



HAL
open science

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

Thomas Iftner, Sonja Eberle, Angelika Iftner, Barbara Holz, Norbert Banik,
Wim Quint, Anja-Natascha Straube

► To cite this version:

Thomas Iftner, Sonja Eberle, Angelika Iftner, Barbara Holz, Norbert Banik, et al.. 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. *Journal of Medical Virology*, 2010, 82 (11), pp.1928. 10.1002/jmv.21910 . hal-00599780

HAL Id: hal-00599780

<https://hal.science/hal-00599780>

Submitted on 11 Jun 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

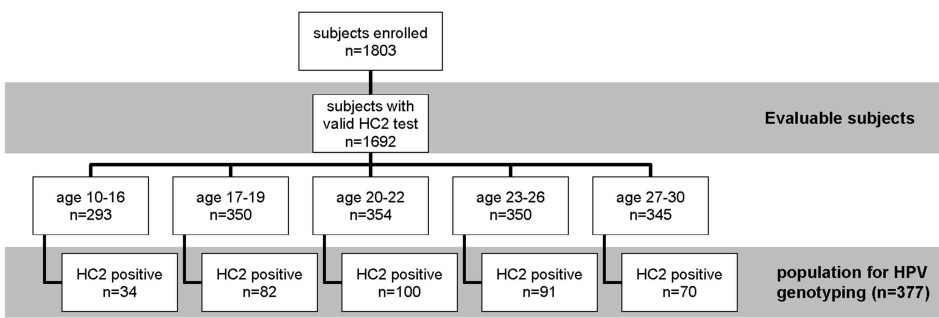


Prevalence of Low-Risk and High-Risk types of Human Papillomavirus and other risk factors for HPV infection in Germany Within Different Age Groups in Women up to 30 Years of Age: An Epidemiological Observational Study

Journal:	<i>Journal of Medical Virology</i>
Manuscript ID:	JMV-09-1687.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	20-May-2010
Complete List of Authors:	Iftner, Thomas; Institute for Medical Virology, Section for Experimental Virology Eberle, Sonja; GlaxoSmithKline GmbH & Co KG Iftner, Angelika; Institute for Medical Virology, Section for Experimental Virology Holz, Barbara; Institute for Medical Virology, Section for Experimental Virology Banik, Norbert; GlaxoSmithKline GmbH & Co KG Quint, Wim; DDL Diagnostic Laboratory Straube, Anja-Natascha; GlaxoSmithKline GmbH & Co KG
Keywords:	HPV prevalence, screening, comparison of tests, HPV vaccination

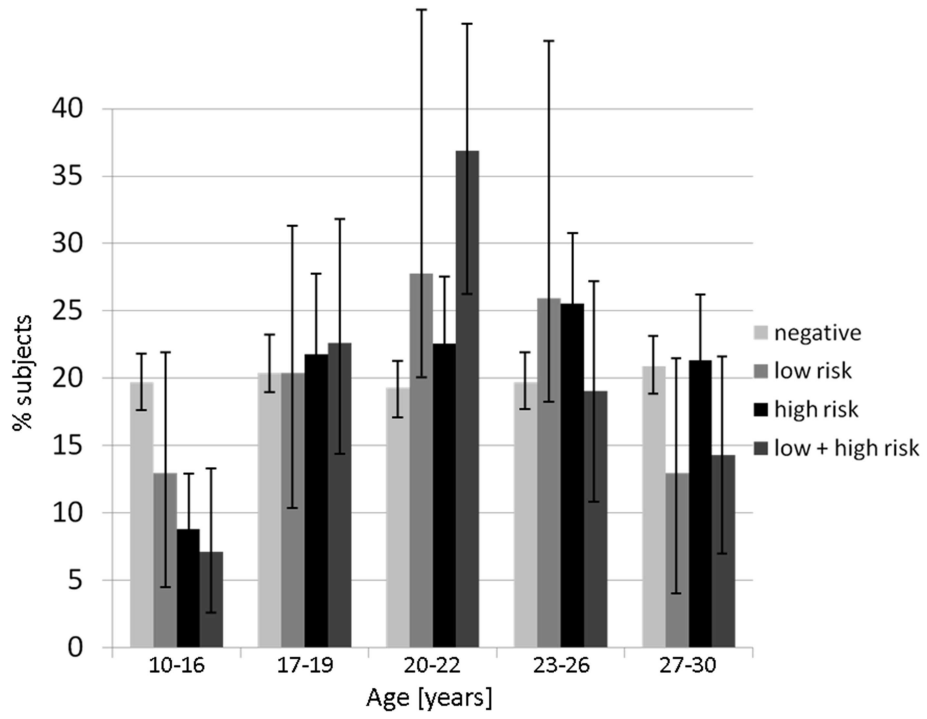


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



254x190mm (307 x 307 DPI)

Review

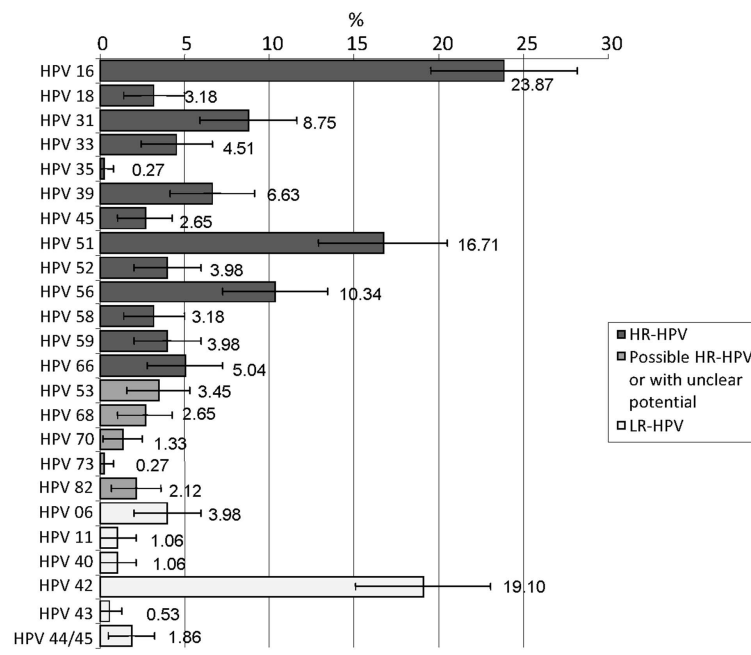


254x190mm (307 x 307 DPI)

review

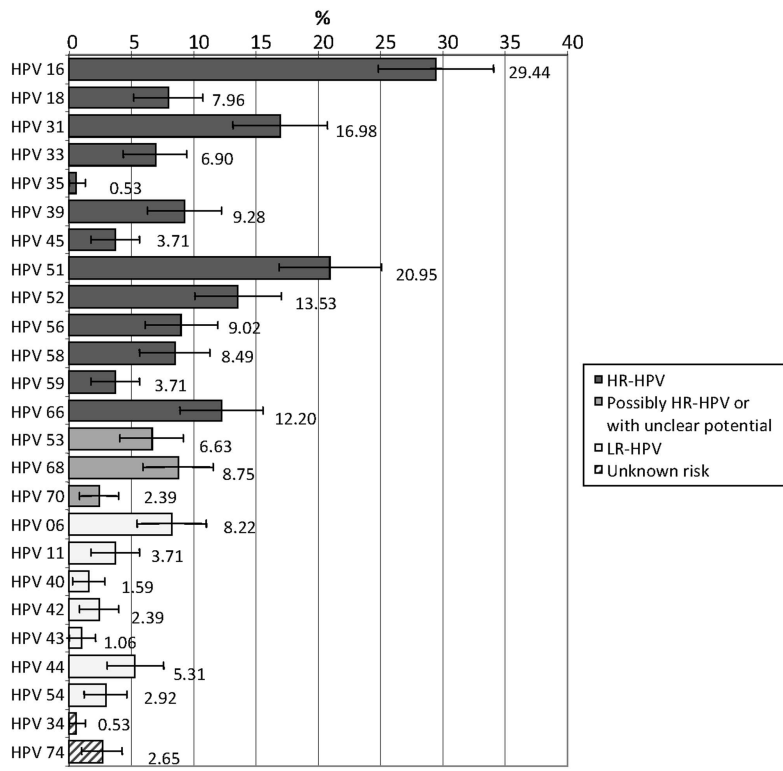
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



