High-risk human papillomavirus detection in self-sampling compared to physician-taken smear in a responder population of the Dutch cervical screening: Results of the VERA study

P.J.W. Ketelaars [a,*], R.P. Bosgraaf [a], A.G. Siebers [c], L.F.A.G. Massuger [a], J.C. van der Linden [d], C.A.P. Wauters [f], J.C. Rahamat-Langendoen [b], A.J.C. van den Brule [e], J. IntHout [g], W.J.G. Melchers [b], R.L.M. Bekkers [a]

[a] Department of Obstetrics and Gynaecology, Radboud University Medical Center, Nijmegen, The Netherlands
[b] Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands
[c] Department of Pathology, Radboud University Medical Center, Nijmegen, The Netherlands
[d] Department of Pathology, Jeroen Bosch Hospital, 's-Hertogenbosch, The Netherlands
[e] Department of Molecular Diagnostics, Jeroen Bosch Hospital, 's-Hertogenbosch, The Netherlands
[f] Department of Pathology, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands
[g] Department for Health Evidence, Radboud Institute of Health Sciences, The Netherlands

ARTICLE INFO

ABSTRACT

In 2017 the cervical cancer screening program in The Netherlands will be revised. Cervical smears will primarily be tested for the presence of high-risk human papillomavirus (hrHPV) instead of cytology, and vaginal self-sampling will be offered to non-responders. This includes a potential risk that part of the women who would otherwise opt for a cervical smear will wait for self-sampling. However, self-sampling for hrHPV in a responder population has never been studied yet. The aim of this study was to investigate the applicability and accuracy of self-sampling in detecting hrHPV in a screening responder population. A total of 2049 women, aged 30–60 years, participating in the screening program in The Netherlands were included from April 2013 to May 2015. After they had their cervical smear taken, women self-collected a cervicovaginal sample with a brush-based device, the Evalyn Brush. Both the cervical smear and self-sample specimen were tested with the COBAS 4800 HPV platform. The hrHPV prevalence was 8.0% (95% CI 6.9–9.2) among the physician-taken samples, and 10.0% (95% CI 8.7–11.3) among the self-samples. There was 96.8% (95% CI 96.0–97.5) concordance of hrHPV prevalence between self-samples and physician-taken samples. Women in our study evaluated self-sampling as convenient (97.1%), user-friendly (98.5%), and 62.8% preferred self-sampling over a physician-taken sampling for the next screening round. In conclusion, self-sampling showed high concordance with physician-taken sampling for hrHPV detection in a responder screening population and highly acceptable to women. Implementation of HPV-self-sampling for the responder population as a primary screening tool may be considered.

1. Introduction

Cervical cancer is the fourth most common cancer in women worldwide, with an estimated 530,000 new cases in 2012 (Globocan, n.d.), and approximately 270,000 deaths annually. Nationwide, cytology based cervical cancer screening programs have proven to be effective in reducing both the incidence and mortality of cervical cancer (Arbyn et al., 2009). Cervical cancer screening was introduced in The Netherlands in 1988, and revised in 1996 in a nationwide cytology based 5-yearly screening program for women aged 30–60 years. Despite the significant decrease in incidence of cervical carcinoma. However, cytology based screening has resulted in many false negative results with a sensitivity of only 30–87% (Nanda et al., 2000).

HPV DNA testing can be performed on physician-taken material, and depending on the particular HPV test and workflow used, on self-sampled material (Eperon et al., 2013; Stanczuk et al., 2015; Arbyn et al., 2014). Moreover, primary high-risk human papillomavirus (hrHPV) testing has been shown to be more sensitive than cytology, and performs better in a population entering the screening program (Ronco et al., 2010; Ronco et al., 2014; Rijkaart et al., 2012; Naucler et al., 2007; Kitchener et al., 2009). Based on extensive and accumulating scientific evidence, the Ministry of Health, Welfare and Sports in the Netherlands has decided to revise the screening program in 2017. Cervical smears

* Corresponding author at: Radboud University Medical Center, Department of Obstetrics & Gynaecology, (internal mail 791), PO Box 9101, 6500 HB Nijmegen, The Netherlands.

E-mail address: Pleun.Ketelaars@Radboudumc.nl (P.J.W. Ketelaars).

will primarily be tested for the presence of hrHPV DNA instead of cytology, with reflex cytology in case of a positive hrHPV test (Standpunt minister van VWS screening op baarmoederhalskanker, 2013).

