Improving cervical cancer screening by HPV self-sampling

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CHAPTER 4

Comparative performance of novel self-sampling methods in detecting high-risk human papillomavirus in 30,130 women not attending cervical screening: a randomised controlled trial

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ABSTRACT

We determined whether the participation rate for a brush-based cervicovaginal self-sampling device is non-inferior to the participation rate for a lavage-based one for testing for hrHPV (high-risk human papillomavirus). Additionally, positivity rates for hrHPV, the detection rates for cervical intraepithelial neoplasia grades 2 and 3 or worse (CIN2+/3+), and user comfort were compared. A total of 35,477 non-responders of the regular cervical screening programme aged 33-63 years were invited to participate. Eligible women (n=30,130) were randomly assigned to receive either a brush-based or a lavage-based device, and a questionnaire for reporting user convenience. Self-sampling responders testing hrHPV-positive were invited for a physician-taken sample for cytology; triage-positive women were referred for colposcopy. A total of 5218 women participated in the brush-based sampling group (34.6**%**) and 4809 women in the lavage-based group (31.9%), i.e. an absolute difference of 2.7% (95%CI 1.8–4.2). The hrHPV-positivity rates in the two groups were identical (8.3%, relative risk (RR) 0.99, 95%CI 0.87–1.13). The detection of CIN2+ and CIN3+ in the brush group (2.0% for CIN2+; 1.3% for CIN3+) was similar to that in the lavage group (1.9% for CIN2+; 1.0% for CIN3+) with a cumulative RR of 1.01, 95%CI 0.83–1.24 for CIN2+ and 1.25, 95%CI 0.92–1.70 for CIN3+. The two self-sampling devices performed similarly in user comfort. In conclusion, offering a brush-based device to non-responders is non-inferior to offering a lavage-based device in terms of participation. The two self-sampling methods are equally effective in detecting hrHPV, CIN2+/CIN3+ and are both well accepted.

INTRODUCTION

The introduction of organised programmes for cervical cancer screening in developed countries has contributed to a significant decrease in the incidence and mortality rate of cervical cancer.[1-5] A major issue concerning the effectiveness of the screening programmes is that many women do not attend the cervical screening (i.e. non-responders). Non-participation women are at increased risk of developing cervical cancer; therefore, it is important to reach these women.[5-8] Offering self-sampling for testing for high-risk human papillomavirus (hrHPV) is a suitable screening method for previously unscreened or under-screened women. Large-scale cohort studies have demonstrated that about one-third of a non-responder population will participate in the screening programme when offered a self-sampling device.[9-17] This improves population coverage and might further reduce both the incidence and mortality rate of cervical cancer.[18,19] Recent studies have shown a high concordance of hrHPV test results between most vaginal self-samples and physician-taken cervical scrapes. Even more, vaginal self-samples and physician-taken samples show similar test accuracy in detecting cervical intraepithelial neoplasia grade 2 or worse (CIN2+), provided that the test and the self-sampling device have been validated both individually and in a combined method.[20-22]

Although various self-sampling devices have been investigated in research settings, no large-scale population-based studies have compared different self-sampling devices in a randomised setting and considered the participation rates, prevalence of hrHPV, and detection of CIN. Most prospective studies have compared a self-sampling approach with a re-invitation to the regular screening programme.[9,10,12-14,16] Before self-sampling devices are used in population-based screening,[23] it is important to explore their acceptability and user-friendliness. No large population-based studies have evaluated this aspect yet.[24-28]

Here, we present the results of a randomised controlled trial that took place within the setting of a national screening programme in the Netherlands. We have compared the performance of brush-based and lavage-based self-sampling devices (in the PROHTECT-3B trial). The primary outcome was the participation rate, and the secondary outcomes were detection of hrHPV and the yields of CIN2+ and CIN3+. We used a questionnaire to investigate the acceptability and user-friendliness of both self-sampling devices and the participants' preference for either self-sampling or a physician-taken smear for cytological testing in the next screening round. The results of this study can be used in developing future screening programmes in which self-sampling may play an important role.

METHODS

Study population

Cervical screening in the Netherlands is organised in a nationwide programme in which the screening organisations invite women aged 30–60 years for a cervical smear at 5-year intervals. Women who do not attend regular screening are registered as 'non-responders' in the databases of the screening organisations. In the current study, 35,477 who did not respond in 2008 and who were living in regions of North Holland, Flevoland, Utrecht, and Gelderland were invited to participate in the PROHTECT-3B (Protection by Offering HPV Testing on self-sampled Cervicovaginal specimens Trial-3B) study between October 2011 and February 2012. All the eligible women received a pre-invitation letter and could 'opt out' of this trial. The exclusion criteria were previous hysterectomy, being followed up by a gynaecologist because of a previous abnormal cytological test result less than 2 years before inclusion, and a current pregnancy. Those wishing to opt out could do so by returning a form by regular post, sending an e-mail, calling a service desk, or opting out via the study website (http://www.thuistesthpv.nl). Figure 1 shows the study design.

