Cancer Epidemiology, Biomarkers & Prevention

Research Article

High-Throughput Monitoring of Human Papillomavirus Type Distribution

Anna Söderlund-Strand[1] and Joakim Dillner[1,2]

Abstract

Background: There is a need for a rapid and cost-effective evaluation of the effects of different human papillomavirus (HPV) vaccination strategies. Sexually active adolescents are a preferred target group for monitoring, as effects on HPV prevalence would be measurable shortly after implementation of vaccination programs.

Methods: The Swedish *Chlamydia trachomatis* testing program offers free *Chlamydia trachomatis* testing and reaches a majority of all adolescents in the population. We anonymized the 44,146 samples submitted for *Chlamydia trachomatis* testing in Southern Sweden during March to November 2008 and conducted HPV genotyping using PCR followed by mass spectrometry.

Results: The HPV positivity peaked at 54.4% [95% confidence interval (CI), 52.2–56.6] among 21-year-old women and at 15.0% (95% CI, 12.4–17.6) among 23-year-old men. The HPV positivity was 37.8% (95% CI, 37.3–38.3) for women and 11.2% (95% CI, 10.6–11.8) for men. The most prevalent types among women were HPV 16 (10.0%; 95% CI, 9.7–10.3) and HPV 51 (6.0%; 95% CI, 5.7–6.3) and, among men, HPV 16 (2.1%; 95% CI, 1.8–2.4) and HPV 6 and HPV 51 (1.7%; 95% CI, 1.5–1.9).

Conclusion: The high HPV prevalences seen in the *Chlamydia trachomatis* screening population enables monitoring of the HPV type distribution among sexually active adolescents at high precision.

Impact: Effectiveness of HPV vaccination programs in terms of reducing HPV infections has been difficult to measure because of logistic constraints. We describe a system for high-throughput monitoring of HPV type-specific prevalences using samples from the *Chlamydia trachomatis* screening program. *Cancer Epidemiol Biomarkers Prev; 22(2); 242–50. ©2012 AACR.*

Introduction

During recent years, human papillomavirus (HPV) vaccines targeting the HPV types associated with highest risk for cervical cancer have been introduced and proven to efficiently prevent the high-grade cervical lesions caused by these HPV types (1). For monitoring the impact of HPV vaccination policies, outcomes such as cervical cancer incidence and incidence of high-grade cervical lesions cannot provide timely feedback on strategy effectiveness because of the long incubation times between infection and disease (2). The earliest outcome of HPV vaccination that can be monitored is changes in the HPV type-specific prevalences. The vaccines have some cross-protection against phylogenetically related HPV types not included in the vaccines (1, 3, 4), which might affect circulation of these related HPV types in vaccinated populations (5). It is also of interest to monitor whether the reduction of the vaccine types in the population may lead to increases in HPV prevalence of other HPV types ("type replacement"; ref. 5).

One available infrastructural option for monitoring of the HPV-vaccination impact would be HPV analysis of the samples obtained from cervical screening. However, as cervical screening does not start until age 23 or 25 in many countries, the effectiveness of the vaccination strategy selected would not be measurable until many years after onset of organized vaccination. In addition, cervical screening samples will not provide any data regarding sex-specific changes of HPV prevalence. An alternative is to use the samples from the *Chlamydia trachomatis* screening programs, which in many countries has a high coverage among sexually active teenagers, and would provide a more rapid evaluation of HPV vaccination impact, with data also being provided on HPV prevalences among both sexes. The *Chlamydia trachomatis* testing in Sweden is strongly promoted, for example, at youth clinics and in the media. In Southern Sweden, 1 single laboratory conducts the *Chlamydia trachomatis* analysis on all of the approximately 80,000 samples collected annually, and more than half of the tested individuals are in the age group of 14 to 24.

Authors' Affiliations: [1]Department of Clinical Microbiology, Skåne University Hospital, Malmö; and [2]Departments of Laboratory Medicine, Medical Epidemiology, and Biostatistics, Karolinska Institutet and Karolinska Hospital, Stockholm, Sweden

Corresponding Author: Joakim Dillner, WHO LabNet Global Reference Laboratory, Clinical Microbiology, Skåne University Hospital Entrance 78, S-20502 Malmö, Sweden. Phone: 46-7-688-71126; Fax: 464-033-7312; E-mail: joakim.dillner@ki.se

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In Sweden, HPV-vaccination targeting 11- to 12-year-old girls and catch-up vaccination of 13- to 17-year-old girls started only recently. HPV vaccines were, however, given a public subsidy for 13- to 17-year-old girls already in 2006 (6), but were still not widely used in 2008.

We developed a high-throughput, high-precision, and cost-effective strategy for monitoring of effectiveness of HPV-vaccination and report the baseline data on HPV prevalence for Southern Sweden in the year 2008, when the vaccine coverage was still low.

