

# Experience with high-risk human papillomavirus testing on vaginal brush-based self-samples of non-attendees of the cervical screening program

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We evaluated the effect of offering brush-based vaginal self-sampling for high-risk human papillomavirus (hrHPV) testing to non-attendees of the cervical screening program on response rate, compliance to follow-up and cervical intraepithelial neoplasia grade 2 or 3 (CIN2+/CIN3+) yield. In addition, concordance of hrHPV test results between physician-taken cervical scrapes and vaginal self-samples was determined. A total of 26,409 nonattending women were randomly assigned to receive a vaginal brush device for hrHPV testing by Hybrid Capture-2<sup>®</sup> method (*i.e.*, self-sampling group,  $n = 26,145$ ) or a reinvitation for regular cytology-based screening (*i.e.*, recall control group,  $n = 264$ ). hrHPV-positive self-sampling responders were invited for a physician-taken scrape for cytology and blinded hrHPV testing. If cytology was abnormal, women were referred for colposcopy. Response rate in the self-sampling group was significantly increased compared to the recall control group (30.8% *versus* 6.5%;  $p < 0.001$ ). The concordance rate between hrHPV detection in self-samples and corresponding physician-taken cervical scrape samples was 68.8%. Amongst women with CIN3+ and CIN2+, the concordance rates in hrHPV positivity between both samples were 95.5% and 93.8%, respectively. Adherence at baseline to cytology triage of hrHPV-positive self-sampling women (89.1%) and colposcopy referral of those with abnormal cytology (95.8%) was high. The CIN2+/CIN3+/carcinoma yields were 1.5%, 1.0% and 0.1%, respectively, in self-sampling responders. In conclusion, offering hrHPV testing on self-sampled vaginal material with a brush device to non-attendees significantly increases the attendance to the regular screening program, yields hrHPV test results that are in very good concordance with those of physician-taken scrapes in women with CIN2+/CIN3+, and is effective in detecting CIN2+/CIN3+.

**Key words:** population-based cervical screening, non-attendees, human papillomavirus, hybrid capture 2, cervical intraepithelial neoplasia, cervical carcinoma, self-sampling

