HPV-based Cervical Cancer Screening on Self-samples in the Netherlands: Challenges to Reach Women and Test Performance Questions

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ABSTRACT

In 2017, cervical cancer screening in the Netherlands switched from cytology to human papillomavirus (HPV) testing using the validated PCR-based cobas 4800. Women could order and subsequently received a free self-sampling kit (Evalyn Brush) at their home address instead of clinician sampling. In the laboratory, the shipped brush was placed into 20 mL of PreservCyt fluid, before testing. In the first 2 years of the new program, only 7% of screening tests were performed on a self-sample. Those who chose self-sampling versus clinician sampling were more likely to have never been screened previously and differed also with respect to sociodemographic factors. Subsequent more active promotion and increasing the ease to obtain kits increased the proportion opting for self-sampling (16% in 2020). HPV positivity and detection rate of precancer (CIN3+) were lower in the self-sampling compared with the clinician-sampling group (adjusted ORs of 0.65 and 0.86, respectively). Although population differences may partially explain these results, self-samples may have been too dilute, thereby reducing the analytic and clinical sensitivity. The Dutch findings demonstrate the importance of optimizing outreach, specimen handling and testing protocols for self-samples to effectively screen the target population and reach in particular the women at highest risk for cervical cancer.

See related article by Aitken et al., p. 183

Given the strong evidence regarding the superior performance of high-risk human papillomavirus (hrHPV)-based over cytology-based screening for the prevention of cervical cancer (1, 2), more and more countries have switched or are in the phase of switching toward virological screening (3). Testing for hrHPV DNA has the additional advantage that it can be performed on self-sampled vaginal specimens, with similar accuracy for detection of cervical precancer as on provider-taken cervical samples under the condition that a validated PCR-based assay is used (4). Moreover, offering self-sampling kits to women who do not regularly participate in screening has been shown in randomized trials to trigger higher response rates compared with conventional invitations for a clinical visit to take a cervical specimen (5).

The Netherlands has a long-standing tradition of organized cervical cancer screening with particular interest in balancing benefits against harms (6). In 2017, it was one of the first countries that changed from using liquid-based cytology to a validated hrHPV assay (7, 8) as primary stand-alone screening test, with the option of self-sampling. The Dutch program targets women ages 30–60 years for screening every 5 years (9). For women ages 40 and 50 years, who test hrHPV negative, the screening interval is extended to 10 years due to the low subsequent risk of invasive cervical cancer. Women ages 65 years receive an extra invitation when they are hrHPV positive and cytology negative at age 60 years.

The choice of the test [cobas 4800 assay (Roche Molecular Systems)], collection device [Evalyn-Brush for self-sampling (Rovers Medical Devices)], transport medium [PreservCyt (Hologic Inc)] and selection of the five screening laboratories were determined via an European tender procedure, resulting in uniform sample handling and test protocols and a low overall cost (9). In the first invitation letter and the reminder letter (if no screening followed the first invitation), women had the option to order a self-sampling kit if they felt uncomfortable to have a cervical specimen taken by a clinician. If they opted for self-sampling, an Evalyn-Brush was sent to their home address along with instructions. After self-collection, the dry brush was placed in a prepaid return envelope that was addressed to one of the testing laboratories. After arrival in the laboratory, the dry brush was transferred into 20 mL of PreservCyt solution, which is a widely used storage medium that preserves cells and nucleic acids.

In the current issue of *Cancer Epidemiology Biomarkers & Prevention*, Aitken and colleagues (10) compared the sociodemographic characteristics of 779,474 women (93%) who had a cervical specimen taken by their general practitioner (GP) and 58,126 women (7%) who chose a vaginal self-sample in the first 2 years of the Dutch HPV-based screening program (2017–2018). The following population categories were more likely to self-sample than undergo clinician sampling: never screened women (29.0% vs. 11.9%, in the self-sampling and clinician-sampling groups, respectively, ratio = 2.43); the youngest ages 30–39 years (ratio = 1.29) and oldest group ages 60 years versus the middle age groups (ratio = 1.06); unemployed and supported by social welfare versus employed (ratio = 1.12); live in a one-person household versus multi-person household (ratio = 1.43); and the highest income quartile versus the lowest quartile (ratio = 1.09). Interestingly, Inturrisi and colleagues (same author group) previously reported that the hrHPV positivity was lower and the positive predictive value (proportion referred hrHPV-positive women with biopsies who had histologically confirmed CIN2/3+) was