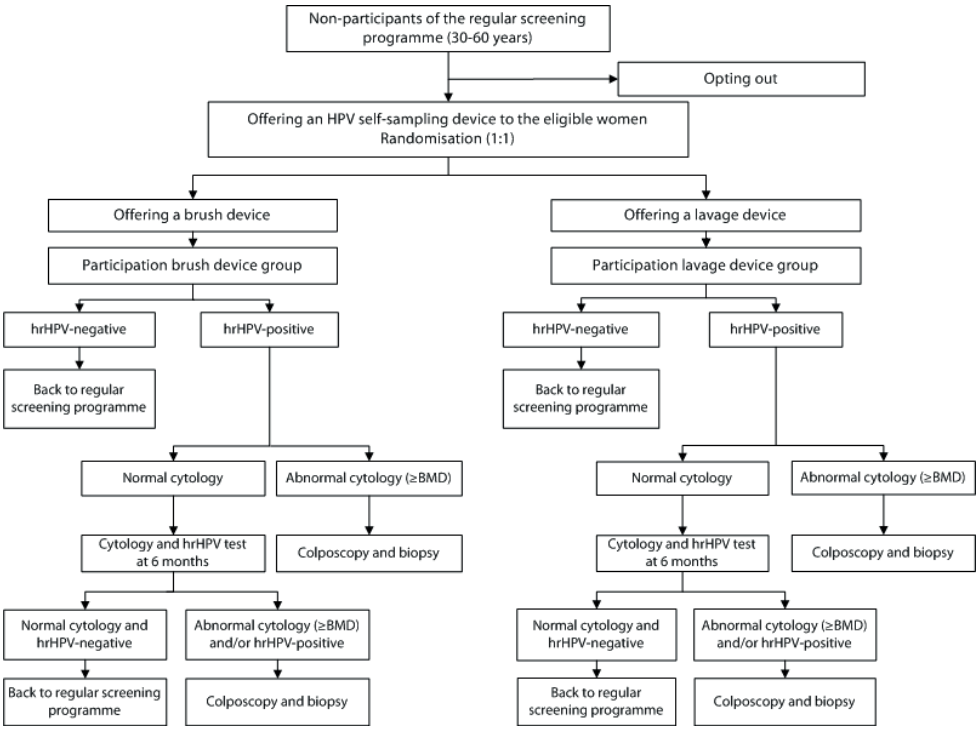


Figure 1. Study design

BMD = borderline or mildly dyskaryotic; hrHPV = high-risk human papillomavirus

Randomisation

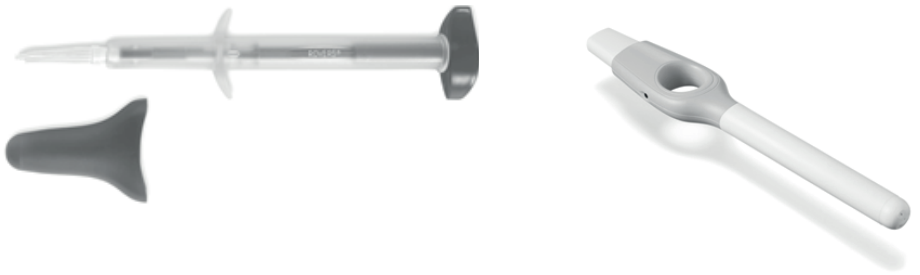
We randomised the invited women who did not opt out within 3 weeks in a 1:1 ratio. We used a computer number generator to determine who would receive a self-sampling brush device (Evalyn Brush, Rovers Medical Devices, Oss, The Netherlands, Figure 2a) and who would receive a self-sampling lavage device (second generation Delphi Screener, Delphi Bioscience, Scherpenzeel, The Netherlands, Figure 2b).

The randomisation was stratified for age (seven age cohorts) and degree of urbanisation. Population areas with fewer than 100,000 inhabitants were considered low-level urban areas and those with more than 100,000 inhabitants, high-level urban areas. Researchers and health professionals were blinded to the randomisation, which was performed by an independent statistician. All patient data were entered and managed within a password-protected web-based database approved for 'good clinical practice' (Infermed MACRO, London, UK). The national ethics committee (Ministry of Public Health No 2010/04WBO) approved this study, the trial was registered in the trial register (Trialregister.nl, NTR3350) and all participants gave written informed consent.