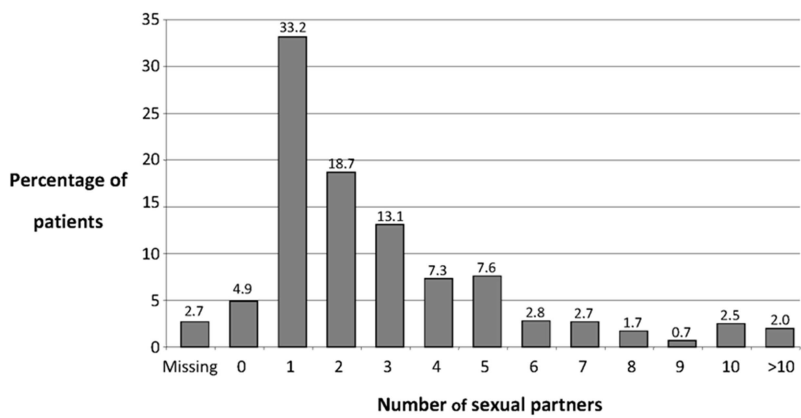
190x274mm (284 x 284 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



190x274mm (284 x 284 DPI)

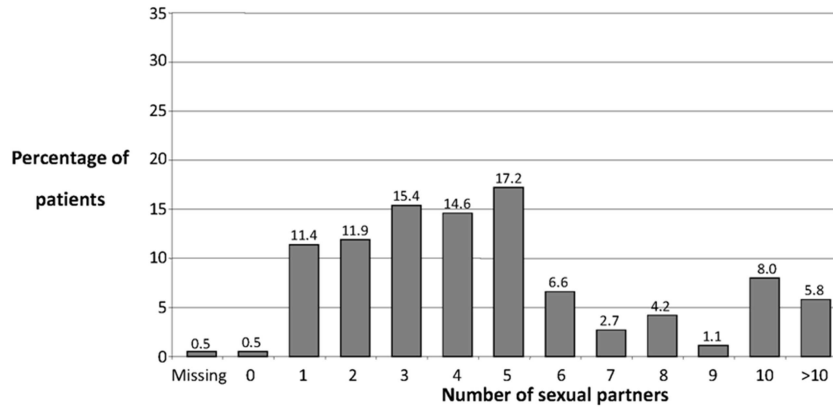
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



274x190mm (284 x 284 DPI)

Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



274x190mm (284 x 284 DPI)

Review

1
2
3 1 Prevalence of Low-Risk and High-Risk types of Human Papillomavirus and other risk
4
5 2 factors for HPV infection in Germany Within Different Age Groups in Women up to 30
6
7
8 3 Years of Age: An Epidemiological Observational Study
9
10 4

11
12 5 1) Thomas Iftner^{1*}

13
14 6 2) Sonja Eberle²

15
16 7 3) Angelika Iftner¹

17
18 8 4) Barbara Holz¹

19
20 9 5) Norbert Banik²

21
22 10 6) Wim Quint³

23
24 11 7) Anja-Natascha Straube²

25
26
27
28
29
30 13 ¹Dept of Experimental Virology, Universitaetsklinikum, Tuebingen, Germany,

31
32 14 ²GlaxoSmithKline GmbH & Co KG, Munich, Germany

33
34 15 ³DDL Diagnostic Laboratory, Voorburg, The Netherlands

35
36
37
38 17 *Correspondence to:

39
40
41 18 Thomas Iftner, Section of Experimental Virology, Institute of Medical Virology, Hospital of the

42
43 19 University of Tuebingen, Elfriede Aulhorn Straße 6, D-72076 Tuebingen, Germany.

44
45
46 20 E-mail: thomas.iftner@med.uni-tuebingen.de

47
48
49 21 **Shortened Title:**

50
51
52 22 HPV Prevalence in Young Women in Germany
53
54
55
56
57
58
59
60

23 **ABSTRACT**

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

43 **Keywords:**

44 HPV prevalence, screening, comparison of tests, HPV vaccination

45 **Abbreviations:**

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

47 HR-HPV, high-risk human papillomavirus; LR-HPV, low-risk human papillomavirus

For Peer Review

48 INTRODUCTION

49 Persistent infection with human papillomavirus (HPV) is a necessary risk factor for cervical cancer
50 [Munoz et al., 1992; Schiffman et al., 1993, Bosch, 2002], which is the second most prevalent tumour
51 in women worldwide. In Germany, the incidence of cervical cancer is one of the highest compared to
52 other Western countries, with about 6,200 newly diagnosed cases and more than 1,600 deaths per
53 year. Among women younger than 50 years, it is still the second most common cause of mortality in
54 Germany [Robert Koch-Institute, 2008]. HPV DNA is detectable in almost all cervical tumours
55 [Walboomers et al. 1999]. Cervical cancer progresses slowly from cervical intraepithelial neoplasia
56 (CIN) to invasive cancer, and precursor lesions can be detected by cytological screening. The
57 mainstay of screening programs has been the Pap smear. However, considerable numbers of false-
58 negative Pap smears may occur with the traditional Pap technique, due mostly to sampling errors
59 [Nanda et al., 2002]. The prevalence of HPV in Europe is estimated to be between 8-15% throughout
60 all age groups, but is considerable higher among younger women aged 20-24 years, with up to 50.2%
61 in Denmark [De Sanjosé et al., 2007; Bosch et al., 2006, Kjaer et al., 2008]. Infection occurs mostly in
62 adolescence and early adulthood: 74% of all infections are diagnosed in women aged 15 to 24 years
63 [Weinstock et al., 2004]. Estimations in Finland and in the USA give a lifetime incidence of 70-80%
64 [Syrjänen et al., 1990; Myers et al., 2000]. However, in the majority (70-90%) of cases, the infection is
65 transient and HPV DNA is not detectable 1 to 2 years after infection [Ho et al., 1995; Schiffman et al.,
66 2007]. With increasing age, prevalence rates decline but the relative contribution of persistent
67 infections increase.'

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

1
2
3 73 cervical tumour samples, HPV 18 in 10-20%, and HPV16/18 in approximately 70% of all cervical
4
5 74 cancer cases [Munoz et al., 2003 and 2004]. At present, the Hybrid Capture 2 (HC2) assay (Qiagen,
6
7
8 75 Hildesheim, Germany) is one of two tests approved by the US Food and Drug Administration for the
9
10 76 detection of HPV DNA in cervical cancer screening. The HC2 assay uses an RNA probe cocktail to
11
12 77 detect 13 high-risk and five low-risk HPV types, but in fact detects many more types through cross
13
14
15 78 reactivity [Castle et al., 2002]. However, the test does not distinguish individual genotypes and
16
17 79 cannot identify infection with multiple genotypes. Several PCR based genotyping tests for HPV types
18
19 80 are available, but the tests differ with respect to sensitivity for certain types, and there is currently
20
21 81 no "gold standard" [Klug et al., 2008].
22
23

24
25 82 Since March 2007, the German Standing Committee on Vaccination (STIKO) has recommended
26
27 83 vaccination against HPV types 16 and 18 for all female adolescents aged 12 to 17 years.
28
29 84 Unfortunately, data on the incidence of genital HPV infections and HPV prevalence in younger age
30
31 85 groups in Germany are scarce. However, such information is vital for estimating the burden of HPV
32
33 86 infection, the HPV-type distribution and the putative impact of HPV vaccination.
34
35

