Extended HPV Genotyping to Compare HPV Type Distribution in Self- and Provider-Collected Samples for Cervical Cancer Screening

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ABSTRACT

Background: Primary high-risk human papillomavirus (hr-HPV) testing of self-collected cervico-vaginal swabs could increase cervical cancer screening coverage, although triage strategies are needed to reduce unnecessary colposcopies. We evaluated the use of extended hr-HPV genotyping of self-collected samples for cervical cancer screening.

Methods: We recruited women ages 25–65 years at two colposcopy clinics in North Carolina between November 2016 and January 2019, and obtained self-collected cervico-vaginal samples, provider-collected cervical samples, and cervical biopsies from all enrolled women. Self- and provider-collected samples were tested for 14 hr-HPV genotypes using the Onclarity Assay (Becton Dickinson). We calculated hr-HPV genotype–specific prevalence and assessed agreement between results in self- and provider-collected samples. We ranked the hr-HPV genotypes according to their positive predictive value (PPV) for the detection of cervical intraepithelial neoplasia (CIN) grade 2 or higher (CIN2+).

Results: A total of 314 women participated (median age, 36 years); 85 women (27%) had CIN2+. More women tested positive for any hr-HPV on self-collected (76%) than on provider-collected samples (70%; $P = 0.009$) with type-specific agreement ranging from substantial to almost perfect. HPV-16 was the most common genotype in self-collected (27%) and provider-collected samples (20%), and HPV-16 prevalence was higher in self- than provider-collected samples ($P < 0.001$). In self- and provider-collected samples, HPV-16 had the highest PPV for CIN2+ detection.

Conclusions: Overall sensitivity for CIN2+ detection was similar for both sample types, but the higher HPV-16 prevalence in self-collected samples could result in increased colposcopy referral rates.

Impact: Additional molecular markers might be helpful to improve the triage of women who are hr-HPV positive on self-collected samples.

Introduction

Almost all cervical cancer cases are caused by oncogenic high-risk (hr) human papillomavirus (HPV) types (1). However, not all hr-HPV types have the same potential for causing progression to cervical cancer (2–5). In 2018, the U.S. Preventive Services Task Force proposed primary hr-HPV testing or a combination of Pap and hr-HPV testing (cotesting) every 5 years for women ages 30–65 years (6). Current guidelines of the American Society for Colposcopy and Cervical Pathology recommend immediate referral to colposcopy for women who are cytology negative for intraepithelial lesion or malignancy (NILM) if they are infected with hr-HPV genotypes 16 or 18, irrespective of HPV vaccination status (7). This screening algorithm was designed because HPV-16 and HPV-18 are the two most common types associated with cervical cancer, accounting for approximately 60% and 10%–15% of cervical cancers, respectively (3, 8). HPV types 31, 33, 45, 52, and 58 combined account for another 19% of cervical cancer cases (9). However, the introduction of HPV vaccinations has led to a shift in the HPV genotype distributions with lower HPV-16/18 prevalence among younger immunized women as compared with unimmunized women (10, 11). Extended hr-HPV genotyping beyond HPV-16/18 could serve as a triage strategy and aid in optimizing the detection of clinically important high-grade cervical dysplasia while simultaneously minimizing unnecessary colposcopy referrals for cases of transient or nonprogressing HPV infection (5, 12–14).

Most cervical cancer cases occur in underscreened women (15–17). Main barriers to care include limited access to costly screening, lack of transportation, and personal reasons including embarrassment, fear of finding cancer, and anxiety about undergoing a pelvic examination (18). Many of these barriers could be addressed by accurate and low-cost hr-HPV self-sampling strategies. Most women prefer self-collected cervico-vaginal brushes/swabs over provider-collected

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samples for hr-HPV testing (19, 20). However, self-collection for hr-HPV testing is not yet FDA approved for clinical use.

Many different HPV tests exist, but currently, only the cobas 4800 Assay (Roche Diagnostics) and the Onclarity Assay (Becton Dickinson) are FDA approved with a clinical indication for primary hr-HPV screening. The cobas assay provides individual hr-HPV results for HPV-16 and 18, but not for the other hr-HPV types. The Onclarity Assay has the potential to provide extended genotyping results by simultaneously detecting DNA from 14 hr-HPV types: six types are individually genotyped (HPV-16, 18, 31, 45, 51, and 52) and the remaining eight types in three groups (33/58, 56/59/66, and 33/39/68; ref. 21).

In this study, we evaluated the potential use of extended hr-HPV genotyping of self-collected cervico-vaginal samples for cervical cancer screening. Specifically, using the Onclarity Assay, we compared the hr-HPV type distributions in self- and provider-collected samples stratified by cervical lesion grade, assessed agreement between self- and provider-collected samples for hr-HPV positivity, and computed positive predictive values (PPV) of different hr-HPV genotypes for the detection of cervical intraepithelial neoplasia (CIN) grade 2 or higher (CIN2+).

Materials and Methods

Study population

Between November 2016 and January 2019, we recruited a convenience sample of women ages 25–65 years attending colposcopy clinics at either the University of North Carolina (UNC) Women's Hospital (Chapel Hill, NC) or Duke University Hospital (Durham, NC) for one of the following reasons: (i) abnormal cytology results, (ii) infection with HPV-16 or 18, (iii) persistent infection with other hr-HPV genotypes, or (iv) treatment for CIN2+. In addition, we invited women to participate in the study if they were NILM on cytology, but positive for hr-HPV genotypes other than 16 or 18 at their routine screening. This group was referred to as "research only," because immediate referral for colposcopy is currently not recommended for these women (7).

Potentially eligible women were selected through a review of electronic medical records and were contacted via phone or during their clinic visit to ask whether they would participate. Women were excluded from participation if they were pregnant or had their cervix removed; additionally, women in the "research only" group were excluded if they were taking blood thinners or if the enrollment date was not within 3 months of their original hr-HPV diagnosis. Women were not asked to abstain from sexual intercourse before the study visit. Written informed consent was obtained from each eligible woman willing to participate, and the study was approved by the Institutional Review Boards (IRB) of UNC (Chapel Hill, NC, IRB# 15-2872) and of Duke University (Durham, NC, IRB# Pro00083075).