Material and Methods

All samples taken in Southern Sweden from March to November in 2008 for analysis of *Chlamydia trachomatis* were tested. The HPV vaccination coverage among girls who were 13 to 17 years of age in 2008 and living in the study area increased from approximately 8% to approximately 17% during this period (excerpt of the Swedish HPV vaccination registry). The study population was the population of the Skåne region in Southern Sweden, with 1.2 million inhabitants. The samples for *Chlamydia trachomatis* testing were systematically collected among attendants at several types of clinics, for instance, gynecology clinics, youth clinics, venereology clinics, and primary care units. All samples were anonymized. The ethical review board in Lund, Sweden, decided that informed consent was not required.

All samples were extracted and analyzed for *Chlamydia trachomatis* using the Abbott m2000sp system according to the manufacturer's instructions (Abbott Molecular). The residual extracted DNA that remained after *Chlamydia trachomatis* analysis was stored at 4°C before analysis for HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68, using PCR followed by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS). As a first step of the MS genotyping method, a consensus PCR is conducted, followed by a mass extend (ME) reaction with ME primers that are specific for each genotype and checks that each has a distinct mass. If the amplified template for a certain type is present, the corresponding ME primer will be elongated by a single base generating a certain mass that is unique for that genotype. In the final MS detection step, the ME-primers are separated by mass. Presence of specific elongated ME-primers shows the presence and identity of the specific genotypes, while the presence of ME-primers that were not elongated show the absence of that specific genotype. Unless otherwise specified, all procedures regarding mass extension and MS were conducted with protocols and materials from SEQUENOM (Hamburg, Germany). In short, a consensus PCR was conducted using the MGP primer system as previously described but with reaction mixes of 6 μL containing 2 μL DNA template (7). The mixes were robotically pipetted with disposable tips and amplified in 384-well plates; after this, the PCR products were dephosphorylated with shrimp alkaline phosphatase according to the SEQUENOM protocol. A mass-extend reaction was then carried out using the SEQUENOM i-plex MassARRAY technology according to the manufacturer's instructions and protocols, with some modifications. The ME primers with a molecular mass of more than 6,500 Da were added to the i-plex mix at a concentration of 1.25 μmol/L in the final reaction volume of 10 μL, while the ME primers with lower molecular weight had a concentration of 0.625 μmol/L. The sequences of the ME primers were (in the 5′ to the 3′ direction) GTGTATGTGGAAGATGTAGTTAC for HPV 6, GTGTATGTAGCAGATTTAGACAC for HPV 11, GTAGTTTCTGAAGTAGATATG for HPV 16, CATCATATTGCCCAGGT for HPV 18, ATGTAGTATCACTGTTTGC for HPV 31, TGTCACTAGTTACTTGT for HPV 33, GACACAGCAGAACAC for HPV 35, AGAAGGTATGGAAGACTC for HPV 39, TACTTGGCACAGGATTT for HPV 45, TGCTTAAAGTTACTTGGAGT for HPV 51, GCTTTCCTTTTTAACC for HPV 52, GTCTAAGGTACTGATTAATTT for HPV 56, TCAACATGACGTACA for HPV 58, AGGAATAGAAGAAGTAGTAGA for HPV 59, GATTGATTTCACGGGCA for HPV 66, and CTGTAGTAGTGGACAATGTA for HPV 68. The thermocycle conditions for the mass-extend reaction were 94°C for 1 minute followed by 40 cycles of 94°C for 7 seconds with 5 internal cycles of 52°C for 7 seconds and 72°C for 5 seconds in each of the 40 cycles, and a final step of 72°C for 3 minutes. After desalting, 15 nL of each ME product was applied to a 384-spot SpectroChip, and the MS analysis was carried out and interpreted using the MassARRAY Typer Software.

One large and 1 small set of positive controls were used in the MS analysis. The large set was the 2008 WHO Global HPV LabNet HPV DNA typing proficiency panel, consisting of 43 samples with HPV plasmid dilutions in defined amounts (traceable to the International Standard for HPV DNA). For HPV 16 and 18, the panel samples contain 1, 10, and 100 copies per microliter; for the 12 other oncogenic HPV types and for HPV 6 and 11 there were samples with 10 and 100 copies per microliter input volume. The remaining 8 panel samples contain a mix of plasmids of 4 different HPV types with 10 and 100 copies per microliter input volume of each type. The small set of positive controls was the 8 mixed samples from the proficiency panel. In each MS run, both sets of positive controls were included; however, because the input volume for the MS method is 2 μL, the copy numbers were 2, 20, and 200 copies/2 μL. As nontemplate controls, 10 ng/μL human DNA in Tris-EDTA buffer was used. The criterion for proficiency as defined by the WHO Global HPV LabNet is that all 16 HPV types should be detectable at 500 copies, except for HPV 16 and 18 that should be detectable at 50 copies (8). The MS method was also tested for proficiency using the 2010 WHO Global HPV LabNet HPV DNA typing proficiency panel.

