urine of male subjects according to estimated risk of being infected. The size of the bubbles is proportional to the number of subjects tested in each group, with the largest bubble representing 313 subjects and the smallest bubble three subjects. Bubbles with a *dotted outline* represent data from substandard studies with anticipated lower detection rates, i.e., low rates of human DNA detected or HPV PCR using less sensitive primers



Graph 2 HPV in urine of female subjects according to estimated risk of being infected. The size of the bubbles is proportional to the number of subjects tested in each group, with the largest bubble representing 3,262 subjects and the smallest bubble 12 subjects. Bubbles with a *dotted outline* represent data from substandard studies with anticipated lower detection rates, i.e., low rates of human DNA detected or HPV PCR using less sensitive primers

There is a trend that cohorts with high-grade lesions or cancer have a higher prevalence of HPV DNA in urine, while the prevalence is more scattered among the different studies in cohorts with no or low-grade lesions. Detection rates and concordance among paired urine and cervical samples was described by Sehgal et al., who showed that symptomatic patients attending gynecology outpatient departments/cancer clinics or those attending colposcopy clinics had a high degree of concordance with the same type of HPV in paired samples, indicative of the "contamination" of urine with infected exfoliated cells [65].

To date, in most cases, the prevalence or viral load of HPV DNA in urine has been reported to be lower than in corresponding cervical samples. Only Jong et al. found in



Graph. 3 HPV DNA detection in urine of women according to diagnosed lesions of the cervix. The size of the bubbles is proportional to the number of subjects tested in each group, with the largest bubble representing 315 subjects and the smallest bubbles two subjects

HIV-positive women that the HPV DNA prevalence in urine and cervical smear samples was 81.5 and 51.9%, respectively [37]. Improving analytical sensitivity may reduce the concordance with clinical sensitivity in cervical cancer screening.

Alternative sampling can be instrumental in improving a cervical cancer screening program. A pilot study in France showed that the response rate of women having been invited for a cervical smear examination substantially increased when they were asked to provide a self-sampled urine specimen via mail [44]. Urine samples have been successfully used in the post-treatment follow-up of cervical cancer [22]. Furthermore, HPV DNA testing of urine samples can also be a convenient tool in vaccine trials to compare pre- and post-vaccination prevalence, to detect and follow-up incident infections, to determine the circulation of the virus, and to study possible herd immunity. Two interesting studies with women with a low risk of infection (and a high threshold for cervical sampling) surprisingly showed that 24% (7/29) of the group of preadolescent girls and 6% (6/100) of the sexually inexperienced girls produced HPV DNA-positive urine [45, 46].

Conclusion

The available studies dealing with human papillomavirus (HPV) DNA prevalence in urine were found to be highly diverse in their setup and applied methodology. Many studies did not properly report on sampling, storage, sample preparation and DNA extraction; however, the detection of HPV DNA in urine is not as straightforward as that assumed by many authors. The standards used for HPV DNA detection in cervical smears may not lead to satisfactory results when applied to urine.

The need to improve and standardize urine sampling methods, storage conditions, sample preparation, DNA extraction, and DNA amplification is a prerequisite for combining data and performing meta-analyses, since all of these factors may have an important impact on the HPV DNA detection rates and, probably, also on the form of viral DNA that is detected. Papers need to provide in-depth information on the applied methods and further elaborate on uniform testing conditions.

It is clear that HPV DNA detection in urine is a feasible practice and a useful tool in future research, but further optimization and standardization is required. Different settings may require different methodologies.

Many unresolved issues remain in the field of HPV. Since urine samples can be easily obtained by a non-invasive self-sampling method, testing urine for HPV DNA will definitely become a valuable approach for further investigations in the HPV field.

Acknowledgments This work was financially supported by the Industrial Research Fund (with the participation of GlaxoSmithKline Biologicals) of the University of Antwerp (IOF/SBO 3501/3494). The funding sources had no role in the study design, collection of data, analysis, and interpretation of the data; in the writing of the report; and in the decision to submit the paper for publication.

Conflicts of interest Pierre van Damme is the promoter, and Margareta Ieven and Johannes Bogers are the co-promoters of this research project, for which the University obtains a 10% financial contribution from GSK Biologicals. Alex Vorsters, Isabel Micalessi, and Joke Bilcke have no conflicts of interest.

