

Prevalence of Low-Risk and High-Risk types of Human Papillomavirus and other risk factors for HPV infection in Germany Within Different Age Groups in Women up to 30 Years of Age: An Epidemiological Observational Study

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Prevalence of Low-Risk and High-Risk types of Human Papillomavirus and other risk factors for HPV infection in Germany Within Different Age Groups in Women up to 30 Years of Age: An Epidemiological Observational Study

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| | | subjects enrolled n=1803 | | | |
|--------------------|--------------------|---|--------------------|--------------------|--------------------|
| | | subjects with valid HC2 test n=1692 | | | Evaluable subjects |
| age 10-16 n=293 | age 17-19 n=350 | age 20-22 n=354 | age 23-26 n=350 | age 27-30 n=345 | |
| HC2 positivo | HC2 positive | HC2 positive | HC2 positive | HC2 positive | population for HPV |

254x190mm (307 x 307 DPI)

negative

Iow risk

high risk

Iow + high risk



40

35

30

25

20

15

10

5

0

10-16

17-19

20-22

Age [years]

254x190mm (307 x 307 DPI)

23-26

27-30

% subjects

%

10.34

8.75 ا

16.71

23.87

HPV 16

HPV 18

HPV 31

HPV 33

HPV 39

HPV 45

HPV 51

HPV 52

HPV 56

HPV 35 🕂

0.27

3.18

┥2.65

- 3.98

➡ 4.51

⊣ 6.63



190x274mm (284 x 284 DPI)



- 59
- 60





190x274mm (284 x 284 DPI)







- 56
- 57 58
- 59 60



274x190mm (284 x 284 DPI)

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| 2 3 4 | 1 | Prevalence of Low-Risk and High-Risk types of Human Papillomavirus and other risk |
| 5 6 | 2 | factors for HPV infection in Germany Within Different Age Groups in Women up to 30 |
| 7 8 | 3 | Years of Age: An Epidemiological Observational Study |
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| 59 60 | | |

23 ABSTRACT

HPV infection is frequent in young women and persistent infection may lead to cervical cancer. Therefore, vaccination against HPV is recommended for young women in the age group from 12-17 years in Germany. However, epidemiological data on the prevalence of HPV types and risk factors for infection for younger women in Germany is scarce. To address this, an observational study was performed in Germany including 1,692 women aged 10-30 years. After a routine Pap smear, cervical swabs were tested for high-risk and low risk HPV, respectively, using the Hybrid Capture 2 test, and genotyped using the PCR-based tests SPF₁₀/LiPA₂₅ and PapilloCheck[®]. In addition, the women were interviewed regarding their medical history and lifestyle factors. 377 (22.28%) women had positive HC2 results. The proportion of HPV positive women was highest in the 20-22 age group with 28.3%. Predominant HPV types were HPV 16,42,51 and HPV 16,51,31 as defined by PapilloCheck[®] and SPF₁₀/LiPA₂₅, respectively. 95.8% of women did not show signs of any cervical lesion. Adjusted analysis identified number of sexual partners (OR:1.105; 95% CI:[1.069-1.142]), smoking (OR:1.508;[1.155–1.968]) and vaccination against HPV (OR:0.589;[0.398–0.872]) rather than increasing age as risk associated with HPV infection. Comparison of the genotyping assays showed that they correspond well regarding the high-risk HPV types but less well for low-risk HPV types. This epidemiological study shows that high-risk HPV infection is common in young women in Germany. According to our data, vaccination of young women could have a potential impact on the prevention of HPV infection and cervical disease.

Keywords:

Abbreviations:

HPV, Human papillomavirus; CIN, cervical intraepithelial neoplasia; WHO, World Health Organisation;

HPV prevalence, screening, comparison of tests, HPV vaccination

| 1 2 | | |
|----------|----|---|
| 3 4 | 47 | HR-HPV, high-risk human papillomavirus; LR-HPV, low-risk human papillomavirus |
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48 INTRODUCTION

Persistent infection with human papillomavirus (HPV) is a necessary risk factor for cervical cancer [Munoz et al., 1992; Schiffman et al., 1993, Bosch, 2002], which is the second most prevalent tumour in women worldwide. In Germany, the incidence of cervical cancer is one of the highest compared to other Western countries, with about 6,200 newly diagnosed cases and more than 1,600 deaths per year. Among women younger than 50 years, it is still the second most common cause of mortality in Germany [Robert Koch-Institute, 2008]. HPV DNA is detectable in almost all cervical tumours [Walboomers et al. 1999]. Cervical cancer progresses slowly from cervical intraepithelial neoplasia (CIN) to invasive cancer, and precursor lesions can be detected by cytological screening. The mainstay of screening programs has been the Pap smear. However, considerable numbers of false-negative Pap smears may occur with the traditional Pap technique, due mostly to sampling errors [Nanda et al., 2002]. The prevalence of HPV in Europe is estimated to be between 8-15% throughout all age groups, but is considerable higher among younger women aged 20-24 years, with up to 50.2% in Denmark [De Sanjosé et al., 2007; Bosch et al., 2006, Kjaer et al., 2008]. Infection occurs mostly in adolescence and early adulthood: 74% of all infections are diagnosed in women aged 15 to 24 years [Weinstock et al., 2004]. Estimations in Finland and in the USA give a lifetime incidence of 70-80% [Syrjänen et al., 1990; Myers et al., 2000]. However, in the majority (70-90%) of cases, the infection is transient and HPV DNA is not detectable 1 to 2 years after infection [Ho et al., 1995; Schiffman et al., 2007]. With increasing age, prevalence rates decline but the relative contribution of persistent infections increase.'

Among more than 100 HPV types, approximately 13 genotypes have been identified as carcinogenic (high-risk [HR] types), whereas others are considered to be low-risk (LR) types or HPV types of unknown risk. According to the WHO, the following HPV types are classified as class I carcinogenic: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66 [Cogliano et al., 2005]. Among these, the prevalence of HPV 16 in cervical cancer cells is predominant. HPV 16 is detectable in 50-60% of all

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cervical tumour samples, HPV 18 in 10-20%, and HPV16/18 in approximately 70% of all cervical cancer cases [Munoz et al., 2003 and 2004]. At present, the Hybrid Capture 2 (HC2) assay (Qiagen, Hildesheim, Germany) is one of two tests approved by the US Food and Drug Administration for the detection of HPV DNA in cervical cancer screening. The HC2 assay uses an RNA probe cocktail to detect 13 high-risk and five low-risk HPV types, but in fact detects many more types through cross reactivity [Castle et al., 2002]. However, the test does not distinguish individual genotypes and cannot identify infection with multiple genotypes. Several PCR based genotyping tests for HPV types are available, but the tests differ with respect to sensitivity for certain types, and there is currently no "gold standard" [Klug et al., 2008].