A subset of samples was also analyzed using the MGP primer system followed by detection using Luminex (7), with some modifications. Luminex detects the same 16 types as MS, but it also detects HPV 26, 30, 40, 42, 43, 53, 54, 61, 67, 69, 70, 73, 74, 81, 82, 83, 86, 87, 89, 90, 91, and 114. The cutoff was the mean of the median fluorescence intensity of the nontemplate controls plus 5 times the SD, with a minimum SD of 1. For HPV 6, 40, 51, 59, 68A, 73, 74, and 82, the cutoff was modified to 1.5 times the cutoff, and, for HPV 30 and 90, the cutoff was modified to 2 times the cutoff. Because the ME primer for HPV 68 showed some cross-reactivity with HPV 70, all samples MS-positive for HPV 68 were also analyzed using the Luminex-based detection with probes for HPV 68A (GenBank accession number DQ080079), HPV 68B (GenBank accession number M73258 for the original sequence ME180), and HPV 70 for confirmation of the results.

The samples of the proficiency panel used as positive controls throughout the study were detected in 98.9% of experiments at the 200 copy level with a specificity of 100%. The MS method was proficient in the blinded 2010 WHO Global HPV LabNet HPV DNA typing proficiency panel.

The reproducibility for the MS method and the Luminex-based method was determined by parallel testing of a subset of 534 samples using both methods and were concordant in 82.2% of tests (range, 75.3–86.5%).

Statistical analysis

Two-sided χ^2 tests for prevalence differences between groups and 95% confidence intervals (CI) for proportions of HPV positivity were calculated using SPSS software (IBM)

Results

We analyzed 44,146 samples, of which 33,137 samples were from women and 11,009 from men (Table 1). Because all samples were anonymized and some subjects may have several Chlamydia tests during a year, the exact number of samples obtained from each individual is unknown. However, as the total number of sampled subjects and the total number of samples collected during 2008 was known, the number of unique subjects tested can be estimated (Table 1). The largest proportion of women (23.0%) in the catchment area population was sampled at the age of 19, whereas the largest proportion of men (7.8%) in the population was sampled at the age of 22.

The HPV positivity peaked at 54.4% (95% CI, 52.2–56.6) among 21-year-old women and at 15% (95% CI, 12.4–17.6) among 23-year-old men (Table 2 and Fig. 1). Stratification of the age-specific HPV prevalences to specific sample types, such as urine samples, found a similar dependence on age (Table 2 and Fig. 1), although samples with only urine uniformly had lower HPV prevalences than samples containing genital swabs (Table 2 and Fig. 1). The general HPV positivity was 37.8% (95% CI, 37.3–38.3) for all

Table 1. Samples ($n = 44{,}146$) collected during March to November 2008 according to age and gender, and in comparison to all inhabitants in the region

	Tested samples (estimated number of unique subjects)		Inhabitants in Skåne region Nov 1, 2008		Percentage of population tested	
Age group, years	Women	Men	Women	Men	Women	Men
0–12	70 (55)	56 (44)	83,036	87,789	0.066	0.050
13	14 (11)	1 (1)	6,782	7,258	0.16	0.014
14	130 (101)	15 (12)	7,227	7,595	1.4	0.16
15	563 (439)	46 (36)	7,647	7,837	5.7	0.46
16	982 (766)	202 (158)	7,811	8,384	9.8	1.9
17	1,715 (1,338)	327 (255)	7,983	8,503	16.8	3.0
18	2,313 (1,804)	546 (426)	8,284	8,497	21.8	5.0
19	2,300 (1,794)	697 (544)	7,808	8,261	23.0	6.6
20	2,237 (1,745)	763 (595)	8,083	8,077	21.6	7.4
21	2,051 (1,600)	765 (597)	7,850	7,762	20.4	7.7
22	2,039 (1,590)	791 (617)	8,002	7,863	19.9	7.8
23	1,849 (1,442)	742 (579)	7,943	7,795	18.2	7.4
24	1,575 (1,228)	648 (505)	7,888	7,832	15.6	6.4
25	1,418 (1,106)	584 (456)	7,643	7,872	14.5	5.8
26–30	5,984 (4,668)	2,164 (1,688)	38,862	40,067	12.0	4.2
31–35	3,829 (2,987)	1,121 (874)	39,788	41,643	7.5	2.1
36–40	2,181 (1,701)	615 (480)	40,526	41,742	4.2	1.1
41–45	1,060 (827)	372 (290)	42,727	44,256	1.9	0.66
46–50	467 (364)	208 (162)	37,411	38,144	1.0	0.42
51–55	195 (152)	147 (115)	36,744	37,119	0.41	0.31
56–60	92 (72)	100 (78)	38,374	37,927	0.19	0.21
61+	73 (57)	99 (77)	154,379	127,875	0.037	0.060
Total	33,137 (25,847)	11,009 (8,587)	612,798	600,098	4.2	1.4

Table 2. HPV prevalences in the tested population (44,146 samples) according to gender and age group