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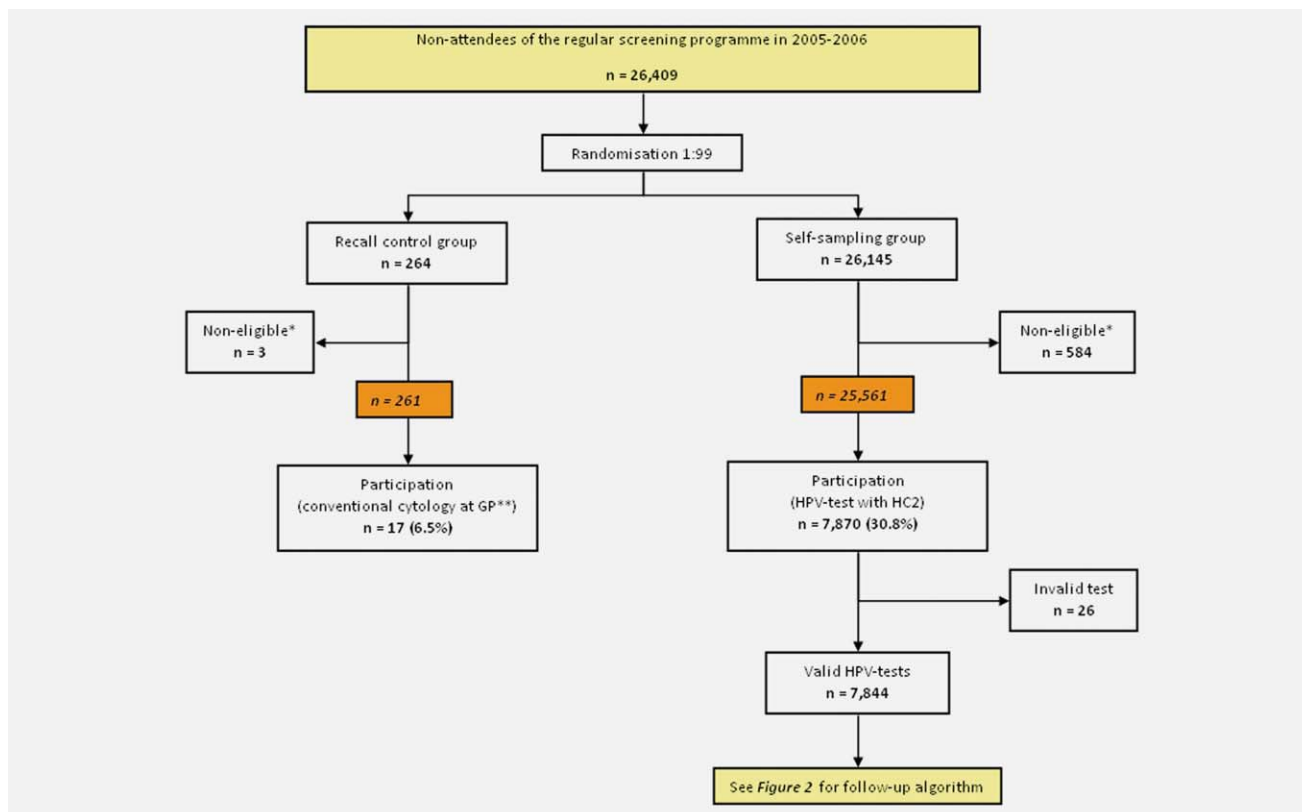
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In The Netherlands, an organised cervical cancer screening program with a call and recall system, targeting women between 30 and 60 years of age every 5 year, is effective since 1996. Each year, 65% of the women attend the screening program.<sup>1</sup> Together with some opportunistic screening this contributes to an overall coverage for cervical screening of 77%, leaving 23% of women unscreened.<sup>1</sup> The effectiveness of the screening program is strongly dependent of the degree of attendance.<sup>2-5</sup> Nonattendance is especially a problem in the youngest and oldest age groups of invitees.<sup>1,5</sup> Women not attending the screening program have an increased risk of cervical carcinoma compared to attending women.<sup>6</sup> We have previously shown in the PROtection by Offering Hpv TEsting on Cervicovaginal specimens Trial (PROTECT-1) trial that offering a self-sampling device for collecting cervico-vaginal lavage material for high-risk human papillomavirus (hrHPV) testing is a feasible and effective alternative for women not attending regular cytological screening, which improves coverage of the screening program significantly.<sup>2,7</sup>

Here, we present data of the PROTECT-2 study, in which we evaluated the performance of brush-based vaginal self-



**Figure 1.** Study design for comparison of compliance rates between the recall control group and self-sampling group. \*Excluded due to prior hysterectomy or meanwhile passed away. \*\*General practitioner. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

sampling for hrHPV testing among non-attendees of the regular screening program. Outcome parameters were response rate, compliance to follow-up, concordance of hrHPV test results between vaginal self-samples and physician-taken cervical scrapes, and yield of cervical intraepithelial neoplasia grade 2 or 3 (CIN2+ or CIN3+), or worse within 18 months of follow-up.

## Methods

### Trial design

For PROHTECT-2, women were recruited who lived in the region Noord-Holland or Flevoland and, according to the database of the Regional Health Council, had not attended the organised cervical screening program in the year 2006 after the regular and reminder invitation. In that year, women who turned in their 30th, 35th, 40th, 45th, 50th, 55th or 60th birth year were invited for the regular program. Women were invited for PROHTECT-2 between November, 2007 and March, 2008.

We randomised the women into a recall control arm and a self-sampling arm. The former did receive a second reminder invitation for regular cytology, whereas the latter received a self-sampling brush device (VibaBrush<sup>®</sup>, Rovers, Oss, The Netherlands) for hrHPV testing by the Hybrid Capture-2<sup>®</sup> method (HC2). Essentially, the same trial design was used as for PROHTECT-1, in which HC2 test positive women were advised to visit a physician for a cervical scrape

for cytology triage (see flowchart in Fig. 1). All eligible women were sent a 1-week prior notice by surface mail to their home address to inform them about the study and alerting them on the possibility of either receiving a package for self-collection or a second reinvitation for regular cytology. After 1 week, women of the self-sampling group received a self-sample kit consisting of an explanatory letter with a brush for self-collection of a vaginal specimen, a collection vial containing 1.5 mL universal collection medium (Qiagen, Gaithersburg, MD), written and drawn instructions, an informed consent form and a return envelope. Women of the control recall group received an official second reminder to visit their physician for regular cytology, an explanatory letter and an informed consent form. A website and telephone desk providing information of the study were available throughout the study period ([http:// www.hpvthuiest.nl](http://www.hpvthuiest.nl)).