Sample collection

During the clinic visit, participating women received detailed verbal and written instructions concerning the study procedures in either English or Spanish. Women self-collected a cervico-vaginal sample by inserting a Viba-Brush (Rovers Medical Devices BV) to the top of the vaginal canal, rotating five times, removing it, and releasing the brush head into a vial prefilled with 6 mL of preservative liquid–based Cytology Media (ThinPrep, Hologic Inc.). A urine pregnancy test was performed when clinically indicated.

Next, women underwent a pelvic examination, during which the clinical provider collected a cervical scraping with two 360° turns in a clockwise fashion of a brush-like cervical cell collector (Wallach Papette, Wallach Surgical Devices). The provider-collected cervical sample was preserved in a standard 20 mL vial of ThinPrep media for subsequent hr-HPV testing. Colposcopy was performed on all participating women, following cervical treatment with 3%–5% acetic acid (followed by Lugol iodine at the Duke site), according to standard clinical procedures. Directed biopsies were taken from visible cervical lesions, and an endocervical curettage (ECC) was performed if the transformation zone or the limits of a lesion near the cervical os could not be fully visualized. If no cervical lesions were observable, one random biopsy at the 12 o'clock position of the cervix was taken and an ECC was performed. Loop electrosurgical excision procedure was performed when clinically indicated. At the end of the visit, women received a gift card for their participation in the study.

Sample processing and laboratory analyses

All samples were placed in a cooler with frozen gel packs within 10 minutes of sample collection and kept cool until they could be further aliquoted the same day. The self- and provider-collected samples were vortexed for 10–30 seconds and 0.5 mL of each was transferred to separate BD Molecular Tubes containing 1.7 mL of an HPV diluent buffer. Initially, samples were not vortexed. When this laboratory error was discovered, samples were realiquoted after vortexing them for 10–30 seconds. However, 69 provider-collected samples and three self-collected samples were not available for realiquoting and, therefore, had to be excluded from the analysis. The tubes were fitted with a pierceable cap to facilitate automated sample processing on the BD Viper LT System. The tubes were stored at −20°C until shipment to BD for hr-HPV testing using the Onclarity Assay (Becton Dickinson). The staff who performed the hr-HPV testing at BD did not have access to any clinical information including cervical histology results of the participants. The BD Onclarity Assay uses PCR and nucleic acid hybridization to detect DNA of 14 hr-HPV genotypes. Six hr-HPV genotypes (16, 18, 31, 45, 51, and 52) are individually genotyped, and the remaining eight are identified in three groups (33/58, 56/59/66, and 35/39/68). Self- and provider-collected specimens were processed using the standard liquid-based cytology workflow on the BD Viper LT System. We used the following established PCR cycle thresholds (Ct) for both self- and provider-collected samples: ≤38.3 for HPV-16 and ≤34.2 for all other hr-HPV genotypes. The remaining provider-collected cervical sample was sent to the UNC cytopathology laboratory for liquid-based ThinPrep cytological analysis, if clinically indicated, or otherwise stored at −20°C.

Cervical biopsies from participating women who underwent a colposcopy as part of their scheduled clinical appointment were sent to UNC (Chapel Hill, NC) or Duke Hospital (Durham, NC) Surgical Pathology Laboratory for histologic evaluation per standard clinical procedures. Pathologists had access to clinical information captured in the electronic medical records, but were unaware of the study hr-HPV test results. Women who underwent a clinically indicated colposcopy were informed of their histologic results by the clinical team per standard of care. Biopsies taken from women in the "research only" group, who underwent the colposcopy for study purposes, were histologically analyzed by a gynecologic pathologist (S. O'Connor) at the UNC (Chapel Hill, NC) translational pathology laboratory, who did not have access to any additional clinical information. Women in the "research only" group were contacted with histologic results by the study team after review by two practicing gynecologists (L. Rahangdale and A.K. Knittel). If CIN2+ was detected, the women were referred to further treatment as per standard of care.

Statistical analyses

We included women with valid hr-HPV test results on both self- and provider-collected samples and valid cervical histology results. We compared sociodemographic characteristics and CIN2+ status between eligible women with and without matched self- and provider-collected samples using Fisher exact test. We calculated hr-HPV genotype–specific prevalence stratified by cervical lesion grade and compared genotype-specific prevalence between self- and provider-collected samples using the McNemar test. We computed the unweighted Cohen kappa and its 95% confidence interval (CI) to assess agreement beyond chance between hr-HPV results in self- and provider-collected samples. We calculated Cohen kappa overall, stratified by cervical lesion grade, and for specific hr-HPV genotypes. We used the following interpretation for Cohen kappa: ≤0, no agreement; 0.01–0.20, slight agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement; and 0.81–1.00, almost perfect agreement (23). We ranked the hr-HPV genotypes detected in self- and provider-collected samples according to their PPVs for the detection of CIN2+ from the highest to the lowest PPVs, excluding women with multiple-type infections. In a sensitivity analysis, we ranked the genotypes on the basis of both single- and multiple-type infections. For this analysis, PPVs were calculated after excluding women who had one of the previously ranked genotypes. We computed cumulative sensitivity and specificity for the detection of CIN2+ based on the sequential addition of genotypes according to the obtained order. In a second sensitivity analysis, we ranked the genotypes on the basis of their PPVs for detection of CIN3+. All analyses were done using SAS/STAT (SAS Institute Inc.), Stata 15 (StataCorp LLC), and R software (R Foundation for Statistical Computing).

In total, 434 women (363 with an indication for colposcopy and 71 in the "research only" group) were enrolled in the study (**Fig. 1**). Of these, 413 women had valid histology results and were available for analysis. However, 70 provider-collected samples and 33 self-collected samples had hr-HPV results that were either invalid or unavailable. Thus, the analyses reported here are based on data from 314 women with valid hr-HPV results for both the self-collected cervico-vaginal and the provider-collected cervical samples. Baseline characteristics, such as age, race/ethnicity, marital status, education, health insurance, and smoking status, as well as CIN2+ status, did not significantly differ (Supplementary Table S1) between eligible women with ($N = 314$) and without ($N = 99$) matched samples.

Data availability

The data that support the findings of this study are available on request from the corresponding author, in accordance to the NIH guidelines on data sharing, as proposed in our original grant submission.

