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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 anonymised the 44146 samples submitted for *Chlamydia trachomatis* testing in Southern Sweden during March 2008-November 2008 and performed 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) 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.

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") (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 chosen would not be measurable until many years after onset of organised vaccination. Also, 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 e.g. at youth clinics and in the media. In Southern Sweden, one single lab performs the *Chlamydia trachomatis*

analysis on all of the about 80 000 samples collected annually, and more than half of the tested individuals are in the ages 14-24.

In Sweden, HPV-vaccination targeting 11-12-year-old girls and catch-up vaccination of 13-17-year-old girls started only recently. HPV vaccines were, however, given a public subsidy for 13-17-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 13-17-years old in 2008 and living in the study area increased from about 8% to about 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 anonymised. The ethical review board in Lund, Sweden, decided that informed consent was not required.

All samples were extracted and analysed for *Chlamydia trachomatis* using the Abbott m2000sp system according to the manufacturer's instructions (Abbott Molecular, Illinois, USA). 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 polymerase chain reaction (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 performed followed by a mass extend (ME) reaction with ME primers that are specific for each genotype and 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 demonstrates the presence and identity of the specific genotypes, whereas presence of ME-primers that were not elongated demonstrate the absence of that specific genotype. Unless otherwise specified, all procedures regarding mass extension and

MS were performed with protocols and materials from SEQUENOM (Hamburg, Germany). In short, a consensus PCR was performed 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 which the PCR products were dephosphorylated with shrimp alkaline phosphatase according to SEQUENOM protocol. A mass extend reaction was then performed 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 > 6500 Da were added to the i-plex mix at a concentration of 1.25 µM in the final reaction volume of 10 µl, whereas the ME primers with lower molecular weight had a concentration of 0.625 µM. 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 min followed by 40 cycles of 94°C for 7s with 5 internal cycles of 52°C for 7 s and 72°C for 5 s in each of the 40 cycles, and a final step of 72°C for 3 min. After desalting, 15 nl of each ME product was applied to a 384-spot SpectroChip, and the MS analysis was peformed and interpreted using the MassARRAY Typer Software.

One large and one 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 µl, for the 12 other oncogenic HPV types and for HPV 6 and 11 there were samples with 10 and 100 copies per µl input volume. The remaining 8 panel samples contain a mix of plasmids of 4 different HPV types with 10 and 100 copies per µl 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, but since the input volume for the MS method is 2 µl, the copy numbers were 2, 20, and 200 copies/ 2 µl. As non-template controls, 10 ng/µl human DNA in Tris-EDTA buffer was used. The criterium 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 analysed using the MGP primer system followed by detection using Luminex (7) with some modifications. Luminex detects the same 16 types as MS, but 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 standard deviation, with a minimum standard deviation of 1. For HPV 6, 40, 51, 59, 68A, 73, 74, and 82, the cutoff was modified to 1.5 times cutoff, and for HPV 30 and 90 the cutoff was modified to 2 times cutoff. Since the ME primer for HPV 68 showed some cross-reactivity with HPV 70, all samples MS-positive for HPV 68 were also analysed 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 proficiency panel samples 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 Chi2-tests for prevalence differences between groups and 95% confidence intervals (CI) for proportions of HPV positivity were calculated using SPSS software (IBM, USA).

Results

We analysed 44146 samples, 33137 samples from women and 11009 from men (table 1). Since all samples were anonymised 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, figure 1). Stratification of the age-specific HPV prevalences to specific sample types such as urine samples found a similar dependence on age (table 2, figure 1), although samples with only urine uniformly had lower HPV prevalences than samples containing genital swabs (table 2, figure 1). The general HPV positivity was 37.8% (95% CI 37.3-38.3) for all 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% versus 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 15-18-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), and among men 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.

The high-throughput HPV DNA analysis system used is semi-automated, and has a low reagent cost per sample (about 2 euro/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. Since samples were anonymised, informed consent was not required and cost, management and selection biases induced by non-attendance could be minimised. 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 feed-back 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 before. Baseline data on HPV prevalences before HPV vaccination has previously been established using self-collected cervico-vaginal 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 to 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 20-24-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 15-40 year-old women was 30.0% and 31.3% for non-indigenous 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 15-18 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 15-18 year-old men in Scotland was 2.4% (11), considerably lower than in our 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 18-44 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 18-44 year-old men in the British survey (12) was similar to the 10.8% HPV prevalence found in urine samples among 18-44 year-old men in our study.

