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Validation of the clinical performance and reproducibility of the NeuMoDx HPV assay self-sample workflow

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ABSTRACT

Background: Human papillomavirus (HPV) testing on self-samples is a valid tool for cervical cancer screening. HPV self-sample workflows need to be clinically validated to ensure safe use in screening.
Objective: This study evaluated the fully automated NeuMoDx HPV Assay self-sample workflow that is compiled of the NeuMoDx HPV assay and the NeuMoDx 96/288 Molecular Systems, for clinical performance and reproducibility on Evalyn Brush-collected self-samples.
Methods: The clinical performance of the NeuMoDx HPV Assay self-sample workflow for cervical intraepithelial neoplasia grade 2 or worse (CIN2+) and CIN3+ was evaluated on 987 self-samples obtained from women attending national organized HPV-based cervical cancer screening by a noninferiority analysis relative to reference workflows using either HPV-Risk Assay or high-risk HPV GP5+/6+-PCR. Intra- and inter-laboratory reproducibility of the NeuMoDx HPV Assay self-sample workflow using both NeuMoDx 96 and 288 Molecular Systems was assessed on 520 self-samples in three laboratories.
Results: The clinical sensitivity and specificity of the NeuMoDx HPV Assay self-sample workflow for the detection of CIN2+ and CIN3+ were found to be non-inferior to the reference workflows using either HPV-Risk Assay or high-risk HPV GP5+/6+-PCR, with all p-values <0.034. The NeuMoDx HPV Assay self-sample workflow exhibited an intra-laboratory reproducibility of 94.4 % (95 %CI:92.5–96.1 %) with kappa value 0.86 (95 % CI:0.81–0.91). Inter-laboratory agreement was high (all ≥93.4 % and all kappa values ≥0.83).
Conclusions: The NeuMoDx HPV Assay self-sample workflow demonstrated high clinical accuracy for CIN2+/3+ and high reproducibility. The NeuMoDx HPV Assay self-sample workflow can be considered suitable for cervical cancer screening purposes.

1. Introduction

There is compelling evidence that testing for high-risk human papillomavirus (HPV) DNA has a superior performance over cytology-based screening for the prevention of cervical cancer [1]. This guided several countries worldwide to introduce primary HPV-based cervical cancer screening [2,3]. HPV DNA testing can be accurately applied to both clinician-collected cervical samples and self-collected samples. HPV testing done with a clinically validated PCR-based assay has demonstrated similar clinical accuracy on self-collected and clinician-collected samples in terms of the detection of cervical intraepithelial neoplasia (CIN) grade 2 (CIN2) or worse (CIN2+) and grade 3 (CIN3) or worse (CIN3+) [4–6]. This implies that HPV self-sampling can be a suitable primary screening tool for routine cervical cancer screening [6,7]. Accordingly, several countries have implemented or are considering the introduction of self-sampling as an alternative or additive to clinician-based sampling to improve equitable access to and effectiveness of cervical cancer screening [8].

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A key issue for HPV testing in cervical cancer screening is the use of HPV assays that are clinically validated to ensure an optimal distinction between HPV infections associated with clinically relevant cervical disease, and clinically irrelevant, transient HPV infections to prevent unnecessary referral, excessive diagnostics and overtreatment [9]. Given the relatively low specificity of HPV testing in general, HPV-based screening programs use a secondary method for triaging. Nowadays, the most commonly used triage method is cytology [3]. HPV assays intended for cervical cancer screening must comply with the international guidelines for HPV DNA test requirements [9]. These guidelines delineate a cross-sectional clinical equivalence analysis to compare the performance of the candidate HPV assay head-to-head with a reference assay as well as intra-laboratory reproducibility and inter-laboratory agreement analysis. An increasing number of HPV assays are now considered acceptable for use in primary cervical cancer screening on clinician-collected cervical samples according to these guidelines [10,11]. It is important to note that the use of an HPV assay that is clinically validated for clinician-collected cervical samples may not automatically result in high clinical accuracy when applied to self-collected samples [4,7,12,13]. Therefore, the clinical validation of HPV self-sample workflows (i.e., specific combinations of self-collection device, sample preparation protocol, and HPV assay) remains important prior to their implementation in cervical cancer screening [14,15].

Here, we evaluated the clinical performance and reproducibility of the fully automated NeuMoDx HPV Assay self-sample workflow utilizing the NeuMoDx HPV assay (NeuMoDx™ HPV Test Strip, NeuMoDx molecular, a QIAGEN company, Ann Arbor, MI, USA) and NeuMoDx 96/288 Molecular Systems with self-samples collected with the Evalyn Brush (Rovers Medical Devices, Oss, The Netherlands) by women attending cervical cancer screening as part of the Dutch HPV-based population-based national screening program. The NeuMoDx HPV Assay has previously shown to meet the clinical accuracy and reproducibility criteria of the international guidelines [9] on clinician-collected cervical samples when compared with the standard reference test Hybrid Capture 2 (QIAGEN, Gaithersburg, MD, USA) [16]. In addition, the performance of the NeuMoDx HPV Assay was non-inferior to those of three other clinically validated HPV assay, i.e., HPV-Risk Assay (Self-screen B.V., Amsterdam, The Netherlands), COBAS 4800 HPV test (Roche Molecular Systems, Alameda, CA, USA) and Alinity m HR HPV assay (Abbott Molecular, Des Plaines, IL, USA). The assay demonstrated to be compatible with clinician-collected cervical samples in both the liquid-based cytology media PreservCyt (Hologic Inc., Marlborough, MA, USA) and SurePath (BD Diagnostics–-TriPath, Burlington, NC, USA) [16]. In the current performance evaluation study, the clinical accuracy of the fully automated NeuMoDx HPV Assay self-sample workflow was assessed against reference self-sample workflows using HPV-Risk Assay[17] and high-risk HPV GP5+/6+-PCR [5], and workflow agreement was evaluated on both NeuMoDx 96 and 288 instruments.