Results

Study population

The median age of the 314 participating women was 36 years (interquartile range, 31–45 years). The study population was ethnically and racially diverse with 38% non-Hispanic White ($N = 120$), 29% Hispanic ($N = 92$), 26% non-Hispanic Black ($N = 82$), and 6% women with other racial identities ($N = 20$; **Table 1**). Eighty-five (27%) women were diagnosed with CIN2+.

Hr-HPV prevalence

Overall, more women tested positive for any hr-HPV on self-collected samples ($N = 239$, 76%) than on provider-collected samples ($N = 220$, 70%; $P = 0.009$; **Fig. 2A**). Multiple-type hr-HPV infections were also more common in self-collected samples ($N = 76$, 24%) than in provider-collected samples ($N = 44$, 14%; $P < 0.001$). HPV-16 was the most common hr-HPV type detected in self-collected samples ($N = 85$, 27%) and in provider-collected samples ($N = 62$, 20%). Prevalence of HPV-16 was significantly higher in self-collected cervico-vaginal samples than in provider-collected cervical samples ($P < 0.001$). Especially, prevalence of multiple-type HPV-16 infections was higher in self-collected versus provider-collected samples (**Table 2**). When we restricted the analysis to women with CIN2+ or CIN3+, HPV prevalence did not significantly differ between self-collected cervico-vaginal samples and provider-collected cervical samples (**Fig. 2B**; **Table 2**). Among women with a histologic diagnosis of <CIN2, the pattern of a higher hr-HPV prevalence in self-collected than provider-collected samples was observed across all hr-HPV genotypes, although differences by collection site reached statistical significance only for any hr-HPV ($P = 0.004$), HPV-16 ($P < 0.001$), HPV-33/58 ($P = 0.046$), and HPV-35/39/68 ($P = 0.008$). Supplementary Tables S2 and S3 show prevalence of hr-HPV genotypes in self- and provider-collected samples among all 85 CIN2+ cases, stratified by race/ethnicity and age, respectively.

Agreement between hr-HPV results in self- and provider-collected samples

Overall agreement between self- and provider-collected samples for any hr-HPV was 83% (Supplementary Table S4); agreement beyond chance was moderate with a Cohen kappa value of 0.57 (95% CI, 0.47–0.67). Type-specific agreement beyond chance among all included women ranged from substantial (Cohen kappa, 0.69; 95% CI, 0.60–0.79) for HPV-16 to almost perfect (Cohen kappa, 0.95; 95% CI, 0.87–1.00) for HPV-51 (**Fig. 3A**). When we restricted the analysis to women with CIN2+, agreement beyond chance remained moderate overall (Cohen kappa, 0.51; 95% CI, 0.14–0.89) and ranged from substantial to perfect for specific hr-HPV genotypes (**Fig. 3B**). Among the 85 CIN2+ cases, 31 cases were positive for HPV-16 on both sample types, eight cases on self-collected specimens only, and three cases on provider-collected specimens only.

Cumulative sensitivity and specificity of different hr-HPV genotypes for CIN2+ detection

In both self- and provider-collected samples, positivity for HPV-16 had the highest PPV for the detection of CIN2+ when considering single-type infections only (**Table 3**). However, the subsequent order of hr-HPV genotypes selected to maximize the PPVs among the remaining samples varied slightly between self- and provider-collection. In provider-collected samples, the order was HPV-16, 31, 33/58, 52, 35/39/68, 51, 45, 56/59/66, and 18. In self-collected samples, the order was HPV-16, 31, 51, 33/58, 52, 18, 35/39/68, 45, and 56/59/66. Positivity for HPV-16 alone for the detection of CIN2+ had a sensitivity of 34.4% in provider-collected samples and 40.7% in self-collected samples; specificity was 90.0% and 86.4%, respectively. By adding hr-HPV genotypes to the algorithm in the determined order, the cumulative sensitivity for detection of CIN2+ increased to 91.8% in provider-collected and 88.9% in self-collected specimens when all 14 hr-HPV genotypes were included (**Table 3**). Cumulative specificity decreased to 42.6% in provider-collected and 37.5% in self-collected samples when an algorithm based on all 14 hr-HPV genotypes was used.

When including women with multiple-type infections, we found that HPV-16, 33/58, 51, and 31 had the highest PPVs for CIN2+