36
37 87 An epidemiological, observational study was performed in 77 centres throughout Germany to
38
39 88 determine the prevalence of LR-HPV and HR-HPV in young women up to 30 years of age undergoing
40
41 89 routine consultation in office-based gynaecological practices. Women were sampled at pre-defined
42
43 90 age intervals in a stratified manner. After a routine Pap smear, a cervical swab was taken for HPV
44
45 91 detection and genotyping. Finally, the women were interviewed regarding their medical history and
46
47 92 lifestyle factors. The cervical swabs were tested for high-risk (HR)- and low-risk (LR)-HPV by means of
48
49 93 Hybrid Capture 2 (HC2), and genotyping was performed for HC2 positive samples using both the PCR-
50
51 94 based SPF₁₀/LiPA₂₅ test and the PapilloCheck[®] test. The primary objective of the study was to assess
52
53 95 the distribution of the age-specific HPV types in young women up to 30 years of age in Germany. To
54
55 96 assess the impact of the following additional risk factors on HPV prevalence, women completed a
56
57 97 questionnaire on pregnancy, smoking behaviour, contraceptives, vaccination against HPV, history of
58
59
60

1
2
3 98 cervical cancer, immunosuppressive disease, immunosuppressive drugs, sexually transmitted
4
5 99 diseases and the number of sexual partners. The results of the HC2 assay and the two HPV
6
7
8 100 genotyping tests (SPF₁₀/LiPA₂₅ and PapilloCheck[®]) were also compared to ascertain the degree to
9
10 101 which they corresponded in terms of multiple infection and individual HPV type detection.
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1
2
3 102 **MATERIALS AND METHODS**
4

5
6 103 **Study Population**
7
8

9 104 Women were recruited at office-based gynaecologists when they attended for routine screening.

10 105 Subjects were eligible for inclusion in the study if they were under 30 years of age, had not

11 106 undergone hysterectomy and signed an informed consent form. A routine Pap smear was taken

12 107 before the additional cervical sample was collected. In the case of minors, informed consent had to

13 108 be given by a parent or legal representative. In addition, minors were provided with an adequate

14 109 age-based informed consent form for signature. The study was approved by the Ethics Committee of

15 110 the State Medical Chamber of Bavaria.

16 111 The sample size for this study was based on obtaining a representative sample of subjects in all

17 112 predefined age strata. To achieve a high level of accuracy for prevalence estimators and with an

18 113 expected prevalence of 5% - 25% it was planned to include 2,500 women in the study, 500 per age

19 114 group (≤ 16 years, 17 to ≤ 19 years, 20 to ≤ 22 years, 23 to ≤ 26 years and 27 to ≤ 30 years). The

20 115 subjects were planned to be recruited in approximately 100 gynaecological practices spread all over

21 116 Germany, in total 25 women per practice and 5 women per age group. Administrative difficulties in

22 117 recruiting centres led to a smaller sample size than was planned originally.

23 118 Between February 2007 and August 2008, 1,803 women with informed consent from 79 participating

24 119 centres in Germany were recruited to the study and included in the safety analysis implying

25 120 documentation of adverse events due to the collection of cervical samples. One thousand six

26 121 hundred and ninety-two of those subjects with valid HC2 tests from 77 centres were included in

27 122 statistical analyses (evaluable subjects)(Fig. 1).
28
29
30
31
32
33
34
35
36
37
38
39
40
41

42 123 **Study Objectives**
43
44
45
46
47
48
49
50
51
52
53
54

55 124 The primary objective of the study was to assess the distribution of the age-specific 24 HPV types: 13

56 125 high-risk (HR-) HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66), six low-risk (LR-)

1
2
3 126 HPV types (6, 11, 40, 42, 43 and 44) and five “possible HR or with unclear risk potential“-HPV types
4
5 127 (53, 68, 70, 73 and 82) in young German women up to 30 years of age with a positive Hybrid Capture
6
7
8 128 2 test (HC2) result using the PapilloCheck[®] assay (Greiner Bio-One GmbH, Frickenhausen, Germany)
9
10 129 and the Short PCR Fragment assay SPF₁₀ HPV LiPA₂₅, version 1, (Labo Bio-Medical Products, Rijswijk,
11
12 130 The Netherlands).

13
14
15 131 Secondary objectives were the overall prevalence of the age-specific 13 HR-HPV types and five LR-
16
17 132 HPV types using the HC2 test [Castle et al., 2002] in girls and young women up to 30 years of age in
18
19 133 Germany. In addition, the impact of the following additional risk factors on HPV prevalence was
20
21 134 determined: pregnancy, smoking behavior, contraceptives, vaccination against HPV, history of
22
23 135 cervical cancer, immunosuppressive disease, immunosuppressive drugs, sexually transmitted
24
25 136 diseases, and the number of sexual partners.
26
27
28
29

30 137 **Cytological Material**

31
32
33 138 After routine Pap smear for cytological evaluation using the Second Munich Cytological Classification,
34
35 139 an additional cervical scrape was taken for HPV detection and genotyping. The second scrape was
36
37 140 obtained using a cervical sample device (Qiagen, Hildesheim, Germany) and suspended in 1 ml
38
39 141 specimen transport medium (STM; Qiagen, Hildesheim, Germany) for HPV DNA testing. HPV
40
41 142 detection was performed without knowledge of the cytological diagnosis.
42
43
44
45

46 143 **Cytological Diagnosis**

47
48
49 144 All Pap smears were examined at the routine cytological laboratories normally used by the office-
50
51 145 based gynaecologists. The laboratories were not informed of the study and the slides and
52
53 146 accompanying forms did not contain any mention of the study. The Pap smears were assessed
54
55 147 according to the Second Munich Cytological Classification [Bundesaerztekammer, 1994].
56
57
58

59 148 **HPV Detection**

1
2
3 149 **HC2 test.** The HC2 test (Qiagen, Hildesheim, Germany), using the high-risk and low-risk probe
4
5 150 cocktail, was performed on the second cervical smear taken for all women included in the study. A
6
7
8 151 positive result refers to a subject who is positive for one or more of the 13 high-risk and 5 low-risk
9
10 152 HPV types included. Samples were considered positive if they attained or exceeded the Food and
11
12 153 Drug Administration approved threshold of 1.0 pg HPV DNA/ml, which corresponds to a 1.0 relative
13
14 154 light units coefficient (RLU/CO).

15
16
17 155 **HPV genotyping.** All samples positive by HC2 were genotyped using DNA extracted from the
18
19 156 denatured pretested HC2 sample. Two different genotyping methods for the detection of genital HPV
20
21 157 types were used to compare sensitivity and specificity for HPV-positive, high-risk HPV types:
22
23 158 PapilloCheck[®] (Greiner Bio-One GmbH, Frickenhausen, Germany) and the SPF₁₀ HPV LiPA₂₅, version 1
24
25 159 (Labo Bio-Medical Products, Rijswijk, The Netherlands).

26
27
28
29 160 **DNA extraction** using MagNA Pure LC (MPLC) (Roche Diagnostics, Mannheim, Germany) and all pre-
30
31 161 and post-PCR procedures were carried out in separate rooms and cabinets. Buffer and blank controls
32
33 162 were included at every 30th position to obtain sufficient numbers of negative controls to monitor
34
35 163 contamination events. All samples were tested for integrity of DNA by using the PapilloCheck[®] HPV
36
37 164 genotyping test sample control function.