We computed response rates in the recall control group and the self-sampling group and analysed in the self-sampling arm the CIN2+/CIN3+ yields within a period of 18 months after receipt of the hrHPV test result. Analyses were done *via* record tracking of individual cases as well as *via* query from the nationwide network and registry of histology and cytology database (PALGA; Bunnik, The Netherlands). To verify the follow-up data, the physician was contacted, if necessary.

### Randomisation

To compare response rates, women were assigned to either a self-sampling group or a second reinvitation group for conventional cytology at a 99:1 ratio using computer's "randomize number generator". This skewed ratio was chosen to ensure adequate power to detect a difference in response rate between both study groups, but at the same time to maximize detection of CIN2+/CIN3+ rate among self-sampling responders.<sup>7</sup>

The study was approved by the Ministry of Public Health (no. 2006/01WBO) and registered in the trial register (<http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=1851>) as NTR1851. All participating women gave written informed consent.

### HPV testing of the self-sampled material

Women of the self-sampling group were asked to send the collection vial containing the self-sampled vaginal specimen together with the signed informed consent to the laboratory for hrHPV testing at the department of pathology, VU University Medical Center, Amsterdam. After visual inspection of the liquid samples in the lab, these were tested by HC2 according to the manufacturer's protocol, in an automated format on a rapid capture system.<sup>6,8,9</sup> This test uses a hrHPV cocktail probe, which is designed to detect HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. Results of HC2 were expressed as relative light units per cutoff value. When no clear cell material was visible in the samples beta-globin, polymerase chain reaction (PCR) was performed first.<sup>10</sup> In case of a negative beta-globin, PCR test samples were considered invalid for HC2 testing. In this case, women received a second self-sampling kit with the request to repeat self-sampling at home.

### Cytology reading

Cervical smears were read in local laboratories, and results were reported according to the CISOE-A classification, the standard classification system for cytology in The Netherlands, which can easily be translated into the Bethesda classification.<sup>11</sup> For this analysis, cytology results on the basis of either squamous or columnar abnormalities were grouped as normal, borderline or mild dyskaryosis (BMD; corresponding to Bethesda ASCUS/LSIL), or moderate dyskaryosis or worse (>BMD; corresponding to ASC-H/HSIL or worse).

### The follow-up triage of self-sampling group and control group

All responding women in the self-sampling group received a written test result and explanation by mail. Those who were hrHPV-negative were advised to await the next screening round invitation (*i.e.*, 5 years after 2006). All women who were hrHPV-positive were advised to visit their physician for taking a scrape for cytology triage and blinded HPV testing. They were referred for colposcopy if the smear result was

≥BMD. In case of normal cytology, they were reinvited after 1 year for a physician taken cervical scrape for cytology and hrHPV testing. Women with a positive cytology and/or hrHPV test result at that occasion were referred for colposcopy. In case a woman did not comply with the follow-up protocol at baseline or after 1 year, a reminder letter was sent to them with a copy to their physician. Women who had a double negative test result after 1 year were advised to attend the next screening round.

Women responding in the recall control group received a cytology report of their physician-taken cervical specimen. Those with abnormal cytology were managed according to the guidelines of the national screening program.<sup>8,9</sup> Endometrial abnormalities were excluded.

### Outcome measures

The primary outcome measure of PROTECT-2 was the response rate in both recall control and self-sampling arms. The time span for measuring attendance was 1 year from the moment the study responders received either the recall invitation or self-sampling device.

The secondary outcome measures included the prevalence of hrHPV among self-sampling responders, adherence to cytology triage and referral, and the number of histologically confirmed CIN2+/CIN3+ within a follow-up period of 18 months. Women with normal cytology and a hrHPV-negative cervical sample were not referred for colposcopy, because their risk of CIN2+ was considered that low that the medical ethics committee found it unethical to refer these women for colposcopy. In the analyses, these women were assumed to have no CIN2+.