Since March 2007, the German Standing Committee on Vaccination (STIKO) has recommended vaccination against HPV types 16 and 18 for all female adolescents aged 12 to 17 years. Unfortunately, data on the incidence of genital HPV infections and HPV prevalence in younger age groups in Germany are scarce. However, such information is vital for estimating the burden of HPV infection, the HPV-type distribution and the putative impact of HPV vaccination.

An epidemiological, observational study was performed in 77 centres throughout Germany to determine the prevalence of LR-HPV and HR-HPV in young women up to 30 years of age undergoing routine consultation in office-based gynaecological practices. Women were sampled at pre-defined age intervals in a stratified manner. After a routine Pap smear, a cervical swab was taken for HPV detection and genotyping. Finally, the women were interviewed regarding their medical history and lifestyle factors. The cervical swabs were tested for high-risk (HR)- and low-risk (LR)-HPV by means of Hybrid Capture 2 (HC2), and genotyping was performed for HC2 positive samples using both the PCRbased SPF₁₀/LiPA₂₅ test and the PapilloCheck[®] test. The primary objective of the study was to assess the distribution of the age-specific HPV types in young women up to 30 years of age in Germany. To assess the impact of the following additional risk factors on HPV prevalence, women completed a questionnaire on pregnancy, smoking behaviour, contraceptives, vaccination against HPV, history of

98 cervical cancer, immunosuppressive disease, immunosuppressive drugs, sexually transmitted 99 diseases and the number of sexual partners. The results of the HC2 assay and the two HPV 100 genotyping tests (SPF₁₀/LiPA₂₅ and PapilloCheck[®]) were also compared to ascertain the degree to 101 which they corresponded in terms of multiple infection and individual HPV type detection.

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MATERIALS AND METHODS

Study Population

Women were recruited at office-based gynaecologists when they attended for routine screening. Subjects were eligible for inclusion in the study if they were under 30 years of age, had not undergone hysterectomy and signed an informed consent form. A routine Pap smear was taken before the additional cervical sample was collected. In the case of minors, informed consent had to be given by a parent or legal representative. In addition, minors were provided with an adequate age-based informed consent form for signature. The study was approved by the Ethics Committee of the State Medical Chamber of Bavaria.

The sample size for this study was based on obtaining a representative sample of subjects in all predefined age strata. To achieve a high level of accuracy for prevalence estimators and with an expected prevalence of 5% - 25% it was planned to include 2,500 women in the study, 500 per age group (\leq 16 years, 17 to \leq 19 years, 20 to \leq 22 years, 23 to \leq 26 years and 27 to \leq 30 years). The subjects were planned to be recruited in approximately 100 gynaecological practices spread all over Germany, in total 25 women per practice and 5 women per age group. Administrative difficulties in recruiting centres led to a smaller sample size than was planned originally.

Between February 2007 and August 2008, 1,803 women with informed consent from 79 participating centres in Germany were recruited to the study and included in the safety analysis implying documentation of adverse events due to the collection of cervical samples. One thousand six hundred and ninety-two of those subjects with valid HC2 tests from 77 centres were included in statistical analyses (evaluable subjects)(Fig. 1).

Study Objectives

The primary objective of the study was to assess the distribution of the age-specific 24 HPV types: 13 high-risk (HR-) HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66), six low-risk (LR-)

HPV types (6, 11, 40, 42, 43 and 44) and five "possible HR or with unclear risk potential"-HPV types
(53, 68, 70, 73 and 82) in young German women up to 30 years of age with a positive Hybrid Capture
2 test (HC2) result using the PapilloCheck[®] assay (Greiner Bio-One GmbH, Frickenhausen, Germany)
and the Short PCR Fragment assay SPF₁₀ HPV LiPA₂₅, version 1, (Labo Bio-Medical Products, Rijswijk,
The Netherlands).

Secondary objectives were the overall prevalence of the age-specific 13 HR-HPV types and five LR-HPV types using the HC2 test [Castle et al., 2002] in girls and young women up to 30 years of age in Germany. In addition, the impact of the following additional risk factors on HPV prevalence was determined: pregnancy, smoking behavior, contraceptives, vaccination against HPV, history of cervical cancer, immunosuppressive disease, immunosuppressive drugs, sexually transmitted diseases, and the number of sexual partners.

137 Cytological Material

After routine Pap smear for cytological evaluation using the Second Munich Cytological Classification, an additional cervical scrape was taken for HPV detection and genotyping. The second scrape was obtained using a cervical sample device (Qiagen, Hildesheim, Germany) and suspended in 1 ml specimen transport medium (STM; Qiagen, Hildesheim, Germany) for HPV DNA testing. HPV detection was performed without knowledge of the cytological diagnosis.

143 Cytological Diagnosis

All Pap smears were examined at the routine cytological laboratories normally used by the office-based gynaecologists. The laboratories were not informed of the study and the slides and accompanying forms did not contain any mention of the study. The Pap smears were assessed according to the Second Munich Cytological Classification [Bundesaerztekammer, 1994].

59 148 **HPV Detection** 60

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HC2 test. The HC2 test (Qiagen, Hildesheim, Germany), using the high-risk and low-risk probe
cocktail, was performed on the second cervical smear taken for all women included in the study. A
positive result refers to a subject who is positive for one or more of the 13 high-risk and 5 low-risk
HPV types included. Samples were considered positive if they attained or exceeded the Food and
Drug Administration approved threshold of 1.0 pg HPV DNA/ml, which corresponds to a 1.0 relative
light units coefficient (RLU/CO).