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higher in women who self-sampled compared with those with clinician-taken samples (11). Inturrisi and colleagues found higher mean real-time PCR C_t values (indicating lower viral load) in self-samples versus clinician samples, and this difference was greater by underlying disease status (11). This finding is not surprising and was reported repeatedly in the literature (5). Belinson and colleagues reported a progressively weaker signal for hrHPV by Hybrid Capture 2 (Qiagen) in cervical, upper vaginal, lower vaginal, and perineal samples (12). Likewise, the VALHUDES study observed higher PCR metrics (indicating lower viral concentration) in self-samples compared with clinician-taken samples and demonstrated that the accuracy of hrHPV testing on self-samples (already validated on cervical specimens but with lower sensitivity or specificity on self-samples) could be optimized by modification of the positivity cutoff (13–15). Inturrisi and colleagues hypothesized that resuspension of the Evalyn-Brushes in 20 mL of transport medium may have resulted in a too low viral concentration with consequently a lower clinical sensitivity, higher specificity and higher positive predictive value of hrHPV testing in self-samples versus clinician samples (11).

Table 1. Participation rate in the self-sampling arm and control arms and relative participation in randomized trials that reported data stratified by screening history status and distinguishing opt-in and send-to-all scenarios to offer self-sampling kits [updated from Arbyn et al. BMJ 2018 (5)].

Study	Age (years)	Scenario	Screening history	Participation		Relative Participation (SS/control)	P^c	Ratio opt-in/ mail-to-all
				SS arm	Control arm			
Gok, 2010 (the Netherlands)	30–60	Mail-to all	Screened ≥5 y ago	27.7%	16.6%	1.67 (1.28–2.18)	0.8480	
			Screened >7 y ago	15.8%	9.0%	1.76 (1.09–2.86)		
Broberg, 2014 (Sweden)	30–62	Opt-in	Screened ≤10 y ago	39.7%	16.9%	2.35 (1.88–2.93)	0.1708	
			Screened >10 y ago	20.3%	7.0%	2.89 (2.14–3.89)		
			Never screened	19.1%	9.7%	1.96 (1.50–2.56)		
Cadman, 2015 (Uk)	25–65	Mail-to all	Screened 0–3y ago	29.5%	16.4%	1.81 (0.85- 3.83)	0.1776	
			Screened 3–5y ago	33.1%	15.1%	2.19 (1.71–2.81)		
			Screened 5–10y ago	18.8%	7.5%	2.5 (1.85–3.37)		
			Screened >10y ago	11.3%	2.3%	4.94 (2.60–9.39)		
			Never screened	8.3%	3.1%	2.68 (1.81–3.96)		
Sultana, 2016 (Australia)	30–69	Mail-to all	Screened >2.5 y ago	11.6%	6.4%	1.80 (1.41–2.29)	0.3934	
			Never screened	20.5%	9.4%	2.18 (1.51–3.15)		
Tranberg, 2018 (Denmark) [a]	30–64	Mail-to all	Regularly screened[a]	29.7%	35.7%	0.83 (0.76–0.91)	<0.0001	
			Underscreened	25.3%	14.3%	1.78 (1.45–2.18)		
			Never screened	19.9%	7.2%	2.75 (1.96–3.84)		
		Opt-in	Regularly screened	43.2%	35.7%	1.21 (1.12–1.31)	0.4464	1.45
			Underscreened	20.0%	14.3%	1.40 (1.13–1.73)		0.79
			Never screened	8.7%	7.2%	1.21 (0.82–1.79)		0.44
Ivanus, 2018 (Slovenia)	30–64	Mail-to all	Screened 4–9 y ago	54.8%	33.7%	1.63 (1.49–1.77)	<0.0001	
			Screened ≥10 y ago	22.2%	6.7%	3.32 (2.73–4.06)		
		Opt-in	Screened 4–9 y ago	51.5%	33.7%	1.53 (1.40–1.66)	<0.0001	0.94
			Screened ≥10 y ago	18.3%	6.7%	2.76 (2.26–3.36)		0.82
MacDonald, 2021 (New Zealand)	25–69	Offer at Healthcare Service	Screened 4–5y ago	71.1%	34.2%	2.10 (1.60–2.75)	0.0872	
			Screened 6–10y ago	60.9%	26.0%	2.34 (1.70–3.24)		
			Screened >10y ago	48.6%	10.0%	4.86 (2.41–9.77)		
			Never screened	42.7%	12.7%	3.36 (1.97–5.73)		
Brewer, 2021 (New Zealand) [b]	30–69	Mail-to all	Underscreened[b]	13.5%	2.6%	5.21 (2.57–10.56)	0.9153	
			Never screened	14.6%	3.0%	4.92 (2.19–11.05)		
		Opt-in	Underscreened	6.4%	2.6%	2.49 (1.20–5.17)	0.7642	0.47
			Never screened	6.3%	3.0%	2.10 (0.91–4.87)		0.43
Aasbo, 2022 (Norway)	35–69	Mail-to all	Screened 10–15y ago	32.4%	8.7%	3.73 (2.81–4.96)	0.0001	
			Screened >15y ago	26.0%	4.0%	6.50 (4.37–9.69)		
			Never screened	25.0%	1.9%	13.40 (7.53–23.84)		
		Opt-in	Screened 10–15y ago	22.9%	8.7%	2.64 (1.95–3.55)	0.019	0.71
			Screened >15y ago	17.3%	4.0%	4.33 (2.87–6.54)		0.67
			Never screened	11.4%	1.9%	6.11 (3.35–11.13)		0.46

