Journal of Virological Methods 270 (2019) 18–20

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Increased HPV detection by the use of a pre-heating step on vaginal self-samples analysed by Aptima HPV assay

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ARTICLE INFO

Keywords:
HPV mRNA
Vaginal
Self-sample
Cancer
Screening

ABSTRACT

Background: We recently reported a sensitivity of 85.5% to detect high-grade squamous intraepithelial lesions (HSIL)/adenocarcinoma *in situ* (AIS)/cancer by the use of self-collected vaginal samples analysed by the Aptima mRNA HPV assay (AHPV).
Objectives: To increase detection of HPV among self-samples.
Study design: We used a pre-heating step at 90 °C for 1 h on our previously AHPV-negative self-samples (N = 20) among women with AHPV-positive cervical samples. We also analysed AHPV results before and after the heating among a series of self-samples from women who had not attended cervical screening for ≥ 7 years (N = 173).
Results: After heating, 55% (11/20) of the self-samples became AHPV-positive. By updating our original series 93.1% (121/130, 95% CI: 87.3–96.8) of the self-samples were AHPV-positive among women with AHPV-positive cervical samples, and among women with histologically confirmed cervical intraepithelial neoplasia or worse (CIN2 +) now 95.3% (61/64, 95% CI: 86.9–99.0) of the self-samples were AHPV-positive. Among the 11 AHPV-positive self-samples we detected high-risk HPV types in 10 of the samples (HPV16 3 cases, HPV18 1, HPV31 1, HPV33 1, HPV 45 1, HPV51 2, HPV 56 and 58 1, HPV42 and 90 1 [low risk]) by multiplex PCR and Luminex assay. Among the self-samples from the non-attenders 16% (27/170) and 5.3% (8/152) were AHPV-positive after and before the heating step, respectively (P = 0.0022). Concerning validity of AHPV-results, 99% (170/172) were valid after the heating step compared to 88% (152/172) before the heating step (P < 0.0001).
Conclusions: A pre-heating step on vaginal self-samples increased HPV detection by the AHPV assay.

1. Background

Increased risk of cervical cancer cases is associated with non-participation of routine cervical screening (Andrae et al., 2008; Lonnberg et al., 2012; Sasieni et al., 2003; IARC, 2005). Testing for human papillomavirus (HPV) among self-collected vaginal samples could serve as one option to increase cervical cancer screening attendances. Arbyn et al., reported that HPV testing of vaginal self-samples by PCR based assays, demonstrated similar accuracy as that of cervical samples (Arbyn et al., 2018). Within a referral group of women we recently reported a sensitivity of 85.5% to detect high-grade squamous intraepithelial lesions (HSIL)/adenocanrcinoma *in situ* (AIS)/cancer by the use of self-collected vaginal samples analysed by the Aptima mRNA HPV assay (AHPV, Hologic, Inc, MA, USA) (Asciutto et al., 2018). The AHPV detects mRNA coding for the E6 and E7 proteins of HPV types 16, 18, 31, 33, 35, 38, 39, 45, 51, 52, 56, 58, 59, 66 and 68. The relatively moderate sensitivity raised our concerns of false-negative results of the AHPV assay. High sensitivity of the AHPV assay by the use of vaginal self-samples is a prerequisite for cervical screening. In this study, we evaluated the effect of a pre-heating step on self-collected vaginal samples as an attempt to increase HPV detection by the use of the AHPV assay.

1.1. Objectives

To increase HPV detection among self-collected vaginal samples.

2. Study design

2.1. Previously AHPV-negative self-samples

From women with AHPV-positive cervical samples and AHPV-negative vaginal self-samples originally collected in Aptima Multitest Swab Transport Media (STM) (Hologic Inc, Marlborough, MA, USA)

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https://doi.org/10.1016/j.jviromet.2019.04.015
Received 14 March 2019; Received in revised form 15 April 2019; Accepted 16 April 2019
Available online 16 April 2019

(Asciutto et al., 2018), stored vaginal self-samples (N = 20) at −20 °C were thawed and 300 µL from each sample was transferred to tubes with 2.9 mL Aptima Multitest STM. Tubes were incubated at 90 °C for 1 h in a heating chamber (Termaks AS. Bergen Norway), cooled to room temperature and analysed by the AHPV. Self-samples that became AHPV-positive only after the heating step were further investigated in order to confirm presence of specific HPV types in the self-samples and to exclude that the AHPV-positive results after the heating step were due to targets not related to HPV. For detection of specific HPV types among the AHPV-positive self-samples, the samples were subjected to DNA-extraction (200 µL input and 100 µL output by the use of MagnaLC, Roche) and analyzed for presence of 40 different HPV types by the use of multiplex MGP-PCR and Luminex (Soderlund-Strand et al., 2009; Michael et al., 2011).