HPV genotyping. All samples positive by HC2 were genotyped using DNA extracted from the
denatured pretested HC2 sample. Two different genotyping methods for the detection of genital HPV
types were used to compare sensitivity and specificity for HPV-positive, high-risk HPV types:
PapilloCheck[®] (Greiner Bio-One GmbH, Frickenhausen, Germany) and the SPF₁₀ HPV LiPA₂₅, version 1
(Labo Bio-Medical Products, Rijswijk, The Netherlands).

DNA extraction using MagNA Pure LC (MPLC) (Roche Diagnostics, Mannheim, Germany) and all pre and post-PCR procedures were carried out in separate rooms and cabinets. Buffer and blank controls
 were included at every 30th position to obtain sufficient numbers of negative controls to monitor
 contamination events. All samples were tested for integrity of DNA by using the PapilloCheck[®] HPV
 genotyping test sample control function.

The PapilloCheck[®] HPV genotyping assay recognizes the following HPV types: 6, 11, 16, 18, 31, 33, 35, 39, 40, 42, 43, 44/55, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73 and 82. Thus the test recognizes all 13 HPV types recently classified as class-I carcinogenic with respect to cervical cancer by the WHO (Cogliano et al., 2005), six HPVs (6, 11, 40, 42, 43, 44/55) considered as low-risk types, and five types (53, 68, 70, 73, 82) with proposed high-risk or unclear risk potential. Additionally, the human ADAT1 (t-RNA specific adenosine desaminase1) gene is amplified and used as a control for the integrity of the purified DNA. The PapilloCheck[®] chips and all reagents were provided by Greiner Bio-One GmbH (Frickenhausen, Germany). All PCRs were performed with 5 μ l input DNA in the presence of nucleotides with a fluorescent label in a final volume of 25 µl using the AmpliTaq Gold hot start

polymerase (Roche Molecular Systems, Inc, Somerville, USA). An initial activation step of 95 °C for 10
min was followed by 40 cycles of 30 sec of denaturation at 95 °C, by 25 sec primer annealing at 55 °C
and 45 sec of primer extension at 72 °C. The average size of the amplified product is 350 bp.

After the amplification reaction, an additional 15 cycles of 30 sec at 95 °C and 45 sec at 72 °C were performed to achieve a single-stranded product for the following hybridization step. The PCR was carried out on a MJ Thermocycler PTC200 (Bio-Rad Laboratories, Munich, Germany). Five microlitres of the PCR product and 30 μ l hybridization buffer were used for the hybridization on the low-density microarray plastic HTA12 chip. A volume of 25 µl of the PCR hybridization mix was applied to every well on the chip and incubated for 15 min at room temperature in a humid atmosphere. Twelve hybridization reactions can be performed simultaneously on one chip. Hybridization is followed by three washing steps (the first for 10 sec at room temperature; the second for 60 sec at 50 °C; the third for 10 sec again at room temperature). The chip was dried subsequently under a stream of compressed air. The chip was scanned automatically and analysed using CheckScanner[™] and CheckReport[™] software (Greiner Bio-One, Frickenhausen, Germany) respectively.

SPF₁₀-LiPA₂₅ Assay: A 5-ul aliquot of extracted DNA was used for each SPF10 PCR. The SPF10 PCR primer set was used to amplify at least 54 HPV genotypes, as described earlier [Kleter et al., 1998; Kleter et al., 1999]. Briefly, this primer set amplifies a small fragment of 65 bp from the L1 region of HPV. Reverse primers contain a biotin label at the 5' end. The SPF₁₀ amplimers were used to identify the HPV genotype by reverse hybridization on line probe assay (LiPA₂₅) containing probes for 25 HPV genotypes . The LiPA₂₅ detects HPV types 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, and 74.

All PCRs were performed with 5 μl input DNA and biotinylated primers in a final volume of 50 μl using
 All PCRs were performed with 5 μl input DNA and biotinylated primers in a final volume of 50 μl using
 the AmpliTaq Gold hot start polymerase (Roche Molecular Systems, Somerville, USA) and an initial
 activation step of 94 °C for 9 min, followed by 40 cycles of 30 sec of denaturation at 94 °C, by 45 sec

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of primer annealing at 52 °C and 45 sec of primer extension at 72 °C. The PCR was carried out on a MJ
Thermocycler PTC 200 (Bio-Rad Laboratories, Munich, Germany). The SPF₁₀ PCR line probe array v1
test amplifies a short (65 bp) fragment in the L1 region using four forward and two reverse primers. A
10-μl aliquot of the PCR reaction was taken, denatured and hybridized at 49 °C for 60 min to one
detection strip, followed by multiple washing steps. Detection was performed using the substrate
solutions provided and bands were read manually and interpreted according to the manual.

204 Statistical Analysis

All data were analyzed descriptively using summary statistics for categorical and continuous data. These calculations were performed for all evaluable subjects as well as for age groups and the HPV (HC2 test result). Overall and age specific prevalence with asymptotic 95% confidence interval (CI) was estimated for all three HPV tests. Calculations according to HC2 test were based on evaluable subjects (women with a valid laboratory test), prevalence estimation due to the two genotyping assays and comparisons between the different testing methods were based on those evaluable subjects showing a positive HC2 test result.

Univariate and adjusted multivariate Odds Ratios (OR) with 95% CI were obtained by logistic
 regression models assessing the impact of possible risk factors (especially age) on the HC2 test result.
 Adverse events reported in the context of taking the cervical sample were categorized and described
 by counts and percentages.

216 All statistical analyses were performed using SAS[®] 9.1 software (SAS Institute Inc., Cary, NC, USA)

50 217 **RESULTS**

53 218 **Demography and risk factors**

A total of 1,803 women attending gynaecological practices for routine consultation were enrolled A total of 1,803 women attending gynaecological practices for routine consultation were enrolled into the study. Of these subjects, 1,692 women (93.8%) with appropriate cervical samples for HPV https://www.appropriate.cervical.samples for HPV

DNA testing were included in statistical analyses (evaluable subjects) (Fig.1). The total population was distributed over the predefined five age groups as follows: 10-16 years: 17.3%, 17-19 years: 20.7%, 20-22 years: 20.9%, 23-26 years: 20.7%, and 27-30 years: 20.4%. Of the 293 girls in the age group 10-16 years, 19 (6.5%) were younger than 14 years. In total, mean age was 21.5 ± 4.6 years. 108 (6.4%) women were currently pregnant. 13.3% of women had been pregnant once. Two, three, and up to six pregnancies were documented for 5.9%, 2.1% and 0.8% of women, respectively. In general, the number of pregnancies and births increased with age: 45.8% of the women aged between 27 and 30 years had given birth at least once.