Previous studies have shown that vaginal self-sampling is a well-accepted alternative for a physician-taken cervical smear for non-responders in organized primary hrHPV based screening, and it increases the screening participation rate (Bosgraaf et al., 2014; Gok et al., 2010; Gok et al., 2012a; Gok et al., 2012b; Bosgraaf et al., 2015; Schmeink et al., 2011). 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 (Arbyn et al., 2014; Schmeink et al., 2011; Snijders et al., 2013; Stanczuk et al., 2016). In the revised Dutch screening program vaginal self-sampling will be available for non-responders (Ministry of Health WaS, n.d).

In the responder screening population the preferred method of screening is still unknown, and it remains to be determined whether self-sampling is comparable to physician sampling in detecting cervical abnormalities. Therefore, the VERA (Validation of the Evalyn brush with the Roche cobas 4800 hrHPV Test) study has been designed. The objectives of the VERA study were to investigate concordance in hrHPV positivity and HPV 16/18 genotypes between self-collected samples, using the Evalyn brush, and physician-taken samples, and the acceptability of self-sampling among a responder population of the Dutch cervical screening program.

2. Materials and methods

2.1. Study population

The Dutch screening program is a nationwide program targeting women aged 30–60 years. Women are invited at 5-year intervals for a cervical smear, generally taken by their physician. In the current study, 2460 women, aged 30–60 years and living in the regions of Nijmegen and 's-Hertogenbosch in the Netherlands, participated in the VERA study.

2.2. Clinical specimen collection

The participants had their regular cervical smear taken by their physician as part of the nationwide program. A trained physician obtained a liquid-based cytology sample using a Rovers Cervex-Brush (Rovers Medical Devices B.V., Oss, Netherlands). The Cervex-Brush was rinsed in ThinPrep medium (Hologic, Marlborough, MA) in the Nijmegen region and in SurePath medium (Klinipath BV, Duiven, Netherlands) in the 's-Hertogenbosch region. Cytological examination and classification were performed at the local laboratory according to the CISOE-A (composition, inflammation, squamous epithelium, other and endometrium, endocervical columnar epithelium, and adequacy of the smear) classification, which can easily be translated into the Bethesda 2001 classification (Bulk et al., 2004). The VERA study was not allowed to interfere in the cervical cancer screening program, according to the Dutch law. Therefore, the self sample was always taken after the physician taken sample, and referral was based on the outcome of the cytology assessment, and not of the obtained hrHPV result from the study.

2.3. Self-sampling procedures

After informed consent, and at the time of the appointment with their physician for their scheduled cervical smear, the participants also received a self-sampling kit including a self-sampling device (Evalyn Brush, Rovers Medical Devices B.V., Oss, Netherlands). The Evalyn Brush 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; each rotation generates an audible click. After rotation, the brush can be removed from the vagina, and pulled back into the case. A cap is to be clicked onto the case and the brush can be directly sent to the laboratory. The self-sampling kit also includes an explanatory letter, an informed consent form, user instructions (written and drawn), a short questionnaire, and a return envelope with the address of the laboratory. Women self-collected a cervicovaginal sample with the Evalyn Brush either at home or in the physician's practice, in either case after the physician collected sample was taken. The women were asked to return the dry brush with the self-sampled material, the signed informed consent form, and the questionnaire by regular mail.

2.4. Specimens preparation

Upon arrival of the dry brush devices at the laboratory, the brush tips were suspended in 4.5 ml of Preservcyt medium (Hologic, Marlborough, MA). The vials were vortexed for 3×15 s, stored overnight at 4 °C, and again vortexed for 2×15 s, before the brushes were removed and discarded.

A total of 3 ml was collected from the physician obtained samples, and stored and transported at room temperature to the department of Medical Microbiology, Radboudumc, Nijmegen, Netherlands, for molecular hrHPV DNA testing.

2.5. Cobas 4800 HPV test

The physician-collected residual liquid-based cytology (LBC) specimens and the self-sampled specimens were vortexed before being placed in the cobas 4800 for hrHPV testing using the clinically validated Roche cobas 4800 HPV Test to detect 12 hrHPV types (hrHPV31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68) as a pooled result, and simultaneously provide specific genotyping information for HPV-16 and HPV-18. The cobas 4800 HPV Test features automated sample preparation combined with real-time PCR technology. β-globin from cellular input is used as an internal control to assess specimen adequacy and identify specimens containing factors that inhibit the amplification process.

2.6. Questionnaires

To investigate the acceptability of the Evalyn Brush, all women were asked to fill out a short questionnaire to record their experience, their response to the instructions, and their assessment of the convenience of the Evalyn Brush. Participants were also asked whether they preferred self-sampling or physician sampling for the next screening round. All questionnaires were collected and analyzed centrally. Cardiff Teleform Software (version 10.1, 2010; Cambridge, UK) was used to design the questionnaire and record the data.