Age group		*n* samples	HPV-Positive samples	% HPV Positivity (95% CI)	% HPV Positivity restricted to samples with only urine (95% CI)	% HPV Positivity restricted to samples with cervicovaginal swabs (95% CI)[a]
0–12	Women	70	2	2.9 (0–6.8)	0 (0)	10.0 (0–28.6)
	Men	56	0	0 (0)	0 (0)	N/A
13	Women	14	3	21.4 (0–42.9)	20.0 (0–55.1)	22.2 (0–49.4)
	Men	1	0	0 (0)	0	N/A
14	Women	130	20	15.4 (9.2–21.6)	11.1 (0.84–21.4)	17.2 (9.5–24.9)
	Men	15	0	0 (0)	0	N/A
15	Women	563	121	21.5 (18.1–24.9)	18.9 (13.5–24.3)	23.4 (19.0–27.9)
	Men	46	1	2.2 (0–6.4)	0	N/A
16	Women	982	318	32.4 (29.5–35.3)	29.0 (24.3–33.7)	34.4 (30.6–38.2)
	Men	202	14	6.9 (3.4–10.4)	7.1 (3.5–10.7)	N/A
17	Women	1,715	677	39.5 (37.2–41.8)	34.0 (29.9–38.1)	42.3 (39.5–45.1)
	Men	327	19	5.8 (3.3–8.3)	5.7 (3.1–8.3)	N/A
18	Women	2,313	1,035	44.7 (42.7–46.7)	39.1 (35.5–42.7)	47.3 (44.9–49.7)
	Men	546	56	10.3 (7.8–12.8)	9.8 (7.3–12.3)	N/A
19	Women	2,300	1,154	50.2 (48.2–52.2)	43.2 (39.5–46.9)	53.6 (51.1–56.1)
	Men	697	77	11.0 (8.7–13.3)	11.1 (8.7–13.5)	N/A
20	Women	2,237	1,192	53.3 (51.2–55.4)	43.1 (39.3–46.9)	57.7 (55.2–60.2)
	Men	763	90	11.8 (9.5–14.1)	11.8 (9.4–14.2)	N/A
21	Women	2,051	1,115	54.4 (52.2–56.6)	44.0 (40.1–47.9)	59.7 (57.1–62.3)
	Men	765	106	13.9 (11.4–16.4)	14.1 (11.6–16.6)	N/A
22	Women	2,039	1,094	53.7 (51.5–55.9)	37.6 (33.6–41.6)	60.2 (57.7–62.7)
	Men	791	113	14.3 (11.9–16.7)	13.9 (11.4–16.4)	N/A
23	Women	1,849	892	48.2 (45.9–50.5)	36.1 (32.0–40.2)	53.4 (50.7–56.1)
	Men	742	111	15.0 (12.4–17.6)	13.8 (11.2–16.4)	N/A
24	Women	1,575	705	44.8 (42.3–47.3)	28.2 (24.0–32.4)	51.9 (49.0–54.8)
	Men	648	86	13.3 (10.7–15.9)	12.5 (9.8–15.2)	N/A
25	Women	1,418	586	41.3 (38.7–43.9)	31.1 (26.7–35.5)	46.0 (42.9–49.1)
	Men	584	68	11.6 (9.0–14.2)	10.3 (7.7–12.9)	N/A
26–30	Women	5,984	1,985	33.2 (32.0–34.4)	19.0 (17.3–20.7)	41.1 (39.5–42.7)
	Men	2,164	249	11.5 (10.2–12.8)	9.6 (8.3–10.9)	N/A
31–35	Women	3,829	846	22.1 (20.8–23.4)	13.5 (11.8–15.2	28.4 (26.5–30.3)
	Men	1,121	99	8.8 (7.1–10.5)	7.5 (5.8–9.2)	N/A
36–40	Women	2,181	426	19.5 (17.8–21.2)	13.5 (11.2–15.8)	23.5 (21.2–25.8)
	Men	615	56	9.1 (6.8–11.4)	8.3 (5.9–10.7)	N/A
41–45	Women	1,060	208	19.6 (17.2–22.0)	16.3 (12.2–20.4)	21.2 (18.2–24.2)
	Men	372	27	7.3 (4.7–9.9)	6.1 (3.4–8.8)	N/A
46–50	Women	467	81	17.3 (13.9–20.7)	11.2 (5.5–16.9)	19.9 (15.6–24.2)
	Men	208	22	10.6 (6.4–14.8)	5.9 (2.2–9.6)	N/A
51–55	Women	195	45	23.1 (17.2–29.0)	22.0 (10.5–33.5)	23.5 (16.4–30.6)
	Men	147	17	11.6 (6.4–16.8)	7.6 (2.8–12.4)	N/A
56–60	Women	92	17	18.5 (10.6–26.4)	17.9 (3.7–32.1)	19.0 (8.9–29.1)
	Men	100	19	19.0 (11.3–26.7)	12.7 (5.4–20.0)	N/A
61+	Women	73	12	16.4 (7.9–24.9)	5.6 (0–16.2)	22.4 (10.7–34.1)
	Men	99	5	5.1 (0.77–9.4)	3.9 (0–8.2)	N/A
Total	Women	33,137	12,534	37.8 (37.3–38.3)	26.9 (26.1–27.7)	43.6 (42.9–44.3)
	Men	11,009	1,235	11.2 (10.6–11.8)	10.3 (9.7–10.9)	N/A

[a]Samples combining a cervicovaginal swab with urine are included in this category, as their HPV prevalences were similar to the HPV prevalences in cervicovaginal swabs.
N/A, not applicable.