Besides age and pregnancy, a number of additional risk factors were documented in this study in order to assess their impact on the prevalence of HPV. These factors comprised smoking behavior, immunosuppressive diseases and drugs, use of contraceptives, sexually transmitted diseases and the number of sexual partners as well as vaccination against HPV and history of cervical cancer (Table I).

Nearly half (45.6%) of the subjects had never smoked, 38.4% were reported to be current smokers,
whereas 16.1% were past smokers. Two subjects (0.1%) aged between 27 and 30 years were HIV
positive. Adrenogenital syndrome was a further immunosuppressive disease reported in one women
aged between 17 and 19 years. Immunosuppressive drugs were administered to 17 (1%) subjects, 15
(0.9%) of whom received cortisone.

Nearly all women observed used contraceptives (95.8%), the vast majority used hormonal
contraceptives (84%). As expected, there was a strong correlation between age and duration of use
of hormonal contraceptives as well as between age and number of sexual partners.

Three hundred and nine (18.3%) women were vaccinated against HPV before inclusion in this study. The proportion of vaccinated women decreased with age, from 50.9% in the age group 10-16 years to 0.3% women at the age range 27-30 years. Five subjects (0.3%) reported to have suffered from

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cervical cancer. Twenty-two subjects (1.3%) had suffered previously from a sexually transmitted
disease, including 11 subjects with chlamydial infection and six with anogenital warts.

According to the Pap smear, 1,621 out of 1,692 subjectss (95.8%) showed no signs of cervical abnormalities (PAP I and PAP II). PAP III, PAP IIID, and PAP IVa were documented in six (0.4%), 28 (1.7%) and two (0.1%) subjects, respectively. Sixteen Pap smears (0.9%) were not suitable for diagnosis, 19 (1.1%) without specification of PAP status.

251 Prevalence of HPV Types

In total, 377 subjects (22.3%; 95% CI: [20.3-24.3]) showed a positive HC2 test result, and 1,315 subjects (77.7%; 95% CI: [75.7-79.7]) had negative findings (Table II). The proportion of subjects with positive HPV findings steeply increased from 11.6% in the 10-16 year range to a maximum of 28.3% in the 20-22 year-old group. The prevalence of HPV then declined with increasing age (Table II). According to these findings, the distribution of HR- and LR-HPV positive subjects showed the lowest proportion in the age group 10-16 years (HR only: 7.2%, LR only:2.4%, LR & HR: 2.1%) and the highest proportion in the age groups 20-22 years (HR only: 15.3%, LR only: 4.2%, LR & HR: 8.8%) and 23-26 years (HR only: 17.4%, LR only: 4.0%, LR & HR: 4.6%). In all groups there were more HR-HPV than LR-HPV types detected. Table II shows the number of subjects with a positive HC2 test result in each age group related to the number of subjects included in each age group. In contrast figure 2 depicts the distribution of subjects with a negative LR, HR or both – HC2 test result per age group in relation to all women with a negative LR, HR or both — HC2 test result, respectively.

Cervical samples of the 377 women with a positive HC2 test result were genotyped with the PapilloCheck[®] HPV screening test and the SPF₁₀ HPV LiPA₂₅ assay. HPV genotyping by PapilloCheck[®] confirmed 88. 6% (95% CI: [85.4-91.8]) of the positive HC2 findings. As shown in Figure 3, the prevalence of HR-HPV type 16 measured by PapilloCheck[®] (among 377 HC2-positive subjects) was

predominant in this study, amounting to 23.9% (95% CI: [19.6-28.2]). Other frequent HPV types were HPV 42 (LR; 19.1%), HPV 51 (HR; 16.7%), HPV 56 (HR; 10.3%) and HPV 31 (HR; 8.8%). The rate of HR-HPV type 18, the second most common subtype in cervical cancer, was 3.2% (95% CI: [1.4-5.0]). The SPF₁₀ assay showed a positive result for 94.7% [95% CI: 92.4 - 97.0] of subjects with positive HC2 findings. Among 377 HPV-positive women, the prevalence of HPV 16 measured by SPF₁₀ was 29.4% (95% CI: [24.8-34.1]), followed by HPV 51 (21.0%), HPV 31 (17.0%), HPV 52 (13.5%) and HPV 66 (HR;

12.2%). HPV 18 was detected in 8% (95% CI: [5.2-10.7]) samples (Figure 4).

Correlation Between the HC2 Results and risk Factors

A larger proportion of pregnant women at the time was recorded among the HPV-positive subjects (9%) than in women with a negative test result (5.6%) (Table I).

Nearly half (48.5%) of HPV-positive women were current smokers compared to 35.4% of subjects with negative HC2 test results (HR-HPV only: 50.2%, LR-HPV only: 37.0%, HR & LR: 51.2%). The proportion of women taking contraceptives was similar between the HC2-positive and negative subjects, namely 83.6% and 82.6% respectively. Regarding the occurrence of self-reported cervical cancer and immunosuppressive diseases and the use of immunosuppressive drugs, only minor differences between HC2-positive and HC2-negative subjects were found. As expected, the proportion of subjects who had already been vaccinated against HPV was smaller in subjects with positive HC2 results than in those with negative findings (11.1% vs. 20.3%). 14.8% of subjects with low HPV risk only, 10.9% of subjects who had high HPV risk only, and 9.5% with LR & HR HPV types had been vaccinated.

A larger proportion of subjects with a higher number of sexual partners were recorded in subjects with a positive HC2 test result than in subjects with a negative test result (figure 5a and 5b).

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Stratification by Pap-smear results showed that more HC2-positive subjects were classified as PAP III
and higher compared to subjects without detection of HPV according to the HC2 result (HC2-positive:
7.2% vs. HC2-negative: 0.7%).

