New Microbiologica, 45, 2, 111-114, 2022, ISSN 1121-7138

FULL PAPER

Performance and pre-analytical stability of self-collected samples versus clinician cervical samples for the detection of HPV16, HPV18 and a pool of 12 other HPV types on the Roche Cobas 8800 System

Claudio Ilardo, Melanie Marguerettaz, Alain Breton, Tacfarinas Azzoug, Nelly Schmitt, Remi Fournier, Jeremy Bayette, Guillaume Teissier, Christian Lelarge, Benoit Ponseille

LABOSUD laboratory (Inovie member), Montpellier, France

SUMMARY

This study aimed to validate the agreement between human papillomavirus (HPV) tests self-collected samples versus clinician cervical specimens, and the pre-analytical stability of self-sampling. One hundred and fifty-seven women aged between 25 and 65 years who presented to the gynaecological department of the "CLEMENTVILLE" clinic in Montpellier voluntarily participated in HPV screening by self-sampling. Polymerase chain reaction was used to detect the presence of HPV16, HPV18 and a pool of 12 other HPV types on the Roche Cobas 8800 System. Median age was 40 years (range 20-73 and IQR 31-49 years). The overall HPV prevalence on the population studied was 27%. The agreement between clinician cervical samples and self-collected vaginal presented good agreement (Kappa =0.90) and high sensitivity (0.91) and specificity (0.98). For swabs stored for 7 days at room temperature, the HPV results presented substantial agreement (Kappa =0.89) and high sensitivity (0.97) and specificity (0.93). Our data showed that the HPV assay performed in the self-collected vaginal samples have high consistency of results with the clinician cervical samples. The use of self-collected cervical sample could be a simple and inexpensive approach in cervical cancer screening programs due to their high pre-analytical stability.

Received December 27, 2021 *Accepted* April 19, 2022

INTRODUCTION

Human papillomavirus (HPV) infection is a very common sexually transmitted infection, with approximately 80% of people (both men and women) becoming infected during their lifetime. Most of these infections disappear spontaneously, but in a small proportion of women the HPV persists. If it is a so-called high-risk human papillomavirus (HPV) type, it can induce cervical cancer (Walboomers *et al.*, 1999; Bosch *et al.*, 2002). Currently, pap smear (PS) is the primary cervical cancer screening (CCS) test used to detect precancerous lesions and early-stage cervical cancer (Schiffman *et al.*, 2016). To reduce non-coverage of screening for pre-cancerous lesions, several national cervical screening programs have started to transition from cytology to human papillomavirus (HPV)-based primary testing. An advantage of HPV testing is that, unlike cytology, it enables women to self-sample cervico-vaginal material at home (HPV self-sampling), which may improve cervical cancer screening participation (Verdoodt *et al.*, 2015). In 2021, a French study showed that providing a self-collected HPV test increased the participation of underprivileged women in CCS (Reques *et al.*, 2021). This study aimed to measure concordance in HPV detection between paired self-collected samples using the dry nylon flocked swab device and clinician cervical samples analysed on the Cobas 8800 HPV DNA test. Further, we wanted to evaluate the pre-analytical stability of self-collected samples during seven days at room temperature and provide evidence on the analytical quality of dry self-sampling nylon flocked swab during this time.

Key words:
HPV, Self-collected, Vaginal specimens, Cervical samples, Pre-analytical.

Corresponding author:
Claudio Ilardo
E-mail address: calogero.ilardo@labosud.fr

MATERIALS AND METHODS

Ethical approval

The study was performed on patients in accordance with Article L.1211-2 of the French "Public Health

Code." It used completely anonymized residual clinical swab sample material. All participants were informed of the study's objectives, and their participation was voluntary after providing written informed consent. The laboratory investigations were carried out in accordance with the General Data Protection Regulation (EU Regulation 2016/679 and Directive 95/46/EC) and the French data protection law (Law 78-17 of 6 January 1978 and Decree 2019-536 of 29 May 2019), which does not require a review by an ethics committee for the secondary use of samples collected for health-care purposes. In such case, the use of elements and products of the human body for a medical or scientific purpose other than that for which they were removed or collected is possible (article L.1211-2 of the French Public Health Code). The "Labosud Database" is registered at the French National Commission on Informatics and Liberty, CNIL, under record No. 2073511v0.