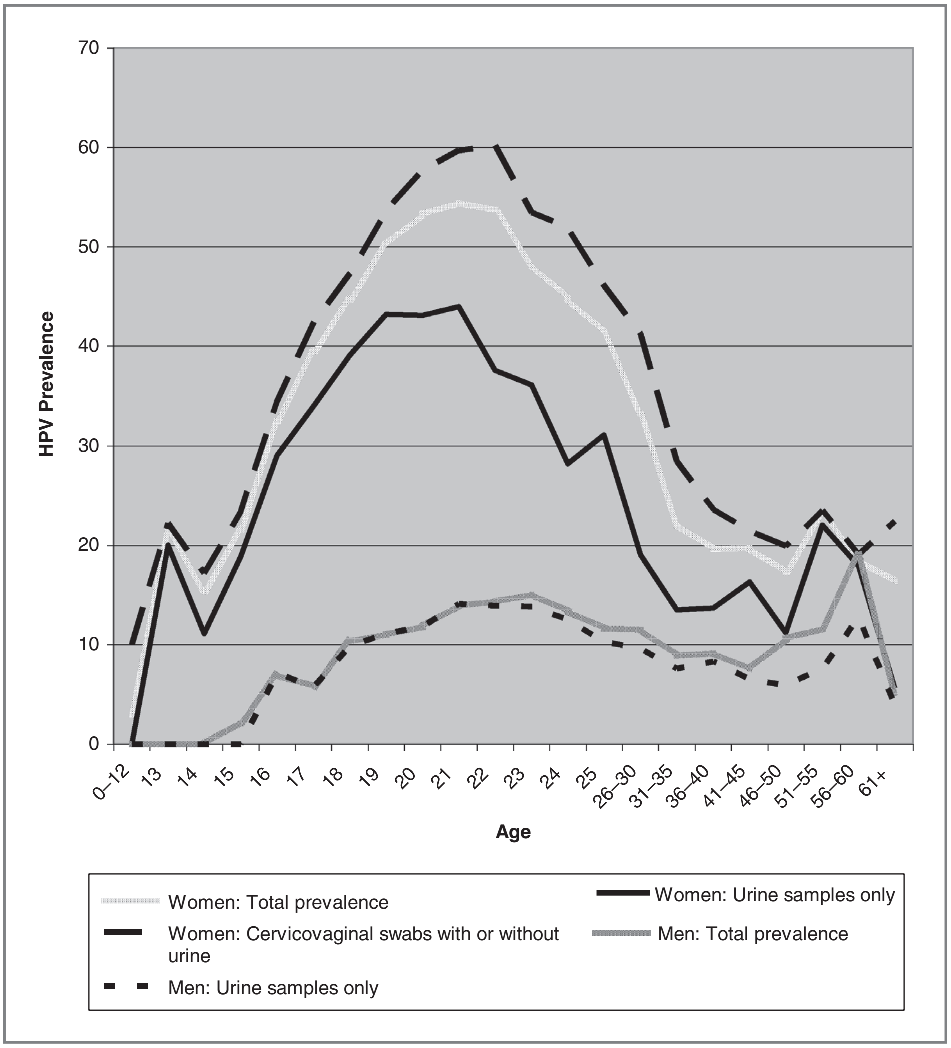

Figure 1. HPV prevalence according to age and gender.

women in the study population and 11.2% (95% CI, 10.6–11.8) for all men (Table 2).

The recommended and most common sample type among women was a genital swab immersed in first-void urine, constituting 41.0% of samples (Table 3). The second most common sample type for women was a first-void urine sample (32.7%), followed by cervical swabs (22.1%). The most common sample type among men was a first-void urine sample (89.0%; Table 3). The highest HPV prevalences among women were found in combined cervical/urethral swabs with a prevalence of 46.0% (95% CI, 42.3–49.7) and in the genital swabs immersed in urine with a prevalence of 44.5% (95% CI, 43.7–45.3; Table 3). The HPV prevalence was slightly higher for cervicovaginal samples combined with urine than for cervicovaginal samples without urine (44.5% vs. 42.0%). The type-specific prevalences were significantly higher in the combined samples for HPV 6, 18, 51, and 66. The highest HPV prevalences among men were found in rectal samples (37.6%; 95% CI, 30.4–44.8) followed by urethral samples (21.8%; 95% CI, 18.5–25.1) and urine samples with an immersed genital swab (21.1%; 95% CI, 10.5–31.7). A significantly higher type-specific prevalence in the rectal samples was found for HPV 11, 16, 18, 45, 56, 59, and 68.