The influence of age on the occurrence of a positive HC2 test result was investigated by logistic regression. The univariate logistic model showed a significant correlation between age and positive HC2 results (odds ratio: 1.028; 95% CI: [1.003-1.054]). According to this univariate model, higher age was significantly associated with higher risk of HPV infection. In order to adjust the age effect for possible confounders, a multivariate analysis was performed including pregnancy, smoking, hormonal contraception, HPV vaccination and the number of sexual partners besides age. Based on this model, the effect of age was no longer statistically significant (odds ratio: 0.981; 95% CI: [0.952-1.011]). Variables showing statistical significance were numbers of sexual partners, HPV vaccination and smoking. Due to the strong positive correlation between age and the number of sexual partners, the significant age effect observed in the univariate model is a result due to confounding. The risk of HPV detection increased with "smoking" (yes vs. never) (OR: 1.508; 95% CI: [1.155-1.968]) and "number of sexual partners" (OR: 1.105; [1.069-1.142]) and declined significantly with "vaccination against HPV" (OR: 0.589; [0.398-0.872]) (table III).

309 Agreement of Test Results

The level of agreement between the different laboratory methods (i.e. HC2, PapilloCheck[®] and SPF₁₀) was analysed in 377 subjects with positive HC2 findings. In the HC2 test, 54 subjects (14.3%) showed LR-HPV types only and 239 subjects (63.4%) only HR-HPV types. 84 subjects (22.3%) were infected with both, LR- and HR-HPV types. 88.6% of subjects were positive both by HC2 and PapilloCheck[®], whereas 11.4% of HC2-positive subjects had negative results when tested by PapilloCheck[®]. 86.6% of subjects with HPV-type HR only according to the HC2 test showed infection with an HR-HPV (only) type using the PapilloCheck[®] screening, whereas 11.3% showed negative results. Of the LR-HPV

317 (only) infected subjects tested by HC2, 68.5% were confirmed by PapilloCheck[®] and 24.1% had
318 negative results For the LR & HR-group the results matched in 52.4% of cases (Table IV).

By comparing HPV detection using HC2 and SPF10, the replication rate of HC2-positive findings with SPF₁₀ was 94.7%. Twenty samples (5.3%) were positive when examined with HC2 and negative when examined with SPF₁₀.. Of the subjects with LR-HPV (only) according to HC2, 31.5% had negative test results with SPF₁₀. It is noteworthy that the LR-HPV positive results (LR only) with HC2 were only confirmed in 14.8% of subjects when tested by SPF₁₀ (Table V).

On comparison, both methods (SPF₁₀ and PapilloCheck[®]) were found to correspond to a large extent as regards HR-HPV types (98.1% with PapilloCheck[®] as reference, 81.3% with SPF₁₀ as reference). However, the degree of correspondence in the detection of LR-HPV types was considerably smaller (39.4% and 45.3% respectively).

A total of six adverse events in six subjects (0.3%), which occurred during the collection of cervical
smears, were reported, five of which were minor bleedings and one narrow cervical canal.

331 DISCUSSION

Epidemiological data especially in young women on the prevalence of HPV and the distribution of HPV types in Germany is lacking. HPV vaccination has become available recently, which may influence the prevalence of certain HPV types in the future. Therefore, it is important to obtain a picture of the *status quo* before the HPV vaccine coverage becomes high in the population.

This German epidemiological study in young women shows that more than one fifth (22%) of the subjects were HPV positive according to the HC2 test. There was an age-dependent prevalence, showing the highest prevalence for women at the age of 20-22 years, and then declining with increasing age. These findings are in accordance with a recent American study on HPV prevalence [0] 100 mine et al., 2007], in which 27% of women were HPV positive, with the highest prevalence (45%) in

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women aged 20-24 years. It is estimated that 74% of all infections are diagnosed in women aged between 15 and 24 years [Weinstock et al., 2004]. In this study 95.8% of women did not show signs of any cervical lesion. Due to the fact that in this observational study women visiting their gynaecologist only for routine reasons were included a possible selection bias might be taken into account.

To reduce the rate of infection with HPV, it is reasonable to vaccinate women prophylacticly against HPV, preferentially as young girls before they become sexually active. This is reflected by the recommendations of the German Standing Committee on Vaccination (STIKO), which advises vaccination against HPV types 16 and 18 for all females aged 12 to 17 years in Germany. In accordance with this recommendation, this study shows the lowest HPV prevalence in the age group 10 to 16 years, in which half of the women were immunized with at least one vaccination dose, followed by the age group 17-19 years, with one third of women immunized. At the present time, HPV vaccination is not a replacement for cervical screening. Routine testing by Pap smears has the disadvantage of low sensitivity in the range of 50-60% for a single test [Nanda et al., 2000; Wentzensen et al., 2008]. To detect persistent HPV infections, which are a necessary risk factor for cervical cancer and to identify and monitor those high-risk women, the use of genotyping tests is being discussed currently. This study revealed that 85.7% of the HPV-positive women were infected with high-risk types according to the HC2 test. Genotyping by PapilloCheck[®] and SPF₁₀ confirmed approximately 90% of these high-risk types. In contrast, HC2 low-risk results were confirmed by PapilloCheck[®] and SPF₁₀ genotypingto a less extent. This underlines the importance of reliable genotyping tests, since HC2 often detects erroneously an infection with low-risk-types when a high-risk infection is present or vice versa.

54363The genotyping tests in this study revealed the highest prevalence for the HR-HPV types 16, 31, 51,555636452 and 56 as well as HPV LR subtype 42, which was the second most common subtype detected by5758365the PapilloCheck[®] assay. In other studies, different HPV types have been described to be prevalent