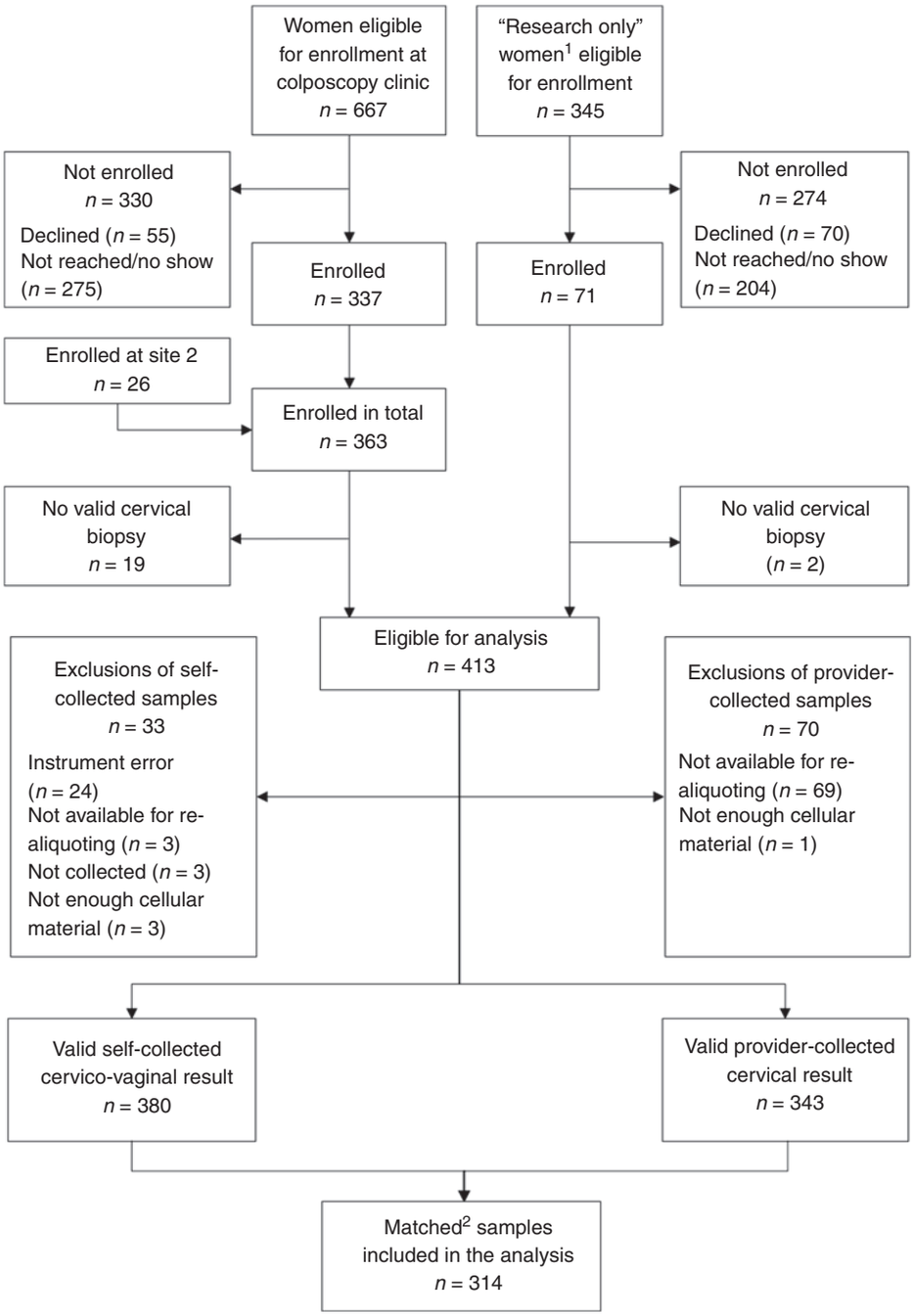

Figure 1.
Study flow diagram. [1] "Research only" women were NILM on cytology, but positive for hr-HPV genotypes other than 16 or 18. [2] Matched self-collected and provider-collected samples available for the same participant.

detection in both provider- and self-collected samples (Supplementary Table S5). In provider-collected samples, the order was HPV-16, 33/58, 51, 31, 52, 35/39/68, 45, 56/59/66, and 18. In self-collected samples, the order was HPV-16, 51, 33/58, 31, 52, 18, 56/59/66, 45, and 35/39/68. Cumulative sensitivity based on all 14 hr-HPV genotypes for single- and multiple-type infections combined was 94.1% in provider-collected samples and 92.9% in self-collected samples. For the detection of CIN3+, HPV-16, 33/58, and 52 had the highest PPVs in both provider- and self-collected samples on the basis of single- and multiple-type infections combined (Supplementary Table S6).

Discussion

Among women with histologically confirmed CIN2+ included in our study, the prevalence of hr-HPV in provider-collected (94%) and self-collected samples (93%) was similar. Among all 314 participants, however, more women tested positive for any hr-HPV on self-collected cervico-vaginal samples (76%) than on provider-collected cervical samples (70%). Overall agreement between self- and provider-collected samples was moderate (kappa = 0.57), but type-specific agreement ranged from substantial (e.g., HPV-16) to almost perfect (e.g., HPV-51). On the basis of single-type infections only, HPV-16

Table 1. Characteristics of and hr-HPV positivity on self- or provider-collected samples among 314 study participants[a].

	Overall *N* = 314 *n* (%)	Self-collection hr-HPV positive[b] *n* = 239 *n* (%)	Provider-collection hr-HPV positive[b] *n* = 220 *n* (%)
Age groups (years)			
25–29	56 (18%)	46 (19%)	45 (20%)
30–39	135 (43%)	110 (46%)	98 (45%)
40–49	69 (22%)	45 (19%)	43 (20%)
50–65	54 (17%)	38 (16%)	34 (15%)
Race and ethnicity			
Hispanic	92 (29%)	69 (29%)	72 (33%)
Non-Hispanic Black	82 (26%)	66 (28%)	51 (23%)
Non-Hispanic White	120 (38%)	88 (37%)	83 (38%)
Other[c]	20 (6%)	16 (7%)	14 (6%)
Marital status			
Married or living with a partner	119 (39%)	87 (38%)	85 (40%)
Divorced, separated, widowed	82 (27%)	64 (28%)	56 (26%)
Single/never married	101 (33%)	80 (35%)	71 (33%)
Missing	12	8	8
Education			
Elementary school or less	27 (9%)	20 (9%)	20 (9%)
High school	93 (31%)	68 (29%)	69 (33%)
Some college	106 (35%)	80 (35%)	69 (33%)
College graduate	77 (25%)	63 (27%)	54 (25%)
Missing	11	8	8
Health insurance			
Private insurance	120 (39%)	91 (38%)	80 (37%)
Medicaid/Medicare/TRICARE	71 (23%)	52 (22%)	43 (20%)
None/uninsured	120 (39%)	94 (40%)	95 (44%)
Missing	3	2	2
Current smoker			
Yes	72 (23%)	55 (23%)	53 (25%)
No	238 (77%)	180 (77%)	163 (75%)
Missing	4	4	4

[a]Study population includes 250 women (80%) with a clinical indication for colposcopy and 64 women (20%) who underwent the colposcopy for study purposes.
[b]Positive for any of the 14 hr-HPV types detected by the Onclarity Assay.
[c]Includes Asian (10), American Indian/Alaskan Native (5), Native Hawaiian/Other Pacific Islander (1), Black Indian (1), Mediterranean (1), and not further specified (2).

and -31 had the highest PPVs for CIN2+ in both self- and provider-collected samples.