38
39
40
41 165 The **PapilloCheck[®] HPV genotyping assay** recognizes the following HPV types: 6, 11, 16, 18, 31, 33,
42
43 166 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
44
45 167 13 HPV types recently classified as class-I carcinogenic with respect to cervical cancer by the WHO
46
47 168 (Cogliano et al., 2005), six HPVs (6, 11, 40, 42, 43, 44/55) considered as low-risk types, and five types
48
49 169 (53, 68, 70, 73, 82) with proposed high-risk or unclear risk potential. Additionally, the human ADAT1
50
51 170 (t-RNA specific adenosine desaminase1) gene is amplified and used as a control for the integrity of
52
53 171 the purified DNA. The PapilloCheck[®] chips and all reagents were provided by Greiner Bio-One GmbH
54
55 172 (Frickenhausen, Germany). All PCRs were performed with 5 µl input DNA in the presence of
56
57 173 nucleotides with a fluorescent label in a final volume of 25 µl using the AmpliTaq Gold hot start
58
59
60

1
2
3 174 polymerase (Roche Molecular Systems, Inc, Somerville, USA). An initial activation step of 95 °C for 10
4
5 175 min was followed by 40 cycles of 30 sec of denaturation at 95 °C, by 25 sec primer annealing at 55 °C
6
7
8 176 and 45 sec of primer extension at 72 °C. The average size of the amplified product is 350 bp.
9

10
11 177 After the amplification reaction, an additional 15 cycles of 30 sec at 95 °C and 45 sec at 72 °C were
12
13 178 performed to achieve a single-stranded product for the following hybridization step. The PCR was
14
15 179 carried out on a MJ Thermocycler PTC200 (Bio-Rad Laboratories, Munich, Germany). Five microlitres
16
17
18 180 of the PCR product and 30 µl hybridization buffer were used for the hybridization on the low-density
19
20 181 microarray plastic HTA12 chip. A volume of 25 µl of the PCR hybridization mix was applied to every
21
22 182 well on the chip and incubated for 15 min at room temperature in a humid atmosphere. Twelve
23
24 183 hybridization reactions can be performed simultaneously on one chip. Hybridization is followed by
25
26
27 184 three washing steps (the first for 10 sec at room temperature; the second for 60 sec at 50 °C; the
28
29 185 third for 10 sec again at room temperature). The chip was dried subsequently under a stream of
30
31
32 186 compressed air. The chip was scanned automatically and analysed using CheckScanner™ and
33
34 187 CheckReport™ software (Greiner Bio-One, Frickenhausen, Germany) respectively.
35
36

37 188 **SPF₁₀-LiPA₂₅ Assay:** A 5-µl aliquot of extracted DNA was used for each SPF10 PCR. The SPF10 PCR
38
39 189 primer set was used to amplify at least 54 HPV genotypes, as described earlier [Kleter et al., 1998;
40
41 190 Kleter et al., 1999]. Briefly, this primer set amplifies a small fragment of 65 bp from the L1 region of
42
43 191 HPV. Reverse primers contain a biotin label at the 5' end. The SPF₁₀ amplimers were used to identify
44
45 192 the HPV genotype by reverse hybridization on line probe assay (LiPA₂₅) containing probes for 25 HPV
46
47 193 genotypes . The LiPA₂₅ detects HPV types 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52,
48
49 194 53, 54, 56, 58, 59, 66, 68/73, 70, and 74.
50
51
52

53
54 195 All PCRs were performed with 5 µl input DNA and biotinylated primers in a final volume of 50 µl using
55
56 196 the AmpliTaq Gold hot start polymerase (Roche Molecular Systems, Somerville, USA) and an initial
57
58 197 activation step of 94 °C for 9 min, followed by 40 cycles of 30 sec of denaturation at 94 °C, by 45 sec
59
60

1
2
3 198 of primer annealing at 52 °C and 45 sec of primer extension at 72 °C. The PCR was carried out on a MJ
4
5 199 Thermocycler PTC 200 (Bio-Rad Laboratories, Munich, Germany). The SPF₁₀ PCR line probe array v1
6
7
8 200 test amplifies a short (65 bp) fragment in the L1 region using four forward and two reverse primers. A
9
10 201 10-µl aliquot of the PCR reaction was taken, denatured and hybridized at 49 °C for 60 min to one
11
12 202 detection strip, followed by multiple washing steps. Detection was performed using the substrate
13
14
15 203 solutions provided and bands were read manually and interpreted according to the manual.
16
17

18 204 **Statistical Analysis**

19
20
21 205 All data were analyzed descriptively using summary statistics for categorical and continuous data.
22
23 206 These calculations were performed for all evaluable subjects as well as for age groups and the HPV
24
25
26 207 (HC2 test result). Overall and age specific prevalence with asymptotic 95% confidence interval (CI)
27
28 208 was estimated for all three HPV tests. Calculations according to HC2 test were based on evaluable
29
30 209 subjects (women with a valid laboratory test), prevalence estimation due to the two genotyping
31
32
33 210 assays and comparisons between the different testing methods were based on those evaluable
34
35 211 subjects showing a positive HC2 test result.
36
37 212 Univariate and adjusted multivariate Odds Ratios (OR) with 95% CI were obtained by logistic
38
39 213 regression models assessing the impact of possible risk factors (especially age) on the HC2 test result.
40
41 214 Adverse events reported in the context of taking the cervical sample were categorized and described
42
43
44 215 by counts and percentages.
45

46 216 All statistical analyses were performed using SAS[®] 9.1 software (SAS Institute Inc., Cary, NC, USA)
47
48

49 50 217 **RESULTS**

51 52 53 218 **Demography and risk factors**

54
55
56 219 A total of 1,803 women attending gynaecological practices for routine consultation were enrolled
57
58 220 into the study. Of these subjects, 1,692 women (93.8%) with appropriate cervical samples for HPV
59
60

1
2
3 221 DNA testing were included in statistical analyses (evaluable subjects) (Fig.1). The total population was
4
5 222 distributed over the predefined five age groups as follows: 10-16 years: 17.3%, 17-19 years: 20.7%,
6
7 223 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-
8
9 224 16 years, 19 (6.5%) were younger than 14 years. In total, mean age was 21.5 ± 4.6 years. 108 (6.4%)
10
11 225 women were currently pregnant. 13.3% of women had been pregnant once. Two, three, and up to six
12
13 226 pregnancies were documented for 5.9%, 2.1% and 0.8% of women, respectively. In general, the
14
15 227 number of pregnancies and births increased with age: 45.8% of the women aged between 27 and 30
16
17 228 years had given birth at least once.

18
19
20
21
22 229 Besides age and pregnancy, a number of additional risk factors were documented in this study in
23
24 230 order to assess their impact on the prevalence of HPV. These factors comprised smoking behavior,
25
26 231 immunosuppressive diseases and drugs, use of contraceptives, sexually transmitted diseases and the
27
28 232 number of sexual partners as well as vaccination against HPV and history of cervical cancer (Table I).

29
30
31
32 233 Nearly half (45.6%) of the subjects had never smoked, 38.4% were reported to be current smokers,
33
34 234 whereas 16.1% were past smokers. Two subjects (0.1%) aged between 27 and 30 years were HIV
35
36 235 positive. Adrenogenital syndrome was a further immunosuppressive disease reported in one women
37
38 236 aged between 17 and 19 years. Immunosuppressive drugs were administered to 17 (1%) subjects, 15
39
40 237 (0.9%) of whom received cortisone.

41
42
43
44 238 Nearly all women observed used contraceptives (95.8%), the vast majority used hormonal
45
46 239 contraceptives (84%). As expected, there was a strong correlation between age and duration of use
47
48 240 of hormonal contraceptives as well as between age and number of sexual partners.

49
50
51 241 Three hundred and nine (18.3%) women were vaccinated against HPV before inclusion in this study.
52
53 242 The proportion of vaccinated women decreased with age, from 50.9% in the age group 10-16 years
54
55 243 to 0.3% women at the age range 27-30 years. Five subjects (0.3%) reported to have suffered from
56
57
58
59
60

1
2
3 244 cervical cancer. Twenty-two subjects (1.3%) had suffered previously from a sexually transmitted
4
5 245 disease, including 11 subjects with chlamydial infection and six with anogenital warts.
6
7

8 246 According to the Pap smear, 1,621 out of 1,692 subjectss (95.8%) showed no signs of cervical
9
10 247 abnormalities (PAP I and PAP II). PAP III, PAP IIID, and PAP IVa were documented in six (0.4%), 28
11
12 248 (1.7%) and two (0.1%) subjects, respectively. Sixteen Pap smears (0.9%) were not suitable for
13
14 249 diagnosis, 19 (1.1%) without specification of PAP status.
15
16
17

