Gynecology and Obstetrics Clinical Medicine 2 (2022) 34–37

Contents lists available at ScienceDirect

Gynecology and Obstetrics Clinical Medicine

journal homepage: www.keaipublishing.com/en/journals/gynecology-and-obstetrics-clinical-medicine

Methdology

Evaluation of dry specimen transport and processing time using an isothermal amplification high-risk human papillomavirus assay

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ARTICLE INFO

Keywords:
Cervical cancer
Screening
High-risk human papillomavirus
Specimen transport
Processing time
Sample preparation

ABSTRACT

Background: Dry specimen transport has shown equivalence to traditional liquid transport using a novel high-risk Human papillomavirus assay. Considering that dry transport might cross obstacles during cervical cancer screening in low and middle resource settings, this study was designed evaluate different processing time of dry specimen transport using the same isothermal amplification hrHPV assay.

Methods: There were 564 women between the ages of 30–55 recruited from colposcopy clinic. For each patient, two endocervical samples were collected and placed into empty collection tubes by physician. Samples were stored at room temperature until analyzed for hrHPV using the AmpFire assay at two time points: 2 days and 2 weeks. 511 of the 564 participants with positive hrHPV were provided colposcopy exam and quadrant biopsy.

Results: A total of 1128 endocervical samples from 564 patients were detected by the Ampfire assay. Good agreement was found between two time periods (Kappa ± Standard error = 0.67 ± 0.04). Sensitivity (2days/2weeks) for CIN2+ was 95.28% (95% CI: 92.14%–98.42%) vs 90.57% (CI (86.65%–94.49%) and specificity (2days/2weeks) was 22.47% (CI 19.33%–25.61%) vs 28.15% (CI 24.23%–32.07%) respectively. The difference for Ampfire HPV detection in sensitivity for CIN2+ for the two time periods was not significant (P = 0.227), while the difference in specificity for CIN2+ was significant (P = 0.001). The difference in Ct values 29.23 (CI 28.15–30.31) and 29.27 (CI 28.19–30.35) between two time points was not significant (P = 0.164).

Conclusion: Processing dry brush specimens can be delayed up to 2 weeks. Using the AmpFire assay platform which supports cervical cancer prevention programs in low-to-middle-income countries (LMICs).

1. Introduction

In 1997 our collaborators began to study self-collection for cervical cancer screening.[1] Since that time, they and others have found that a self-collected vaginal sample tested for high-risk human papillomavirus (hrHPV) by a polymerase chain reaction (PCR)-based assay has a sensitivity for cervical intraepithelial neoplasia (CIN) 3 or cancer equal to that of a practitioner collected endocervical specimen.[2–4] We have developed inexpensive transport cards,[5,6] assisted in the development of faster assays,[7] and have developed community-based models to reach the people most in need.[8,9] Recently with the identification of the new AmpFire assay which amplifies the HPV virus using isothermal

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Publishing services by Elsevier on behalf of KeAi

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https://doi.org/10.1016/j.gocm.2022.01.009
Received 26 December 2021; Received in revised form 20 January 2022; Accepted 28 January 2022
Available online 10 February 2022

technology,[10] there is the opportunity for a simple assay effective with self-collection (similar to "traditional" PCR) that is compatible with dry specimen transport. We have previously demonstrated equivalence between dry and liquid transport.[11] In this study we will determine whether endocervical samples transported dry and tested for high-risk human papillomavirus (hrHPV) with the AmpFire assay two days after collection have similar results to those tested two weeks after collection. Our interest being to further anticipate and explore obstacles that could be encountered with self-collection screening samples in low and middle resource settings.