In addition, we assessed the concordance between hrHPV tests results on physician-taken smears *versus* self-sampled vaginal material of self-sample hrHPV-positive women and evaluated the CIN2+/CIN3+ outcome according to either HPV test result.

## Results

### Study cohort

A flowchart of the study design is given in Figure 1. A total of 26,409 non-attendees were eligible for inclusion in PROTECT-2. After randomisation, 26,145 self-sampling kits were sent to women registered as non-attendees, and 264 non-attendees received a second reminder for regular cytology screening. A total of 584 women in the self-sampling arm and 3 women in the control group did not respond to the study because they reported having had a hysterectomy or meanwhile passed away, leaving 25,561 women in the self-sampling arm and 261 women in the recall control arm, respectively. No statistically significant differences were found between the age distributions in both arms.

### Response rate

Of the self-sampling group, 7,870 women (30.8%; 95% CI 30.2–31.4%) submitted a self-sampled specimen. The

**Table 1.** Response rate, hrHPV-prevalence and CIN2+/CIN3+/carcinoma yield in non-attendees to the regular screening program who responded by self-sampling, stratified by age

Age category	Total (n)	Response rate (%)	HPV-pos	95% CI (HPV-pos)	CIN2+	CIN3+	Carcinoma
29–33 years	4,166	1,163 (27.9%)	182 (15.6%)	13.5–17.7%	42 (3.6%)	31 (2.7%)	2 (0.2%)
34–38 years	4,875	1,501 (30.8%)	164 (10.9%)	9.4–12.6%	35 (2.3%)	21 (1.4%)	3 (0.2%)
39–43 years	4,165	1,342 (32.2%)	102 (7.6%)	6.2–9.0%	16 (1.2%)	11 (0.8%)	–
44–48 years	3,766	1,167 (31.0%)	67 (5.7%)	4.5–7.2%	16 (1.4%)	10 (0.9%)	3 (0.3%)
49–53 years	3,147	986 (31.3%)	48 (4.9%)	3.5–6.2%	3 (0.3%)	3 (0.3%)	–
54–58 years	2,275	811 (29.2%)	48 (5.9%)	4.3–7.5%	4 (0.5%)	3 (0.4%)	–
59–63 years	2,667	900 (33.7%)	41 (4.6%)	3.1–5.8%	3 (0.3%)	2 (0.2%)	–
Total	25,561	7,870 (30.8%)	652 (8.3%) <sup>1</sup>	7.7–8.9% <sup>1</sup>	119 (1.5%) <sup>1</sup>	81 (1.0%) <sup>1</sup>	8 (0.1%) <sup>1</sup>

<sup>1</sup>These percentages are based on 7,844 as denominator (7,870 minus the 26 inadequate HPV self-sampled material).

response rate in the recall control group for cervical cytology was 17 out of 261 (6.5%; 95% CI 9.0–14.4%). The difference in response rate between the two study arms was statistically significant ( $\chi^2 = 71.77$ ;  $p < 0.01$ ). In the self-sampling arm, young women (29–33 years) showed a significantly lower response rate than older women ( $\chi^2$  for linear trend = 10.65;  $p < 0.01$ ), whereas no differences in response rate were found between women in the age strata from 34 to 63 years ( $\chi^2$  for linear trend = 0.63;  $p = 0.43$ ).

#### hrHPV detection rate

Of the women who submitted a self-sampled specimen, 26 (0.3%) had an invalid hrHPV test result, leaving 7,844 women with a valid test (99.7%) (Fig. 1). Among the latter, 652 (8.3%; Table 1) had a positive hrHPV test result. The percentage of hrHPV-positive women decreased with age from 15.6% in women of 29–33 years of age to 4.6% in women aged 59–63 years ( $\chi^2$  for linear trend = 113.14;  $p < 0.01$ ). The proportion of hrHPV-positive women did not decrease with age in women of 44 years and older ( $\chi^2$  for linear trend = 0.69;  $p = 0.406$ ).