References

1. Moscicki AB, Schiffman M, Kjaer S, Villa LL (2006) Chapter 5: Updating the natural history of HPV and anogenital cancer. Vaccine 24(Suppl 3):S3/42–S3/51
2. Einstein MH, Schiller JT, Viscidi RP, Strickler HD, Coursaget P, Tan T, Halsey N, Jenkins D (2009) Clinician's guide to human papillomavirus immunology: knowns and unknowns. Lancet Infect Dis 9(6):347–356
3. Wright TC, Bosch FX, Franco EL, Cuzick J, Schiller JT, Garnett GP, Meheus A (2006) Chapter 30: HPV vaccines and screening in the prevention of cervical cancer; conclusions from a 2006 workshop of international experts. Vaccine 24(Suppl 3):S3/251–S3/261
4. Kyrgiou M, Koliopoulos G, Martin-Hirsch P, Arbyn M, Prendiville W, Paraskevaidis E (2006) Obstetric outcomes after conservative treatment for intraepithelial or early invasive cervical lesions: systematic review and meta-analysis. Lancet 367(9509):489–498
5. zur Hausen H (1991) Viruses in human cancers. Science 254 (5035):1167–1173
6. van Doorn LJ, Molijn A, Kleter B, Quint W, Colau B (2006) Highly effective detection of human papillomavirus 16 and 18 DNA by a testing algorithm combining broad-spectrum and type-specific PCR. J Clin Microbiol 44(9):3292–3298
7. Jenkins D (2008) A review of cross-protection against oncogenic HPV by an HPV-16/18 AS04-adjuvanted cervical cancer vaccine: importance of virological and clinical endpoints and implications for mass vaccination in cervical cancer prevention. Gynecol Oncol 110(3 Suppl 1):S18–S25
8. Sellors JW, Lorincz AT, Mahony JB, Mielzynska I, Lytwyn A, Roth P, Howard M, Chong S, Daya D, Chapman W, Chernesky M (2000) Comparison of self-collected vaginal, vulvar and urine samples with physician-collected cervical samples for human papillomavirus testing to detect high-grade squamous intraepithelial lesions. CMAJ 163(5):513–518
9. Khan G, Kangro HO, Coates PJ, Heath RB (1991) Inhibitory effects of urine on the polymerase chain reaction for cytomegalovirus DNA. J Clin Pathol 44(5):360–365
10. Milde A, Haas-Rochholz H, Kaatsch HJ (1999) Improved DNA typing of human urine by adding EDTA. Int J Legal Med 112 (3):209–210
11. Carder C, Robinson AJ, Broughton C, Stephenson JM, Ridgway GL (1999) Evaluation of self-taken samples for the presence of genital *Chlamydia trachomatis* infection in women using the ligase chain reaction assay. Int J STD AIDS 10(12):776–779
12. Howard M, Sellors J, Kaczorowski J, Lorincz A (2004) Optimal cutoff of the hybrid capture II human papillomavirus test for self-collected vaginal, vulvar, and urine specimens in a colposcopy referral population. J Low Genit Tract Dis 8(1):33–37
13. Baldwin SB, Wallace DR, Papenfuss MR, Abrahamsen M, Vaught LC, Kornegay JR, Hallum JA, Redmond SA, Giuliano AR (2003)