2. Methods

2.1. Study population

A study flowchart is shown in Fig. 1. A total of 107 self-samples from women with histologically confirmed CIN2+ (i.e., 42 CIN2, 59 CIN3, 3 adenocarcinomas in situ, 2 squamous cell carcinomas, 1 adenocarcinoma) were used for clinical sensitivity analysis, and 897 self-samples from women without evidence of CIN2+ (referred to as ≤CIN1) in follow-up monitoring (median of 26 months, range 16–30 months) for clinical specificity analysis [9]. The samples for the sensitivity and specificity analysis were age-matched to represent the screening population. A total of 520 self-samples, of which one third tested HPV positive in cervical cancer screening by COBAS 4800 HPV test, was used for analysis of intra-laboratory reproducibility over time and inter-laboratory agreement [9].

2.2. HPV testing

Residual self-samples were tested for HPV DNA using three different PCR-based assays in this study: NeuMoDx HPV Assay, HPV-Risk Assay, and high-risk HPV GP5+/6+-PCR with enzyme immunoassay (EIA) read out.

Self-samples were used for testing in the NeuMoDx HPV Assay self-sample workflow following the manufacturer's instructions using a For Performance Evaluation Only (PEO) version. The NeuMoDx Molecular Systems and NeuMoDx HPV Assay are CE-marked for clinician-collected cervical samples under Regulation (EU) 2017/746 (IVDR). Current study is intended for extending the coverage of certification to self-samples. The NeuMoDx HPV Assay self-sample workflow combines automated DNA extraction and amplification/detection by real-time PCR (target HPV E7 gene) [16]. The NeuMoDx HPV Assay self-sample workflow ultimately uses an equivalence of 0.83 % of original self-sample as input in PCR. The assay provides individual results for HPV16 and HPV18, along with a pooled result for 13 other HPV types (i.e., HPV 31,33,35,39,45,51,52,56,58,59,66,67 and 68; further referred to as "HPV-other"). Human β-globin is detected separately and serves as a qualitative endogenous sample process control to monitor for issues such as poor sampling, presence of inhibitory substances, and system, process or reagent failures [16].

For the HPV-Risk Assay and high-risk HPV GP5+/6+-PCR, DNA was extracted from the self-samples using a silica-based extraction system (NucleoMag 96 tissue kit, Macherey-Nagel GmbH&Co. KG, Düren, Germany) on a Microlab Star robotic system (Hamilton, Gräfelfing, Germany) according to manufacturer's protocol [17]. Both PCR assays were performed with aliquots of the same DNA isolate. The HPV-Risk Assay (target HPV E7 gene) ultimately uses an equivalence of 0.5 % of original self-sample as input in PCR. The assay identifies and distinguishes HPV16 and HPV18 from a pool of 13 other HPV types (HPV31, 33,35,39,45,51,52,56,58,59,66,67 and 68). Human β-globin is amplified as an internal control to determine the quality of the sample DNA and the presence of potential inhibitory substances [17]. Standardized high-risk HPV DNA detection with the clinically validated GP5+/6+-PCR (target HPV L1 gene) was performed essentially as described before [5] using a lab-developed test under ISO 15,189. The high-risk HPV GP5+/6+-PCR ultimately uses an equivalence of 1.33 % of original self-sample as input in PCR. Genotyping of GP5+/6+-PCR products was performed with a bead-based array [18]. A sample was considered invalid in the reference workflows, when Ct value for β-globin in HPV-Risk Assay was >33 and no HPV was detected.

2.3. Data and statistical analysis

All HPV testing was performed blinded for cervical cancer screening results and histological outcome, and data were correlated afterwards. The clinical performance of the NeuMoDx HPV assay self-sample workflow for detection of CIN3+ (primary endpoint) and CIN2+ (secondary endpoint) were assessed. The clinical sensitivity and specificity values of the NeuMoDx HPV Assay self-sample workflow were compared to the performance of both the HPV-Risk Assay and high-risk HPV GP5+−6+-PCR as the standard comparator workflow using a non-inferiority score test according to Tang et al. [19]. with a relative sensitivity threshold of 90 % and a relative specificity threshold of 98 % [9]. For the intra-laboratory reproducibility and inter-laboratory agreement analyses of the NeuMoDx HPV Assay self-sample workflow, the agreement and kappa values according to Cohen's kappa statistics for samples with valid test results were determined. For acceptable results, the 95 % lower confidence bounds of the intra-laboratory reproducibility and inter-laboratory agreement values should both be ≥87 %, with kappa values of ≥0.5 [9]. In addition, intra- and inter-laboratory genotype agreement were determined for samples that tested double-positive. All statistical calculations were performed using SPSS (version 28) and p-values ≤0.05 were considered statistically