#### Compliance to follow-up of hrHPV-positive women in self-sampling group

A total of 71 of 652 (10.9%) hrHPV-positive women did not adhere to invitations for cytology triage testing, leaving 581 women (89.1%) with a cervical scrape taken by their physician. Of these, 28 women (4.8%) did not have a cervical smear, but only a hrHPV-test. Another 100 women (17.2%) had only a cytology result. The remaining 453 women (78.0%) followed the protocol and had both cytology and hrHPV-test results.

Of the 581 women with follow-up 192 (33.0%) had abnormal cytology (*i.e.*,  $\geq$ BMD), of whom 184 (95.8%) adhered to the advice for direct referral to the gynaecologist for colposcopy. Another 8 women (4.2%) declined further follow-up ( $n = 2$ ), or did not comply to repeated advice for direct referral for colposcopy ( $n = 6$ ).

Of the 389 women without cytological abnormalities (including 28 women who had only a hrHPV test) 226

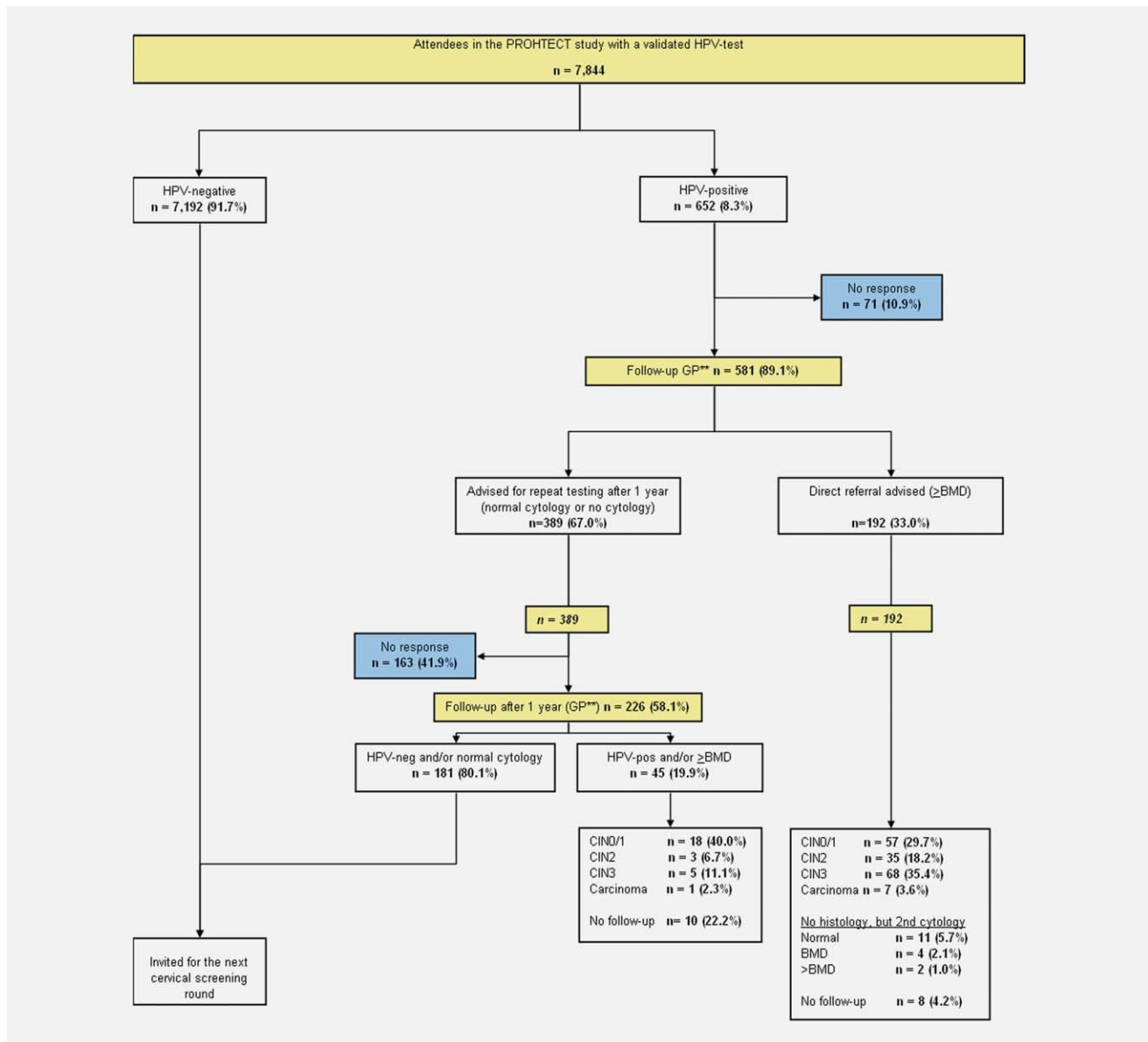
(58.1%) adhered to the follow-up protocol of repeat testing after 1 year. Sixty-nine women (41.9%) declined further follow-up, and 94 women did not adhere to repeated advice for repeat testing at 1 year (in total 163 women). Amongst the 226 women with a repeat test after 1 year, 45 (19.9%) had cytological abnormalities and/or a hrHPV-positive test result. Twenty-seven (60.0%) of these women completed follow-up with histology (Fig. 2).