[Munoz et al. 2003 and 2004], which is due to the rather insensitive methods that have been combined in meta-analyses (Klug etal., 2008; Schopp et al., 2009). In cancer samples, HPV16 was the most prevalent subtype, followed by HPV 18 (Munoz et al., 2003, 2004). Together they account for approximately 70%. In this study, HPV 18 was detected in only approximately 3-8% of HC2 positive cervical samples. This supports the conclusion by others that certain HR-HPV types, such as HPV 18, are more tumourigenic than others [Castle et al., 2005; Lai et al., 2007; Kjaer et al., 2002]. Lai et al. [2007] already demonstrated the independent prognostic significance of HPV 18 positivity in early-stage cervical cancer, and HPV 16 infection was associated with a very high absolute risk of \geq cervical intraepithelial neoplasia grade 3 over a 2-year period [Castle et al., 2005]. HPV45 might be another such candidate. It was detected it in 3-4% of all HC2 positive samples, but it is among the most prevalent types in cervical lesions [Clifford et al., 2006; Bao et al. 2008] These results underline the importance of genotyping tests in addition to high-risk HPV-positive or -negative screening. However, none of the genotyping methods available is yet regarded as standard. The comparison in the study of two genotyping tests, PapilloCheck[®] and SPF₁₀, revealed that their results correspond fairly well regarding the most prevalent HR-HPV types 16, 51 and 31. However, correspondence of the results of the two test methods for the LR-HPV types is less pronounced, mainly due to the very high sensitivity of the PapilloCheck[®] test for HPV subtype 42. The assays differ in sensitivity, which is most probably the result of the different length of the amplified DNA fragment as well as primer specificity used for amplification, as shown in two recent studies [Klug et al, 2008; Schopp et al, 2009]. In this study factors that increase or lower the risk for HPV infection should also be identified. There

seems to be a positive effect of HPV vaccination on the risk of HPV infection. However, the study was
not designed to show a significant effect of vaccination on the risk of HPV infection rather than to
estimate age specific HPV prevalence. There was no further information on the vaccine employed,
such as which vaccine was used, the vaccination scheme and adherence to it. Therefore a firm
conclusion on the effect of HPV vaccination cannot be drawn from this study. Nevertheless, previous

 clinical trials have shown significant effects of HPV vaccination on the occurrence of cervical disease
[Harper et al., 2004; Villa et al., 2005; Paavonen et al., 2009; Brown et al., 2009].

Risk factors for HPV infection were smoking and the number of sexual partners. Smoking as a risk
factor for HPV infection has also been described in recent epidemiological studies in China and Brazil
[Chan et al., 2009; Sarian et al., 2009]. An association between sexual behaviour and the risk of HPV
infection has been reported in other European countries [Lenselink et al., 2008; Ammatuna et al.,
2008; Nielsen et al., 2008].

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TABLE I: Risk factors versus HC2-laboratory results

| | Total | | Positive | | Low Risk only | | High Risk only | | High & Low Risk | |
|-------------------------------------|------------|------|-----------|------|---------------|-------|----------------|------|-----------------|-------|
| | (N=1692) | | (N=377) | | (N=54) | | (N=239) | | (N=84) |) |
| age, mean ± SD | 21.5 ± 4.6 | 5 | 22.0 ± 4. | 0 | 21.4 ± 4.3 | | 22.3 ± 4.1 | L | 21.6 ± 3 | .5 |
| highest graduation | | | | | | | | | | |
| Hauptschule | 395 | 23.3 | 107 | 28.4 | 14 | 25.9 | 71 | 29.7 | 22 | 26.2 |
| secondary school | 1145 | 67.7 | 240 | 63.7 | 34 | 63.0 | 150 | 62.8 | 56 | 66.7 |
| university | 134 | 7.9 | 27 | 7.2 | 6 | 11.1 | 15 | 6.3 | 6 | 7.1 |
| current pregnancy | | | | | | | | | | |
| yes | 108 | 6.4 | 34 | 9.0 | 7 | 13.0 | 18 | 7.5 | 9 | 10.7 |
| no | 1584 | 93.6 | 343 | 91.0 | 47 | 87.0 | 221 | 92.5 | 75 | 89.3 |
| Self-reported cervical cancer | | | | | | | | | | |
| yes | 5 | 0.3 | 1 | 0.3 | 0 | 0.0 | 1 | 0.4 | 0 | 0.0 |
| no | 1687 | 99.7 | 376 | 99.7 | 54 | 100.0 | 238 | 99.6 | 84 | 100.0 |
| vaccination against HPV | | | | | | | | | | |
| yes | 309 | 18.3 | 42 | 11.1 | 8 | 14.8 | 26 | 10.9 | 8 | 9.5 |
| no | 1383 | 81.7 | 335 | 88.9 | 46 | 85.2 | 213 | 89.1 | 76 | 90.5 |
| immunosuppressive disease | | | | | | | | | | |
| yes | 3 | 0.2 | 2 | 0.5 | 0 | 0.0 | 1 | 0.4 | 1 | 1.2 |
| no | 1689 | 99.8 | 375 | 99.5 | 54 | 100.0 | 238 | 99.6 | 83 | 98.8 |
| Immunosuppressive drugs | | | | | | | | | | |
| yes | 17 | 1.0 | 5 | 1.3 | 3 | 5.6 | 2 | 0.8 | 0 | 0.0 |
| no | 1675 | 99.0 | 372 | 98.7 | 51 | 94.4 | 237 | 99.2 | 84 | 100.0 |
| smoking | | | | | | | | | | |
| yes | 649 | 38.4 | 183 | 48.5 | 20 | 37.0 | 120 | 50.2 | 43 | 51.2 |
| no | 272 | 16.1 | 61 | 16.2 | 12 | 22.2 | 35 | 14.6 | 14 | 16.7 |
| never smoked | 771 | 45.6 | 133 | 35.3 | 22 | 40.7 | 84 | 35.1 | 27 | 32.1 |
| previous sexual transmitted disease | | | | | | | | | | |
| yes | 22 | 1.3 | 12 | 3.2 | 1 | 1.9 | 10 | 4.2 | 1 | 1.2 |
| no | 1666 | 98.5 | 365 | 96.8 | 53 | 98.1 | 229 | 95.8 | 83 | 98.8 |
| number of sexual partners | | | | | | | | | | |
| 0 | 67 | 4.0 | 2 | 0.5 | 0 | 0.0 | 2 | 0.8 | 0 | 0.0 |
| 1 | 480 | 28.4 | 43 | 11.4 | 8 | 14.8 | 24 | 10.0 | 11 | 13.1 |
| 2 | 291 | 17.2 | 45 | 11.9 | 6 | 11.1 | 33 | 13.8 | 6 | 7.1 |
| 3 | 230 | 13.6 | 58 | 15.4 | 12 | 22.2 | 33 | 13.8 | 13 | 15.5 |
| 4 | 151 | 8.9 | 55 | 14.6 | 7 | 13.0 | 35 | 14.6 | 13 | 15.5 |
| 5 | 165 | 9.8 | 65 | 17.2 | 8 | 14.8 | 40 | 16.7 | 17 | 20.2 |
| 6 | 62 | 3.7 | 25 | 6.6 | 3 | 5.6 | 15 | 6.3 | 7 | 8.3 |
| | | | | 20 | | | | | | |