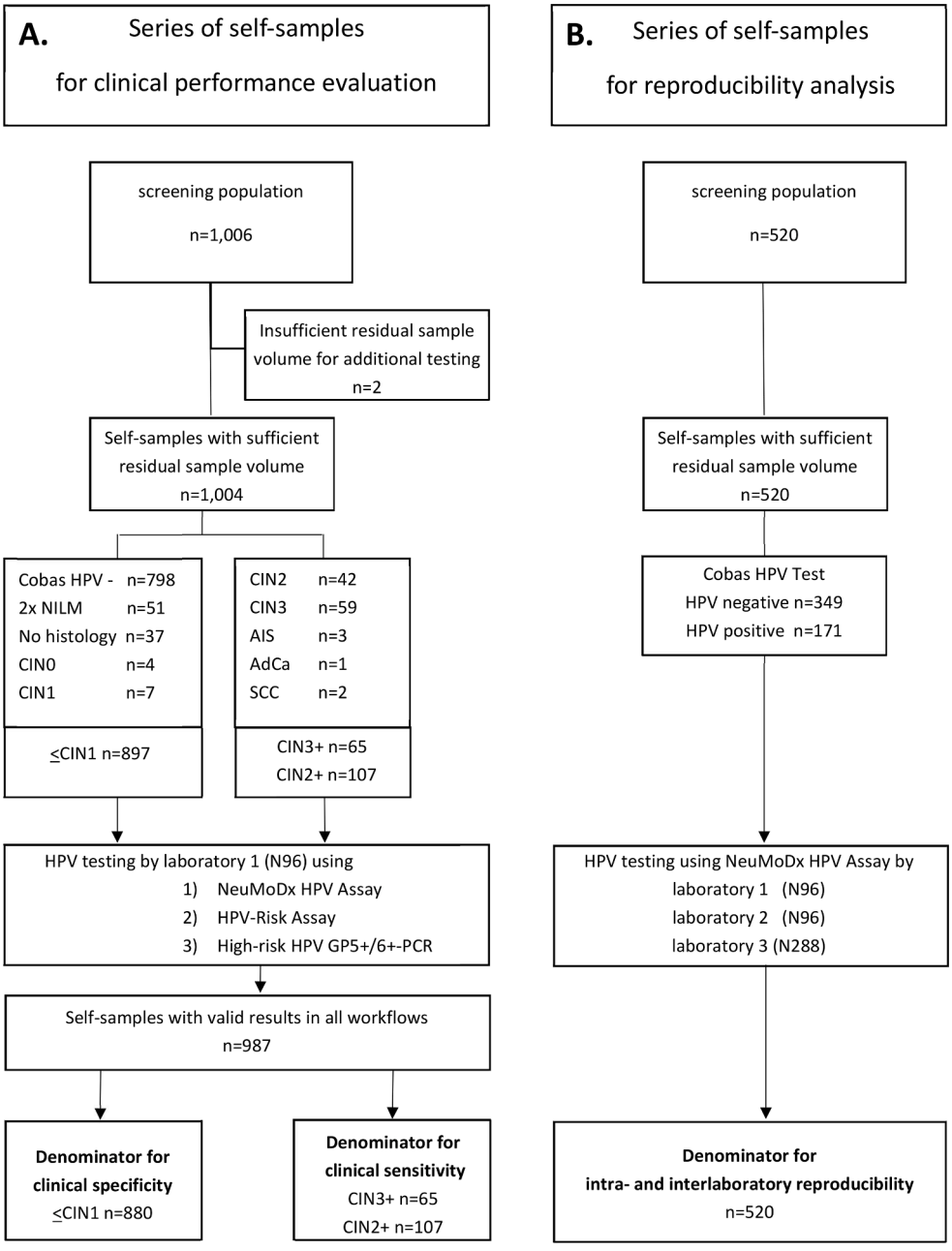

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Fig. 1. Flowchart of the study. The study evaluated the clinical performance (A) and reproducibility (B) of the NeuMoDx HPV Assay self-sample workflow. The study design is based on the international consensus guidelines for HPV DNA tests for primary cervical cancer screening [9], herein applied to a self-sample workflow. The study utilized residual self-collected (cervico-)vaginal samples from women, aged 30 years and older, who attended the Dutch national HPV-based cervical cancer screening program in the region North-Holland of the Netherlands in the period from December 2020 to April 2021. All self-samples were collected using the Evalyn Brush and sent to the screening laboratory (Symbiant B.V., Alkmaar, The Netherlands) by regular post. Upon arrival at the laboratory, dry brushes were resuspended in 20 ml PreservCyt collection medium and according to routine screening protocol tested by COBAS 4800 HPV test following the manufacturer's instructions, with an equivalence of 0.33 % of original self-sample as input in PCR [6]. Residual self-samples were stored prior to testing in the current study, i.e., as per screening protocol for 3 months at room temperature and thereafter at −80 °C for a median of 15 months (range 6–20 months).
Women who were tested HPV positive by COBAS 4800 HPV test in routine screening were triaged by cytology. HPV-positive women with normal cytology (i.e., negative for intraepithelial lesion or malignancy, NILM) at baseline were invited for repeat cytology testing after 6 months. All women who were HPV-positive with atypical squamous cells of undetermined significance (ASC-US) or worse, in the first testing or retesting at 6 months in case of NILM at baseline testing, were referred to colposcopy. Women were referred back for routine screening, if they had NILM at repeat cytology testing (further referred to as 2x NILM). Results of COBAS 4800 HPV test, cytology and histology were available for this study through the Dutch Nationwide Pathology Databank (PALGA).
(A) 1006 self-samples were selected for clinical performance evaluation. Two self-samples had insufficient residual sample volume for applying all testing, leaving 1004 samples for analysis by NeuMoDx HPV assay and comparator assays based on PCR amplification, i.e., HPV-Risk Assay [17] and high-risk HPV GP5+/6+-PCR [5]. Comparative analyses were restricted to samples with valid results in all workflows, i.e., clinical performance evaluation finally comprised 65 self-samples of women with CIN3+ (median age 35 years, min 30 years, max 55 years, 5th percentile 30 years, 95th percentile 50 years), 42 of women with CIN2 (median age 31 years, min 30 years, max 60 years, 5th percentile 30 years, 95th percentile 50 years), and 880 of women without evidence of CIN2+ (referred to as ≤CIN1), median age 42 years, min 30 years, max 67 years, 5th percentile 30 years, 95th percentile 60 years). A total of 98 self-samples of women with ≤CIN1 (11.1 %;95 % CI:9.3–13.3 %) were tested HPV-positive by COBAS 4800 HPV test in routine screening.