Human papillomavirus infection in men attending a sexually transmitted disease clinic. J Infect Dis 187(7):1064–1070
14. Chen Z, Ye Z, Zeng W (2004) Clinical investigation on the correlation between lower urinary tract infection and cystitis glandularis. J Huazhong Univ Sci Technolog Med Sci 24(3):303–304
15. Alameda F, Bellosillo B, Fusté P, Musset M, Mariñoso ML, Mancebo G, Lopez-Yarto MT, Carreras R, Serrano S (2007) Human papillomavirus detection in urine samples: an alternative screening method. J Low Genit Tract Dis 11(1):5–7
16. Astori G, Pipan C, Muffato G, Botta GA (1995) Detection of HPV-DNA in semen, urine and urethral samples by dot blot and PCR. New Microbiol 18(2):143–149
17. Brinkman JA, Jones WE, Gaffga AM, Sanders JA, Chaturvedi AK, Slavinsky J 3rd, Clayton JL, Dumestre J, Hagensee ME (2002) Detection of human papillomavirus DNA in urine specimens from human immunodeficiency virus-positive women. J Clin Microbiol 40(9):3155–3161
18. Brinkman JA, Rahmani MZ, Jones WE, Chaturvedi AK, Hagensee ME (2004) Optimization of PCR based detection of human papillomavirus DNA from urine specimens. J Clin Virol 29(4):230–240
19. Costa FA, da Silva RC, Arruda LB, Duarte AJ, Casseb J (2009) Prevalence of human papillomaviruses in urine samples of male patients infected with HIV-1 in Sao Paulo, Brazil. J Med Virol 81(12):2007–2011
20. Daponte A, Pournaras S, Mademtzis I, Hadjichristodoulou C, Kostopoulou E, Maniatis AN, Messinis IE (2006) Evaluation of high-risk human papillomavirus types PCR detection in paired urine and cervical samples of women with abnormal cytology. J Clin Virol 36(3):189–193
21. Daponte A, Tsezou A, Oikonomou P, Hadjichristodoulou C, Maniatis AN, Pournaras S, Messinis IE (2008) Use of real-time PCR to detect human papillomavirus-16 viral loads in vaginal and urine self-sampled specimens. Clin Microbiol Infect 14(6):619–621
22. D'Hauwers K, Depuydt C, Bogers JP, Stalpaert M, Vereecken A, Wyndaele JJ, Tjalma W (2007) Urine versus brushed samples in human papillomavirus screening: study in both genders. Asian J Androl 9(5):705–710
23. Fambrini M, Penna C, Pieralli A, Bussani C, Fallani MG, Andersson KL, Scarselli G, Marchionni M (2008) PCR detection rates of high risk human papillomavirus DNA in paired self-collected urine and cervical scrapes after laser CO_2 conization for high-grade cervical intraepithelial neoplasia. Gynecol Oncol 109(1):59–64
24. Feng Q, Hawes SE, Stern JE, Dem A, Sow PS, Dembele B, Toure P, Sova P, Laird PW, Kiviat NB (2007) Promoter hypermethylation of tumor suppressor genes in urine from patients with cervical neoplasia. Cancer Epidemiol Biomarkers Prev 16(6):1178–1184
25. Fife KH, Coplan PM, Jansen KU, DiCello AC, Brown DR, Rojas C, Su L (2003) Poor sensitivity of polymerase chain reaction assays of genital skin swabs and urine to detect HPV 6 and 11 DNA in men. Sex Transm Dis 30(3):246–248
26. Forslund O, Hansson BG, Rymark P, Bjerre B (1993) Human papillomavirus DNA in urine samples compared with that in simultaneously collected urethra and cervix samples. J Clin Microbiol 31(8):1975–1979
27. Geddy PM, Wells M, Lacey CJ (1993) Lack of detection of human papillomavirus DNA in male urine samples. Genitourin Med 69(4):276–279
28. Giuliano AR, Nielson CM, Flores R, Dunne EF, Abrahamsen M, Papenfuss MR, Markowitz LE, Smith D, Harris RB (2007) The optimal anatomic sites for sampling heterosexual men for human papillomavirus (HPV) detection: the HPV detection in men study. J Infect Dis 196(8):1146–1152
29. Golijow CD, Pérez LO, Smith JS, Abba MC (2005) Human papillomavirus DNA detection and typing in male urine samples from a high-risk population from Argentina. J Virol Methods 124(1–2):217–220