2.7. Outcome measures

The primary outcome measure of the VERA-study was concordance in hrHPV positivity between self-collected samples, using the Evalyn brush, and physician-taken samples. Furthermore, we investigated hrHPV genotypes 16 and 18, and the acceptability of self-sampling in a Dutch cervical screening responder population. For comparison of the presence of HPV-16, HPV-18 and 12 other hrHPV genotypes between the two samples, we used the following terminology; identical, concordant or discordant. An identical result is determined if results from all three cobas HPV channels (16, 18, and 12 other hrHPV genotypes) were equal; concordance is determined as at least one identical genotype in both samples; and discordance is determined as no similarities in the genotypes.

Follow-up outcomes of all participants were retrieved from the nationwide network and registry of histology and cytology database

(PALGA). The latest diagnosis was registered. When histological and cytological diagnoses were both available in the follow-up period until June 2015, only histological results were included in analysis. All cytological and histological findings recorded before June 2015 were included in our analysis.

2.8. Statistical analysis

We aimed to include 135 hrHPV positive cases in order to reliably assess concordance between physician-taken samples and self-samples. The overall study aim was to target a responder population of 3000 women, with an estimated hrHPV positivity of 4.5%. Analysis of the first 1500 samples revealed an hrHPV positivity rate of 8%, leading to an adjustment of the sample size calculation. With 8% hrHPV positivity only 2000 samples were needed in order to detect concordance with the same reliability. In the analysis of the questionnaires the percentages were calculated for the women who answered the question. Concordance was calculated, and a McNemar test for correlated proportions comparing the hrHPV positive portion of self-sampling with the hrHPV positive portion of physician sampling, was performed on the total group as well as per age group.

3. Results

3.1. Patient characteristics

Samples were collected between April 2013 and September 2014. In total 2460 women participated. From 316 women no cervical smear and/or self-sample was received. Twenty-eight women were excluded because 14 physician-taken specimens, and 14 self-sampling specimens tested β-globin negative. Another 20 cervical smears and 47 self-sampling specimens were excluded because of an invalid test result because of too much blood or mucus. Therefore, in total 2049 women with both a self-sample and physician-taken test result were enrolled.

3.2. HPV prevalence

HrHPV prevalence was 8.0% (163/2049; 95% confidence interval [95% CI], 6.9 to 9.2) among the physician-taken samples, and 10.0% (204/2049; 95% CI, 8.7 to 11.3) among the self-samples (Table 1). The hrHPV prevalence in both the physician-taken samples and the self-samples decreased with age from respectively 18.4%, and 20.0% in women aged 29–33 to 4.7%, and 5.9% in women aged 59–63. (Table 2).

Overall concordance between self-samples and physician-taken samples was 96.8% (Table 1). Using the physician samples result as reference, the proportion of true positive self-samples was 92.6% (151/163; 95% CI, 87.6 to 95.8), while the proportion of true negative self-samples was 97.2% (1833/1886; 95% CI, 96.3 to 97.8). The McNemar test for correlated proportions was highly significant ($p < 0.000002$) indicating that the proportions are not comparable, although this differed between the age groups (Table 2).

3.3. HPV genotyping

The HPV genotypes detected by the cobas 4800 HPV Test in the physician-taken smear were hierarchically based on oncogenicity for cervical cancer. A total of 43 out of 2049 samples (2.1%) showed single or multiple infections with genotype HPV-16; 12 samples (0.6%) showed single or multiple infections with HPV-18 excluding any coinfections with HPV-16. The remaining 108 samples (5.3%) showed single or multiple infections with non-16/18-HPV genotypes.

In 51 self-samples (2.5%), single or multiple infections with genotype HPV-16 were found, and 11 (0.5%) single or multiple infections with HPV-18 (excluding coinfections with HPV-16). In the non-16/18 HPV genotypes 142 (6.9%) samples were detected.

An identical result was found in 96.4% (1976/2049) of the samples. Analysis showed a concordance of 96.8% (1984/2049), and a discordance of 3.2% (65/2049). Forty-two out of 65 discordant samples tested non-16/18 HPV genotype positive in self-sampling, and hrHPV negative in the physician sample. Table 3 shows the results for HPV detection separating HPV 16/18 from the other hrHPV types. The concordance for HPV16 and/or 18 positives is 99.1% (62 (3.0%) HPV 16/18 positives in self-sampling, and 55 (2.7%) in physician sampling), while for hrHPV positives non-HPV16/18 the concordance is 97.0% (164 (8.0%) HPV 16/18 positives in self-sampling, and 128 (6.3%) in physician sampling). The McNemar test for correlated proportions was not significant for HPV16 and/or 18 positives, but highly significant for non-HPV16/18 positive samples, indicating that regarding HPV 16/18 detection, the proportions are equal, and that the differences in proportions are mainly caused by non-HPV 16/18 types.