18 250

21 251 **Prevalence of HPV Types**

22
23
24 252 In total, 377 subjects (22.3%; 95% CI: [20.3-24.3]) showed a positive HC2 test result, and 1,315
25
26 253 subjects (77.7%; 95% CI: [75.7-79.7]) had negative findings (Table II). The proportion of subjects with
27
28 254 positive HPV findings steeply increased from 11.6% in the 10-16 year range to a maximum of 28.3%
29
30 255 in the 20-22 year-old group. The prevalence of HPV then declined with increasing age (Table II).
31
32 256 According to these findings, the distribution of HR- and LR-HPV positive subjects showed the lowest
33
34 257 proportion in the age group 10-16 years (HR only: 7.2%, LR only:2.4%, LR & HR: 2.1%) and the highest
35
36 258 proportion in the age groups 20-22 years (HR only: 15.3%, LR only: 4.2%, LR & HR: 8.8%) and 23-26
37
38 259 years (HR only: 17.4%, LR only: 4.0%, LR & HR: 4.6%). In all groups there were more HR-HPV than LR-
39
40 260 HPV types detected. Table II shows the number of subjects with a positive HC2 test result in each age
41
42 261 group related to the number of subjects included in each age group. In contrast figure 2 depicts the
43
44 262 distribution of subjects with a negative LR, HR or both — HC2 test result per age group in relation to
45
46 263 all women with a negative LR, HR or both — HC2 test result, respectively.
47
48
49
50

51
52 264 Cervical samples of the 377 women with a positive HC2 test result were genotyped with the
53
54 265 PapilloCheck[®] HPV screening test and the SPF₁₀ HPV LiPA₂₅ assay. HPV genotyping by PapilloCheck[®]
55
56 266 confirmed 88. 6% (95% CI: [85.4-91.8]) of the positive HC2 findings. As shown in Figure 3, the
57
58 267 prevalence of HR-HPV type 16 measured by PapilloCheck[®] (among 377 HC2-positive subjects) was
59
60

1
2
3 268 predominant in this study, amounting to 23.9% (95% CI: [19.6-28.2]). Other frequent HPV types were
4
5 269 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-
6
7
8 270 HPV type 18, the second most common subtype in cervical cancer, was 3.2% (95% CI: [1.4-5.0]).
9

10 271
11
12 272 The SPF₁₀ assay showed a positive result for 94.7% [95% CI: 92.4 – 97.0] of subjects with positive HC2
13
14 273 findings. Among 377 HPV-positive women, the prevalence of HPV 16 measured by SPF₁₀ was 29.4%
15
16 274 (95% CI: [24.8-34.1]), followed by HPV 51 (21.0%), HPV 31 (17.0%), HPV 52 (13.5%) and HPV 66 (HR;
17
18 275 12.2%). HPV 18 was detected in 8% (95% CI: [5.2-10.7]) samples (Figure 4).
19
20
21

22 276

25 277 **Correlation Between the HC2 Results and risk Factors**

26
27
28 278 A larger proportion of pregnant women at the time was recorded among the HPV-positive subjects
29
30 279 (9%) than in women with a negative test result (5.6%) (Table I).
31

32
33 280 Nearly half (48.5%) of HPV-positive women were current smokers compared to 35.4% of subjects
34
35 281 with negative HC2 test results (HR-HPV only: 50.2%, LR-HPV only: 37.0%, HR & LR: 51.2%). The
36
37 282 proportion of women taking contraceptives was similar between the HC2-positive and negative
38
39 283 subjects, namely 83.6% and 82.6% respectively. Regarding the occurrence of self-reported cervical
40
41 284 cancer and immunosuppressive diseases and the use of immunosuppressive drugs, only minor
42
43 285 differences between HC2-positive and HC2-negative subjects were found. As expected, the
44
45 286 proportion of subjects who had already been vaccinated against HPV was smaller in subjects with
46
47 287 positive HC2 results than in those with negative findings (11.1% vs. 20.3%). 14.8% of subjects with
48
49 288 low HPV risk only, 10.9% of subjects who had high HPV risk only, and 9.5% with LR & HR HPV types
50
51 289 had been vaccinated.
52
53
54
55

56 290 A larger proportion of subjects with a higher number of sexual partners were recorded in subjects
57
58 291 with a positive HC2 test result than in subjects with a negative test result (figure 5a and 5b).
59
60

1
2
3 292 Stratification by Pap-smear results showed that more HC2-positive subjects were classified as PAP III
4
5 293 and higher compared to subjects without detection of HPV according to the HC2 result (HC2-positive:
6
7 294 7.2% vs. HC2-negative: 0.7%).
8
9

10 295 The influence of age on the occurrence of a positive HC2 test result was investigated by logistic
11
12 296 regression. The univariate logistic model showed a significant correlation between age and positive
13
14 297 HC2 results (odds ratio: 1.028; 95% CI: [1.003-1.054]). According to this univariate model, higher age
15
16 298 was significantly associated with higher risk of HPV infection. In order to adjust the age effect for
17
18 299 possible confounders, a multivariate analysis was performed including pregnancy, smoking,
19
20 300 hormonal contraception, HPV vaccination and the number of sexual partners besides age. Based on
21
22 301 this model, the effect of age was no longer statistically significant (odds ratio: 0.981; 95% CI: [0.952-
23
24 302 1.011]). Variables showing statistical significance were numbers of sexual partners, HPV vaccination
25
26 303 and smoking. Due to the strong positive correlation between age and the number of sexual partners,
27
28 304 the significant age effect observed in the univariate model is a result due to confounding. The risk of
29
30 305 HPV detection increased with "smoking" (yes vs. never) (OR: 1.508; 95% CI: [1.155-1.968]) and
31
32 306 "number of sexual partners" (OR: 1.105; [1.069-1.142]) and declined significantly with "vaccination
33
34 307 against HPV" (OR: 0.589; [0.398-0.872]) (table III).
35
36
37
38
39
40
41
42

43 309 **Agreement of Test Results**

44
45
46 310 The level of agreement between the different laboratory methods (i.e. HC2, PapilloCheck[®] and SPF₁₀)
47
48 311 was analysed in 377 subjects with positive HC2 findings. In the HC2 test, 54 subjects (14.3%) showed
49
50 312 LR-HPV types only and 239 subjects (63.4%) only HR-HPV types. 84 subjects (22.3%) were infected
51
52 313 with both, LR- and HR-HPV types. 88.6% of subjects were positive both by HC2 and PapilloCheck[®],
53
54 314 whereas 11.4% of HC2-positive subjects had negative results when tested by PapilloCheck[®]. 86.6% of
55
56 315 subjects with HPV-type HR only according to the HC2 test showed infection with an HR-HPV (only)
57
58 316 type using the PapilloCheck[®] screening, whereas 11.3% showed negative results. Of the LR-HPV
59
60

1
2
3 317 (only) infected subjects tested by HC2, 68.5% were confirmed by PapilloCheck[®] and 24.1% had
4
5 318 negative results For the LR & HR-group the results matched in 52.4% of cases (Table IV).

6
7 319 By comparing HPV detection using HC2 and SPF10, the replication rate of HC2-positive findings with
8
9 320 SPF₁₀ was 94.7%. Twenty samples (5.3%) were positive when examined with HC2 and negative when
10
11 321 examined with SPF₁₀. Of the subjects with LR-HPV (only) according to HC2, 31.5% had negative test
12
13 322 results with SPF₁₀. It is noteworthy that the LR-HPV positive results (LR only) with HC2 were only
14
15 323 confirmed in 14.8% of subjects when tested by SPF₁₀ (Table V).

16
17 324 On comparison, both methods (SPF₁₀ and PapilloCheck[®]) were found to correspond to a large extent
18
19 325 as regards HR-HPV types (98.1% with PapilloCheck[®] as reference, 81.3% with SPF₁₀ as reference).
20
21 326 However, the degree of correspondence in the detection of LR-HPV types was considerably smaller
22
23 327 (39.4% and 45.3% respectively).
24
25
26
27
28

29 328
30
31 329 A total of six adverse events in six subjects (0.3%), which occurred during the collection of cervical
32
33 330 smears, were reported, five of which were minor bleedings and one narrow cervical canal.
34
35
36

37 331 **DISCUSSION**

38
39 332 Epidemiological data especially in young women on the prevalence of HPV and the distribution of
40
41 333 HPV types in Germany is lacking. HPV vaccination has become available recently, which may
42
43 334 influence the prevalence of certain HPV types in the future. Therefore, it is important to obtain a
44
45 335 picture of the *status quo* before the HPV vaccine coverage becomes high in the population.
46
47
48

49 336 This German epidemiological study in young women shows that more than one fifth (22%) of the
50
51 337 subjects were HPV positive according to the HC2 test. There was an age-dependent prevalence,
52
53 338 showing the highest prevalence for women at the age of 20-22 years, and then declining with
54
55 339 increasing age. These findings are in accordance with a recent American study on HPV prevalence
56
57 340 [Dunne et al., 2007], in which 27% of women were HPV positive, with the highest prevalence (45%) in
58
59
60

1
2
3 341 women aged 20-24 years. It is estimated that 74% of all infections are diagnosed in women aged
4
5 342 between 15 and 24 years [Weinstock et al., 2004]. In this study 95.8% of women did not show signs
6
7 343 of any cervical lesion. Due to the fact that in this observational study women visiting their
8
9 344 gynaecologist only for routine reasons were included a possible selection bias might be taken into
10
11 345 account.