The type-specific HPV prevalence during the first 2 months of sampling among 15- to 18-year-old girls was compared with the last 3 months of sampling, but the prevalence did not change significantly for any HPV type during the study period (data not shown).

The most prevalent types among women were HPV 16 (10.0%; 95% CI, 9.7–10.3), HPV 51 (6.0%; 95% CI, 5.7–6.3), HPV 31 (5.2%; 95% CI, 5.0–5.4), and HPV 18 and HPV 66 (5.1%; 95% CI, 4.9–5.3); among men, the most prevalent types were HPV 16 (2.1%; 95% CI, 1.8–2.4), HPV 6 and HPV 51 (1.7%; 95% CI, 1.5–1.9), HPV 18 and 66 (1.4%; 95% CI, 1.2–1.6), and HPV 31 (1.3%; 95% CI, 1.1–1.5; Table 4).

Discussion

We have developed a high-throughput, high-precision strategy for monitoring the type-specific HPV prevalences among young, sexually active subjects of both sexes.

Table 3. HPV prevalence according to sample type in the tested population of 33,137 samples from women and 10,997 samples from men (12 samples from men with missing sample type are excluded)

Sample type		*n* samples	HPV-positive samples	% HPV positivity (95% CI)
Urine	Women	10,840	2,919	26.9 (26.1–27.7)
	Men	9,787	1,009	10.3 (9.7–10.9)
Combined urine and genital swab sample[a]	Women	13,574	6,047	44.5 (43.7–45.3)
	Men	57	12	21.1 (10.5–31.7)
Vagina	Women	53	16	30.2 (17.8–42.6)
	Men	N/A	N/A	N/A
Cervix	Women	7,333	3,058	41.7 (40.6–42.8)
	Men	N/A	N/A	N/A
Combined cervical and urethral sample	Women	681	313	46.0 (42.3–49.7)
	Men	N/A	N/A	N/A
Urethra	Women	45	10	22.2 (10.1–34.3)
	Men	597	130	21.8 (18.5–25.1)
Rectum	Women	10	3	30.0 (1.6–58.4)
	Men	173	65	37.6 (30.4–44.8)
Eye	Women	108	3	2.8 (0–5.9)
	Men	97	3	3.1 (0–6.5)
Pharynx	Women	82	2	2.4 (0–5.7)
	Men	270	14	5.2 (2.6–7.8)
Other	Women	411	163	39.7 (35.0–44.4)
	Men	16	1	6.3 (0–18.2)

[a]Genital swab: for women, includes cervical, vaginal, or unspecified genital swabs; for men, unspecified genital swabs. N/A, not applicable.

The high-throughput HPV DNA analysis system used is semi-automated, and has a low reagent cost per sample (~2 euro per sample). The use of residual extracted DNA from *Chlamydia trachomatis* testing provides already extracted DNA samples in a plate format, ready to use for HPV testing. Because samples were anonymized, informed consent was not required and cost, management, and selection biases induced by nonattendance could be minimized. As the samples were obtained from a sexually active and mostly young population, they are not representative of the general population. However, our selection of samples has targeted a maximally relevant population to answer the question of whether HPV vaccination strategies will impact HPV prevalences in the sexually active populations that are most affected by HPV infections. Furthermore, as the sampled population overlaps in age range with the same population to be targeted by HPV vaccination programs and as HPV DNA becomes detectable shortly after infection, the monitoring strategy should allow a very rapid feedback on whether the HPV vaccination strategies used are effective in controlling the spread of HPV infections.

Large-scale use of residual extracted DNA from *Chlamydia trachomatis* screening for monitoring of effectiveness of HPV vaccination strategies has, to our knowledge, not been described earlier. Baseline data on HPV prevalences before HPV vaccination has previously been established using self-collected cervicovaginal samples, urine samples, or cervical cytology samples (9). We provided a very large-scale description of how HPV prevalences are dependent on the type of genital sample obtained. The fact that prevalences were considerably higher among women sampled with cervicovaginal swabs compared with sampling with only urine highlights that comparisons of HPV prevalences between studies will need to consider the type of sample used. Female high-risk HPV prevalence in cervicovaginal swab samples in the United States among 20- to 24-year-olds has been estimated to be 43.4% (9), slightly lower than what we found in the corresponding age group (56.9%). In Australia, the age-adjusted baseline high-risk HPV prevalence in cervical swab samples among 15- to 40-year-old women was 30.0% and 31.3% for nonindigenous and indigenous Australian women, respectively (10). This is also slightly lower than what we found in the corresponding age group (45.2%). In Scotland, the prevalence of high-risk HPV in urine from 15- to 18-year-old women was 12.6% (11), considerably lower than what we find in the same age group (33.3%). The HPV prevalence in urine among 15- to 18-year-old men in Scotland was 2.4% (11), considerably lower than in our