Self-sampling procedures

The self-sampling kit consisted of either a brush device or a lavage device. The brush device (Figure 2a) is about 20 cm long and consists of a transparent case with wings that control the depth of insertion into the vagina. After the device has been inserted up to its wings, pushing the plunger toward the casing will push the brush out into the vagina. The brush needs to be rotated five times; at each rotation there is an audible click. After rotation, the brush can be pulled back into the case and removed from the vagina. A cap is to be clicked onto the case and the brush can be directly sent by regular mail. The lavage device (Figure 2b) is 22.5 cm long and is pre-filled with 3 ml of sterile saline. After the participant removes the seal covering the top, she inserts the device into the vagina until she feels resistance. Pressing the white button at the back releases the sterile saline, which will spread around the cervical area. When the button is released, the fluid from the cervix and vagina will flow back into the device. The device is then removed from the vagina and the fluid is transferred to a test tube for sending by regular mail.

The self-sampling kit also included an explanatory letter, an informed consent form, user instructions (written and drawn), a questionnaire, and a return envelope with the address of the laboratory. The women were asked to return the used brush device or the lavage device's collection vial, the signed informed consent form, and the questionnaire. For both groups, the self-sampling kits contained similar content except for the type of device and the accompanying user instructions.



a. Brush-based device (Evalyn Brush) b. Lavage-based device (Delphi Screener)

Figure 2. Self-sampling devices

Testing of the self-sampled material

Upon arrival of the dry brush devices at the laboratory, they were resuspended in 1.5 ml of Preservcyt medium (Hologic, Marlborough, Mass.). The vials were vortexed for 3 x 15 s, stored overnight at 4°C, and again vortexed for 2 x 15 s. The vials were visually inspected and scored on a small pellet or no pellet. When the lavage-based specimens arrived at the laboratory, they were visually inspected the same way as the brush specimens were. The lavage specimens were centrifuged to concentrate the cell material. Then the supernatant was removed before the pellet was resuspended in 1.5 ml of Preservcyt medium.

The brush and lavage specimens were tested for hrHPV by means of the clinically validated hrHPV GP 5+/6+ PCR (EIA HPV GP HR kit, Diassay, Voorburg, The Netherlands) according to the manufacturer's instructions. A PCR for the β-globin gene was used to check for the presence of amplifiable DNA in all samples with a small or no pellet on arrival at the lab and in an additional random 5% of the samples.[29] Samples were considered invalid if the β-globin PCR test was negative. In such cases, the participants were requested to send a new sample. If no new sample was received, their results were classified as inadequate for evaluation. All participants were notified of the test result. Those who tested negative for hrHPV were advised to participate in the next regular screening round, and women who tested positive were advised to have an additional cervical cytological smear taken by the general practitioner for colposcopy triage.

Follow-up algorithm

All liquid-based cytological specimens were taken with the Cervex brush (Rovers Medical Devices, Oss, The Netherlands), stored in PreservCyt and sent to the Department of Pathology at the Radboud University Medical Center. The monolayer slides were then prepared and classified according to the Dutch CISOE-A classification system, which can easily be translated into the Bethesda nomenclature.[30] The cytological test results were grouped as normal, borderline or mildly dyskaryotic (BMD; corresponding to Bethesda ASCUS/LSIL), or moderately dyskaryotic or worse (>BMD; corresponding to Bethesda ASC-H/HSIL or worse).

Participants with abnormal cytological test results (threshold BMD) were referred to a gynaecologist for colposcopy, and participants with cytologically normal smears were invited for cytology testing and hrHPV retesting after 6 months. If either of these tests was abnormal (threshold BMD and/or hrHPV-positive), the participants were referred to a gynaecologist for colposcopy. Those with both a normal cytological test result and a hrHPV-negative result at 6 months were advised to participate in the next regular screening round. Three months later, a reminder letter was sent to all women and their general practitioners who did not comply with the follow-up protocol.

At colposcopy, cervical lesions were biopsied and/or treated according to the standard procedure in the Netherlands.[31,32] If no abnormalities were seen at colposcopy, the gynaecologist was advised to take two random biopsies. Firstly, we decided for two biopsies in this subgroup to ensure the same study end point for all participants and secondly, because of the fact that colposcopy has only a 60-80% positive predictive value. That means, 20-40% of all cases are false negative. In addition, the positive predictive rate of the colposcopic impression in cases with minor grades of CIN is lower than in cases with severe dysplasia.[33] Histopathological analysis took place according to the current guidelines. The results were recorded in the nationwide network and registry of histopathology and cytopathology in the Netherlands (PALGA, Utrecht, The Netherlands).