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| 7 | 46 | 2.7 | 10 | 2.7 | 2 | 3.7 | 6 | 2.5 | 2 | 2.4 |
|----------------------------|------|------|-----|------|----|------|-----|------|----|------|
| 8 | 39 | 2.3 | 16 | 4.2 | 5 | 9.3 | 6 | 2.5 | 5 | 6.0 |
| 9 | 13 | 0.8 | 4 | 1.1 | 0 | 0.0 | 3 | 1.3 | 1 | 1.2 |
| 10 | 63 | 3.7 | 30 | 8.0 | 3 | 5.6 | 21 | 8.8 | 6 | 7.1 |
| >10 | 48 | 2.8 | 22 | 5.8 | 0 | 0.0 | 20 | 8.4 | 2 | 2.4 |
| contraceptives | | | | | | | | | | |
| none | 117 | 6.9 | 29 | 7.7 | 4 | 7.4 | 17 | 7.1 | 8 | 9.5 |
| hormonal* | 1421 | 84.0 | 315 | 83.6 | 42 | 77.8 | 201 | 84.1 | 72 | 85.7 |
| other* | 178 | 10.5 | 37 | 9.8 | 8 | 14.8 | 25 | 10.5 | 4 | 4.8 |
| PAP smear result | | | | | | | | | | |
| not suitable for diagnosis | 16 | 0.9 | 2 | 0.5 | 1 | 1.9 | 1 | 0.4 | 0 | 0.0 |
| PAPI | 185 | 10.9 | 40 | 10.6 | 9 | 16.7 | 20 | 8.4 | 11 | 13.1 |
| PAPII | 1436 | 84.9 | 307 | 81.4 | 42 | 77.8 | 198 | 82.8 | 67 | 79.8 |
| PAPIII | 6 | 0.4 | 2 | 0.5 | 0 | 0.0 | 2 | 0.8 | 0 | 0.0 |
| PAPIIID | 28 | 1.7 | 23 | 6.1 | 2 | 3.7 | 16 | 6.7 | 5 | 6.0 |
| PAPIVa | 2 | 0.1 | 2 | 0.5 | 0 | 0.0 | 2 | 0.8 | 0 | 0.0 |

*multiple response

Table II: HPV prevalence by age strata according to the HC2-, PapilloCheck[®]- and SPF₁₀-Test

| | | | | | | age st | rata | | | | | |
|---------------------------|-------|------------------|-------|------------------|-----|------------------|------|------------------|-----|------------------|-----|------------------|
| | total | | 10-16 | | | 17-19 | | 20-22 | | 23-26 | | 27-30 |
| | N | % [95% CI] | Ν | % [95% CI] | Ν | % [95% CI] | Ν | % [95% CI] | Ν | % [95% CI] | Ν | % [95% CI] |
| HC2 | 1692 | | 293 | | 350 | | 354 | | 350 | | 345 | |
| | | | | | | | | | | | | |
| - positive | 377 | 22.3 [20.3-24.3] | 34 | 11.6 [7.9-15.3] | 82 | 23.4 [19.0-27.9] | 100 | 28.3 [23.5-33.0] | 91 | 26.0 [21.4-30.6] | 70 | 20.3 [16.0-24.6] |
| - low risk only | 54 | 3.2 [2.4-4.0] | 7 | 2.4 [0.6-4.1] | 11 | 3.1 [1.3-5.0] | 15 | 4.2 [2.1-6.3] | 14 | 4.0 [2.0-6.1] | 7 | 2.0 [0.5-3.5] |
| - high risk only | 239 | 14.1 [12.5-15.8] | 21 | 7.2 [4.2-10.1] | 52 | 14.9 [11.1-18.6] | 54 | 15.3 [11.5-19.0] | 61 | 17.4 [13.5-21.4] | 51 | 14.8 [11.0-18.5] |
| - low & high risk | 84 | 5.0 [3.9-6.0] | 6 | 2.1 [0.4-3.7] | 19 | 5.4 [3.1-7.8] | 31 | 8.8 [5.8-11.7] | 16 | 4.6 [2.4-6.8] | 12 | 3.5 [1.5-5.4] |
| PapilloCheck [®] | 377 | | 34 | | 82 | | 100 | | 91 | | 70 | |
| (only HC2 positive women) | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| - positive | 334 | 88.6 [85.4-91.8] | 29 | 85.3 [72.8-97.8] | 74 | 90.2 [83.7-96.8] | 96 | 96.0 [92.1-99.9] | 79 | 86.8 [79.7-93.9] | 56 | 80.0 [70.4-89.6] |
| - low risk only | 50 | 13.3 [9.8-16.7] | 8 | 23.5 [9.3-37.8] | 9 | 11.0 [4.2-17.7] | 17 | 17.0 [9.6-24.4] | 9 | 9.9 [3.8-16.0] | 7 | 10.0 [3.0-17.0] |
| - high risk only* | 234 | 62.1 [57.2-67.0] | 17 | 50.0 [33.2-66.8] | 53 | 64.6 [54.3-75.0] | 59 | 59.0 [49.4-68.6] | 60 | 65.9 [56.2-75.7] | 45 | 64.3 [53.1-75.5] |
| - low & high risk | 49 | 13.0 [9.6-16.4] | 4 | 11.8 [0.9-22.6] | 11 | 13.4 [6.0-20.8] | 20 | 20.0 [12.2-27.8] | 10 | 11.0 [4.6-17.4] | 4 | 5.7 [0.3-11.2] |