2. Methods

This study was approved by Shanxi Dayi Hospital medical ethics committee (YXLL-2019-4). Women between the ages of 30–55 years of age attending our colopscopy clinic were asked to participate in the study. Attendees were from several referral clinics and had tested HPV positive *and/or* were reported to have abnormal cytology. Additionally, patients were referred because the cervix looked "unusual", even though they had negative screening tests (HPV and cytology). There were 564 women who agreed to participate and signed the informed consent. For each patient, the cervix was visualized with a bivalve speculum and two endocervical samples were collected by a physician. The protocol for obtaining the samples was making 2 turns with each sampling brush and then placing the brush head into an empty collection tube. The two samples from each patient were stored at room temperature (within standard of 10–30℃) until they were analyzed for hrHPV using the AmpFire assay at two time points: 2 days and 2 weeks.

Using isothermal amplification, the AmpFire assay directly detects HPV DNA from raw samples (deoxyribonucleic acid (DNA) extraction is not required). This real time fluorescent methodology was developed in the USA by Atila BioSystems, Inc, Mountain View, CA. Samples can be collected and transported by dry brush and stored without transport media. Less than 1 h is required for processing. Amplification Cycle numbers are expressed as Ct values.

The assay was performed according to manufacturer's instructions.[12] Fifteen specific HPV genotypes were detected in a multiplex assay, type 16, type 18, and a thirteen-type high risk HPV pool. This flexible platform can process specimens individually, simultaneously (batched) or sequentially based on requirements. The Chinese Food and Drug Administration approved the AmpFire assay in December 2015 and Conformité Européenne (European Community) CE marked this assay in 2017. The assay has received regulatory approval in several other European and Asian countries and has been adopted by 8 African countries for population screening.

511 of the 564 participants agreed to have a colposcopy exam with biopsies according to the study protocol. The quadrant biopsy procedure followed the Preventive Oncology International micro-biopsy protocol.[13] Enough biopsies were taken from any suspicious area to adequately assess the detected lesion. Negative cervical quadrants were biopsied at the squamocolumnar junction (SCJ) at 2, 4, 8, or 10 o'clock depending on the quadrant. An endocervical curettage (ECC) was also performed. 106 patients were diagnosed with cervical intraepithelial neoplasia grade 2 or above (CIN2+) and 405 patients were with diagnosis of CIN1 or lower (Fig. 1).

Statistics - SPSS 23.0 software was used for statistic analysis. P-value of less than 0.05 was considered significant. Agreement was measured by Cohen's Kappa coefficient. Ct value is the number of cycles required to detect the HPV (therefore the higher the Ct value the lower the titer). Ct of 65 was the company determined positive/negative cut-point. Power calculation was performed in comparing paired proportions by McNemar Z test in two sides. Power (1-β) was calculated with 0.95, which was based on type I error rate ($\alpha = 0.05$), sample size (564), proportions of no event-event (0.03) and event-no event (0.08) (Table 1). McNemar Chi-square tests were applied for sensitivity and specificity analysis (Table 2). Descriptive statistics was applied for comparative data for the 11 cases of CIN2+ with discordant results (Table 3).

3. Results

3.1. HPV detection efficiency in two time periods using Ampfire HPV detection

A total of 1128 dry brush endocervical samples collected from 564 patients were analyzed using the Ampfire assay at the two study time points (within 2 days and 2 weeks of collection). Good agreement was found between two time periods (Kappa ± Standard error = 0.67 ± 0.04). There was a difference in HPV detection (80% vs 75%) between two time periods respectively. ($P \leq 0.001$) (Table 1).

3.2. Sensitivity and specificity for CIN2+ in two time periods using Ampfire HPV detection

Among the CIN2+ patients there were 64 CIN2, 33 CIN3, and 9 cancers. The sensitivity at the two time points (2 days and 2 weeks) for CIN2+ was 95.28% (95% CI, 92.14%–98.42%) and 90.57% (CI (86.65%–94.49%) respectively, the difference had no statistically significance ($P = 0.227$). The specificity at the two time points for CIN2+ was 22.47% (CI 19.33%–25.61%) and 28.15% (CI 24.23%–32.07%) respectively, with significant difference ($P = 0.001$). The negative predictive values (NPV) at the two time points were 94.79% (CI 91.65%–97.93%) and 91.94% (CI 88.02%–95.86%) respectively; and the positive predictive values (PPV) at the two time points were 24.34% (CI 21.20%–27.48%) and 24.81% (CI 20.89%–28.73%) respectively (Table 2). (Table 2). The mean Ct values at 2 days and 2 weeks were 29.23 (CI 28.15–30.31) and 29.27 (CI 28.19–30.35) respectively, which had no significant difference ($P = 0.164$).