HPV prevalence was somewhat higher in self-collected cervico-vaginal compared with provider-collected cervical samples in this study, which is consistent with some studies (24–27), although other studies have shown comparable (28, 29) or slightly lower HPV prevalence in self-collected samples (30, 31). However, when summarized in a systematic review and meta-analysis, HPV prevalence in self- and provider-collected samples is similar when highly sensitive HPV assays are used (32). In-line with others (28, 33), we also found a higher prevalence of multiple-type infections in self-collected cervico-vaginal as compared with provider-collected cervical samples. The sampling order could have played a role in finding a higher HPV prevalence in self-collected samples, as the sample obtained first might have yielded more exfoliated cells and, therefore, more HPV DNA, than the second sample. The sequence of sample collection in our study was not randomized, with self-collection always occurring before provider-collection. However, a randomized clinical trial found that hr-HPV detection was not affected by the sequence in which self- and provider-collection were performed (34). We provided participating women with comprehensive self-collection instructions in English and Spanish, but incorrect performance of self-collection might still have affected the hr-HPV test results obtained for self-collected samples. Although, in an acceptability study based on the same study population, we found that women generally thought that the self-collection was easy to perform (35). Furthermore, in our study, the self-collected sample was approximately 3.3-fold more concentrated than the provider-collected sample (resuspended in 6 vs. 20 mL ThinPrep), which might also have contributed to the higher HPV prevalence in self-collected samples. The unequal sample concentrations may have affected genotypes differentially, given that the Ct cutoff to determine positivity was higher for HPV-16 (≤38.3) than for all other hr-HPV genotypes (≤34.2). However, we used the same established Ct cutoffs for both self- and provider-collected samples. Another potential explanation for the higher HPV prevalence in self-collected samples is that vaginal HPV infections may be acquired earlier in time than cervical infections (36), and that not all vaginal HPV infections will go on to infect the cervix.

In-line with other studies (25, 28), we have shown good type-specific agreement, indicating that HPV detected in cervico-vaginal samples is representative of cervical HPV infections. High type-specific agreement is essential for self-sampling to be a valid alternative to provider-based hr-HPV testing for cervical cancer screening, as the ultimate goal is to prevent cervical disease. Our finding that only few HPV-16 infections among CIN2+ cases detected in provider-collected cervical samples were missed by the self-collected samples is reassuring, as this is the hr-HPV genotype most commonly associated with cervical

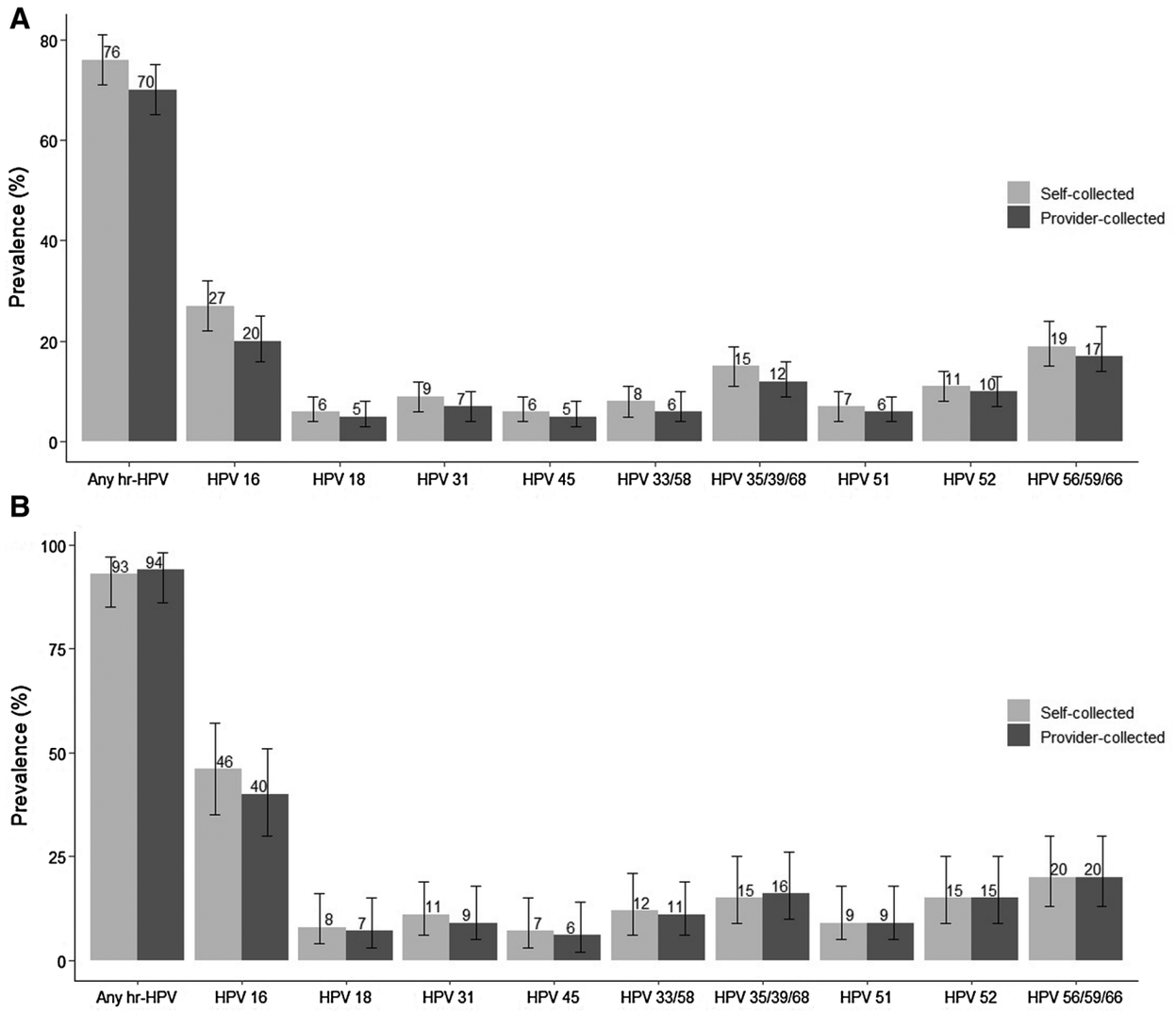

Figure 2.
Prevalence of any hr-HPV and specific genotypes with 95% CIs in all (**A**) self-collected cervico-vaginal and provider-collected cervical samples and among CIN2+ cases (**B**).

cancer (3, 8). However, the higher observed HPV-16 prevalence in self- compared with provider-collected samples would also result in higher colposcopy referral rates. Additional triage strategies should be considered to avoid unnecessary referrals and overtreatment among women who are positive for HPV-16 on self-collected cervico-vaginal samples, but do not have CIN2+.