Questionnaire

The self-sampling kit contained a questionnaire about the self-sample device. The participants were asked about the overall convenience, user comfort, and their perceptions during self-sampling about shame, feeling at ease, usability, stress, comfort, pain, and trust in completing the test correctly. They were asked which test – self-sampled or physician-sampled – they would prefer for the next screening round. The participants sent the questionnaires and their self-samples to the laboratory. All questionnaires were collected and analysed centrally. Cardiff Teleform Software (version 10.1, 2010; Cambridge, UK) was used to design the questionnaire and to record the data.

Outcome measures

The primary outcome measure of PROHTECT-3B was the participation rate, i.e. the percentage of randomised women who returned a self-sample. All women who submitted self-samples between October 2011 and December 2012 were counted as self-sampling responders. The secondary outcome measures were hrHPV positivity, the number of histologically confirmed CIN2+ and CIN3+ lesions, and user comfort as the questionnaire reported.

The participants reached a study end point if (1) their self-samples tested HPV-negative, (2) they were hrHPV-negative and had normal cytology at the 6-month follow-up, or (3) if there was a positive or negative histopathological result. Women with a negative colposcopy without biopsy did not meet the study end point and this was considered as incomplete follow-up. The most severe diagnosis was registered when more histological diagnoses were available in the follow-up period. All cytological and histological findings recorded before June 2013 were included in our analysis. At this point, the database was complete after a mean follow-up of 15 months for

both devices (range 6–18 months). Follow-up outcomes of all participants were retrieved from the nationwide network and registry of histology and cytology database (PALGA) was used, and if necessary, obtained from general practitioner or gynaecologists.

Statistical analysis

The primary objective of the study was to compare the participation rates of women who received the brush device and those who received the lavage device. A difference in participation of at most 1.4% was defined as the non-inferiority margin: if the lower limit of the 95% CI for the difference (brush group vs. lavage group) was above -1.4%, the participation rate of the brush group could be considered non-inferior to the participation rate of the lavage group. We selected the non-inferiority margin on the basis of clinically important differences, costs, and feasibility of the national cervical cancer screening. To achieve a power of 80% while assuming a participation rate of 27%,[9, 34] we had to invite at least 16,500 women for each arm.

We analysed the data with SPSS (Statistical Package for the Social Sciences, version 20.0.1 for Windows). Wald confidence intervals for proportions were presented. The association between the self-sampling group and categorical outcomes was tested with the Pearson chi-square test, except for the association between age and the proportion of hrHPV-positive women, which was tested with the chi-square test for linear trend. We used risk ratios (RRs) to compare detection ratios. We fitted them by means of a log-binomial model, and adjusted for age group and degree of urbanisation. Differences with a two-sided $p<0.05$ were considered significant. The CONSORT (CONsolidated Standards Of Reporting Trials) Statement on the reporting of non-inferiority trials was followed.[35]

RESULTS

Patient characteristics and participation rate

A total of 35,477 non-responding women were approached to participate in the current study; 5347 (15.1%) opted out. A total of 3149 out of 5347 women who opted out met the inclusion criteria, but decided not to participate. The remaining 30,130 women were randomly assigned to receive either the self-sampling brush device or the self-sampling lavage device in a ratio of 1:1. A total of 5218 women (34.6%, 95% CI 33.9–35.4) in the brush group (mean age 44.5 years, range 33–63 years) and 4809 women (31.9%, 95% CI 31.2–32.7) in the lavage group (mean age 44.8 years, range 33–63 years) returned self-sampled material and a signed informed consent form (absolute difference 2.7%, 95% CI 1.8–4.2). As a result, the participation rate in the brush group was non-inferior to, and higher than the participation in the lavage group. The self-sampling participation rates in the different age strata ranged from 31.3% to 37.8% in the brush group and from 30.1% to 34.7% in the lavage group (Table 1). The overall participation rate of all non-responders who met the inclusion criteria in this study was 30.1% (10,027 participants out of 33,279 women who met the inclusion criteria). Figure 3 shows the trial flowchart.