Inclusion of participants

From August 2021 to September 2021, one hundred and fifty-seven women aged 25 to 65 years who presented to the gynaecological department of the "Clémentville" clinic in Montpellier, France for a pap smear (PS) cervical cancer screening (CCS) test, were offered the option to self-collect a vaginal sample for HPV testing before the gynaecological check-up. If they agreed, they were given a self-sampling kit comprising two dry nylon flocked swabs (FLOQSwab® 5E089N, Copan SPA, Brescia, Italy), with written and picture-based user instructions showing how to collect the cervico-vaginal sample with the device. The clinician was available in case the participant had any questions about collecting the sample. After self-collection, the gynaecologist practitioner conducted a pap smear. The exclusion criterion was collecting the self-sample in menstrual cycle due to potential interference, in that the presence of blood at levels above 10% could inhibit the PCR.

Vaginal self-samples: storage and analysis

Upon arrival at the laboratory, the first dry nylon flocked swab was placed in 20 ml of cell medium (Roche Cell Collection Medium reference 07994745190, Roche Diagnostics, Mannheim, Germany) and stored at room temperature until the day of analysis. The second dry nylon flocked swab was stored for 7 days at room temperature before being placed in 20 ml of cell medium. The protocol using this medium did not require any pre-treatment before performing the HPV. Only HPV testing was performed from the self-sampling collection.

Clinician-collected samples: storage and analysis

For the clinician-collected samples, the brush head was placed in 10 ml of SurePath medium (reference 4911253, BD Diagnostics, Burlington, NC) and stored at room temperature until the day of analysis. For the SurePath medium, a denaturation step of the medium at 95°C for 20 minutes was required before proceeding with the HPV test. As HPV testing is recommended only in women over 30 years of age, and in order not to be restricted to this age category, we added a free HPV test in addition to the cytology study for patients under 30 years of age.

Biological analyses

The HPV test selected for the study was the Roche Real-Time High-Risk HPV test (real-time PCR) reference 07460155190, which identifies the DNA of the L1 gene of HPV16 and HPV18 separately and the pool of 12 other hrHPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) with the Cobas® 8800 (Roche Molecular System, Pleasanton, CF, USA).

Statistical analyses

Percent agreement and kappa coefficients (Cohen, 1960) with a 95% confidence interval (CI) were calculated to estimate the agreement of evidence and recommendation levels between all paired samples. According to Landis and Koch (1997), kappa coefficients can be interpreted as one of the following six degrees of agreement: poor (<0), slight (0.01-0.20), fair (0.21-0.40), moderate (0.41-0.60), substantial (0.61–0.80), and almost perfect (0.81-1.00).

The percent agreement between the paired samples was calculated as the proportion of concordant sample sets divided by the total number of samples. The sensitivity and specificity of HPV detection in the self-samples with corresponding 95% CI is based on the binomial distribution using the clinician-collected samples as reference standard.

The concordance between dry nylon flocked swab placed in cell medium immediately (D0) and seven days after collection (D7) was assessed using the same statistical analysis.

RESULTS

Participants' characteristics

157 women were enrolled. The median age was 40 years (range 20 -73 and IQR 31-49 years). The overall HPV prevalence in the population studied was 27%. *Table 1* shows age-specific prevalence (of any HPV

Table 1 - *Demographic characteristics of study participants (N=157) and prevalence of HPV results.*

Age (years)	*N (%)*	*HPV negative prevalence %*	*HPV positive prevalence %*
20-30	37 (24)	57	43
31-40	44 (28)	80	20
41-50	43 (27)	93	7
51-60	27 (17)	85	15
>61	6 (4)	100	0

type) that changed with age, from a peak of 43% in women aged 20-30 years to less than 20% in the older age categories (31-60 years).