3.4. Detection rate of CIN2 or worse (CIN2 +)

Follow-up outcomes were retrieved from the nationwide network and registry of histology and cytology database (PALGA). All 21 women with HSIL/CIN2 +, had hrHPV identified on their physician-obtained sample, whereas 19 (90.5%) also had hrHPV detected on their self-sampled material (Table 4). Two CIN3 results in group D were missed by hrHPV testing on self-sampled material (both women between 30 and 35 years). Both women did not report any problems with performing the self-sample.

3.5. Questionnaires

A total of 2194 out of 2460 questionnaires (89.2%) were returned. The mean age of the women who returned a questionnaire was 43.4 years (range, 29–61 years).

Women had the opportunity to do the self-sample at the physician's practice (7.1%; 153/2166) or at home (92.9%; 2013/2166). The user friendliness of the self-sampling device was rated good to excellent by 98.5% of the women.

>95.8% of the women experienced no problems during the use of the self-sampling device. A total of 17 women (0.8%) reported uncertainty about performing the test correctly. In 99.2% of the cases women were confident in performing the self-sampling correctly.

Table 1
Concordance for hrHPV between self-sampling and physician sampling.

		Self-sampling		Total (%)
		hrHPV positive (%)	hrHPV negative (%)	
Physician sampling	hrHPV positive (%)	151 (7.4)	12 (0.6)	163 (8.0)
	hrHPV negative (%)	53 (2.6)	1833 (89.5)	1886 (92.0)
Total (%)		204 (10.0)	1845 (90.0)	2049 (100.0)

Note: This study was conducted in the Netherlands from April 2013 to May 2015.
McNemar test for correlated proportions comparing hr-HPV positive portion of self sampling with positive portion of physician sampling, $p < 0.000001$.

Table 2
Participation rate and prevalence of hrHPV, categorized by age.

Age category (in years)	Participants per age group	Physician sampling		Self-sampling		P-value[*]
		hrHPV Positive (%)	hrHPV Negative (%)	hrHPV Positive (%)	hrHPV Negative (%)	
29–33	245	45 (18.4)	200 (81.6)	49 (20.0)	196 (80.0)	0.34
34–38	240	20 (8.3)	219 (91.3)	28 (11.7)	212 (88.3)	0.02
39–43	316	22 (7.0)	288 (91.1)	30 (9.5)	286 (90.5)	0.07
44–48	401	31 (7.7)	370 (92.3)	40 (10.0)	361 (90.0)	0.01
49–53	416	24 (5.8)	392 (94.2)	30 (7.2)	386 (92.8)	0.07
54–58	327	16 (4.9)	311 (95.1)	22 (6.7)	305 (93.3)	0.18
59–63	85	4 (4.7)	81 (95.3)	5 (5.9)	80 (94.1)	1.00
Unknown	19	–	19 (100.0)	–	19 (100.0)	1.00
Total	2049	163 (8.0)	1886 (92.0)	204 (10.0)	1845 (90.0)	<0.000001

Note: This study was conducted in the Netherlands from April 2013 to May 2015.

 [*] McNemar test for correlated proportions.

Overall, most women (62.7%) preferred the self-sampling over a physician-taken smear, 24.8% preferred a physician-taken smear for the next screening round and 11.9% had no preference for either self-sampling or physician sampling. There were no notable differences between the age groups (Table 5).

The convenience of self-sampling in comparison with a physician-taken sample was rated good to excellent by 97.1% of the women (2100/2163).

4. Discussion

This study shows a high concordance in hrHPV prevalence between self-sampling material and physician sampling material, especially with regard to HPV 16 and/or 18 positivity. Furthermore, the brush-based Evalyn device provided a well-accepted self-sampling method for women participating in the cervical screening program. We showed that self-sampling for hrHPV DNA in a Dutch responder screening population is well accepted, and is rated as highly user-friendly. Over 95% of enrolled women found self-sampling convenient and user-friendly. Most women (62.8%) preferred self-sampling over a physician-taken smear for the next screening round. To the best of our knowledge, no other studies investigated hrHPV concordance between a validated brush-based self-sampling device and physician-taken sample in a responder screening population with a clinically validated and automated PCR platform (Meijer et al., 2009).