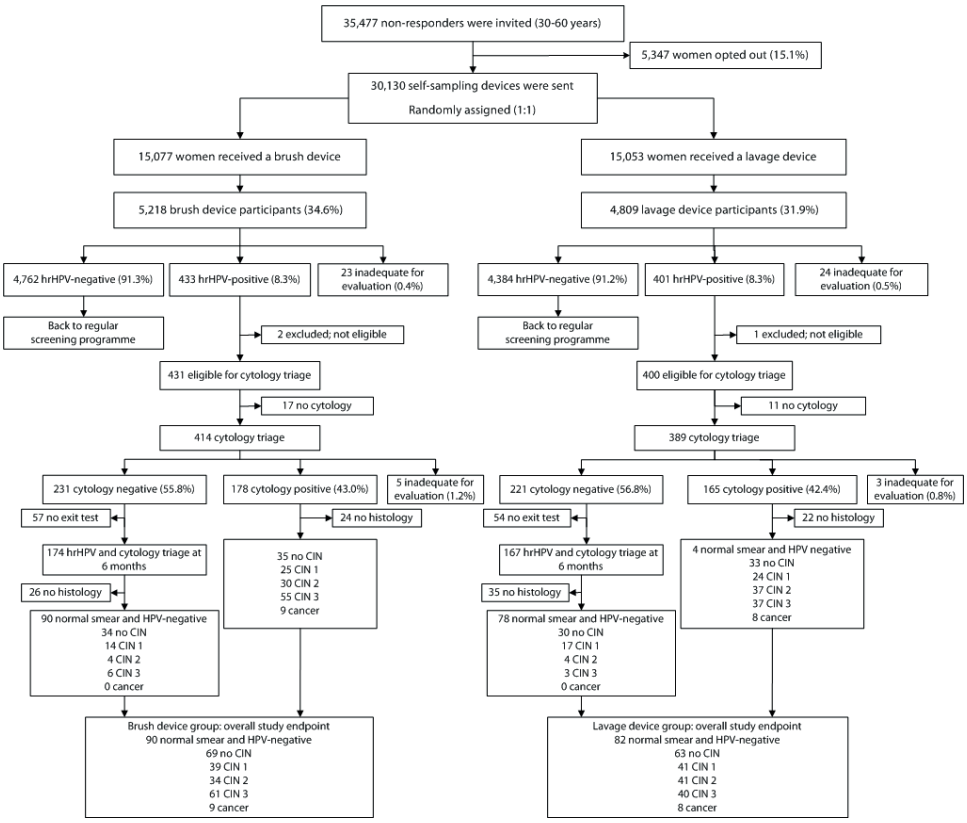


Figure 3. Trial profile
hrHPV = high-risk human papillomavirus; CIN = cervical intraepithelial neoplasia

HPV test positivity rate

While 433 (8.3%) brush samples were hrHPV-positive, 4762 (91.3%) were hrHPV-negative and 23 (0.4%) were inadequate for evaluation. Of the lavage samples, 401 (8.3%) were hrHPV-positive, 4384 (91.2%) were hrHPV-negative, and 24 (0.5%) were inadequate for evaluation (Figure 3). The hrHPV positivity rate did not differ between the brush group and the lavage group; RR 0.99 (95% CI 0.87–1.13). The proportion of hrHPV-positive women decreased with age from 12.2% at age 33 years to 5.3% at age 63 years (Pearson chi-square for linear trend = 79.81; $p<0.01$) and was similar for the two devices (Table 1).

Table 1. Participation rate, prevalence of hrHPV and cumulative CIN2+, CIN3+, and carcinoma yield, categorised by age and device type

Age in years	Type of device	Total randomised	Total participants	Participation rate (in %)	HPV-positive (%)	CIN2+ (%)	CIN3+ (%)	Cancer (%)
33	Evalyn Brush	3260	1036	31.8	133 (12.8)	36 (3.5)	24 (2.3)	3 (0.3)
	Delphi Screener	3266	986	30.2	114 (11.6)	30 (3.0)	19 (1.9)	2 (0.2)
38	Evalyn Brush	3354	1209	36	122 (10.1)	38 (3.1)	26 (2.2)	2 (0.2)
	Delphi Screener	3344	1035	31	106 (10.2)	29 (2.8)	15 (1.4)	2 (0.2)
43	Evalyn Brush	2246	848	37.8	65 (7.7)	15 (1.8)	10 (1.2)	3 (0.4)
	Delphi Screener	2236	738	33	61 (8.3)	15 (2.0)	9 (1.2)	3 (0.4)
48	Evalyn Brush	1917	674	35.2	41 (6.1)	6 (0.9)	2 (0.3)	-
	Delphi Screener	1915	628	32.8	38 (6.1)	8 (1.3)	3 (0.5)	1 (0.2)
53	Evalyn Brush	1610	587	36.5	26 (4.4)	8 (1.4)	7 (1.2)	1 (0.2)
	Delphi Screener	1622	563	34.7	33 (5.9)	4 (0.7)	-	-
58	Evalyn Brush	1429	469	32.8	29 (6.2)	1 (0.2)	1 (0.2)	-
	Delphi Screener	1396	475	34	25 (5.3)	3 (0.6)	2 (0.4)	-
63	Evalyn Brush	1361	395	31.3	17 (4.3)	-	-	-
	Delphi Screener	1274	384	30.1	24 (6.3)	-	-	-
Total of Evalyn Brushes		15077	5218	34.6	433 (8.3)	104 (2.0)	70 (1.3)	9 (0.2)
Total of Delphi Screeners		15053	4809	31.9	401 (8.3)	89 (1.9)	48 (1.0)	8 (0.2)
Grand total		30130	10027	33.3	834 (8.4)	193 (1.9)	118 (1.2)	17 (0.2)