#### Cervical carcinoma, CIN3+ and CIN2+ yield among hrHPV-positive self-sampling responders

Among 185 self-sampling responders with  $\geq$ BMD who visited a gynaecologist at baseline 7 cervical squamous cell carcinomas (3.6%), 68 CIN3 (35.4%) and 35 CIN2 (18.2%) lesions were detected. Most CIN2+ lesions were found in the youngest age group (29–33 years).

Of the 27 women who underwent colposcopy after one year, 1 had cervical carcinoma (3.8%), 5 had CIN3 (18.5%) and 3 had CIN2 (11.1%). All of these 9 women had abnormal cytology and those tested for hrHPV ( $n = 3$ ) were hrHPV positive. At baseline, 8 of these 9 women had normal cytology and 1 had no cytology result. The 5 women with CIN3 and 1 woman with CIN2 had a hrHPV-positive physician-taken scrape, whereas 2 remaining women with CIN2 had an invalid baseline hrHPV test on the physician-taken scrape. Strikingly, in the woman with cervical cancer, the hrHPV test performed on the physician taken smear at baseline was negative.

The cumulative 18-month CIN3+ and CIN2+ yields in women with a hrHPV-positive self-sampling test were 1.0% (81 of 7,844) and 1.5% (119 of 7,844), respectively (see Table 1 and Fig. 2). After stratification into age groups, the CIN2+/CIN3+ yields appeared significantly higher in young women (aged 29–33 years) compared to older women (aged 34–63 years; CIN2+: 3.6% vs. 1.1%, respectively;  $p < 0.001$ , and for CIN3+: 2.7% vs. 0.7%, respectively;  $p < 0.001$ ). Also, significant differences were found when comparing women aged 29–38 years to women of 39–63 years (CIN2+: 2.9% vs. 0.8%, respectively;  $p < 0.01$ ; CIN3+: 2.0% vs. 0.8%, respectively;  $p < 0.01$ ).



**Figure 2.** Study design for evaluation of CIN2+ yield in women of the self-sampling group. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

**Table 2.** HPV test results on physician-taken cervical scrapes of 652 women with hrHPV-positive self-samples in relation to histological outcome

	≤ CIN 1	CIN 2	CIN 3	CxCa	No hist fup	Total
HPV positive	50 (80.6%)	27 (90.0%)	61 (96.8%)	3 (75.0%)	188 (58.9%)	329 (68.8%)
HPV negative	12 (19.4%)	3 (10.0%)	2 (3.2%)	1 (25.0%)	131 (41.1%)	149 (31.2%)
Invalid HPV test <sup>1</sup>	0	2	–	–	1	3
No HPV-test performed <sup>2</sup>	13	6	10	4	138	171
Total	75 (100%)	38 (100%)	73 (100%)	8 (100%)	458 (100%)	652 (100%)

<sup>1</sup>Samples inadequate for HPV testing on physician-taken cervical scrape are not included in the given percentages. <sup>2</sup>Women without an HPV test on physician-taken cervical scrape are not included in the given percentages.