12
13
14 346 To reduce the rate of infection with HPV, it is reasonable to vaccinate women prophylactically against
15
16 347 HPV, preferentially as young girls before they become sexually active. This is reflected by the
17
18 348 recommendations of the German Standing Committee on Vaccination (STIKO), which advises
19
20 349 vaccination against HPV types 16 and 18 for all females aged 12 to 17 years in Germany. In
21
22 350 accordance with this recommendation, this study shows the lowest HPV prevalence in the age group
23
24 351 10 to 16 years, in which half of the women were immunized with at least one vaccination dose,
25
26 352 followed by the age group 17-19 years, with one third of women immunized. At the present time,
27
28 353 HPV vaccination is not a replacement for cervical screening. Routine testing by Pap smears has the
29
30 354 disadvantage of low sensitivity in the range of 50-60% for a single test [Nanda et al., 2000;
31
32 355 Wentzensen et al., 2008]. To detect persistent HPV infections, which are a necessary risk factor for
33
34 356 cervical cancer and to identify and monitor those high-risk women, the use of genotyping tests is
35
36 357 being discussed currently. This study revealed that 85.7% of the HPV-positive women were infected
37
38 358 with high-risk types according to the HC2 test. Genotyping by PapilloCheck[®] and SPF₁₀ confirmed
39
40 359 approximately 90% of these high-risk types. In contrast, HC2 low-risk results were confirmed by
41
42 360 PapilloCheck[®] and SPF₁₀ genotyping to a less extent. This underlines the importance of reliable
43
44 361 genotyping tests, since HC2 often detects erroneously an infection with low-risk-types when a high-
45
46 362 risk infection is present or *vice versa*.

47
48 363 The genotyping tests in this study revealed the highest prevalence for the HR-HPV types 16, 31, 51,
49
50 364 52 and 56 as well as HPV LR subtype 42, which was the second most common subtype detected by
51
52 365 the PapilloCheck[®] assay. In other studies, different HPV types have been described to be prevalent
53
54
55
56
57
58
59
60

1
2
3 366 [Munoz et al. 2003 and 2004], which is due to the rather insensitive methods that have been
4
5 367 combined in meta-analyses (Klug et al., 2008; Schopp et al., 2009). In cancer samples, HPV16 was the
6
7 368 most prevalent subtype, followed by HPV 18 (Munoz et al., 2003, 2004). Together they account for
8
9 369 approximately 70%. In this study, HPV 18 was detected in only approximately 3-8% of HC2 positive
10
11 370 cervical samples. This supports the conclusion by others that certain HR-HPV types, such as HPV 18,
12
13 371 are more tumourigenic than others [Castle et al., 2005; Lai et al., 2007; Kjaer et al., 2002]. Lai et al.
14
15 372 [2007] already demonstrated the independent prognostic significance of HPV 18 positivity in early-
16
17 373 stage cervical cancer, and HPV 16 infection was associated with a very high absolute risk of \geq cervical
18
19 374 intraepithelial neoplasia grade 3 over a 2-year period [Castle et al., 2005]. HPV45 might be another
20
21 375 such candidate. It was detected it in 3-4% of all HC2 positive samples, but it is among the most
22
23 376 prevalent types in cervical lesions [Clifford et al., 2006; Bao et al. 2008] These results underline the
24
25 377 importance of genotyping tests in addition to high-risk HPV-positive or -negative screening. However,
26
27 378 none of the genotyping methods available is yet regarded as standard. The comparison in the study
28
29 379 of two genotyping tests, PapilloCheck[®] and SPF₁₀, revealed that their results correspond fairly well
30
31 380 regarding the most prevalent HR-HPV types 16, 51 and 31. However, correspondence of the results
32
33 381 of the two test methods for the LR-HPV types is less pronounced, mainly due to the very high
34
35 382 sensitivity of the PapilloCheck[®] test for HPV subtype 42. The assays differ in sensitivity, which is most
36
37 383 probably the result of the different length of the amplified DNA fragment as well as primer specificity
38
39 384 used for amplification, as shown in two recent studies [Klug et al, 2008; Schopp et al, 2009].

40
41 385 In this study factors that increase or lower the risk for HPV infection should also be identified. There
42
43 386 seems to be a positive effect of HPV vaccination on the risk of HPV infection. However, the study was
44
45 387 not designed to show a significant effect of vaccination on the risk of HPV infection rather than to
46
47 388 estimate age specific HPV prevalence. There was no further information on the vaccine employed,
48
49 389 such as which vaccine was used, the vaccination scheme and adherence to it. Therefore a firm
50
51 390 conclusion on the effect of HPV vaccination cannot be drawn from this study. Nevertheless, previous
52
53
54
55
56
57
58
59
60

1
2
3 391 clinical trials have shown significant effects of HPV vaccination on the occurrence of cervical disease
4
5 392 [Harper et al., 2004; Villa et al., 2005; Paavonen et al., 2009; Brown et al., 2009].
6
7
8 393 Risk factors for HPV infection were smoking and the number of sexual partners. Smoking as a risk
9
10 394 factor for HPV infection has also been described in recent epidemiological studies in China and Brazil
11
12 395 [Chan et al., 2009; Sarian et al., 2009]. An association between sexual behaviour and the risk of HPV
13
14 396 infection has been reported in other European countries [Lenselink et al., 2008; Ammatuna et al.,
15
16
17 397 2008; Nielsen et al., 2008].
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

TABLE I: Risk factors versus HC2-laboratory results

	Total (N=1692)		Positive (N=377)		Low Risk only (N=54)		High Risk only (N=239)		High & Low Risk (N=84)	
age, mean \pm SD	21.5 \pm 4.6		22.0 \pm 4.0		21.4 \pm 4.3		22.3 \pm 4.1		21.6 \pm 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

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 strata											
	total		10-16		17-19		20-22		23-26		27-30	
	N	% [95% CI]	N	% [95% CI]	N	% [95% CI]	N	% [95% CI]	N	% [95% CI]	N	% [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® (only HC2 positive women)	377		34		82		100		91		70	
- 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	-
SPF₁₀ (only HC2 positive women)	377		34		82		100		91		70	
- 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

Self-reported Factor	Univariate		multivariate	
	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®. Only HC2 positive subjects were tested.

PapilloCheck®	HC2					
	Low risk only		High risk 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

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

† 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.