(B) 520 self-samples were used for reproducibility experiments. The NeuMoDx HPV Assay was performed twice, in a blinded manner, at a six-week interval at the same laboratory (Amsterdam UMC location Vrije Universiteit Amsterdam, Pathology, Amsterdam, The Netherlands; hereafter referred to as laboratory 1) using N96 System. A third analysis was performed in a blinded manner using a N96 System located at the second laboratory (Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; referred to as laboratory 2) and a fourth analysis was performed in a blinded manner using a N288 System located at the third laboratory (Molecular Diagnostics Unit, Medical Microbiology, Maasstad Ziekenhuis, Rotterdam, The Netherlands; referred to as laboratory 3).
Abbreviations: AdCa, adenocarcinoma; AIS, adenocarcinoma in situ; CIN, cervical intraepithelial neoplasia; Cobas HPV-, negative for human papillomavirus by COBAS 4800 HPV test in cervical cancer screening program, n, number of; 2x NILM, twice negative for intraepithelial lesion or malignancy; no histology, no histology result or follow-up information available by PALGA; SCC, squamous cell carcinoma.

significant.

3. Results

3.1. Clinical accuracy of the NeuMoDx HPV assay self-sample workflow

Clinical sensitivity and specificity of the NeuMoDx HPV Assay self-sample workflow were assessed on a series of 1004 self-samples (Fig. 1). A valid result with the NeuMoDx HPV Assay self-sample workflow was obtained for 1002 (99.8 %) self-samples. In the reference workflows with HPV-Risk Assay and high-risk HPV GP5+6+-PCR, 987 samples (98.3 %) yielded valid test results. Further comparative analyses were restricted to samples with valid results in all workflows, i.e., $n = 987$ including 65 CIN3+, 42 CIN2 and 880 ≤CIN1.

The clinical sensitivity for CIN3+ and CIN2+ of the NeuMoDx HPV Assay self-sample workflow and the reference workflow with HPV-Risk Assay or high-risk HPV GP5+/6+-PCR are presented in Table 1. Cross-tabulations of NeuMoDx HPV Assay self-sample workflow with HPV-Risk assay and high-risk HPV GP5+/6+-PCR results are presented in Table 2. Three self-samples of women with CIN3 and 3 self-samples of women with CIN2 tested negative for high-risk HPV with the NeuMoDx HPV Assay self-sample workflow. The results with HPV-Risk Assay, high-risk HPV GP5+/6+-PCR and COBAS 4800 HPV test for these 6 cases are shown in Table 3A. The relative sensitivity of the NeuMoDx HPV Assay self-sample workflow compared to the reference workflows is presented in Table 4. Both clinical sensitivity for CIN3 + and CIN2 + of the NeuMoDx HPV Assay self-sample workflow were found to be non-inferior to that of the reference workflow with HPV-Risk assay (P_{ni}=0.0046 and P_{ni}=0.0030, respectively) and to that of the reference workflow with high-risk HPV GP5+/6+-PCR (non-inferiority score test, P_{ni}=0.0333 and P_{ni}=0.0110, respectively).

The clinical specificity of the NeuMoDx HPV Assay self-sample workflow and the reference workflows with HPV-Risk Assay and high-risk HPV GP5+/6+-PCR is presented in Table 1. Ninety-two self-samples of control women were positive with the NeuMoDx HPV Assay self-sample workflow. The HPV-Risk Assay, high-risk HPV GP5+/6+-PCR and COBAS 4800 HPV test confirmed HPV-positivity in 74 of these 92 self-samples. For the remaining 18 samples, the results with HPV-Risk

Table 1
Sensitivity and specificity of the NeuMoDx self-sample workflow (A), and the reference workflows with HPV-Risk Assay (B) or high-risk HPV GP5+6+-PCR (C) for detection of CIN3+ and CIN2+.