Table 3 shows the data for the 11 cases of CIN2+ where we found discordant results. This includes the result (pos/neg), genotype, Ct number (surrogate for titer), pathological diagnosis, and number of cervical quadrants involved (a surrogate for lesion size). There were 11 cases with discordant HPV results (10/11 were HPV 16). There were 93 cases with concordant HPV results (53/93 were HPV 16). The mean Ct values for the discordant results were 24.20 and 26.42 at 2 days and 2 weeks. respectively. The mean Ct values for the concordant results were 21.95 and 22.44 at 2 days and 2 weeks respectively.

4. Discussion

Individual technologies are critical to the development of effective

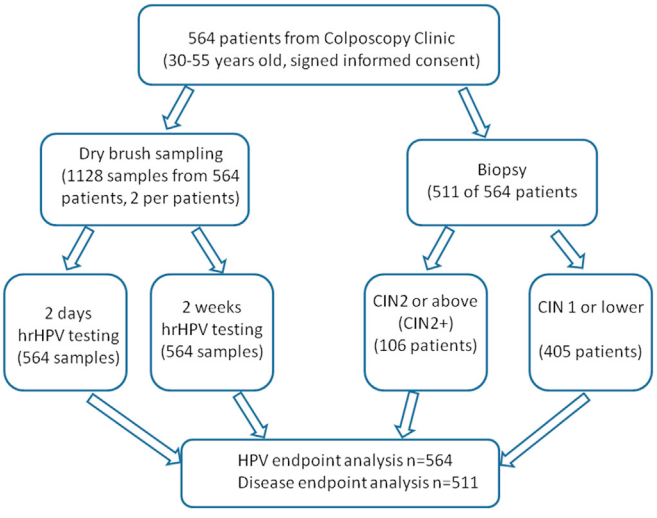

Fig. 1. Flowchart of this study.

Table 1
Comparison of Ampfire HPV detection in two time periods n (%).

2 days	2 weeks		Chi-square value	P value	Kappa value[a]	Standardized error
	HPV positive	HPV negative				
HPV positive	406 (71.99)	47 (8.33)	14.06	<0.001	0.67	0.037
HPV negative	17 (3.01)	94 (0.71)				

Binomial distribution used.
[a] Interpretation of Kappa. Poor agreement: less than 0.20; Fair agreement: 0.20 to 0.40; Moderate agreement: 0.40 to 0.60; Good agreement: 0.60 to 0.80; Very good agreement: 0.80 to 1.00.

Table 2
Sensitivity and specificity to CIN2+ processed at two time periods with Ampfire HPV detection n (%).

HPV at two time periods	Pathology		Sensitivity (95%CI)	Specificity (95%CI)	Negative predictive value (95% CI)	Positive predictive value (95% CI)
	CIN2+n (%)	≤CIN1 n (%)				
Two days						
HPV positive	101 (19.77)	314 (61.45)	95.28	22.47	94.79 (91.65–97.93)	24.34 (21.20–27.48)
HPV negative	5 (0.98)	91 (17.81)	(92.14–98.42)	(19.33–25.61)		
Two weeks						
HPV positive	96 (18.79)	291 (56.95)	90.57	28.15	91.94 (88.02–95.86)	24.81 (20.89–28.73)
HPV negative	10 (1.96)	114 (22.31)	(86.65–94.49)	(24.23–32.07)		
P value			0.227	0.001	0.436	0.878

Table 3
Comparative data for the 11 cases of CIN2+ with discordant results.