SPF ₁₀	HC2					
	Low risk only		High 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

1
2
3 Figure 1: Flow of participants enrolled in the study
4
5

6 Figure 2: Percental distribution of age groups with negative and positive HC2 test results. Subjects
7 with positive HC2 test results are subdivided in low risk, high risk, and both low and high risk groups.
8 The figure shows the distribution of subjects with a negative LR, HR or both — HC2 test result per age
9 group in relation to all women with a negative LR, HR or both — HC2 test result, respectively.
10
11

12 Figure 3: HPV genotyping according to the PapilloCheck[®] test (24 HPV types) among women testing
13 HPV-positive with the HC2 test. Multiple responses possible, bars indicate the 95% confidence
14 interval.
15
16
17
18

19 Figure 4: HPV genotyping according to the SPF₁₀ assay (26 HPV types) among women testing HPV-
20 positive with the HC2 test. Multiple responses possible, bars indicate the 95% confidence interval.
21
22
23
24

25 Figure 5: Number of sexual partners in women with (a) negative and (b) positive HC2 test
26
27

28 (a) Women with negative HC2 test
29
30

31 (b) Women with positive HC2 test
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 **ACKNOWLEDGMENTS**
4

5
6 The study was sponsored by GlaxoSmithKline Inc.
7

8
9 T.I. has received speaker honorarium from GlaxoSmithKline GmbH & Co KG.
10

11 We thank Heike von Krempelhuber for writing assistance of this article.
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1
2
3 **1 REFERENCES**
4

- 5
6
7 2 Ammatuna P, Giovannelli L, Matranga D, Ciriminna S, Perino A. 2008. Prevalence of genital human
8
9 3 papilloma virus infection and genotypes among young women in Sicily, South Italy. *Cancer Epidemiol*
10
11 4 *Biomarkers Prev* 17:2002-2006.
12
13
14
15 5 Antila A, Aoki D, Arbyn M, Austoker J, Bosch X, Chirenje Z. M., Cuzick J, Day N. E., Denny L. A., Fonn S,
16
17 6 Franco E, Iftner T, Krickler A, Lawson H, Lynge E, Marrett L. D., McGoogan E, Meijer C. J., Miller A. B.,
18
19 7 Patnick J, Robles S. C., Ronco G, Schiffman, M. H., Sellors J. W., Singer A, Suba E. J., Wright T. C. 2005.
20
21 8 IARC (International Agency for Research on Cancer) Handbooks of Cancer Prevention Vol. 10: Cervix
22
23 9 cancer screening. IARC Press, Lyon 2005.
24
25
26
27 10 Bao YP, Li N, Smith JS, Qiao YL; ACCPAB members. 2008. Human papillomavirus type distribution in
28
29 11 women from Asia: a meta-analysis. *Int J Gynecol Cancer* 18:71-79.
30
31
32
33 12 Becker N, Brenner H, Klug S, Schilling F, Spix C. 2006. Beiträge der Epidemiologie zur
34
35 13 Krebsfrüherkennung. *Der Onkologe* 12:1136-1145.
36
37
38
39 14 Bosch, FX, Lorincz A, Munoz N, Meijer C J, Shah KV. 2002. The causal relation between human
40
41 15 papillomavirus and cervical cancer. *J Clin Pathol* 55:244–265.
42
43
44 16 Bosch FX, de Sanjosé S, Castellsagué X, Moreno V, Muñoz N: Chapter 3: Epidemiology of human
45
46 17 papillomavirus infections and associations with cervical cancer: New opportunities for prevention. In:
47
48 18 Papillomavirus Research: From Natural History To Vaccines and Beyond. Caister Academic Press,
49
50 19 2006.
51
52
53
54 20 Brown DR, Kjaer SK, Sigurdsson K, Iversen OE, Hernandez-Avila M, Wheeler CM, Perez G, Koutsky LA,
55
56 21 Tay EH, Garcia P, Ault KA, Garland SM, Leodolter S, Olsson SE, Tang GW, Ferris DG, Paavonen J,
57
58 22 Steben M, Bosch FX, Dillner J, Joura EA, Kurman RJ, Majewski S, Muñoz N, Myers ER, Villa LL, Taddeo
59
60

- 1
2
3 23 FJ, Roberts C, Tadesse A, Bryan J, Lupinacci LC, Giacoletti KE, Singhs HL, James M, Hesley TM, Barr E.
4
5 24 2009. The impact of quadrivalent human papillomavirus (HPV; types 6, 11, 16, and 18) L1 virus-like
6
7
8 25 particle vaccine on infection and disease due to oncogenic nonvaccine HPV types in generally HPV-
9
10 26 naive women aged 16-26 years. *J Infect Dis* 199:926-935.
11
12
13 27 Bundesärztekammer. 1994. Leitlinie der Bundesärztekammer zur Qualitätssicherung zytologischer
14
15
16 28 Untersuchungen im Rahmen der Früherkennung des Zervixkarzinoms. *Deutsch. Aerzteblatt* 91, B298-
17
18 29 B300.
19
20
21 30 Castle PE, Schiffman M, Burk RD, Wacholder S, Hildesheim A, Herrero R, Bratti MC, Sherman ME,
22
23 31 Lorincz A. 2002. Restricted cross-reactivity of hybrid capture 2 with nononcogenic human
24
25 32 papillomavirus types. *Cancer Epidemiol Biomarkers Prev* 11:1394-1399.
26
27
28
29 33 Castle, P. E., D. Solomon, M. Schiffman, and C. M. Wheeler. 2005. Human papillomavirus type 16
30
31 34 infections and 2-year absolute risk of cervical precancer in women with equivocal or mild cytologic
32
33 35 abnormalities. *J Natl Cancer Inst* 97:1066-1071.
34
35
36
37 36 Chan PK, Ho WC, Wong MC, Chang AR, Chor JS, Yu MY. 2009. Epidemiologic risk profile of infection
38
39 37 with different groups of human papillomaviruses. *J Med Virol.* 81:1635-1644.
40
41
42
43 38 Clifford G, Franceschi S, Diaz M, Muñoz N, Villa LL. 2006. HPV type-distribution in women with and
44
45 39 without cervical neoplastic disease. *Vaccine* 24 (Suppl. 3):26-34.
46
47
48 40 Cogliano V, Baan R, Straif K, Grosse Y, Secretan B, El GF. 2005. Carcinogenicity of human
49
50 41 papillomaviruses. *Lancet Oncol* 6:204.
51
52
53 42 De Sanjosé S, Diaz M, Castellsagué X, Clifford G, Bruni L, Muñoz N, Bosch FX. 2007. Worldwide
54
55 43 prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal
56
57 44 cytology: a meta-analysis. *Lancet Infect Dis* 7:453-459.
58
59
60

- 1
2
3 45 Dunne EF, Unger ER, Sternberg M, McQuillan G, Swan DC, Patel SS, Markowitz LE. 2007. Prevalence
4
5 46 of HPV infection among females in the United States. *JAMA* 297:813–819.
6
7
8
9 47 Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A, Zahaf T, Innis B, Naud P, De
10
11 48 Carvalho NS, Roteli-Martins CM, Teixeira J, Blatter MM, Korn AP, Quint W, Dubin G; GlaxoSmithKline
12
13 49 HPV Vaccine Study Group. 2004. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of
14
15 50 infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial.
16
17 51 *Lancet* 364:1757-1765.
18
19
20
21 52 Ho GY, Burk RD, Klein S, Kadish AS, Chang CJ, Palan P, Basu J, Tachezy R, Lewis R, Romney S. 1995.
22
23 53 Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. *J*
24
25 54 *Natl Cancer Inst* 87:1365–1371.
26
27
28
29 55 Iftner T. 2006. Zervixkarzinom. Chancen und Risiken des Früherkennungsprogramms. *Geburtsh*
30
31 56 *Frauenheilk* 66:196-198.
32
33
34
35 57 Kjaer, S. K., A. J. van den Brule, G. Paull, E. I. Svare, M. E. Sherman, B. L. Thomsen, M. Suntum, J. E.
36
37 58 Bock, P. A. Poll, and C. J. Meijer. 2002. Type specific persistence of high risk human papillomavirus
38
39 59 (HPV) as indicator of high grade cervical squamous intraepithelial lesions in young women:
40
41 60 population based prospective follow up study. *BMJ* 325:572–576.
42
43
44
45 61 Kjaer SK, Breugelmans G, Munk C, Junge J, Watson M, Iftner T. 2008. Population-based prevalence,
46
47 62 type- and age-specific distribution of HPV in women before introduction of an HPV-vaccination
48
49 63 program in Denmark. *Int J Cancer* 123:1864-1870.
50
51
52
53 64 Kleter B, van Doorn LJ, ter Schegget J, Schrauwen L, van Krimpen K, Burger M, ter Harmsel B, Quint
54
55 65 W. 1998. Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital
56
57 66 human papillomaviruses. *Am J Pathol* 153:1731-1739.
58
59
60