A recent meta-analysis showed that PCR-based hr-HPV assays were similarly accurate for the detection of CIN2+ in self- and in provider-collected samples indicating that self-collected samples can substitute for provider-collected samples to reach underscreened women (37). In high-risk study participants enriched for CIN2+ outcomes, the meta-analysis reported sensitivities of approximately 90% and specificities of approximately 50% for PCR-based hr-HPV testing in self- and provider-collected samples for the detection of CIN2+ (37). In our study, we found similar sensitivities of approximately 90% for CIN2+ detection, but lower specificities of 30%–40%. Our specificity estimates increased substantially to approximately 85% when we used a primary screening algorithm based on HPV-16 only, consistent with other studies (38, 39), although our sensitivity estimates dropped to 40%–46% using this strategy. We and others (40, 41) found HPV-33, 16, and 31 to be among the genotypes with the highest PPVs for CIN2+ detection. This is also in-line with studies that identified HPV-16 and 31 as the genotypes with the highest risk for CIN2+ among women with normal (11) or low-grade cervical cytology (14). HPV-51 had a relatively high PPV for CIN2+ detection in our study, especially when multiple-type infections were included in a sensitivity analysis, but was considered intermediate risk for CIN2+ in other studies (40, 41). Of note, one of these studies was cross-sectional (40), like ours, whereas the other used histologic findings obtained over a 3-year follow-up period (41). Interestingly, HPV-18 and 45, two hr-HPV genotypes commonly associated with cervical cancer (3, 8, 9), ranked low in our ordering of genotypes based on PPVs for CIN2+. This might be partly attributable to a more rapid trajectory for progression given infection with these two HPV types (42). Alternatively, HPV-18 and 45, both of

Table 2. Counts and prevalence of hr-HPV types, stratified by cervical lesion grade, sample collection method, and infection type (single- vs. multiple-type).

hr-HPV and infection type	<CIN2[a] (*N* = 229)			CIN2+[b] (*N* = 85)			CIN3+[c,d] (*N* = 49)		
	Self *n* (%)	Provider *n* (%)	*P*[e]	Self *n* (%)	Provider *n* (%)	*P*[e]	Self *n* (%)	Provider *n* (%)	*P*[e]
Any hr-HPV[f]	160 (70%)	140 (61%)	0.004	79 (93%)	80 (94%)	0.65	47 (96%)	47 (96%)	1.00
Single	115 (50%)	120 (52%)		48 (56%)	56 (66%)		26 (53%)	31 (63%)	
Multiple	45 (20%)	20 (9%)		31 (36%)	24 (28%)		21 (43%)	16 (33%)	
16	46 (20%)	28 (12%)	<0.001	39 (46%)	34 (40%)	0.13	29 (59%)	24 (49%)	0.06
Single	25 (11%)	21 (9%)		22 (26%)	21 (25%)		16 (33%)	14 (29%)	
Multiple	21 (9%)	7 (3%)		17 (20%)	13 (15%)		13 (27%)	10 (20%)	
18	11 (5%)	9 (4%)	0.41	7 (8%)	6 (7%)	0.56	4 (8%)	4 (8%)	1.00
Single	6 (3%)	7 (3%)		2 (2%)	0 (0%)		0 (0%)	0 (0%)	
Multiple	5 (2%)	2 (1%)		5 (6%)	6 (7%)		4 (8%)	4 (8%)	
31	18 (8%)	13 (6%)	0.10	9 (11%)	8 (9%)	0.32	4 (8%)	4 (8%)	1.00
Single	7 (3%)	8 (3%)		5 (6%)	6 (7%)		2 (4%)	3 (6%)	
Multiple	11 (5%)	5 (2%)		4 (5%)	2 (2%)		2 (4%)	1 (2%)	
45	13 (6%)	11 (5%)	0.41	6 (7%)	5 (6%)	0.56	4 (8%)	3 (6%)	0.32
Single	10 (4%)	10 (4%)		2 (2%)	2 (2%)		1 (2%)	2 (4%)	
Multiple	3 (1%)	1 (<1%)		4 (5%)	3 (4%)		3 (6%)	1 (2%)	
33/58	15 (7%)	11 (5%)	0.046	10 (12%)	9 (11%)	0.32	6 (12%)	6 (12%)	1.00
Single	11 (5%)	10 (4%)		5 (6%)	7 (8%)		2 (4%)	4 (8%)	
Multiple	4 (2%)	1 (<1%)		5 (6%)	2 (2%)		4 (8%)	2 (4%)	
35/39/68	34 (15%)	23 (10%)	0.008	13 (15%)	14 (16%)	0.56	5 (10%)	6 (12%)	0.32
Single	19 (8%)	17 (7%)		4 (5%)	8 (9%)		1 (2%)	2 (4%)	
Multiple	15 (7%)	6 (3%)		9 (11%)	6 (7%)		4 (8%)	4 (8%)	
51	13 (6%)	11 (5%)	0.16	8 (9%)	8 (9%)	1.00	4 (8%)	4 (8%)	1.00
Single	3 (1%)	8 (3%)		2 (2%)	2 (2%)		1 (2%)	1 (2%)	
Multiple	10 (4%)	3 (1%)		6 (7%)	6 (7%)		3 (6%)	3 (6%)	
52	20 (9%)	17 (7%)	0.26	13 (15%)	13 (15%)	1.00	9 (18%)	8 (16%)	0.32
Single	12 (5%)	11 (5%)		4 (5%)	6 (7%)		3 (6%)	4 (8%)	
Multiple	8 (3%)	6 (3%)		9 (11%)	7 (8%)		6 (12%)	4 (8%)	
56/59/66	43 (19%)	40 (17%)	0.37	17 (20%)	17 (20%)	1.00	10 (20%)	10 (20%)	1.00
Single	22 (10%)	28 (12%)		2 (2%)	4 (5%)		0 (0%)	1 (2%)	
Multiple	21 (9%)	12 (5%)		15 (18%)	13 (15%)		10 (20%)	9 (18%)	