### hrHPV test results of physician-taken cervical scrapes versus self-samples in relation to histological outcome

In total 481 out of 652 (73.8%) women with a hrHPV-positive vaginal self-sample test also had a hrHPV-test performed on their physician-taken cervical scrape at baseline. The mean time interval between the self-sampling test result and that of the physician-taken scrape at baseline was 76 days (median: 58 days). Three smears (0.6%) had invalid hrHPV results, leaving 478 samples (99.4%) with a valid test result. The concordance between the hrHPV test result of physician-taken cervical scrape and vaginal self-sample was 68.8% (329 out of 478 women) (95% CI 64.7% to 73.0%; see Table 2).

The mean time interval between the self-sampling test result and that of the physician-taken scrape at baseline of the 329 women with an hrHPV positive physician sample was 68 days (range 6–332 days) with 28 women (8.5%) having their physician-taken sample  $\geq 4$  months after the self-sampling result. In contrast for the 149 women with a hrHPV-negative physician-taken sample, the mean time interval between the self-sampling test result and the physician-taken scrape at baseline was 92 days (range 16–337 days) with 38 women (25.5%) having their physician-taken scrape taken  $\geq 4$  months ( $p < 0.001$ ). This longer time interval in women with an hrHPV-negative physician-taken scrape suggest that these women have cleared their HPV infection in the time interval between self-sampling and the physician-taken scrape. In women with CIN2+ and CIN3+, the concordance rate in hrHPV-positivity between both samples was very high (CIN2+: 91/97 (93.8%), and CIN3+: 64/67 (95.5%; see Table 2).

The histological outcome in relation to the hrHPV test result on the physician-taken scrapes in women with hrHPV positive self-samples at baseline is shown in Table 2. Of the 8 women with carcinoma, 4 (50.0%) had a hrHPV-test performed on a physician-taken scrape, of which 3 (75.0%) were hrHPV-positive. This hrHPV-negative woman also had normal cytology at baseline and was detected by abnormal cytology after 1 year. Of the women with CIN3, 61 of 63 (96.8%) of the physician-taken scrapes were hrHPV-positive. Of the 30 cervical scrapes of women with CIN2, 27 (90.0%) were hrHPV-positive. A total of 80.6% (50/62) of the physician-taken cervical scrapes of self-sample hrHPV-positive women with  $\leq$  CIN1, were hrHPV-positive as well. The remaining 188 women with hrHPV positive physician-taken scrapes had no histology follow-up data.

### Discussion

In our study, we showed that offering a brush for vaginal self-sampling to non-attendees of the regular screening programme significantly increases the response rate in the cervical screening programme compared to a repeat reminder for a physician-taken scrape. Together with the high 18 month yield of CIN2+ (1.5%; 119 of 7844) and CIN3+ (1.0%; 81 of 7,844) obtained following hrHPV HC2 testing of these

samples, this indicates that vaginal self-sampling using a brush is an attractive approach to increase the effectiveness of the cervical screening program, both in terms of response rate and high-grade lesion yield.

The adherence of the responders with a positive hrHPV test to a cytology triage test was high (89.1%), and that to direct colposcopy referral after abnormal cytology even higher (95.8% at baseline). However, young (age 29–33 years) and older (age  $> 53$  years) women with hrHPV-positive self-sampled material showed a lower adherence to cytology triage compared to women aged 34–53 years. Such an age difference was also observed in previous studies on attendance to screening programs.<sup>1,5,12</sup> We noticed that sending reminders to hrHPV-positive women and their physicians explaining the consequences of the test result increased the adherence to cytology triage at baseline. Janerich *et al.* also showed that the use of patient reminder systems for adherence to follow-up procedures can greatly reduce the number of women with a delayed diagnosis of CIN2+.<sup>12</sup> Conversely, loss to follow-up after a one year repeat testing advice of women without abnormal cytology at baseline was substantial (58.1% of these women complied after 1 year). A similar compliance with 1 year follow-up (*i.e.*, 57.4%) was obtained in the PROHTECT-1 study.<sup>7</sup> Therefore, we expect that the yield of CIN2+/CIN3+ lesions detected in this study is still underestimated. The positive effect on compliance of sending reminders to women and their physicians strongly argue for adding this approach in a recall system of these women.