- 1
2
3 67 Kleter B, van Doorn LJ, Schrauwen L, Molijn A, Sastrowijoto S, ter Schegget J, Lindeman J, ter Harmsel
4
5 68 B, Burger M, Quint W. 1999. Development and clinical evaluation of a highly sensitive PCR-reverse
6
7
8 69 hybridization line probe assay for detection and identification of anogenital human papillomavirus. J
9
10 70 Clin Microbiol 37:2508-2517.
11
12
13 71 Klug SJ, Molijn A, Schopp B, Holz B, Iftner A, Quint W, J F Snijders P, Petry KU, Krüger Kjaer S, Munk C,
14
15
16 72 Iftner T. 2008. Comparison of the performance of different HPV genotyping methods for detecting
17
18 73 genital HPV types. J Med Virol 80:1264-1274.
19
20
21 74 Lai CH, Chang CJ, Huang HJ, Hsueh S, Chao A, Yang JE, Lin Ct, Huang SL, Hong JH, Chou HH, Wu TI,
22
23 75 Huang KG, Wang CC, Chang TC 2007 Role of human papillomavirus genotype in prognosis of early-
24
25 76 stage cervical cancer undergoing primary surgery. J Clin Oncol 25:3628-3634.
27
28
29 77 Lenselink CH, Melchers WJ, Quint WG, Hoebbers AM, Hendriks JC, Massuger LF, Bekkers RL. 2008.
30
31 78 Sexual behaviour and HPV infections in 18 to 29 year old women in the pre-vaccine era in the
32
33 79 Netherlands. PLoS One. 2008;3:e3743. Epub 2008 Nov 17.
34
35
36
37 80 Muñoz N, Bosch FX, de Sanjosé S, Tafur L, Izarzugaza I, Gili M, Viladiu P, Navarro C, Martos C, Ascunce
38
39 81 N. 1992. The causal link between human papillomavirus and invasive cervical cancer: A population-
40
41 82 based case-control study in Colombia and Spain. Int J Cancer 52:743-749.
42
43
44
45 83 Muñoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJ, Meijer CJ;
46
47 84 International Agency for Research on Cancer Multicenter Cervical Cancer Study Group: Epidemiologic
48
49 85 classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003; 348:
50
51 86 518-527.
52
53
54
55 87 Muñoz N, Bosch FX, Castellsagué X, Diaz M, de Sanjose S, Hammouda D, Shah KV, Meijer CJ: Against
56
57 88 which human papillomavirus types shall we vaccinate and screen? The international perspective. Int J
58
59 89 Cancer 2004; 111: 278-285.
60

- 1
2
3 90 Myers ER, McCroly DC, Nanda K, Bastian L, Matchar DB. 2000. Mathematical model for the natural
4
5 91 history of human papillomavirus infection and cervical carcinogenesis. *Am J Epidemiol* 151:1158–
6
7 92 1171.
8
9
10
11 93 Nanda K, McCroly DC, Myers ER, Bastian LA, Hasselblad V, Hickey JD, Matchar DB. 2000. Accuracy of
12
13 94 the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic
14
15 95 review. *Ann Intern Med* 132:810-819.
16
17
18
19 96 Nielsen A, Kjaer SK, Munk C, Iftner T. 2008. Type-specific HPV infection and multiple HPV types:
20
21 97 prevalence and risk factor profile in nearly 12,000 younger and older Danish women. *Sex Transm Dis*
22
23 98 35:276-282.
24
25
26
27 99 Paavonen J, Naud P, Salmerón J, Wheeler C, Chow SN, Apter D, Kitchener H, Castellsague X, Teixeira J,
28
29 100 Skinner S, Hedrick J, Jaisamrarn U, Limson G, Garland S, Szarewski A, Romanowski B, Aoki F, Schwarz
30
31 101 T, Poppe W, Bosch F, Jenkins D, Hardt K, Zahaf T, Descamps D, Struyf F, Lehtinen M, Dubin G; for the
32
33 102 HPV PATRICIA Study Group. 2009. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted
34
35 103 vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final
36
37 104 analysis of a double-blind, randomised study in young women. *Lancet*;374:301-314.
38
39
40
41 105 Robert Koch-Institute: Krebs in Deutschland 2003 – 2004 Häufigkeiten und Trends. Robert Koch-
42
43 106 Institute & Gesellschaft der epidemiologischen Krebsregister in Deutschland e.V (Eds.). 6th Edition,
44
45 107 Berlin, 2008: www.ekr.med.uni-erlangen.de/GEKID/Doc/kid2008.pdf
46
47
48
49
50 108 Sarian LO, Hammes LS, Longatto-Filho A, Guarisi R, Derchain SF, Roteli-Martins C, Naud P, Erzen M,
51
52 109 Branca M, Tatti S, de Matos JC, Gontijo R, Maeda MY, Lima T, Costa S, Syrjänen S, Syrjänen K. 2009.
53
54 110 Increased risk of oncogenic human papillomavirus infections and incident high-grade cervical
55
56 111 intraepithelial neoplasia among smokers: experience from the Latin American screening study. *Sex*
57
58 112 *Transm Dis* 36:241-248.
59
60

- 1
2
3 113 Schenck U, von Karsa L. 2000. Cervical cancer screening in Germany. *Eur J Cancer* 36:2221–2226.
4
5
6
7 114 Schiffman MH, Bauer HM, Hoover RN, Glass AG, CadellDM, Rush BB, Scott DR, Sherman ME, Kurman
8
9 115 RJ, Wacholder S. 1993. Epidemiologic evidence showing that human papillomavirus infection causes
10
11 116 most cervical intraepithelial neoplasia. *J Natl Cancer Inst* 85:958–964.
12
13
14 117 Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. 2007. Human papillomavirus and
15
16 118 cervical cancer. *Lancet* 370:890–907.
17
18
19
20 119 Schopp B, Holz B, Zago M, Stubenrauch F, Petry K-U, Krüger-Kjaer S, Iftner T. 2009. Evaluation of the
21
22 120 performance of the novel PapilloCheck HPV genotyping test by comparison with two other
23
24 121 genotyping systems and the HC2 test. *J Med Virol*; in press
25
26
27
28 122 Sherman ME, Lorincz AT, Scott DR, Wacholder S, Castle PE, Glass AG, Mielzynska-Lohnas I, Rush BB,
29
30 123 Schiffman M. 2003. Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a
31
32 124 10 year cohort analysis. *J Natl Cancer Inst* 95:46-52.
33
34
35
36 125 Syrjänen K, Hakama M, Saarikoski S, Väyrynen M, Yliskoski M, Syrjänen S, Kataja V, Castrén O. 1990.
37
38 126 Prevalence, incidence, and estimated life-time risk of cervical human papillomavirus infections in a
39
40 127 nonselected Finnish female population. *Sex Transm Dis* 17:15–19.
41
42
43
44 128 Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer
45
46 129 CJ, Munoz N. 1999. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide.
47
48 130 *J Pathol* 189:12–19.
49
50
51
52 131 Weinstock H, Berman S, Cates W. 2004. Sexually transmitted diseases among American youth:
53
54 132 incidence and prevalence estimates, 2000. *Perspect Sex Reprod Health* 36:6–10.
55
56
57 133 Wentzensen N, Klug SJ. 2008. Early detection of cervical carcinomas. Finding an overall approach.
58
59 134 *Dtsch Ärztebl Int* 105:617-622.
60

- 1
2
3 135 Villa LL, Costa RL, Petta CA, Andrade RP, Ault KA, Giuliano AR, Wheeler CM, Koutsky LA, Malm C,
4
5 136 Lehtinen M, Skjeldestad FE, Olsson SE, Steinwall M, Brown DR, Kurman RJ, Ronnett BM, Stoler MH,
6
7 137 Ferenczy A, Harper DM, Tamms GM, Yu J, Lupinacci L, Railkar R, Taddeo FJ, Jansen KU, Esser MT,
8
9
10 138 Sings HL, Saah AJ, Barr E. 2005. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and
11
12 139 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled
13
14 140 multicentre phase II efficacy trial. *Lancet Oncol* 56:271-278.
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review