[a]<CIN2, less than CIN grade 2.
[b]CIN2+, CIN grade 2 or higher.
[c]CIN3+, CIN grade 3 or higher.
[d]CIN2/3 cases were analyzed as CIN3+ cases.
[e]*P* value from McNemar test, which assessed difference between the hr-HPV prevalence in self- and provider-collected samples for a given HPV type or group.
[f]Positive for any of the 14 hr-HPV types detected by the Onclarity Assay.

which belong to the alpha-7 HPV species, are associated with glandular lesions and cervical adenocarcinoma (43). These endocervical lesions are harder to detect by conventional colposcopy, and thus, some of these lesions might have been missed in our study. However, given that we performed an ECC if the transformation zone or the limits of a lesion near the cervical os could not be fully visualized, the risk of missing endocervical lesions is low in our study. Furthermore, although provider-collection is more likely to sample from the endocervix and thus, detect glandular lesions, HPV-18 and 45 prevalence and the genotype ranking appeared to be similar in self- and provider-collected samples. In general, our genotype ranking should be interpreted with caution because of the relatively low number of samples positive for specific genotypes, such as HPV-51, 18, and 45, and, therefore, the limited precision of our estimates for these genotypes.

The majority of cervical cancer cases occur in underscreened women (15–17). Primary hr-HPV testing on self-collected cervico-vaginal samples may help increase cervical cancer screening coverage, as women generally prefer self-collection over provider-collection for hr-HPV testing (19, 20). However, most hr-HPV infections clear within one year, and only a small proportion develops into cervical cancer (44, 45). Therefore, a triage strategy is needed to reduce the number of unnecessary colposcopy referrals among cases of transient or non-progressing hr-HPV infection identified through primary hr-HPV testing. Biomarkers for triage of hr-HPV infections can be broadly categorized into morphological and molecular biomarkers (46). Extended hr-HPV genotyping beyond HPV-16/18 could serve as a molecular triage strategy (5, 12–14, 46). We found type-specific agreement between self- and provider-collected samples to range from substantial to almost perfect, indicating that extended hr-HPV genotyping algorithms identified in provider-collected samples may also be valid for self-collected samples. For HPV-16 we found a significantly higher prevalence in self- versus provider-collected samples, which may result in higher colposcopy referral rates when self-collected samples are used. However, when we restricted the analysis to women with CIN2+ or CIN3+, hr-HPV prevalence did not significantly differ between self-collected cervico-vaginal samples and provider-collected cervical samples. DNA-based extended hr-HPV genotyping tests are unable to discriminate between transient and persistent hr-HPV infections at a cross-sectional timepoint (46).

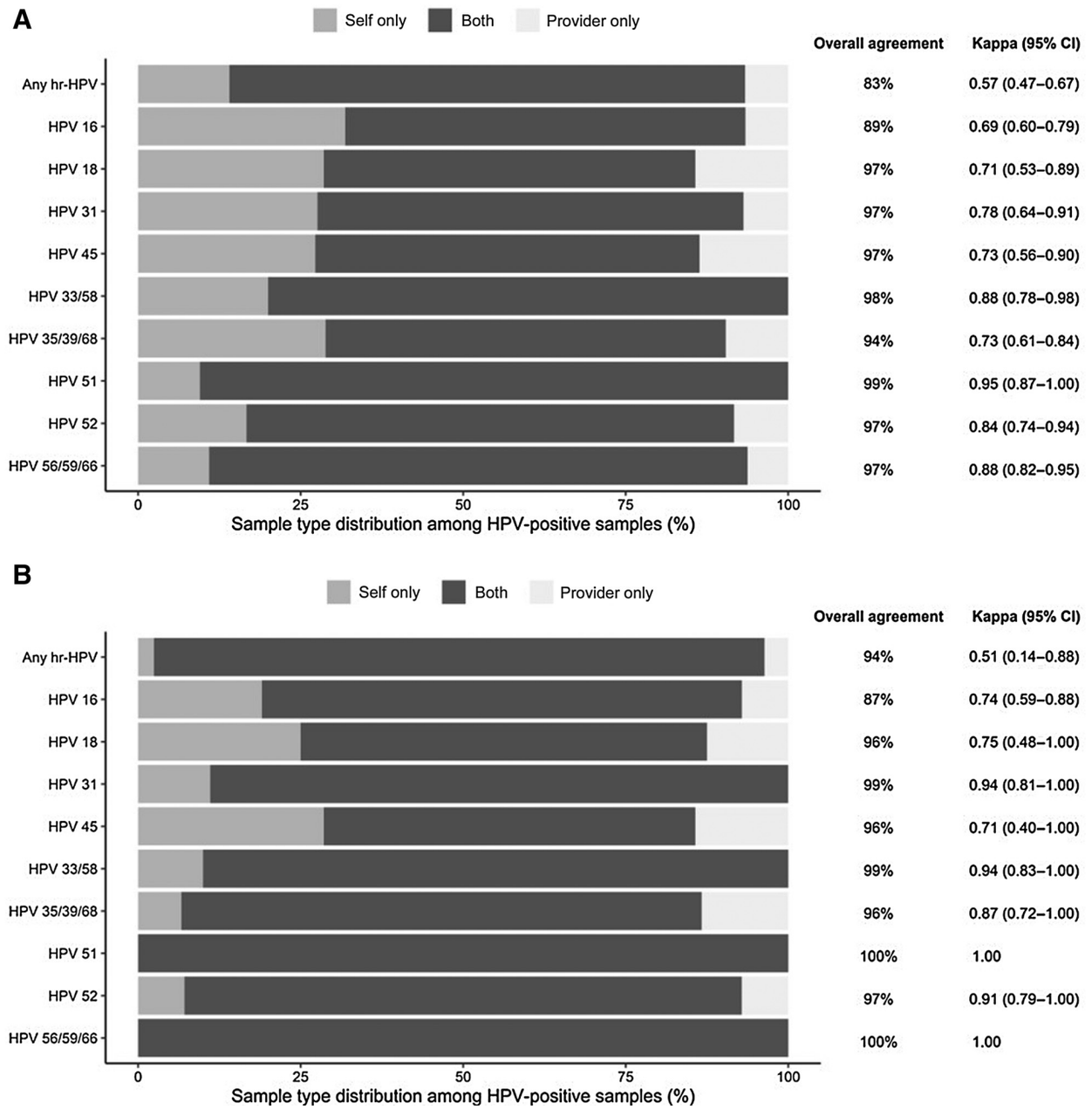

Figure 3.
HPV type–specific agreement between all (**A**) self-collected cervico-vaginal and provider-collected cervical samples and among CIN2+ cases (**B**).