ID	HPV 2 days	Genotype	Ct value	HPV 2 weeks	Genotype	Ct value	Pathology	Numbers of quadrant
SY173	–			+	16	24.55	Cancer	4
SY296	–			+	16	23.18	CIN2	1
SY543	–			+	16	31.52	CIN2	1
SY078	+	16	19.41	–			Cancer	4
SY083	+	13 combo[†]	59.44	–			CIN3	1
SY166	+	16	21.23	–			CIN2	1
SY228	+	16	32.90	–			CIN2	1
SY288	+	16	20.01	–			CIN2	2
SY309	+	16	27.34	–			CIN2	1
SY313	+	16	16.79	–			Cancer	4
SY446	+	16	31.74	–			CIN3	1

Note:– : HPV negative; + HPV positive, †thirteen-type high risk pool.

screening programs. However, they must fit into a system, or a system be built around them for the programs to function efficiently and accurately. Clearly self-collection can screen large populations quickly.[14] Remote sites or simple laboratory overload is a predictable problem that can lead to prolonged storage and processing delays. With dry transport and storage of a lower genital tract specimens, our major concern was bacterial overgrowth resulting in competitive enzymes that might affect the AmpFire assay, or simple specimen degradation.[15] Over the two week period, neither seemed to occur as evidenced by the titer and kappa data. These findings were noted for up to 32 weeks by Ejegod et al.,[16] using the Evalyn Brush and Onclarity assay (Becton, Dickinson and Company, Sparks, MD, USA. The duplication of these findings using the very simple, fast and inexpensive AmpFire assay combined with a simple, inexpensive nylon brush, further supports the expansion of population-based HPV screening into low-to-middle-income countries (LMICs).

It was interesting in our data to note the dominance of type 16 in the discordant results compared to the concordant cases. In addition, the Ct values are higher for the discordant HPV 16 cases compared to the concordant HPV 16 cases. It should be recognized that the Ampfire assay has a higher analytical sensitivity for type 16, which results in Ct values < 24 reflecting significantly lower titers than for the other 14 HPV genotypes detected by the assay, and for Ct values > 24 even lower.[17] Therefore the most likely explanation for these results are based on these Ct values, which were simply very low titer samples (possibly 1–3 viral copies) so they were very sensitive to sampling error. In the future we plan make 4 turns for our single sampling brush in our protocol when employed in population based screening programs. In conclusion, a system of self-collection, dry brush transport and the AmpFire assay platform is very compatible with cervical cancer prevention programs applied to LMICs where processing delays due to transport logistics are commonly encountered.

Our study data is limited by the total number of CIN2+ patients. In addition, the outer time-period of two weeks that we selected may seem too short. However, we do not consider this a serious limitation since over the past 24 years of doing population based clinical trials, transport to a laboratory from the study sites for processing has never exceeded 2 weeks.

5. Conclusion

Our data shows that over 90% of high-grade disease can be detected even when the processing of dry brush transported specimens is delayed up to 2 weeks. using the AmpFire assay platform. This would support cervical cancer prevention programs applied to low-to-middle-income countries where the logistics of specimen transport may be problematic.

Author contributions

HL contributes to manuscript preparation. WS contributes to manuscript editing, concept, design, and definition of intellectual content. SH contributes to data analysis and statistical analysis. J B contributes to study design, manuscript review and definition of intellectual content. LJ

contributes to literature search. WX contributes to experimental studies. HY contributes to data acquisition. SJ contributes to manuscript editing, concept, design, definition of intellectual content, and takes responsibility for the integrity of the work as a whole from inception to published article.

Conflict of interest statement

All authors state that there is no conflict of interest.

Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This work was supported by grants from the Science and Technology Research Project Foundation of Shanxi Province, China (201803D421049).

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