hrHPV = high-risk human papillomavirus; CIN = cervical intraepithelial neoplasia

Cytology triage testing

No cervical sample was taken for cytological testing from 17 of the 431 eligible women (3.9%) who were hrHPV-positive in the brush group. Of the remaining 414, 178 women (43.0%) had abnormal smears; 231 (55.8%), a normal smear; and 5 (1.2%), an invalid result. No cervical sample was taken for cytological testing from 11 of 400 eligible women (2.8%) who were hrHPV-positive in the lavage group. Of the remaining 389 women, 165 (42.4%) had abnormal smears; 221 (56.8%), normal smears; and 3 (0.8%), invalid results. The overall compliance for cytological triage after a hrHPV-positive result was 97% (803 of 831); 96% in the brush group and 97% in the lavage group (Figure 3).

CIN2+ and CIN3+ detection rates

Baseline

Of the 178 women in the brush group with abnormal results in the cytological triage test, 94 (52.8%) were diagnosed with CIN2+, of whom 55 with CIN3, 6 with squamous cell carcinoma, and 3 with adenocarcinoma (64 CIN3+), while 60 women (33.7%) had CIN1 or less (35 no CIN and 25 CIN1). Of the 165 women in the lavage group with abnormal cytology triage results, 82 (49.7%) were diagnosed with CIN2+, of whom 37 with CIN3, 7 with squamous cell carcinoma, and 1 with adenocarcinoma (45 CIN3+); further, 57 women (34.5%) had CIN1 or less (33 no CIN and 24 CIN1; Figure 3).

Follow-up

Follow-up of 231 women with hrHPV-positive results and normal cytology at baseline in the brush group yielded 10 women (4.3%) with CIN2+ (4 CIN2, 6 CIN3), 14 (6.1%) with CIN1, 34 (14.7%) with no CIN, 90 (39.0%) with normal cytology and hrHPV-negative results, and 83 (35.9%) did not return for follow-up testing or had no histopathological end point. Follow-up of 221 women with hrHPV-positive results and normal cytology at baseline in the lavage group yielded 7 women (3.2%) with CIN2+ (4 CIN2 and 3 CIN3), 17 (7.7%) with CIN1, 30 (13.6%) with no CIN, 78 (35.3%) who had normal cytology and were hrHPV-negative, and 89 (40.3%) did not return for follow-up or had histopathological end point (Figure 3).

Overall, 17 cancers (0.2%), 118 (1.2%) CIN3+, and 193 (1.9%) CIN2+ were detected in this study. In the brush group, 104 CIN2+ (2.0%, 95% CI 1.7–2.4), of which 70 CIN3+ (1.3%, 95% CI 1.1–1.7) were detected. In the lavage group, 89 CIN2+ (1.9%, 95% CI 1.5–2.3), of which 48 CIN3+ (1.0%, 95% CI 0.8 – 1.3) were detected. The detection of CIN2+ and CIN3+ in the brush group was similar to that in the lavage group. The RR of the cumulative CIN2+ and CIN3+ detection in the brush group compared to the lavage group was 1.01 (95% CI 0.83–1.24; p=0.87) and 1.25 (95% CI 0.92–1.70; p=0.16), respectively (Table 1 and Figure 3).

Questionnaires

A total of 9484 questionnaires (94.6%) were returned for analysis; 4855 (93.0%) in the brush group and 4629 (96.3%) in the lavage group. Overall, 9302 women rated their self-sample device; 97%

(4619 of 4769) of the brush users and 98% (4445 of 4533) of the lavage users rated their devices as good to very good (p>0.05). The participants felt no shame at all in most cases; 93% (3764 of 4060) in the brush group and 87% (3465 of 3994) in the lavage group. Feeling at ease and usability were rated as moderate to very good in 75% of the brush group and 84% of the lavage group. More than 70% of the women experienced no discomfort, and more than 80% experienced no pain during the use of the self-sampling device. In each group, 20% of the participants were concerned about taking the self-sample correctly. No notable differences were observed between the groups in any of the categories (Table 2).

Overall, most women (80.5%, 7533 of 9360) preferred the self-sampling over a physician-taken smear for a next screening round, while 13% (1216 of 9360) had no preference. No statistically significant differences were found between the brush group and the lavage group.