Abbreviation: SS, self-sampling.

[a]Definitions of categories of screening history in Tranberg, 2018:

- Regularly screened:
  Age 30–34 y; 56–64y: ≥2 cervical cytology samples were registered
  Age 35–55y: ≥3 cervical cytology samples were registered
- Underscreened:
  Age 30–34 y; 56–64y: only one cervical cytology sample registered
  Age 35–55y: 1 or 2 cervical cytology samples were registered
- Never screened: no cervical cytology sample was registered (registry covers 15 years of screening)

[b]Definition of category of screening history in Brewer, 2021:

- Underscreened: no screening recorded for at least the last 5 years

[c]*P* values for heterogeneity by category of screening history (computed according Mantel-Haenszel method).

Aitken and colleagues confirmed the findings of lower hrHPV positivity and lower detection of cervical precancer lesions among women that used self-sampling compared with those who had clinician-collected samples, after controlling for sociodemographic factors and follow-up compliance: adjusted ORs of 0.65 [95% confidence interval (CI), 0.63–0.68] for hrHPV positivity, 0.76 (CI, 0.70–0.82) for CIN2+ detection, and 0.86 (CI, 0.78–0.95) for CIN3+ detection (10). Unfortunately, the authors did not report the absolute hrHPV positivity by population category within the self-sampling and clinician-sampling groups. However, the possibility of differential exposure to hrHPV among respective population categories, which may explain the lower test positivity and precancer detection in the self-sampling group, cannot be ruled out. For instance, the self-sampling population could contain mainly women at lower risk (yielding lower hrHPV positivity than in the clinician-sampling group) and a minority of higher risk women (explaining the higher positive predictive value and ORs for disease nearer to unity). Nevertheless, the Inturrisi hypothesis (excessive dilution by too great resuspension volume; ref. 11) is still plausible. To note, the lower viral load in self-samples may be compensated, at least to some extent, by defining a lower positivity cutoff (higher C_t value; ref. 15).

The rather low proportion of screened Dutch women opting for self-sampling in 2017 to 2018 (~7%) might be explained by the low level of promotion for self-collection and the barrier associated with the effort required to order a kit. Like in other countries, the COVID-19 pandemic led to overcoming barriers triggering increased use of self-sampling as a safe collection method for cervical cancer screening in the Netherlands (16, 17). As a result, a substantially greater proportion of screened women opted for self-sampling (16% in 2020; ref. 10). In 2021, the Dutch Health Council advised sending self-sampling kits to all eligible women as the main outreach approach as already recommended in Sweden (*medRxiv* 2022.07.19. 22277806). The switch from opt-in to send-to-all invitational scenarios is supported by a meta-analysis demonstrating consistently higher response rates in participation trials involving the send-to-all scenario (5). An extremely interesting finding was the increased participation (in self-sampling vs. clinician-sampling arms) by longer delay since last screening (**Table 1**). The meta-analysis of participation trials (5) highlighted important geographic differences and interscenario contrasts in the response to the offer of self-sampling kits. This heterogeneity in the magnitude of effect warrants implementation research or piloting before general rollout of strategies taking into account the attitudes of women and stakeholders and providing tailored education.