		Sensitivity							Specificity						
		n	/	N	%	95 % CI			n	/	N	%	95 % CI		
A	**NeuMoDx self-sample workflow**														
	CIN3+	62	/	65	95.4 %	(87.1	–	99.0%)							
	CIN2+	101	/	107	94.4 %	(88.1	–	97.5%)							
	≤CIN1*								788	/	880	89.5 %	(87.3	–	91.5%)
B	**HPV-Risk Assay**														
	CIN3+	61	/	65	93.8 %	(85.0	–	98.3%)							
	CIN2+	102	/	107	95.3 %	(89.3	–	98.0%)							
	≤CIN1*								791	/	880	89.9 %	(87.7	–	91.8%)
C	**GP5+6+-PCR**														
	CIN3+	64	/	65	98.5 %	(89.9	–	99.8%)							
	CIN2+	104	/	107	97.2 %	(91.7	–	99.1%)							
	≤CIN1*								783	/	880	89.0 %	(84.6	–	92.2%)

* women without evidence of CIN2+ in follow-up monitoring
HPV = Human Papillomavirus; CIN3+(2+) = cervical intraepithelial neoplasia grade 3 or worse (2 or worse); n = number of cases; N = total number of cases; CI = confidence interval.

Table 2
Comparison between the NeuMoDx self-sample workflow and the reference workflows with HPV-Risk Assay or high-risk HPV GP5+6+-PCR, stratified by clinical outcome.

	NeuMoDx self-sample workflow	HPV-Risk Assay Positive	Negative	Total	GP5+6+-PCR Positive	Negative	Total
CIN3+							
	Positive	60	2	62	62	0	62
	Negative	1	2	3	2	1	3
	Total	61	4	65	64	1	65
CIN2+							
	Positive	99	2	101	100	1	101
	Negative	3	3	6	4	2	6
	Total	102	5	107	104	3	107
≤CIN1*							
	Positive	79	13	92	78	14	92
	Negative	10	778	788	19	769	788
	Total	89	791	880	97	783	880

* women without evidence of CIN2+ in follow-up monitoring
HPV = Human Papillomavirus; CIN3+(2+) = cervical intraepithelial neoplasia grade 3 or worse (2 or worse).

Table 3
Comparison between the NeuMoDx self-sample workflow, the reference workflows with HPV-Risk Assay or high-risk HPV GP5+6+-PCR and the COBAS 4800 HPV test for (A) case women who tested HPV-negative by NeuMoDx self-sample workflow, and (B) control women who tested positive by NeuMoDx self-sample workflow. As per screening protocol, all case women were positive with COBAS 4800 HPV test. Genotype information of COBAS 4800 HPV test in screening was not disclosed.

	NeuMoDx self-sample workflow	Genotype	HPV-Risk Assay	Genotype	GP5+6+-PCR	Genotype	COBAS 4800 HPV
A.							
CIN3	–	–	–	–	–	–	Positive
CIN3	–	–	–	–	Positive	HPV33	Positive
CIN3	–	–	Positive	Other	Positive	HPV45	Positive
CIN2	–	–	–	–	–	–	Positive
CIN2	–	–	Positive	Other	Positive	HPV31	Positive
CIN2	–	–	Positive	HPV16	Positive	HPV16	Positive
B.							
≤CIN1*	Positive	HPV18	–	–	–	–	–
≤CIN1*	Positive	Other	–	–	–	–	–
≤CIN1*	Positive	Other	–	–	–	–	–
≤CIN1*	Positive	Other	–	–	–	–	–
≤CIN1*	Positive	Other	–	–	–	–	–
≤CIN1*	Positive	Other	–	–	–	–	–
≤CIN1*	Positive	HPV18	–	–	–	–	–
≤CIN1*	Positive	Other	–	–	–	–	–
≤CIN1*	Positive	Other	Positive	Other	–	–	–
≤CIN1*	Positive	Other	Positive	Other	–	–	–
≤CIN1*	Positive	Other	Positive	Other	–	–	–
≤CIN1*	Positive	Other	Positive	Other	–	–	Positive
≤CIN1*	Positive	Other	Positive	Other	–	–	Positive
≤CIN1*	Positive	Other	–	–	Positive	HPV18	–
≤CIN1*	Positive	Other	–	–	Positive	HPV31	Positive
≤CIN1*	Positive	Other	–	–	Positive	HPV52	Positive
≤CIN1*	Positive	Other	–	–	Positive	HPV59	Positive
≤CIN1*	Positive	Other	–	–	–	-	Positive

* women without evidence of CIN2+ in follow-up monitoring
HPV = Human Papillomavirus; CIN3 (2) = cervical intraepithelial neoplasia grade 3 (2).