DISCUSSION

This study shows that the participation rate in the brush based self-sampling device group was higher than in the lavage based group. While 34.6% of the non-responders to invitations to organised cervical screening participated when offered a vaginal self-sampling brush device, 31.9% participated when offered a lavage device. The prevalences of hrHPV and the cumulative CIN2+ and CIN3+ yields were similar in the two groups, and the devices provided equally well-accepted self-sampling methods.

The participation rates associated with the devices in this study are similar to those reported in most other European studies of self-sampling for hrHPV testing among non-responders to organised cervical screening.[9-12,36] One of the devices used in this study was a new brush-based self-sampling device (the Evalyn Brush), and was specifically developed for cervicovaginal self-sampling. Our study used it in a screening population for the first time. Two previous, large, self-sample studies with a brush device developed for vaginal, ectocervical and endocervical sampling (VibaBrush) have shown participation rates between 30.8% and 34.2%.[10,36] Studies with lavage devices (Delphi Screener) have shown participation rates between 27.5% and 31.5%.[9,11,12] Population-based studies in other European countries and with other self-sampling device types have reported participation rates in a wider range (8.7–39.1%).[13-17,21] In general, and across studies, HPV self-sampling results in better participation than a recall for regular cytological testing.[9-12,36]

Increasing the participation rate is considered the simplest and best way to improve the effectiveness of organised screening programmes.[19] We have shown that self-sampling can reach those who do not respond to invitations to a regular screening programme.[37] Our study also shows that self-sampling improves participation rates in all age categories to almost the same extent. The participation rates varied marginally with age in the brush group (31.3%–37.8%) and the lavage group (30.1%–34.7%). In contrast, participation rates in the current regular Dutch screening programme depend greatly on age: participation in the category of 50–55 years is about 20% greater than in the category of 30–35 years.[38]

Table 2. Questionnaire results from users of the brush and lavage devices

User comfort of the self-sample devices		Not at all n (%)*	A little n (%)*	Moderate n (%)*	Very much n (%)*	Total	Missing
a) Shame	Evalyn Brush	3764 (92.7)	253 (6.2)	23 (0.6)	20 (0.5)	4060	795
	Delphi Screener	3465 (86.8)	275 (6.9)	28 (0.7)	26 (0.7)	3794	835
b) Feeling at ease	Evalyn Brush	441 (10.4)	582 (13.8)	1264 (29.9)	1942 (45.9)	4229	626
	Delphi Screener	400 (10.0)	590 (14.8)	1301 (32.6)	1703 (42.6)	3994	635
c) Usability	Evalyn Brush	361 (8.4)	318 (7.4)	1310 (30.6)	2299 (53.6)	4288	567
	Delphi Screener	372 (9.3)	308 (7.7)	1309 (32.6)	2029 (50.5)	4018	611
d) Stress	Evalyn Brush	1902 (46.6)	1518 (37.2)	507 (12.4)	157 (3.8)	4084	771
	Delphi Screener	1649 (42.8)	1508 (39.1)	540 (14.0)	159 (4.1)	3856	773
e) Discomfort	Evalyn Brush	2990 (73.5)	865 (21.3)	135 (3.3)	80 (2.0)	4070	785
	Delphi Screener	2855 (74.6)	806 (21.1)	108 (2.8)	56 (1.5)	3825	804
f) Pain	Evalyn Brush	3326 (81.7)	586 (14.4)	102 (2.5)	56 (1.4)	4070	785
	Delphi Screener	3306 (87.3)	418 (11.0)	40 (1.1)	25 (0.7)	3789	840
g) Trust in completing the test correctly	Evalyn Brush	301 (7.0)	573 (13.4)	1651 (38.5)	1761 (41.1)	4286	569
	Delphi Screener	271 (6.7)	586 (14.5)	1653 (40.9)	1528 (37.8)	4038	591

* Percentages are based on the number of completed responses to the sub-question.

The compliance to triage after an hrHPV-positive test is high in the current study (97%). This is higher than in previous PROHTECT-1 and 2 studies with a comparable triage strategy (89-90%).[9,10] Also the compliance to follow-up after a negative triage test is higher in the current study (75%), than in PROHTECT 1 and 2 (57-58%). There are some minor differences in study methodology, which may explain these different compliance rates. Firstly, in the above-mentioned studies

reminders were sent only to the participants. In our study, to motivate women to comply with the study, we sent reminders to both the participating women and their physicians. This is in line with the recently published PROHTECT-3 study.[39] In this study, also a high triage follow-up rate was achieved; 99% of the hrHPV-positive women had a cytological triage test taken. Secondly, in our study protocol we used a 6 months follow-up algorithm instead of a 1-year follow-up algorithm in PROHTECT 1 and 2. The rationale of these 6 months is that with a period of 1 year, the sense of urgency might decrease and more women might be lost to follow up. This time period of 6 months is also determined in the new protocol for primary HPV screening, which will be introduced in the Netherlands in 2016.[23]