Current and prior studies have shown that self-sampling for hrHPV DNA is a well-accepted screening method for women non-participating in the regular cervical screening program. Bosgraaf et al. showed in a previous large self-sampling study among non-responders that 80.5%

preferred self-sampling with a brush-based device over a physician-taken smear (Bosgraaf et al., 2015). Szarewski et al. demonstrated in an English screening population that 73% preferred to use the self-sampling at home rather than come to the clinic (Szarewski et al., 2007). The only limitation of self-sampling is the uncertainty about performing the self-sampling correctly.

In previous studies, HPV self-sampling was offered to non-responder women, i.e. women who were never or irregularly screened. In these studies the hrHPV prevalence was 8.3 to 10.3% (Gok et al., 2010; Gok et al., 2012b; Bosgraaf et al., 2015). The hrHPV prevalence of 8.0% in the physician-taken smears in the current study is similar to the prevalence in the non-responder studies in the Netherlands (Gok et al., 2010; Gok et al., 2012b; Bosgraaf et al., 2015; Verhoef et al., 2014a). This is surprising as former studies found generally a prevalence of 5% in a responder population (Rijkaart et al., 2012). This can be due to the technique used (GP5+/6 + -PCR versus cobas hrHPV Test), to demographic factors or to a possible selection bias in the population analyzed.

The difference in higher prevalence of hrHPV in self-samples (10%) compared with physician-taken samples (8%) is in line with previous studies showing higher HPV prevalence in self-sampling compared to physician collected sample (Salmeron et al., 2003; Holanda et al., 2006; Girianelli et al., 2006). Indeed, the significant result of the McNemar test for correlated proportions indicate a true difference in HPV positivity. Additional analysis showed that this difference in proportion is mainly caused by detection of non-HPV 16/18 types in self samples. With self-sampling both the cervical and vaginal area are sampled. This may result in a higher non-HPV 16/18 detection rate and thus self-sampling may not always be a true reflection of the cervical hrHPV flora.

Table 3
Concordance for HPV 16/18, and non-16/18 hrHPV.

		Self-sampling		Total (%)
		HPV 16/18 positive	HPV 16/18 negative	
Physician sampling	HPV 16/18 positive	50	5	55 (2.7)
	HPV 16/18 negative	12	1982	1994 (97.3)
Total (%)		62 (3.0)	1987 (97.0)	2049 (100.0)
		Non-16/18 hrHPV positive	Non-16/18 hrHPV negative	
Physician sampling	Non-16/18 hrHPV positive	117	11	128 (6.3)
	Non-16/18 hrHPV negative	47	1874	1921 (93.7)
Total (%)		164 (8.0)	1885 (92.0)	2049 (100.0)

Concordance for HPV 16/18 positive is 2032/2049 = 99.1%.
Concordance for non-16/18 hrHPV positive is 1991/2049 = 97.0%.
McNemar test for correlated proportions HPV 16/18 positive samples $p = 0.14$.
McNemar test for correlated proportions non-16/18 hrHPV positive samples $p < 0.000002$.

Table 4
Aggregated results per group.

	CS+/SSD+	CS-/SSD-	CS-/SSD+	CS+/SSD-	Total
Cytology					
Invalid	–	3	1	–	4
NILM	112	1801	47	9	1969
ASC-US/AGC	6	9	2	–	17
LSIL	2	–	–	1	3
HSIL	4	–	–	–	4
Histology					
No CIN	7	8	9	–	17
CIN1	5	–	–	–	5
CIN2	4	–	–	–	4
CIN3	11	–	–	2	13
Unknown	–	12	1	–	13
Total	151	1833	53	12	2049

Note: This study was conducted in the Netherlands from April 2013 to May 2015.
CS = hrHPV testing on a physician-taken cervical smear.
SSD = hrHPV testing on a self-sampling device.

High hrHPV prevalence rates may lead to a high referral rates. For this reason a triage test is often suggested to reduce referral rates and prevent overtreatment. It has been shown that cytology triage is not possible on self-sampled material (Arbyn et al., 2012). HrHPV genotyping during cervical cancer screening might improve cervical cancer screening to become more effective, because it might identify women with a higher risk of cervical cancer (Kjaer et al., 2010). HPV-16 and HPV-18 are the most oncogenic hrHPV genotypes (Kjaer et al., 2010). Using immediate genotyping with the COBAS platform to make a distinction between a non-16/18 HPV (hrHPV31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68, a pooled result), and a HPV-16 and/or HPV-18 test result, may positively influence the referral rate. This study shows that especially HPV-16 and 18 are equally well detected with self-sampling compared to physician sampling, as the McNemar test indicated identical proportions.