2.2. Vaginal self-samples from non-attenders

To further explore the heating step we compared AHPV results of vaginal self-samples (in STM tubes) before and after heating. Vaginal self-samples (N = 172) were collected as previously described (Asciutto et al., 2018), during September 2018 among women who had not participated in cervical screening for seven years or more in Skåne, Sweden. The median age of the women was 49 years (range 30–70 years). Samples were tested by the AHPV and the tubes were then re-capped and anonymised, incubated at 90 °C for 1 h and re-tested.

Validity of results were automatically determined by the software of the AHPV assay. A valid AHPV test result for each sample is based on relative light units (RLU) of an internal control target (IC) incorporated to monitor nucleic acid capture, amplification and detection by the AHPV-instrument.

In order to investigate stability of HPV mRNA after our heating approach we incubated (90 °C for 1 h) five AHPV-positive self-samples (AHPV-positive prior to heating) that were then re-tested by the AHPV assay. The samples were from a different series of screening women who had not participated in cervical screening for seven years.

2.3. Laboratory environmental samples

To investigate if the self-samples could have been contaminated during the process in the laboratory we analysed 10 STM tubes, without any human materials, before and after the heating step. In addition four environmental samples were tested before and after the heating step, where different sites were swabbed with cotton tips (sample rack, top on the reagent-vials of the Panther instrument, on samples in the Panther instrument, on the surface of lid of sample rack).

2.4. Statistical analysis

Confidence intervals (95%) were calculated using GraphPad Software (https://www.graphpad.com/

quickcalcs/contingency2/). Fisher's exact test was used for analysis of differences between distribution of HPV and of valid results before and after heating using GraphPad Software. P-values < 0.05 were considered significant.

3. Results

After heating, 55% (11/20) of the previously AHPV-negative self-samples became AHPV-positive. By updating our original series (Asciutto et al., 2018) with these results 93.1% (121/130, 95% CI: 87.3–96.8) of the vaginal self-samples were AHPV-positive among women with AHPV-positive cervical samples, and among women with histologically confirmed CIN2+ now 95.3% (61/64, 95% CI: 86.9–99.0) of the self-samples were AHPV-positive. Furthermore, to support specificity of the 11 AHPV-positive results after the heating step we detected high-risk HPV types in 10 of the self-samples (HPV16 3 cases, HPV18 1 case, HPV31 1 case, HPV33 1 case, HPV 45 1 case, HPV51 2 cases, HPV 56 and 58 1 case, HPV42 and 90 1 case [low risk]) by multiplex HPV PCR (Soderlund-Strand et al., 2009) and a Luminex assay (Schmitt et al., 2006).

Table 1
HPV prevalence of vaginal self-samples before and after a heating step, tested by the Aptima mRNA HPV assay.

	VALID[a] n	HPV positive n	HPV positive %
Before heating	152	8	5.3
After heating	170	27	15.9

Chi2 test showed p = 0.0022 between before and after heating.

[a] A valid test result for each sample is automatically determined by the software of the Aptima mRNA HPV assay. A valid or invalid test result is based on relative light units (RLU) of an internal control (IC) target incorporated to monitor nucleic acid capture, amplification and detection by the instrument of the Aptima mRNA HPV assay.

Among the non-attenders with valid results of self-samples, 16% (27/170) and 5.3% (8/152) were AHPV-positive after and before the heating step, respectively (P = 0.0022) (Table 1). Concerning the validity of AHPV-results, 99% (170/172) were valid after the heating step compared to 88% (152/172) before the heating step (P < 0.0001) (Table 2).

Concerning stability of HPV mRNA after incubation at 90 °C for 1 h, a series of five samples remained AHPV-positive after the heating step (Table 3). Three of these samples, as compared with HPV signal-to-cut-off ratio (S/CO) before heating, had increased S/CO values representing fold changes of 4.44, 6.83 and 14.6. Whereas for two of the samples similar HPV S/CO values were observed before and after heating (Table 3). All the 14 environmental samples were AHPV-negative both before and after the heating step, thus assuring that the procedure used for the heating step did not affect the results of our comparative study above.

4. Discussion

Inclusion of a pre-heating step at 90 °C for 1 h of vaginal self-samples demonstrated an increased detection of HPV mRNA for the AHPV assay. In addition, the proportion of valid AHPV-results increased substantially after the pre-heating step. This indicate that pre-heating increase the reliability of the AHPV assay for detection of HPV among vaginal self-samples.

By updating our self-sample series with the new results after the heating step we achieved a sensitivity of 95.3% (95% CI: 87.3–96.8) for the AHPV to detect CIN2+ cases. Szarewski, et al., reported a similar sensitivity of 95.3% (95% CI: 92.5–97.2) for the AHPV to detect CIN2+ among cervical samples of women referred to colposcopy clinics (Szarewski et al., 2012). Furthermore, such high sensitivity for detection of high-grade lesions is similar to that of cervical screening samples

Table 2
Proportion of valid vaginal self-samples before and after a heating step, tested by the Aptima mRNA HPV assay.