30. Gupta A, Arora R, Gupta S, Prusty BK, Kailash U, Batra S, Das BC (2006) Human papillomavirus DNA in urine samples of women with or without cervical cancer and their male partners compared with simultaneously collected cervical/penile smear or biopsy specimens. J Clin Virol 37(3):190–194
31. Hernandez BY, Wilkens LR, Zhu X, McDuffie K, Thompson P, Shvetsov YB, Ning L, Goodman MT (2008) Circumcision and human papillomavirus infection in men: a site-specific comparison. J Infect Dis 197(6):787–794
32. Hillman RJ, Botcherby M, Ryait BK, Hanna N, Taylor-Robinson D (1993) Detection of human papillomavirus DNA in the urogenital tracts of men with anogenital warts. Sex Transm Dis 20(1):21–27
33. Hillman RJ, Ryait BK, Botcherby M, Taylor-Robinson D (1993) Human papillomavirus DNA in the urogenital tracts of men with gonorrhoea, penile warts or genital dermatoses. Genitourin Med 69(3):187–192
34. Hillman RJ, Ryait BK, Botcherby M, Walker MM, Taylor-Robinson D (1993) Human papillomavirus DNA in the urogenital tracts of men with genital dermatoses: evidence for multifocal infection. Int J STD AIDS 4(3):147–154
35. Iwasawa A, Hiltunen-Back E, Reunala T, Nieminen P, Paavonen J (1997) Human papillomavirus DNA in urine specimens of men with condyloma acuminatum. Sex Transm Dis 24(3):165–168
36. Jacobson DL, Womack SD, Peralta L, Zenilman JM, Feroli K, Maehr J, Daniel RW, Shah KV (2000) Concordance of human papillomavirus in the cervix and urine among inner city adolescents. Pediatr Infect Dis J 19(8):722–728
37. Jong E, Mulder JW, van Gorp EC, Wagenaar JK, Derksen J, Westerga J, Tol A, Smits PH (2008) The prevalence of human papillomavirus (HPV) infection in paired urine and cervical smear samples of HIV-infected women. J Clin Virol 41(2):111–115
38. Jong E, van Gorp EC, Mulder JW, Tol A, Smits PH (2009) Effect of HIV viral load, CD4 cell count and antiretroviral therapy on human papillomavirus prevalence in urine samples of HIV-infected men. Int J STD AIDS 20(4):262–264
39. Lazcano-Ponce E, Herrero R, Muñoz N, Hernandez-Avila M, Salmerón J, Leyva A, Meijer CJ, Walboomers JM (2001) High prevalence of human papillomavirus infection in Mexican males: comparative study of penile-urethral swabs and urine samples. Sex Transm Dis 28(5):277–280
40. Manhart LE, Holmes KK, Koutsky LA, Wood TR, Kenney DL, Feng Q, Kiviat NB (2006) Human papillomavirus infection among sexually active young women in the United States: Implications for developing a vaccination strategy. Sex Transm Dis 33(8):502–508
41. Melchers WJ, Schift R, Stolz E, Lindeman J, Quint WG (1989) Human papillomavirus detection in urine samples from male patients by the polymerase chain reaction. J Clin Microbiol 27(8):1711–1714
42. Nakazawa A, Inoue M, Fujita M, Tanizawa O, Hakura A (1991) Detection of human papillomavirus type 16 in sexual partners of patients having cervical cancer by polymerase chain reaction. Jpn J Cancer Res 82(11):1187–1190
43. Payan C, Ducancelle A, Aboubaker MH, Caer J, Tapia M, Chauvin A, Peyronnet D, Le Hen E, Arab Z, Legrand MC, Tran A, Postec E, Tourmen F, Avenel M, Malbois C, De Brux MA, Descamps P, Lunel F (2007) Human papillomavirus quantification in urine and cervical samples by using the Mx4000 and LightCycler general real-time PCR systems. J Clin Microbiol 45(3):897–901
44. Payan C, Tran A, Foll Y, Vallon C, Poulhazan E, Lacut K, Charles F, Herry F, Postec E, Collet M (2009) Evaluation of a new strategy for cervix cancer screening in women who do not access to pap smear screening in West Brittany, using a urine test for human papilloma-