Urine is a convenient, non-invasive 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 anatomical differences of the urethra between genders are likely to affect the HPV prevalences in urine, but we can not rule out the possibility that the difference may have epidemiological 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 multi-center 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 non-oncogenic types among men who have sex with men and 9.0% for oncogenic types and 12.5% for non-oncogenic 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 one of our most important results, as we demonstrate 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 analyse 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. Since robotic pipetting was used at all steps and the throughput is high, it was possible to analyse 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 in case 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 towards 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 15-18 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 44000 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.

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Table 1. Samples (N=44146) collected during March-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 on 2008-11-01**		**Percent of population tested**	
Age group, years	**Women**	**Men**	**Women**	**Men**	**Women**	**Men**
0-12	70 (55)	56 (44)	83036	87789	0.066	0.050
13	14 (11)	1 (1)	6782	7258	0.16	0.014
14	130 (101)	15 (12)	7227	7595	1.4	0.16
15	563 (439)	46 (36)	7647	7837	5.7	0.46
16	982 (766)	202 (158)	7811	8384	9.8	1.9
17	1715 (1338)	327 (255)	7983	8503	16.8	3.0
18	2313 (1804)	546 (426)	8284	8497	21.8	5.0
19	2300 (1794)	697 (544)	7808	8261	23.0	6.6
20	2237 (1745)	763 (595)	8083	8077	21.6	7.4
21	2051 (1600)	765 (597)	7850	7762	20.4	7.7
22	2039 (1590)	791 (617)	8002	7863	19.9	7.8
23	1849 (1442)	742 (579)	7943	7795	18.2	7.4
24	1575 (1228)	648 (505)	7888	7832	15.6	6.4
25	1418 (1106)	584 (456)	7643	7872	14.5	5.8
26-30	5984 (4668)	2164 (1688)	38862	40067	12.0	4.2
31-35	3829 (2987)	1121 (874)	39788	41643	7.5	2.1
36-40	2181 (1701)	615 (480)	40526	41742	4.2	1.1

41-45	1060 (827)	372 (290)	42727	44256	1.9	0.66
46-50	467 (364)	208 (162)	37411	38144	1.0	0.42
51-55	195 (152)	147 (115)	36744	37119	0.41	0.31
56-60	92 (72)	100 (78)	38374	37927	0.19	0.21
61+	73 (57)	99 (77)	154379	127875	0.037	0.060
Total	33137 (25847)	11009 (8587)	612798	600098	4.2	1.4

Table 2. HPV prevalences in the tested population (44146 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**	**1715**	**677**	**39.5 (37.2-**	**34.0 (29.9-**	**42.3 (39.5-**

				41.8)	**38.1)**	**45.1)**
	Men	327	19	5.8 (3.3-8.3)	5.7 (3.1-8.3)	N/A
18	**Women**	**2313**	**1035**	**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**	**2300**	**1154**	**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**	**2237**	**1192**	**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**	**2051**	**1115**	**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**	**2039**	**1094**	**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**	**1849**	**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**	**1575**	**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**	**1418**	**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**	**5984**	**1985**	**33.2 (32.0-34.4)**	**19.0 (17.3-20.7)**	**41.1 (39.5-42.7)**
	Men	2164	249	11.5 (10.2-12.8)	9.6 (8.3-10.9)	N/A
31-35	**Women**	**3829**	**846**	**22.1 (20.8-23.4)**	**13.5 (11.8-15.2**	**28.4 (26.5-30.3)**
	Men	1121	99	8.8 (7.1-10.5)	7.5 (5.8-9.2)	N/A
36-40	**Women**	**2181**	**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**	**1060**	**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-	6.1 (3.4-	N/A

				9.9)	8.8)	
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**	**33137**	**12534**	**37.8 (37.3-38.3)**	**26.9 (26.1-27.7)**	**43.6 (42.9-44.3)**
	Men	11009	1235	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.

Table 3. HPV prevalence according to sample type in the tested population of 33137 samples from women and 10997 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**	**10840**	**2919**	**26.9 (26.1-27.7)**
	Men	9787	1009	10.3 (9.7-10.9)
Combined urine and genital swab sample[a]	**Women**	**13574**	**6047**	**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**	**7333**	**3058**	**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.

Table 4. HPV type-specific results from the MALDI-TOF analysis of 44146 samples collected during March-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**	**1361**	**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**	**3298**	**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**	**1698**	**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**	**1736**	**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-	0.24 (0.14-0.34)	N/A

			0.34)		
39	**Women**	**1216**	**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**	**1054**	**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**	**1984**	**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**	**1550**	**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**	**1338**	**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**	**1681**	**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.

Figure legend

Figure 1: HPV prevalence according to age and gender.

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Figure 1. HPV prevalence according to age and gender.