Therefore, it is likely that a combination of biomarkers involved in different stages of the cervical carcinogenesis are required to obtain adequate clinical sensitivity and specificity. Novel molecular biomarker including DNA methylation and cell cycle markers may become particularly relevant as HPV vaccination coverage increases on the population level, resulting in changes of hr-HPV genotype distribution among screen-eligible women and decreases in type-specific PPVs for CIN2+ (47). Other triage methods such as machine learning–based automated visual examination of cervical images may also be useful (48). Further studies are needed to evaluate extended hr-HPV testing strategies in self-collected samples among vaccinated and unvaccinated primary screening populations, to quantify the effect of sample collection method on colposcopy referral rates, and to assess the performance of other molecular biomarkers for triage of hr-HPV–positive women.

To our knowledge, this is the first study to compare hr-HPV prevalence and agreement between self- and provider-collected samples at the same timepoint using the Onclarity Assay. All women included in the analysis provided both a self-collected cervico-vaginal and a provider-collected cervical sample for testing. Testing was done under standardized conditions in the same laboratory, and the staff performing the hr-HPV testing was unaware of the related clinical and

Table 3. Classification of hr-HPV genotypes according to PPVs for CIN2+ detection, excluding women with multiple-type infections.

hr-HPV type	*N* at risk	CIN2+/ hr-HPV+	PPV	Cumulative	
				Sensitivity	Specificity
Provider-collected samples					
HPV-16	270	21/42	50.0%	34.4%	90.0%
HPV-31	228	6/14	42.9%	44.3%	86.1%
HPV-33/58	214	7/17	41.2%	55.7%	81.3%
HPV-52	197	6/17	35.3%	65.6%	76.1%
HPV-35/39/68	180	8/25	32.0%	78.7%	67.9%
HPV-51	155	2/10	20.0%	82.0%	64.1%
HPV-45	145	2/12	16.7%	85.2%	59.3%
HPV-56/59/66	133	4/32	12.5%	91.8%	45.9%
HPV-18	101	0/7	0.0%	91.8%	42.6%
Self-collected samples					
HPV-16	238	22/47	46.8%	40.7%	86.4%
HPV-31	191	5/12	41.7%	50.0%	82.6%
HPV-51	186	2/5	40.0%	53.7%	81.0%
HPV-33/58	170	5/16	31.3%	63.0%	75.0%
HPV-52	154	4/16	25.0%	70.4%	68.5%
HPV-18	146	2/8	25.0%	74.1%	65.2%
HPV-35/39/68	123	4/23	17.4%	81.5%	54.9%
HPV-45	111	2/12	16.7%	85.2%	49.5%
HPV-56/59/66	87	2/24	8.3%	88.9%	37.5%

histopathologic information. We also have to acknowledge several limitations. Women in our study self-collected cervico-vaginal samples at the clinic, although the target setting for a future rollout would likely be home-based sample self-collection (37). Nevertheless, our results are also likely to apply to that setting, as our recent study in North Carolina showed comparable detection of CIN2+ in home- and clinic-collected self-samples (49). Our study population mostly consisted of women with abnormal screening results referred for colposcopy, and our findings might not necessarily be generalizable to a primary screening population. Importantly, however, we found agreement of hr-HPV test results between self- and provider-collected samples to be similar among women with and without advanced cervical disease. We did not collect individual-level data on HPV vaccination status, but given the median age of our study population was 36 years and HPV vaccination was introduced in the United States in 2006, most included women are expected to have not been vaccinated. Thus, our findings are not generalizable to a population of vaccinated women. Extended hr-HPV genotyping will remain important to assess nonvaccine genotypes and their associated type-specific risk of high-grade cervical precancer and cancer.

To conclude, HPV-16 had the highest PPV for detection of CIN2+ in both self-collected cervico-vaginal and provider-collected cervical samples, and overall sensitivity for CIN2+ detection was similar for both sample types. However, HPV-16 prevalence was significantly higher in self- versus provider-collected samples, which could result in increased colposcopy referral rates and overtreatment. In the future, additional molecular markers, such as DNA methylation, might be helpful to improve the triage of women positive for hr-HPV on self-collected samples.

Disclosure of Potential Conflicts of Interest

E. Rohner reports grants from Swiss Cancer Research Foundation during the conduct of the study. B. Faherty reports paid employment with Becton Dickinson. L.S. Romocki reports grants from NIH (U54 grant) during the conduct of the study. J.A.E. Nelson reports grants from NIH during the conduct of the study, as well as grants from NIH outside the submitted work. M.G. Hudgens reports grants from NIH during the conduct of the study. A.K. Knittel reports grants from Lupin Pharmaceutical outside the submitted work. J.S. Smith reports grants from NIH and nonfinancial support from Becton Dickinson (donation of specimen transport tubes and HPV testing), Rovers Medical Devices BV (donation of self-collection brushes), and Hologic Corporation (donation of cytology media) during the conduct of the study, as well as grants and personal fees from Becton Dickinson and Hologic outside the submitted work. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

E. Rohner: Formal analysis, methodology, writing–original draft. **C. Edelman:** Investigation, writing–original draft. **B. Sanusi:** Data curation, formal analysis, methodology, writing–review and editing. **J.W. Schmitt:** Investigation, writing–review and editing. **A. Baker:** Investigation. **K. Chesko:** Investigation, writing–review and editing. **B. Faherty:** Investigation, writing–review and editing. **S.M. Gregory:** Investigation, writing–review and editing. **L.S. Romocki:** Conceptualization, writing–review and editing. **V. Sivaraman:** Conceptualization, writing–review and editing. **J.A.E. Nelson:** Investigation, writing–review and editing. **S. O'Connor:** Investigation, writing–review and editing. **M.G. Hudgens:** Methodology, writing–review and editing. **A.K. Knittel:** Investigation, writing–review and editing. **L. Rahangdale:** Conceptualization, investigation, writing–review and editing. **J.S. Smith:** Conceptualization, supervision, funding acquisition, methodology, writing–original draft.

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