Table 4. HPV type-specific results from the MALDI-TOF analysis of 44,146 samples collected during March to November 2008

HPV	Gender	*n* Positive samples	% Positive samples (95% CI)	% HPV Positivity restricted to samples with only urine (95% CI)	% HPV Positivity restricted to samples with cervicovaginal swabs (95% CI)[a]
6	Women	1,361	4.1 (3.9–4.3)	2.9 (2.6–3.2)	4.7 (4.4–5.0)
	Men	183	1.7 (1.5–1.9)	1.6 (1.4–1.8)	N/A
11	Women	289	0.87 (0.77–0.97)	0.65 (0.50–0.80)	1.0 (0.87–1.1)
	Men	48	0.44 (0.32–0.56)	0.37 (0.25–0.49)	N/A
16	Women	3,298	10.0 (9.7–10.3)	6.6 (6.1–7.1)	11.7 (11.3–12.1)
	Men	228	2.1 (1.8–2.4)	1.9 (1.6–2.2)	N/A
18	Women	1,698	5.1 (4.9–5.3)	3.5 (3.2–3.8)	6.0 (5.7–6.3)
	Men	157	1.4 (1.2–1.6)	1.3 (1.1–1.5)	N/A
31	Women	1,736	5.2 (5.0–5.4)	3.6 (3.2–4.0)	6.1 (5.8–6.4)
	Men	147	1.3 (1.1–1.5)	1.2 (0.98–1.4)	N/A
33	Women	785	2.4 (2.2–2.6)	1.5 (1.3–1.7)	2.8 (2.6–3.0)
	Men	55	0.50 (0.37–0.63)	0.52 (0.38–0.66)	N/A
35	Women	548	1.7 (1.6–1.8)	1.1 (0.90–1.3)	2.0 (1.8–2.2)
	Men	27	0.25 (0.16–0.34)	0.24 (0.14–0.34)	N/A
39	Women	1,216	3.7 (3.5–3.9)	2.4 (2.1–2.7)	4.3 (4.0–4.6)
	Men	65	0.59 (0.45–0.73)	0.53 (0.39–0.67)	N/A
45	Women	1,054	3.2 (3.0–3.4)	2.1 (1.8–2.4)	3.7 (3.4–4.0)
	Men	101	0.92 (0.74–1.1)	0.72 (0.55–0.89)	N/A
51	Women	1,984	6.0 (5.7–6.3)	4.2 (3.8–4.6)	6.9 (6.6–7.2)
	Men	183	1.7 (1.5–1.9)	1.6 (1.4–1.8)	N/A
52	Women	1,550	4.7 (4.5–4.9)	3.0 (2.7–3.3)	5.5 (5.2–5.8)
	Men	78	0.71 (0.55–0.87)	0.69 (0.53–0.85)	N/A
56	Women	1,338	4.0 (3.8–4.2)	2.9 (2.6–3.2)	4.6 (4.3–4.9)
	Men	74	0.67 (0.52–0.82)	0.61 (0.46–0.76)	N/A
58	Women	817	2.5 (2.3–2.7)	1.8 (1.5–2.1)	2.9 (2.7–3.1)
	Men	53	0.48 (0.35–0.61)	0.48 (0.34–0.62)	N/A
59	Women	859	2.6 (2.4–2.8)	1.9 (1.6–2.2)	2.9 (2.7–3.1)
	Men	44	0.40 (0.28–0.52)	0.31 (0.20–0.42)	N/A
66	Women	1,681	5.1 (4.9–5.3)	3.6 (3.2–4.0)	5.8 (5.5–6.1)
	Men	153	1.4 (1.2–1.6)	1.3 (1.1–1.5)	N/A
68	Women	284	0.86 (0.76–0.96)	0.51 (0.38–0.64)	1.0 (0.87–1.1)
	Men	13	0.12 (0.055–0.18)	0.061 (0.012–0.11)	N/A

[a]Samples combining a cervicovaginal swab with urine are included in this category, as their HPV prevalences were similar to the HPV prevalences in cervicovaginal swabs.
N/A, not applicable.

population (7.7%). Selection of more sexually active boys is the most likely explanation. A British survey reported a 15.9% prevalence of high-risk HPV in urine among sexually active 18- to 44-year-old women (12), considerably lower than the 27.0% HPV prevalence in urine in the corresponding group in our study. However, the 9.6% prevalence of high-risk HPV in urine reported among 18- to 44-year-old men in the British survey (12) was similar to the 10.8% HPV prevalence found in urine samples among 18- to 44-year-old men in our study.