Amongst all outcome measures analysed in this study only response rate in the self-sampling group and and hrHPV positivity differed slightly with those of PROHTECT-1. The self-sampling response rate was slightly higher (30.5% vs. 27.4%) and hrHPV positivity somewhat lower (8.3% vs. 10.3%) in our study compared to PROHTECT-1. We do not have a good explanation for these findings. However, the total yield of CIN2+ and CIN3+ among participated women did not differ (PROHTECT-1: 99/7,384 (1.3%) and PROHTECT-2: 119/7,844 (1.5%) for CIN2+, and PROHTECT-1: 76/7,384 (1.0%) and PROHTECT-2: 81/7,844 (1.0%) for CIN3+). This indicates that for the detection of high-grade CIN lesions and cervical carcinomas by hrHPV testing both self-sampling devices show good results. However, we noticed that the amount of cells collected by the brush self-sampler device is at least three times lower than obtained by the Delphi cervico-vaginal lavage self-sampler (data not shown). Together with the fact that brush samples primarily contain vaginal cells, this makes brush sampled material less suited for additional molecular tests for disease markers.

The overall concordance between hrHPV-positive physician-taken scrapes and self-samples at baseline was 68.8%. The hrHPV-negative results on the physician-taken scrapes may in part be explained by the presence of vaginal HPV infections detectable by HC2.<sup>7</sup> In addition, 25.5% of women with a hrHPV-negative physician-collected sample visited their physician for a cervical scrape at least 4 months after self-sampling, and might have cleared the hrHPV infection in

between. By comparison, only 8.5% women with a hrHPV-positive physician-obtained sample waited  $\geq 4$  months to visit their physician for a cervical scrape. Importantly, the concordance between hrHPV test results on physician-taken scrapes and self-sampled vaginal material of women with high-grade CIN or cervical cancer, and consequently a persistent HPV infection, was very high ( $>95\%$  for CIN3+).

The strengths of our study are its large size and performance within the setting of the regular screening program. The data confirm those of the earlier study performed with a lavage self-sampler (PROHTECT-1) conducted in the same region.<sup>7</sup> In both studies, the response rate was  $\sim 30\%$ , indicating that a similar proportion of non-attendees can be reached by offering hrHPV testing on self-sampled material collected by both devices. Even more important is that in both studies similar yields of high-grade CIN and cervical cancer were obtained.

The poor adherence to follow-up testing after 1 year is a weakness of this study (58.1%), especially when compared to the high compliance rate at baseline (89.1%). As pointed out before, this has a likely negative effect on CIN2+/CIN3+ yields. Striking was that even women with abnormal cytology after 1 year showed a relatively poor adherence to referral for colposcopy (60.0% vs. 95.8% at baseline). Although we do not have an explanation for this finding, we had the impression that better education of the women as well as physicians about the possible screening results might help to improve the compliance to visit the physician after 1 year.

Many studies, often performed on small numbers of women and with different self-sampling devices and hrHPV detection techniques, have compared self- versus physician-sampling, though mostly at the level of HPV test performance rather than the yield of CIN2+/CIN3+.<sup>13-16</sup> Generally, a high level of concordance between the HPV test results on self- versus physician-taken samples was obtained.<sup>13,14</sup> As we have shown earlier offering HPV testing on self-sampled cervical material should not only be evaluated at the level of HPV test performance but also the level of CIN2+/CIN3+ yield.<sup>16</sup> In fact, the whole chain of self-sampling, HPV testing with a clinically validated test,<sup>17</sup> follow-up of HPV-positive women and the CIN2+/CIN3+ yield of the referred women

should be evaluated before self-sampling can be introduced in routine screening. Non-attendees of the regular screening program form an ideal group of women to test the performance of HPV self-sampling, not only because the prevalence of high-grade cervical lesions is higher than in women who participate in regular screening program<sup>2</sup> but also because there is no other effective alternative for non-attendees.

Together, the results of both PROHTECT-1 and PROHTECT-2 are so encouraging that efforts are warranted to study offering self-sampling as a more pleasant alternative for a physician-taken smear to women (age 30–60 years) invited for regular screening programs.

In summary, offering hrHPV testing on self-sampled vaginal material with a brush device to non-attendees of the regular screening program significantly increases the attendance to the regular screening program, results in HPV test results that are in good concordance with those on physician-taken scrapes in women with CIN2+/CIN3+ and is effective in detecting CIN2+/CIN3+.

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