The discordance between physician collected and self-sampled specimens was particularly notable for non HPV-16/HPV-18 positive samples in the self-sampling group versus negative in physician-taken group. No earlier research has reported on the differences in non HPV-16/HPV-18 between different genital regions. An explanation for the discordance of hrHPV could be that with self-sampling a different anatomic area is sampled. With self-sampling both the cervical and vaginal area are sampled, compared to only the cervical area in the physician-taken sample.

Another direct triage strategy could be DNA methylation. Recent studies have shown that DNA methylation analysis directly on self-sampled brush-based and lavage-based specimens is feasible and showed a high correlation with matched physician-taken samples (Boers et al., 2014; Verhoef et al., 2014b). DNA methylation could be an attractive alternative to cytology. Since, the need for an extra physician-taken cervical smear can be eliminated. This could lead to more compliance and less loss to follow-up. Further research is necessary to investigate which triage strategy is most effective in the new cervical cancer screening program.

In this study, two cases with CIN 3 were not detected with self-sampling, but were hrHPV positive on a physician taken sample. This could be explained because women always had to take the self sample after the physician taken smear, which may have negatively influenced the self sample results. However, CIN detection relied heavily on cytology only, so it is unclear how many CIN 3 may have been present in the self-sampling positive/physician taken sample negative group only in a randomized trial, which would include patient management based on the hrHPV result, instead of cytology may elucidate this issue. However, a recent meta-analysis in screening populations showed a lower pooled sensitivity of HPV testing on self-samples than HPV testing on a physician-taken sample (ratio 0.84 [0.77–0.92], for CIN2+ detection.(6) Most studies in this meta-analysis were done using Hybrid Capture 2 (HC2) for HPV detection. It has been shown before that hybridization performs worse on self samples than PCR assays. Therefore more comparison studies are needed to resolve this issue.

4.1. Strengths and limitations

Strengths of this study include the fact that hrHPV testing was performed both on self-sampled and physician-sampled material of the same women, with a combination of a clinically validated self-sampling device as well as a clinically validated HPV assay. This study also measured the acceptability of the self-sampling device, and assessed attitudes toward self-sampling among responders in a Dutch real-world cervical cancer screening setting.

A limitation of our study is that the self-sample was always the second sample to be collected. This may have affected the concordance given that the initial physician collected sample could have depleted the amount of available hrHPV. With this sample consecution there was an exclusion of 121 out of 2170 samples, because these samples contained too much blood for reliable testing. In other studies the invalid rate of a brush-based self-sampling device is <0.5%. Additionally, the follow-up was not based on the hrHPV result. Therefore, the follow-up outcomes retrieved from PALGA were unable to show how many physician-taken samples missed CIN2+.

5. Conclusion

Self-sampling with the Evalyn Brush compared with the cobas 4800 platform showed a high concordance with physician-taken sampling for hrHPV detection in women participating in the regular cervical screening program, especially regarding HPV 16/18 detection. With self-sampling more non-HPV 16/18 infections are detected. Self-sampling is highly acceptable to women, and a well-accepted alternative to physician-taken samples in a responders screening population. On the basis of the results of this study, implementation of HPV-self-sampling in a responder population as a primary screening tool may be considered.

Conflict of interests

All the authors declare that they do not have any conflict of interest.

Details of ethics approval

The Committee for Human Research approved the VERA study. Registration number 2011/331 NL nr.: 37385.091.11.

Table 5
Preference of screening method in the next screening round, categorized by age.

Age category (in years)	Self-sampling (%)	Physician-taken smear (%)	No preference (%)	No test next screening round (%)	Total
29–33	174 (65.4)	59 (22.2)	32 (12.0)	1 (0.4)	266
34–38	156 (61.7)	65 (25.7)	31 (12.3)	1 (0.4)	253
39–43	211 (62.4)	79 (23.4)	47 (13.9)	1 (0.3)	338
44–48	286 (63.3)	122 (27.0)	44 (9.7)	–	452
49–53	263 (61.4)	113 (26.4)	51 (11.9)	1 (0.2)	428
54–58	201 (61.5)	80 (24.5)	45 (13.8)	1 (0.3)	327
59–63	53 (67.1)	14 (17.7)	5 (6.3)	7 (8.9)	79
Total	1344 (62.7)	532 (24.8)	255 (11.9)	12 (0.6)	2143

Note: This study was conducted in The Netherlands from April 2013 to May 2015.

Acknowledgments

We thank the participating physicians, their assistants, and the women who have participated in this study.