Assay, high-risk HPV GP5+/6+-PCR and COBAS 4800 HPV test are shown in Table 3B. The NeuMoDx HPV Assay self-sample workflow demonstrated for twelve of these 18 samples signals around the assay threshold for HPV-other. The relative specificity of the NeuMoDx HPV Assay self-sample workflow compared to the two reference workflows is presented in Table 4. The clinical specificity of the NeuMoDx HPV Assay self-sample workflow was found to be non-inferior to that of the reference workflow with HPV-Risk Assay (P_{ni}=0.0088) and to that of the reference workflow with high-risk HPV GP5+/6+-PCR (P_{ni}=0.0005).

3.2. Intra-laboratory reproducibility and inter-laboratory agreement of the NeuMoDx HPV assay self-sample workflow

The intra-laboratory reproducibility over time and inter-laboratory agreement of the NeuMoDx HPV Assay self-sample workflow were determined on 520 self-samples (Fig. 1B). A valid test result was obtained for 99.8 % (519/520) and 99.8 % (519/520) of the samples tested in the two runs performed in laboratory 1 using N96 System, for 99.4 % (517/520) of the samples tested in laboratory 2 using N96 System, and for 99.0 % (515/520) of the samples tested in laboratory 3 using N288 System. The intra-laboratory reproducibility over time was determined to be 94.4 % (489/518;95 %CI:92.5–96.1 %) with a kappa value of 0.86 (95 %CI:0.81–0.91; Table 5). The inter-laboratory agreement (Table 5) was found to be acceptable in all analyses, with 95 % lower confidence bounds of 91.4 % or higher and kappa values of 0.83 or above. In addition, the intra- and inter-laboratory genotyping agreements were found to be adequate (details provided in Table 6).

4. Discussion

In this performance evaluation study, the NeuMoDx HPV Assay self-sample workflow was evaluated in a large series of self-samples from

Table 4
Relative sensitivities for CIN3+ and CIN2+ and relative specificities for ≤CIN1 of the NeuMoDx self-sample workflow versus the reference workflow with HPV-Risk Assay (A); and NeuMoDx self-sample workflow versus the reference workflow with high-risk HPV GP5+6+-PCR (B).

		Relative sensitivity (95 % CI)		Relative specificity (95 % CI)		Pni
A.						
	CIN3+	1.016	(0.962–1.074)			0.0046
	CIN2+	0.990	(0.948–1.034)			0.0030
	≤CIN1*			0.996	(0.984–1.008)	0.0088
B.						
	CIN3+	0.969	(0.927–1.012)			0.0333
	CIN2+	0.971	(0.931–1.014)			0.0110
	≤CIN1*			1.006	(0.992–1.021)	0.0005

* women without evidence of CIN2+ in follow-up monitoring

HPV = Human Papillomavirus; CIN3+(2+) = cervical intraepithelial neoplasia grade 3 or worse (2 or worse); CI = confidence interval; P_{ni}, Non-inferiority p-value.

women participating in routine organized HPV-based screening, and demonstrated high reproducibility and similar accuracy as the reference HPV self-sample workflows using HPV-Risk Assay or high-risk HPV GP5+/6+-PCR. Based on these findings, the NeuMoDx HPV Assay self-sample workflow that utilizes the NeuMoDx HPV assay and NeuMoDx 96/288 Molecular Systems with self-samples collected with the Evalyn Brush, can be considered validated for cervical cancer screening purposes.

Clinical evidence and performance evaluation are critical components in legal regulations for in vitro diagnostic (IVD) medical devices, such as Regulation (EU) 2017/746 (IVDR). Various HPV tests are validated for cervical cancer screening following international consensus guidelines [9] and/or VALGENT validation criteria [20] on clinician-collected cervical samples. However, HPV assays validated for use on self-collected samples in a study sufficiently powered and using samples representative of a screening population, are scarce [5,6,21,22]. Ideally, HPV self-sample workflows would be validated by comparing with clinician-collected cervical samples, where same HPV assay is applied to both sample types collected from the same women. This approach would be rather impracticable within screening setting. Previous studies have therefore mainly used self-samples obtained from women referred for colposcopy [17,23-25]. Since the HPV self-sample workflow using high-risk HPV GP5+/6+-PCR had been clinically validated in a large randomized, non-inferiority trial that was performed within the setting of the Dutch cervical screening programme [5], we herein used this workflow as a reference. The robust validation principles that are established for HPV testing on clinician-based sampling [9] were applied in a validation-extension-design from GP5+/6+-PCR to the NeuMoDx HPV self-sample workflow. This can be considered an acceptable approach for validation in screening setting when matched clinician-collected cervical samples are not available. In addition, comparative analysis included HPV-Risk Assay and COBAS 4800 HPV test. The HPV-Risk Assay has previously been evaluated on self-collected samples from women visiting an outpatient clinic, with high agreement value for HPV (genotype) detection compared to paired clinician-collected samples [17,26]. Given that women in the Dutch screening program were managed based on COBAS 4800 HPV testing, comparison of the clinical sensitivity of the NeuMoDx HPV Assay self-sample workflow with that of COBAS 4800 HPV test cannot be accurately performed. The NeuMoDx HPV Assay self-sample workflow demonstrated non-inferior specificity compared with COBAS 4800 HPV test (P_{ni}=0.0002).