| - unknown risk† | 1 | 0.3 [0.0-0.8] | 0 | - | 1 | 1.2 [0.0-3.7] | 0 | - | 0 | - | 0 | - |
|---------------------------|-----|------------------|----|-------------------|----|------------------|-----|------------------|----|------------------|----|------------------|
| SPF ₁₀ | 377 | | 34 | | 82 | | 100 | | 91 | | 70 | |
| (only HC2 positive women) | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| - positive | 357 | 94.7 [92.4-97.0] | 31 | 91.2 [81.1-100.0] | 78 | 95.1 [90.4-99.9] | 96 | 96.0 [92.1-99.9] | 86 | 94.5 [89.7-99.3] | 66 | 94.3 [88.7-99.9] |
| - low risk only | 11 | 2.9 [1.2-4.6] | 2 | 5.9 [0.0-13.8] | 3 | 3.7 [0.0-7.7] | 4 | 4.0 [0.2-7.8] | 1 | 1.1 [0.0-3.2] | 1 | 1.4 [0.0-4.2] |
| - high risk only* | 254 | 67.4 [62.6-72.1] | 17 | 50.0 [33.2-66.8] | 56 | 68.3 [58.2-78.4] | 70 | 70.0 [61.0-79.0] | 62 | 68.1 [58.6-77.7] | 49 | 70.0 [59.3-80.7] |
| - low & high risk | 75 | 19.9 [15.9-23.9] | 10 | 29.4 [14.1-44.7] | 17 | 20.7 [12.0-29.5] | 19 | 19.0 [11.3-26.7] | 20 | 22.0 [13.5-30.5] | 9 | 12.9 [5.0-20.7] |
| - unknown risk† | 17 | 4.5 [2.4-6.6] | 2 | 5.9 [0.0-13.8] | 2 | 2.4 [0.0-5.8] | 3 | 3.0 [0.0-6.4] | 3 | 3.3 [0.0-7.0] | 7 | 10.0 [3.0-17.0] |

* includes also possible HR-HPV types or types with unclear potential

⁺ positive without feasible specification or types 34 or 74 (unknown risk, SPF₁₀ only), respectively

TABLE III: Univariate and multivariate analysis showing the dependence of HC2 –positivity on self-reported/risk factors

| | Uni | variate | multivariate | | | |
|---|------------|---------------|--------------|---------------|--|--|
| Self-reported Factor | Odds Ratio | 95% CI | Odds Ratio | 95% CI | | |
| Age | 1.028 | 1.003 - 1.054 | 0.981 | 0.952 – 1.011 | | |
| Current pregnancy (yes vs. no) | 1.662 | 1.089 – 2.538 | 1.559 | 0.996 – 2.442 | | |
| Smoking behavior (no vs. never smoked) | 1.387 | 0.986 – 1.950 | 1.114 | 0.778 – 1.594 | | |
| Smoking behavior (yes vs. never smoked) | 1.884 | 1.462 – 2.427 | 1.508 | 1.155 – 1.968 | | |
| Hormonal contraception (yes vs. no) | 0.960 | 0.704 - 1.308 | 0.995 | 0.715 – 1.385 | | |
| Vaccination against HPV (yes vs. no) | 0.492 | 0.348 – 0.697 | 0.589 | 0.398 – 0.872 | | |
| Number of sexual partners | 1.120 | 1.086 - 1.155 | 1.105 | 1.069 - 1.142 | | |

TABLE IV: Degree of correspondence in HPV high-risk and low-risk types obtained from HC2 and

| PapilloCheck [®] | HC2 | | | | | | | | | |
|---------------------------|-----|-----------|--------|----------|-----------------|-------|--|--|--|--|
| | Low | risk only | High r | isk only | Low & high risk | | | | | |
| | N | Col % | N | Col % | N | Col % | | | | |
| Negative | 13 | 24.1 | 27 | 11.3 | 3 | 3.6 | | | | |
| Low risk only | 37 | 68.5 | 1 | 0.4 | 12 | 14.3 | | | | |
| High risk only* | 2 | 3.7 | 207 | 86.6 | 25 | 29.8 | | | | |
| Low & high risk | 2 | 3.7 | 3 | 1.3 | 44 | 52.4 | | | | |
| Unknown† | 0 | 0.0 | 1 | 0.4 | 0 | 0.0 | | | | |

PapilloCheck[®]. Only HC2 positive subjects were tested.

* includes also possible HR-HPV types or types with unclear potential , Wr.

+ positive without feasible specification

TABLE V: Degree of correspondence in HPV high-risk and low-risk types obtained from HC2 and SPF₁₀.

Only HC2 positive women were tested.

| | | HC2 | | | | | | | | | |
|-------------------|-----|-----------|--------|-----------|-----------------|-------|--|--|--|--|--|
| SPF ₁₀ | Low | risk only | High r | risk only | Low & high risk | | | | | | |
| | N | Col % | N | Col % | N | Col % | | | | | |
| Negative | 17 | 31.5 | 3 | 1.3 | 0 | 0.0 | | | | | |
| Low risk only | 8 | 14.8 | 1 | 0.4 | 2 | 2.4 | | | | | |
| High risk only* | 5 | 9.3 | 197 | 82.4 | 52 | 61.9 | | | | | |
| Low & high risk | 15 | 27.8 | 32 | 13.4 | 28 | 33.3 | | | | | |
| Unknown† | 9 | 16.7 | 6 | 2.5 | 2 | 2.4 | | | | | |

* includes also possible HR-HPV types or types with unclear potential

+ positive without feasible specification or types 34 or 74 (unknown risk), respectively

Figure 1: Flow of participants enrolled in the study

Figure 2: Percental distribution of age groups with negative and positive HC2 test results. Subjects with positive HC2 test results are subdivided in low risk, high risk, and both low and high risk groups. The figure shows the distribution of subjects with a negative LR, HR or both — HC2 test result per age group in relation to all women with a negative LR, HR or both — HC2 test result, respectively.

Figure 3: HPV genotyping according to the PapilloCheck[®] test (24 HPV types) among women testing

HPV-positive with the HC2 test. Multiple responses possible, bars indicate the 95% confidence

interval.

Figure 4: HPV genotyping according to the SPF₁₀ assay (26 HPV types) among women testing HPV-

positive with the HC2 test. Multiple responses possible, bars indicate the 95% confidence interval.

Figure 5: Number of sexual partners in women with (a) negative and (b) positive HC2 test

(a) Women with negative HC2 test

(b) Women with positive HC2 test

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