A self-sampling device must be user friendly if we wish to benefit optimally from self-sampling and achieve high participation rates. Only small studies have yet explored the acceptability of different self-sampling devices, and the outcomes are conflicting.[26,27] We explored the acceptability of two self-sampling methods for HPV detection that were both designed with consideration of qualitative feedback from women who used the methods. In our population-based study, the participants found both devices very acceptable. The overall rating was good to very good in more than 95% of the cases. However, in each group, 20% of the women were concerned about doing the self-sampling properly. This is in line with findings of previous studies.[28,40-45] This issue will need to be addressed if self-sampling is to be integrated with the primary invitation to cervical cancer screening.

This is the first study that directly compares the accuracy of different devices in a population-based setting of non-responders in a randomised controlled manner. Because different devices could have diverse effects with respect to attendance and disease detection, direct comparative studies are important. Our study compares a brush device and a lavage device, with similar results. The cumulative incidence of CIN2+ was 2.0% (95% CI 1.7–2.4) in the brush group, and 1.9% (95% CI 1.5–2.3) in the lavage group.

The CIN2+ yield was slightly greater than in previous PROHTECT studies with a comparable triage strategy (PROHTECT-1: 99 of 7384 (1.3%) and PROHTECT-2: 119 of 7844 (1.5%) for CIN2+)[9,10] and much greater than in the regular cytological screening programme (0.9%).[34] This likely reflects the increased risk of non-responders because they form an under-screened population. Their high CIN2+ yield also parallels a relatively high HPV-positivity rate in the non-responder population (8.3%). This is almost twice that of the normal Dutch screening population (4%–5%).[46,47] Moreover, cytological testing is the primary screening tool in the Dutch screening programme, and cytological testing is considerably less sensitive in detecting CIN2+ than HPV testing.[48] However, Gök et al.'s study showed similarly increased RRs for CIN2+ after their analysis was restricted to women with abnormal cytological test results at baseline.[34] Therefore, the increased RR of self-sampling responders cannot be solely attributed to a more sensitive screening test.

The main strengths of our study are its large size and its setting in the regular screening programme in the Netherlands. Therefore, the outcome provides a reliable and representative image of self-

sampling among non-responders to organised screening. Another strength is the use of an opt-out approach in the study invitation. This approach modestly reduced the waste and costs of unused devices compared to those of other studies.[9,10] Furthermore, a better selection of the eligible non-responders was possible because ineligible women could opt out.

In the Dutch colposcopy standards, it is defined that if the colposcopy is satisfactory and normal, no biopsies are advised, but follow-up is needed. In our study, colposcopies were performed by a large number of gynaecologists in different clinics, therefore, we advised to take two biopsies if no abnormalities were seen at colposcopy. This ensured a histological study end point in all participants and could equalize differences in quality of colposcopic assessment between clinics as much as possible.

A limitation of our study is the number of women (38.1%, 172 of 452) without a study end point, i.e. they had no exit test or no histopathological result after they tested positive for hrHPV and received normal baseline cytological test results. The overall CIN2+ and CIN3+ yield detected in this study may be an underestimation because histological abnormalities may also occur among women lost to follow-up, and they remain undetected in this study. The relatively low follow-up rates are possibly due to relief because of a normal cytological test result at baseline after a positive hrHPV test, or perhaps these rates can be explained by the presumption that these former non-responders may be more prone to abandon follow-up. It is vitally important that the follow-up be acceptable to the participants, especially reluctant ones. More efficient triage techniques could reduce this loss to follow-up. Promising triage strategies performed directly on the self-sampled specimens have already been described; they include DNA methylation and HPV genotyping.[49-52] The delay or loss to follow-up of women who need to have one or more cervical smears taken by their general practitioner may be circumvented with a direct triage test on self-collected cervicovaginal material.

In conclusion, offering a brush-based device to non-responders of the cervical screening programme is non-inferior to offering a lavage-based device in terms of participation. In clinical performance, the two self-sampling methods are equally effective in detecting hrHPV, CIN2+, and CIN3+, and they are equally well accepted. On the basis of these results, self-sampling can now be used in the development of future HPV-based screening programmes. Given the outcome of our study, aspects other than participation, clinical performance, and user friendliness may be important in choosing the device to be used in a future screening programme. For example, whether new and more efficient triage strategies are equally applicable to these devices is being investigated. Such an aspect, or the costs of the device, may prove to be the deciding factor in choosing which device is to be used in a screening programme.

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