Discrepant results between the NeuMoDx HPV self-sample workflow and the reference HPV self-sample workflows were not related to a specific HPV type and were mostly due to test results around the assay threshold, which is known to be prone to variation [27]. The level of disagreement in results from different HPV assays on primary screening

Table 5
Intra- and inter-laboratory agreement of NeuMoDx self-sample workflow.

lab 1/run 1 N96	**lab 1/run 2 N96** Invalid	Negative	Positive	Total
Invalid	–	1	–	1
Negative	–	363	11	374
Positive	1	18	126	145
Total	1	382	137	520
intra-laboratory agreement	94.4 % (489/518; 95 %CI 92.5–96.1); kappa value 0.86 (95 %CI:0.81–0.91)			
lab 1/run 1 N96	**lab 2 N96** Invalid	Negative	Positive	Total
Invalid	–	1	–	1
Negative	2	357	15	374
Positive	1	19	125	145
Total	3	377	140	520
inter-laboratory agreement	93.4 % (482/516; 95 %CI 91.4–95.3; kappa value 0.83 (95 %CI:0.78–0.89)			
lab 1/run 2 N96	**lab 2 N96** Invalid	Negative	Positive	Total
Invalid	1	–	–	1
Negative	1	365	16	382
Positive	1	12	124	137
Total	3	377	140	520
inter-laboratory agreement	94.6 % (489/517; 95 %CI 92.7–96.2; kappa value 0.86 (95 %CI:0.81–0.91)			
lab 1/run 1 N96	**lab 3 N288** Invalid	Negative	Positive	Total
Invalid	–	1	–	1
Negative	5	360	9	374
Positive	–	23	122	145
Total	5	384	131	520
inter-laboratory agreement	93.8 % (482/514; 95 %CI 91.8–95.6; kappa value 0.84 (95 %CI:0.79–0.89)			
lab 1/run 2 N96	**lab 3 N288** Invalid	Negative	Positive	Total
Invalid	–	1	–	1
Negative	5	367	10	382
Positive	–	16	121	137
Total	5	384	131	520
inter-laboratory agreement	94.9 % (488/514; 95 %CI 93.1–96.5; kappa value 0.87 (95 %CI:0.82–0.92)			
lab 3 N288	**lab 2 N96** Invalid	Negative	Positive	Total
Invalid	–	5	–	5
Negative	3	364	17	384
Positive	–	8	123	131
Total	3	377	140	520
inter-laboratory agreement	95.1 % (487/512; 95 %CI 93.3–96.7; kappa value 0.87 (95 %CI:0.83–0.92)			

samples from women above 30 years of age is in line with earlier observations [27]. Intra- and interlaboratory agreements, overall and at the genotype level, were adequate and workflow reproducibility was demonstrated for both N96 and N288 Systems.

The main strengths of this study are the use of self-samples that are representative of an organised HPV-based cervical cancer screening setting, and a sample size that provides sufficient power to verify clinical accuracy and reproducibility [9]. A possible limitation of the study may be that our results could be biased because no histological endpoint was obtained for a number of HPV-positive control women. However, it is unlikely that this would have had a marked impact on the outcome of this study since most women without a histology endpoint were reported with normal cytology results.

In conclusion, the NeuMoDx HPV self-sample workflow demonstrated a high sensitivity and a high specificity for the detection of CIN2+/3+, along with a high intra- and inter-laboratory reproducibility. Based on the study findings, the NeuMoDx HPV self-sample workflow can be considered suitable for cervical cancer screening

Table 6
Intra- and inter-laboratory genotype agreement of NeuMoDx self-sample workflow.
Concordant genotype findings were defined as complete agreement, compatible as having at least one genotype category in common, and discordant as no similarity between detected genotype categories.