References

Arbyn, M., Raifu, A.O., Weiderpass, E., Bray, F., Anttila, A., 2009. Trends of cervical cancer mortality in the member states of the European Union. Eur. J. Cancer 45 (15), 2640–2648.

Arbyn, M., Ronco, G., Anttila, A., Meijer, C.J., Poljak, M., Ogilvie, G., et al., 2012. Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. Vaccine 30 (Suppl. 5), F88–F99.

Arbyn, M., Verdoodt, F., Snijders, P.J., Verhoef, V.M., Suonio, E., Dillner, L., et al., 2014. Accuracy of human papillomavirus testing on self-collected versus clinician-collected samples: a meta-analysis. The Lancet Oncol. 15 (2), 172–183.

Boers, A., Bosgraaf, R.P., van Leeuwen, R.W., Schuuring, E., Heideman, D.A., Massuger, L.F., et al., 2014. DNA methylation analysis in self-sampled brush material as a triage test in hrHPV-positive women. Br. J. Cancer 111 (6), 1095–1101.

Bosgraaf, R.P., Ketelaars, P.J., Verhoef, V.M., Massuger, L.F., Meijer, C.J., Melchers, W.J., et al., 2014. Reasons for non-attendance to cervical screening and preferences for HPV self-sampling in Dutch women. Prev. Med. 64, 108–113.

Bosgraaf, R.P., Verhoef, V.M., Massuger, L.F., Siebers, A.G., Bulten, J., de Kuyper-de Ridder, G.M., et al., 2015. Comparative performance of novel self-sampling methods in detecting high-risk human papillomavirus in 30,130 women not attending cervical screening. Int. J. Cancer 136 (3), 646–655 Journal international du cancer.

Bulk, S., Van Kemenade, F.J., Rozendaal, L., Meijer, C.J., 2004. The Dutch CISOE-A framework for cytology reporting increases efficacy of screening upon standardisation since 1996. J. Clin. Pathol. 57 (4), 388–393.

Eperon, I., Vassilakos, P., Navarria, I., Menoud, P.A., Gauthier, A., Pache, J.C., et al., 2013. Randomized comparison of vaginal self-sampling by standard vs. dry swabs for human papillomavirus testing. BMC Cancer 13, 353.

Girianelli VR, Thuler LC, Szklo M, Donato A, Zardo LM, Lozana JA, et al. Comparison of human papillomavirus DNA tests, liquid-based cytology and conventional cytology for the early detection of cervix uteri cancer. Eur. J. Cancer Prev.: The Official Journal of the European Cancer Prevention Organisation. 2006;15(6):504–10.

Globocan. Available from: http://globocan.iarc.fr/Default.aspx

Gok, M., Heideman, D.A., van Kemenade, F.J., Berkhof, J., Rozendaal, L., Spruyt, J.W., et al., 2010. HPV testing on self collected cervicovaginal lavage specimens as screening method for women who do not attend cervical screening: cohort study. BMJ 340, c1040.

Gok, M., Heideman, D.A., van Kemenade, F.J., de Vries, A.L., Berkhof, J., Rozendaal, L., et al., 2012a. Offering self-sampling for human papillomavirus testing to non-attendees of the cervical screening programme: characteristics of the responders. Eur. J. Cancer 48 (12), 1799–1808.

Gok, M., van Kemenade, F.J., Heideman, D.A., Berkhof, J., Rozendaal, L., Spruyt, J.W., et al., 2012b. Experience with high-risk human papillomavirus testing on vaginal brush-based self-samples of non-attendees of the cervical screening program. Int. J. Cancer 130 (5), 1128–1135 Journal international du cancer.

Holanda Jr., F., Castelo, A., Veras, T.M., de Almeida, F.M., Lins, M.Z., Dores, G.B., 2006. Primary screening for cervical cancer through self sampling. Int. J. Gynaecol. Obstet. 95 (2), 179–184 The Official Organ of the International Federation of Gynaecology and Obstetrics.

Kitchener, H.C., Almonte, M., Thomson, C., Wheeler, P., Sargent, A., Stoykova, B., et al., 2009. HPV testing in combination with liquid-based cytology in primary cervical screening (ARTISTIC): a randomised controlled trial. The Lancet Oncol. 10 (7), 672–682.

Kjaer, S.K., Frederiksen, K., Munk, C., Iftner, T., 2010. Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. J. Natl. Cancer Inst. 102 (19), 1478–1488.

Meijer, C.J., Berkhof, J., Castle, P.E., Hesselink, A.T., Franco, E.L., Ronco, G., et al., 2009. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. Int. J. Cancer 124 (3), 516–520 Journal international du cancer.