virus (HPV) detection on a large scale plateform combining EasyMag extractor and real-time PCR LightCycler system (the PAPU29 PHASE 1 study). J Clin Virol 46(Suppl 1):S12

45. Powell J, Strauss S, Gray J, Wojnarowska F (2003) Genital carriage of human papilloma virus (HPV) DNA in prepubertal girls with and without vulval disease. Pediatr Dermatol 20 (3):191–194
46. Prusty BK, Kumar A, Arora R, Batra S, Das BC (2005) Human papillomavirus (HPV) DNA detection in self-collected urine. Int J Gynaecol Obstet 90(3):223–227
47. Rintala MA, Pöllänen PP, Nikkanen VP, Grénman SE, Syrjänen SM (2002) Human papillomavirus DNA is found in the vas deferens. J Infect Dis 185(11):1664–1667
48. Smits PH, Bakker R, Jong E, Mulder JW, Meenhorst PL, Kleter B, van Doorn LJ, Quint WG (2005) High prevalence of human papillomavirus infections in urine samples from human immunodeficiency virus-infected men. J Clin Microbiol 43(12):5936–5939
49. Stanczuk GA, Kay P, Allan B, Chirara M, Tswana SA, Bergstrom S, Sibanda EN, Williamson AL (2003) Detection of human papillomavirus in urine and cervical swabs from patients with invasive cervical cancer. J Med Virol 71(1):110–114
50. Strauss S, Jordens JZ, McBride D, Sonnex C, Edwards S, Desselberger U, Watt P, Gray JJ (1999) Detection and typing of human papillomavirus DNA in paired urine and cervical scrapes. Eur J Epidemiol 15(6):537–543
51. Vossler JL, Forbes BA, Adelson MD (1995) Evaluation of the polymerase chain reaction for the detection of human papillomavirus from urine. J Med Virol 45(3):354–360
52. Weaver BA, Feng Q, Holmes KK, Kiviat N, Lee SK, Meyer C, Stern M, Koutsky LA (2004) Evaluation of genital sites and sampling techniques for detection of human papillomavirus DNA in men. J Infect Dis 189(4):677–685
53. Zambrano A, Kalantari M, Simoneau A, Jensen JL, Villarreal LP (2002) Detection of human polyomaviruses and papillomaviruses in prostatic tissue reveals the prostate as a habitat for multiple viral infections. Prostate 53(4):263–276
54. Johnson DJ, Calderaro AC, Roberts KA (2007) Variation in nuclear DNA concentrations during urination. J Forensic Sci 52(1):110–113
55. Baker T (2009) Methods and reagents for preservation of DNA in bodily fluids. United States Patent application publication, pp 1–24
56. Bierau K (2008) Improved urine sample collecting and processing. WIPO International Application published under the patent cooperation treaty, pp 1–91
57. McMillian RA (2003) Method for preservation of cells and nucleic acid targets. United States Patent application publication, pp 1–9
58. Su YH, Wang M, Brenner DE, Norton PA, Block TM (2008) Detection of mutated K-ras DNA in urine, plasma, and serum of patients with colorectal carcinoma or adenomatous polyps. Ann N Y Acad Sci 1137:197–206
59. Su YH, Wang M, Brenner DE, Ng A, Melkonyan H, Umansky S, Syngal S, Block TM (2004) Human urine contains small, 150 to 250 nucleotide-sized, soluble DNA derived from the circulation and may be useful in the detection of colorectal cancer. J Mol Diagn 6(2):101–107
60. Shekhtman EM, Anne K, Melkonyan HS, Robbins DJ, Warsof SL, Umansky SR (2009) Optimization of transrenal DNA analysis: detection of fetal DNA in maternal urine. Clin Chem 55(4):723–729
61. Birch NC, Stickle DF, Young A, Medina P, Hinrichs SH (2003) Evaluation of urine specimen integrity in a public health STD screening program. Am J Clin Pathol 119(4):516–521
62. Middleton K, Peh W, Southern S, Griffin H, Sotlar K, Nakahara T, El-Sherif A, Morris L, Seth R, Hibma M, Jenkins D, Lambert P, Coleman N, Doorbar J (2003) Organization of human papillomavirus productive cycle during neoplastic progression provides a basis for selection of diagnostic markers. J Virol 77(19):10186–10201
63. Bryzgunova OE, Skvortsova TE, Kolesnikova EV, Starikov AV, Rykova EY, Vlassov VV, Laktionov PP (2006) Isolation and comparative study of cell-free nucleic acids from human urine. Ann N Y Acad Sci 1075:334–340
64. D'Hauwers KW, Tjalma WA (2009) Screening for human papillomavirus: is urine useful? Indian J Cancer 46(3):190–193
65. Sehgal A, Gupta S, Parashari A, Sodhani P, Singh V (2009) Urine HPV-DNA detection for cervical cancer screening: prospects and prejudices. J Obstet Gynaecol 29(7):583–589
66. Song ES, Lee HJ, Hwang TS (2007) Clinical efficacy of human papillomavirus DNA detection in urine from patients with various cervical lesions. J Korean Med Sci 22(1):99–104