lab 1/run 1 N96	**lab 1/run 2 N96**							
	HPV16	HPV18	HPV Other	HPV16, 18	HPV16, Other	HPV18, Other	HPV16, 18, Other	Total
HPV16	9	–	2	–	1	–	–	12
HPV18	–	13	–	–	–	1	–	14
HPV Other	–	–	72	–	1	–	–	73
HPV16, 18	–	–	–	1	–	–	–	1
HPV16, Other	2	–	4	–	7	–	–	13
HPV18, Other	–	–	–	–	–	8	1	9
HPV16, 18, Other	–	–	–	–	–	–	4	4
Total	11	13	78	1	9	9	5	126
Genotype agreement: concordant 90.5 % (114/126); compatible 7.9 % (10/126); discordant 1.6 % (2/126)								
lab 1/run 1 N96	**lab 2 N96**							
	HPV16	HPV18	HPV Other	HPV16, 18	HPV16, Other	HPV18, Other	HPV16, 18, Other	Total
HPV16	9	–	–	–	2	–	–	11
HPV18	–	12	–	–	–	1	–	13
HPV Other	–	–	73	–	2	–	–	75
HPV16, 18	–	–	–	–	–	–	1	1
HPV16, Other	3	–	4	–	5	–	–	12
HPV18, Other	–	–	–	–	–	8	1	9
HPV16, 18, Other	–	–	–	–	–	–	4	4
Total	12	12	77	0	9	9	6	125
Genotype agreement: concordant 88.8 % (111/125); compatible 11.2 % (14/125); discordant 0 % (0/125)								
lab 1/run 2 N96	**lab 2 N96**							
	HPV16	HPV18	HPV Other	HPV16, 18	HPV16, Other	HPV18, Other	HPV16, 18, Other	Total
HPV16	10	–	–	–	1	–	–	11
HPV18	–	11	–	–	–	1	–	12
HPV Other	–	–	75	–	3	–	–	78
HPV16, 18	–	–	–	–	–	–	1	1
HPV16, Other	2	–	1	–	5	–	–	8
HPV18, Other	–	1	–	–	–	8	–	9
HPV16, 18, Other	–	–	–	–	–	–	5	5
Total	12	12	76	0	9	9	6	124
Genotype agreement: concordant 91.9 % (114/124); compatible 8.1 % (10/124); discordant 0 % (0/124)								
lab 1/run 1 N96	**lab 3 N288**							
	HPV16	HPV18	HPV Other	HPV16, 18	HPV16, Other	HPV18, Other	HPV16, 18, Other	Total
HPV16	9	–	–	–	–	–	–	9
HPV18	–	14	–	–	–	–	–	14
HPV Other	–	–	68	–	3	1	–	72
HPV16, 18	–	–	–	1	–	–	–	1
HPV16, Other	4	–	4	–	5	–	–	13
HPV18, Other	–	2	–	–	–	7	–	9
HPV16, 18, Other	–	–	–	–	–	–	4	4
Total	13	16	72	1	8	8	4	122
Genotype agreement: concordant 88.5 % (108/122); compatible 11.5 % (14/122); discordant 0 % (0/122)								
lab 1/run 2 N96	**lab 3 N288**							
	HPV16	HPV18	HPV Other	HPV16, 18	HPV16, Other	HPV18, Other	HPV16, 18, Other	Total
HPV16	9	–	–	–	–	–	–	9
HPV18	–	13	–	–	–	–	–	13
HPV Other	1	–	70	–	3	1	–	75
HPV16, 18	–	–	–	1	–	–	–	1
HPV16, Other	3	–	1	–	5	–	–	9
HPV18, Other	–	3	–	–	–	6	–	9
HPV16, 18, Other	–	–	–	–	–	1	4	5
Total	13	16	71	1	8	8	4	121
Genotype agreement: concordant 89.3 % (108/121); compatible 9.9 % (12/121); discordant 0.8 % (1/121)								
lab 3 N288	**lab 2 N96**							
	HPV16	HPV18	HPV Other	HPV16, 18	HPV16, Other	HPV18, Other	HPV16, 18, Other	Total
HPV16	9	–	–	–	4	–	–	13
HPV18	–	13	–	–	–	3	–	16
HPV Other	1	–	73	–	–	–	–	74
HPV16, 18	–	–	–	–	–	–	1	1
HPV16, Other	–	–	2	–	5	–	–	7
HPV18, Other	–	–	1	–	–	6	1	8
HPV16, 18, Other	–	–	–	–	–	–	4	4
Total	10	13	76	0	9	9	6	123
Genotype agreement: concordant 89.4 % (110/123); compatible 9.8 % (12/123); discordant 0.8 % (1/123)								

purposes.

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Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki. The National Institute for Public Health and Environment was informed about the protocol before start of the study and declared no objection. The institutional review board granted ethical approval for the study (Medical Ethics Committee of Amsterdam UMC, location VUmc, number U2022–364).

Informed consent statement

Patient consent was waived for the use of anonymized residual samples procured during regular screening. For the Dutch screening program women have been informed that residual material can be used for anonymous research and they have the opportunity to opt out. Only self-samples of women who did not opt out were used.

Data availability

The data presented in this study are available upon reasonable request from the corresponding author.

CRediT authorship contribution statement

D.A.M. Heideman: Writing – review & editing, Writing – original draft, Resources, Methodology, Conceptualization. **J. Berkhof:** Writing – review & editing, Methodology, Conceptualization. **L. Verhoef:** Writing – review & editing, Data curation. **C. Ouwerkerk:** Writing – review & editing, Investigation, Data curation. **P.W Smit:** Writing – review & editing, Resources, Investigation. **A. Oštrbenk Valenčak:** Writing – review & editing, Resources. **J. Mlakar:** Writing – review & editing, Investigation. **M. Poljak:** Writing – review & editing, Resources. **R.D.M. Steenbergen:** Writing – review & editing, Resources, Conceptualization. **M.C.G. Bleeker:** Writing – review & editing, Resources, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

(1) DAMH and RDMS are minority shareholders of Self-screen B.V., a spin-off company of VUmc (currently known as Amsterdam UMC, Vrije Universiteit Amsterdam); Self-screen B.V. develops, manufactures and licenses high-risk HPV and methylation marker assays for cervical cancer screening and holds patents on these tests;

(2) JB had financial support from the European Commission (RISCC, grant number 847845);

(3) AOV has received reimbursement of travel expenses for attending conferences and honoraria for speaking from Abbott Molecular, Qiagen and Seegene;

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(5) MP and JM declare no personal conflicts of interest. MP's, AOV's and JM's institution received research funding, free-of-charge reagents, and consumables to support research in the last 3 years from Qiagen, Seegene, Abbott, and Roche, all paid to their employer; and

(6) all other authors declare no conflicts of interest.

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