Ministry of Health WaS, d. Letter to the House of Representatives of the Dutch Parliament: Improvement of the Dutch cervical cancer Screening [Brief aan de Tweede Kamer: Verbetering bevolkingsonderzoek baarmoederhalskanker]. Available from. http://www.rijksoverheid.nl/documenten-en-publicaties/kamerstukken/2013/10/17/kamerbrief-oververbetering-bevolkingsonderzoek-baarmoederhalskanker.html.

Nanda, K., McCrory, D.C., Myers, E.R., Bastian, L.A., Hasselblad, V., Hickey, J.D., et al., 2000. Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. Ann. Intern. Med. 132 (10), 810–819.

Naucler, P., Ryd, W., Tornberg, S., Strand, A., Wadell, G., Elfgren, K., et al., 2007. Human papillomavirus and Papanicolaou tests to screen for cervical cancer. N. Engl. J. Med. 357 (16), 1589–1597.

Rijkaart, D.C., Berkhof, J., Rozendaal, L., van Kemenade, F.J., Bulkmans, N.W., Heideman, D.A., et al., 2012. Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial. The Lancet Oncol. 13 (1), 78–88.

Ronco, G., Giorgi-Rossi, P., Carozzi, F., Confortini, M., Dalla Palma, P., Del Mistro, A., et al., 2010. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. The lancet oncology. 11 (3), 249–257.

Ronco, G., Dillner, J., Elfstrom, K.M., Tunesi, S., Snijders, P.J., Arbyn, M., et al., 2014. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet 383 (9916), 524–532.

Salmeron, J., Lazcano-Ponce, E., Lorincz, A., Hernandez, M., Hernandez, P., Leyva, A., et al., 2003. Comparison of HPV-based assays with Papanicolaou smears for cervical cancer screening in Morelos state, Mexico. Cancer Causes Control 14 (6), 505–512 CCC.

Schmeink, C.E., Bekkers, R.L., Massuger, L.F., Melchers, W.J., 2011. The potential role of self-sampling for high-risk human papillomavirus detection in cervical cancer screening. Rev. Med. Virol. 21 (3), 139–153.

Snijders, P.J., Verhoef, V.M., Arbyn, M., Ogilvie, G., Minozzi, S., Banzi, R., et al., 2013. High-risk HPV testing on self-sampled versus clinician-collected specimens: a review on the clinical accuracy and impact on population attendance in cervical cancer screening. Int. J. Cancer 132 (10), 2223–2236 Journal international du cancer.

Stanczuk, G.A., Currie, H., Baxter, G., Foster, A., Gibson, L., Graham, C., et al., 2015. Cobas 4800 HPV detection in the cervical, vaginal and urine samples of women with high-grade CIN before and after treatment. J. Clin. Pathol. 68 (7), 567–570.

Stanczuk, G., Baxter, G., Currie, H., Lawrence, J., Cuschieri, K., Wilson, A., et al., 2016. Clinical validation of hrHPV testing on vaginal and urine self-samples in primary cervical screening (cross-sectional results from the papillomavirus Dumfries and Galloway-PaVDaG study). BMJ Open 6 (4), e010660.

Standpunt minister van VWS screening op baarmoederhalskanker, 31 December 2013. Available from:. www.rivm.nl/documenten_en_publicaties/Algemeen_Actueel/Uitgaven/Preventie_ziekte_zorg/baarmoederhalskankerscreening/Standpunt_minister_van_VWS_screening_op_baarmoederhalskanker.

Szarewski, A., Cadman, L., Mallett, S., Austin, J., Londesborough, P., Waller, J., et al., 2007. Human papillomavirus testing by self-sampling: assessment of accuracy in an unsupervised clinical setting. J. Med. Screen. 14 (1), 34–42.

Verhoef, V.M., Bosgraaf, R.P., van Kemenade, F.J., Rozendaal, L., Heideman, D.A., Hesselink, A.T., et al., 2014a. Triage by methylation-marker testing versus cytology in women who test HPV-positive on self-collected cervicovaginal specimens (PROHTECT-3): a randomised controlled non-inferiority trial. The Lancet oncology. 15 (3), 315–322.

Verhoef, V.M., Heideman, D.A., van Kemenade, F.J., Rozendaal, L., Bosgraaf, R.P., Hesselink, A.T., et al., 2014b. Methylation marker analysis and HPV16/18 genotyping in high-risk HPV positive self-sampled specimens to identify women with high grade CIN or cervical cancer. Gynecol. Oncol. 135 (1), 58–63.