	SAMPLES n	VALID[a] n	INVALID[a] n	VALID %	INVALID %
Before heating	172	152	20	88	11.6
After heating	172	170	2	99	1.2

Chi2 test showed p < 0.0001 between before and after heating.

[a] A valid or invalid test result for each sample is automatically determined by the software of the Aptima mRNA HPV assay. A valid or invalid AHPV test result for each sample is based on relative light units (RLU) of an internal control (IC) target incorporated to monitor nucleic acid capture, amplification and detection by the AHPV-instrument.

Table 3
Detection of HPV mRNA before and after incubation at 90 °C among five vaginal self-samples positive for HPV by Aptima mRNA HPV assay.

Sample ID	Before heating			After heating 90 °C, 1 h			Fold change of HPV S/CO after heating
	IC RLU[a]	HPV RLU[b]	HPV S/CO[c]	IC RLU	HPV RLU	HPV S/CO	
1	168093	225979	**2.27**	139266	1553538	**15.50**	6.83[d]
2	310714	1679280	**16.90**	348905	1451257	**14.48**	0.86
3	279071	1049260	**10.56**	232208	1073752	**10.71**	1.01
4	218082	246808	**2.48**	221501	1103134	**11.01**	4.44
5	248971	135853	**1.36**	361337	1991005	**19.87**	14.6

[a] IC RLU = Internal control relative light unit.
[b] HPV RLU = HPV relative light unit.
[c] HPV signal-to-cut-off ratio (S/CO).
[d] Fold change of HPV S/CO after heating, calculated by dividing the HPV S/CO value by the HPV S/CO value before heating.

with an average sensitivity of 95.7% (range 87.8%–100%) for the AHPV assay to detect CIN2+ (Wu et al., 2010; Monsonego et al., 2011; Iftner et al., 2015; Nieves et al., 2013; Cuzick et al., 2013). However, in comparison to our "heat treated" self-sample series with a sensitivity of 95.3% for CIN2+, other studies reported somewhat lower sensitivity by the use of self-samples combined with the AHPV assay for detection of severe lesions. Chernesky and collegues reported 86.7% (95% CI: 70.3–94.7) sensitivity for the AHPV to detect CIN2+ (Chernesky et al., 2014). Johnson et al., reported a sensitivity of 75% (95% CI: 35.6–95.5) for the AHPV to detect cytological HSIL or SCC (Johnson et al., 2014). Whereas Nieves et al., reported an AHPV sensitivity of 62.5% (95% CI: 35.4–84.8) for detection of CIN3+, where the self-sample brush was placed in PreservCyt transport medium (Nieves et al., 2013).

Concerning our vaginal self-samples series from the non-attenders we observed improvements for both detection of HPV and for the proportion of valid results by the use of the heating step. The invalid rate of 1.2% by AHPV on our heat-treated self-samples is similar to that of HPV DNA test where on average 0.7% (range 0.0%–2.7%) of self-samples showed invalid results (Arbyn et al., 2018). After the heating-step we observed an AHPV positivity of 15.9% which is comparable to HPV-DNA assays where on average 11.1% (range 6.0%–29.4%) of self-samples were HPV-positive among under-screened women (Arbyn et al., 2018).

The HPV mRNA was intact in the Aptima (STM) solution after incubation at 90 °C for 1 h, as demonstrated for the five originally AHPV-positive samples. Notably for three of these samples the HPV S/CO values increased substantially after the heating-step. The molecular reasons for the increased HPV detection by the heating-step are unknown to us and need to be further studied. However, the heating step may release interactions between compounds present in the vaginal tract and the HPV mRNA allowing for improved performance of the AHPV assay on vaginal self-samples.

In conclusion a pre-heating step on vaginal self-samples increased detection of HPV by the use of the AHPV assay. Therefore, we suggest that vaginal self-samples should be heat-treated prior to analysis by the Aptima mRNA HPV assay.

Competing interests

Ola Forslund has received a speech honorarium from Hologic and his laboratory department (Laboratory Medicine, Region of Skåne) has ongoing contracts with Hologic. Christer Borgfeldt has received a grant from Hologic.

Ethical approval

The study was approved by the Ethical Review Board in Lund (Dnr 2013/390) and addendum (Dnr 2018/466).

Acknowledgement

We thank laboratory staff at Medical Microbiology and Pathology departments for technical assistance.

Funding

This work was supported by grants from Governmental funding of clinical research within the Swedish NHS (ALF), Region of Scania R&D funding, The Foundation of the University Hospital of Lund. The funding sources were not involved in the study design of interpretation of results.

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