The forest plot in **Fig. 1** contains data from an updated meta-analysis comparing the clinical accuracy to detect CIN2+ of PCR-based

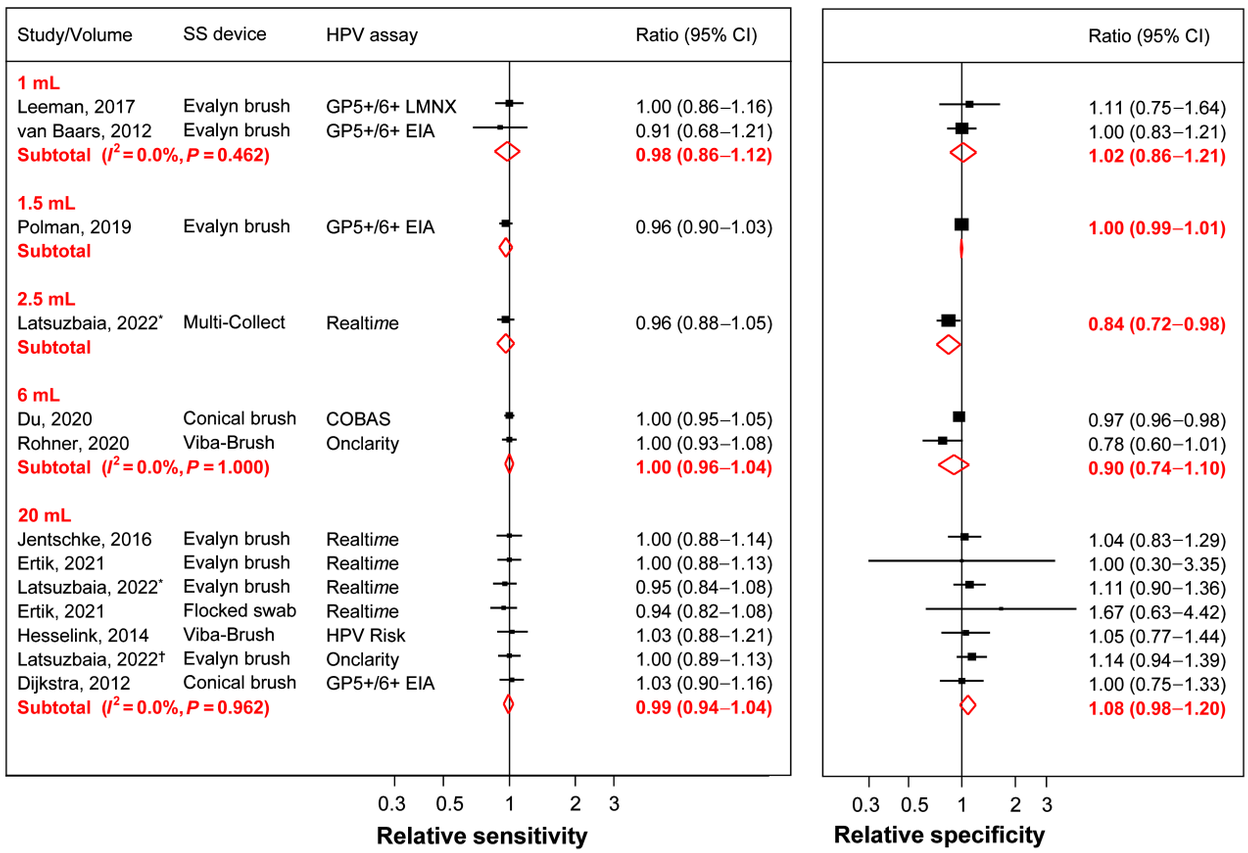

Figure 1.