Urine is a convenient, noninvasive sample that can also be obtained by self-sampling, but has (particularly for men) somewhat lower sensitivity for detection of HPV infections than genital swabs (13). We provide a large-scale description of significant differences in HPV prevalences in urine samples according to gender. The anatomic differences of the urethra between genders are likely to affect the HPV prevalences in urine, but we cannot rule out the possibility that the difference may have epidemiologic explanations. Several studies using male urine samples report HPV positivity rates of 6% or lower (14–16). We found an overall HPV prevalence in male urine samples of 10.3%, considerably lower than in combined male urine and genital swab samples (21.1%). A multicentric study using swab samples from men found HPV prevalences of 29.7% (17). The highest HPV prevalence among men in the present study was found in rectal samples (37.6%). A recent study reported an anal HPV prevalence of 41.7% for oncogenic types and 54.5% for nononcogenic types among men who have sex with men and 9.0% for oncogenic types and 12.5% for nononcogenic types among men who have sex with women (18), which seems comparable to our results.

Our large-scale estimation of HPV prevalences in different types of clinical samples is 1 of our most important results, as we show that, in particular, urine samples have significantly lower HPV prevalences than genital swab samples. Comparisons of results from different HPV-monitoring projects in the world will, therefore, need to consider which sample types have been used if direct comparisons are to be made. When monitoring using a clinical setting such as ours, it will, of course, be essential to continue recording the exact type of clinical samples used and, in subsequent follow-up, analyze any possible changes in HPV prevalences stratified by the type of clinical sample used.

The system for high-throughput monitoring of HPV type-specific prevalences described here was used for a comprehensive analysis of all samples obtained for Chlamydia testing, regardless of sample type and age of the study subjects. Because robotic pipetting was used at all steps and the throughput is high, it was possible to analyze a large number of samples at low cost and with little hands-on time. In other settings, it is possible that restricting the testing to the most informative ages and sample types would be less costly. The present study provides large-scale data on dependence of age and sample type on results, thus enabling an informed choice of optimal age groups and sample types if lower volumes of HPV testing would be desired.

A major reason for the fact that most samples came from women is the fact that women have more *Chlamydia trachomatis*-testing opportunities than men, for instance, at prescription of oral contraceptives. Women are also more positive toward being tested for *Chlamydia trachomatis*, which could explain why we, during the 8 months of the study, were able to test 23% of all 19-year-old women in our region, but only 7.8% of all resident 22-year-old men.

The fact that the type-specific HPV prevalences among 15- to 18-year-old women did not change during the study was expected as there was only limited change in HPV vaccination coverage during this time (from 8% to 17%). However, the stability of type-specific prevalences we found suggests that there is limited random or seasonal fluctuation.

The most common type among women was HPV 16 followed by (in descending order) HPV 51, HPV 31, HPV 18 and 66, and HPV 52. This agrees only in part with a recent meta-analysis where HPV 16, 31, 18, 39, 33, and 66 were the most common types among European women (19). Our finding that HPV 51 was the second most common HPV type after HPV 16 is in accordance with the Australian survey (10). HPV-type distributions among men have been variable, with HPV 59, 16, 52, and 51 being most common among Mexican men (20), and HPV 6, 16 and 59, 52, and 39 among men from the United States (21). Major reasons for difference include different populations, different sample types, and different assays. The WHO HPV LabNet proficiency panel testing has found that different assays differ in their sensitivity for different HPV types and that only a minority of laboratories proficiently detect all the 16 major genital HPV types (8).

The WHO recommends the establishment of sentinel surveillance to monitor the impact of the HPV vaccination on HPV prevalence (22). The baseline HPV prevalence established in the present study is based on the analysis of more than 44,000 samples from a sexually active population, which provided a narrow 95% CI around the prevalence estimates. As the method used was found proficient for all the HPV types tested for, our results can be internationally compared with those of any study using a method that is also proficient for these HPV types. Our HPV monitoring system used the infrastructure available in Sweden, but similar *Chlamydia trachomatis* screening programs exist in many countries. Furthermore, as HPV monitoring will not be possible in many parts of the world where HPV vaccination is introduced, countries that are able to launch such systems may provide internationally useful data on effectiveness of different HPV vaccination strategies that may also inform strategy choices in other countries, as proposed by the WHO in 2008 (23).

In conclusion, a high-throughput HPV-monitoring system has provided reliable and large-scale baseline data on HPV prevalences among men and women in Southern Sweden in 2008. Monitoring of HPV prevalences among young, sexually active individuals, where effectiveness of HPV vaccination is likely to be seen soon after launch of successful vaccination strategies, could open new possibilities for rapid development of evidence-based improvements in vaccination program policies.

Disclosure of Potential Conflicts of Interest

J. Dillner has acted as consultant for and received research grant from Merck and also received commercial research support from Sanofi Pasteur MSD. No potential conflicts of interest was disclosed by the other author.

Authors' Contributions

Conception and design: A. Söderlund-Strand, J. Dillner
Development of methodology: A. Söderlund-Strand
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Söderlund-Strand
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Söderlund-Strand, J. Dillner
Writing, review, and/or revision of the manuscript: A. Söderlund-Strand, J. Dillner
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Söderlund-Strand
Study supervision: A. Söderlund-Strand, J. Dillner

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