Meta-analysis of the relative sensitivity (left) and specificity (right) of validated PCR-based hrHPV DNA tests on vaginal self-taken versus clinician-collected cervical samples, by resuspension volume of transport medium added to the self-collection device. Note: SS: self-sample; COBAS: cobas 4800 (Roche Molecular System); GP5+/6+ EIA: GP5+/6+ PCR-EIA (Diassay); GP5+/6+-LMNX: LMNX Genotyping Kit GP HPV (Diassay B.V.); HPV Risk: HPV-Risk Assay (Self-Screen BV); Onclarity: Onclarity HPV Assay (BD Diagnostics); Realtime: Abbott Realtime High-Risk HPV (Abbott). *Latsuzbaia et al., Microbiol Spectrum 2022 (15); † Latsuzbaia et al., Cancer Epidemiol Biom Prev 2022 (14).

Table 2. Parameters that may influence the accuracy of hrHPV testing on vaginal self-samples.

Vaginal self-sample collection procedure:
- Collection device
- Recommended depth of device insertion during collection/device shaft length
- Procedure for self-collection (i.e., number of vaginal rotations during collection)
- Usage of vaginal products (creams, gels, etc.) preceding sample collection (these may result in PCR inhibition)
Self-sample transport:
- Sample resuspended immediately following collection or transported dry to the laboratory
- Sample stability and storage conditions (temperature and time from collection to laboratory processing)
- Transport medium
- Volume of transport medium used for vaginal sample resuspension
- Sample vortexing (intensity and duration) prior to processing
Preanalytical workflow following vaginal sample resuspension:
- Sample starting volume selected for further processing
- Sample centrifugation with resuspension of cellular pellet versus sample starting volume to be used for nucleic acid extraction
- Nucleic acid extraction method: rapid extraction (i.e., rapid heat extraction) versus standard extraction protocols
- Elution volume—volume used for eluting extracted nucleic acids present in the sample starting volume following standard extraction methods
Analytic procedure—HPV testing using PCR-based methods
- Volume of extracted nucleic acids used in PCR mix
- Positivity criterion in terms of C_t value, viral concentration using standard curve analysis, normalized viral load (viral concentrations adjusted for number of cells per sample)
- Sample's endogenous control to evaluate sample adequacy and/or "internal" control to check for PCR inhibition, or other metrics.

hrHPV DNA tests validated on clinician-taken samples (7) with self-samples taken with dry brushes, stratified by the volume of diluent to resuspend the brush heads of the self-sample devices. The meta-analysis contains studies of a previous systematic review that provided details regarding the resuspension volume (5), updated with newer publications (14, 15, 18–21). Resuspension volumes of diluent varied from 0.5 to 20 mL. Significant intergroup heterogeneity was observed for the relative specificity ($P < 0.01$) but not for sensitivity ($P = 0.91$; ref. 22). The meta-analysis in **Fig. 1** supports Inturrisi's interpretation of higher relative clinical specificity in self-samples diluted in 20 mL of transport medium compared with clinician sampling, but not the interpretation of lower sensitivity. However, only few studies included in the meta-analysis (see **Fig. 1**) reported the volume of diluent, and this and several other sample handling procedures may influence virological and clinical accuracy (23).

The lack of standardization of the preanalytical laboratory processing of self-samples for HPV testing may result in different analytic and clinical performance. To refine knowledge on the influence of self-sampling devices, transport media, transport conditions, and other sample handling issues on test accuracy, we are currently updating and completing the previous meta-analysis (5), in which authors of included studies will be asked for more detailed data (23). **Table 2** contains a short list of potentially influential steps in the work-up of self-specimens from collection to testing. It is expected that in the future, regulatory agencies and decision makers will request for on-label validated protocols for HPV testing on self-samples. Very recently, the Dutch Screening Organization issued a European tender procedure, resulting in new contracts with providers and opted for the validated Onclarity HPV assay (BD Diagnostics) including an on-label testing protocol for self-samples taken with FLOQSwab (Copan; personal communication with Sandra Van Dijk-de Bruin).

To conclude, the screening program in the Netherlands has nicely demonstrated that the offer of self-sample kits can reach more women never screened before. However, the challenge remains to reach as many women as possible especially those at highest risk. Careful monitoring allowed for adjusting the way the self-kits are offered and resulted in higher acceptance rates. The Dutch findings also highlight the need to optimize and standardize procedures for handling and testing of dry self-samples to assure the best possible accuracy to detect cervical (pre)cancer and approaching that of clinician sampling.

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Disclaimer

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