Prevalence of HPV types

The most common HPV types in cytologically normal women were as follows: Other HPV (67%), HPV 16 (17%), HPV 18 (11%) and HPV16/Other HPV (6%). The prevalence HPV types in cytologically abnormal women were Other HPV (53%), HPV 16 (13%), HPV 16/ HPV18 (13%), HPV 16/Other HPV (13%) and HPV 18/Other HPV (7%).

Agreement between HPV results and cytology diagnosis

All cervical samples from patients under 30 years old or with a positive HPV result were reviewed by a cytotechnologist for the presence of normal and abnormal cells. The threshold for an abnormal/positive sample was set as atypical squamous cells of unknown significance or greater using the Bethesda Nomenclature (Apgar *et al*., 2003). The abnormal/positive sample rate in the study population was 10.2%. The agreement rate between the cytology diagnosis, clinician cervical samples and self-collected vaginal sample were comparable. Compared to the cytology results, HPV testing showed high sensitivity (>0.90) and lower specificity (around 0.50) (*Table 2*).

Agreement between clinician cervical samples and self-collected vaginal

The HPV results presented good agreement (Kappa =0.90) and high sensitivity (0.91) and specificity (0.98) (*Table 3*).

Pre-analytic stability of self-sampling

The rate of invalid results was 0% for the flocked swab placed in cell medium immediately (D0) and 6.5% (N=10) for those placed seven days after the collection (D7). In 7.2% (N=11) of the samples, the 7-day time limit was exceeded, and they were excluded from the study. For swabs stored for 7 days at room temperature, the HPV results presented good

Table 2 - *Agreement between positive HPV results and cytological diagnosis.*

Cytology diagnosis	*No (%)*	*Clinician cervical samples*			*Self-collected vaginal sample (Day 0)*			*Self-collected vaginal sample (Day 7)*		
		HPV positive (No)	*HPV negative (No)*	*Invalid Results or Exceeding the pre-analytical time (No)*	*HPV positive (No)*	*HPV negative (No)*	*Invalid Results or Exceeding the pre-analytical time (No)*	*HPV positive (No)*	*HPV negative (No)*	*Invalid Results or Exceeding the pre-analytical time (No)*
NILM	39 (24,8)	18	21	0	17	22	0	19	18	2
ASC-US	5 (3.2)	4	1	0	5	0	0	5	0	0
ASC-H	1 (0.6)	1	0	0	0	1	0	0	0	1
LSIL	7 (4.5)	7	0	0	7	0	0	6	0	1
HSIL	3 (1.9)	3	0	0	3	0	0	2	0	1
Agreement rate (%)		65.5			67.3			63.3		
Kappa (95% CI])		0.36 (0.13-0.59)			0.39 (0.15-0.62)			0.35 (0.11-0.59)		
Sensitivity (95% CI])		0.94 (0.82-1.00)			0.94 (0.82-1.00)			1.00 (1.00-1.00)		
Specificity (95% CI])		0.54 (0.38-0.70)			0.55 (0.40-0.71)			0.49 (0.32-0.65)		

Legend: NILM (negative for intraepithelial lesions or malignancy); ASC-US (atypical squamous cells of undetermined significance); ASC-H (atypical squamous cells, cannot exclude high grade squamous intraepithelial lesion); LSIL (low-grade squamous intraepithelial lesion); HSIL (positive for high-grade squamous intraepithelial lesion or carcinoma.

Table 3 - *Agreement of HPV results between the clinician cervical samples (CCS) and the self-collected vaginal samples (SCS) according to pre-analytical time.*

Storage time after collection	*CCS sample negative and SCS samples negative*	*CCS sample negative and SCS samples positive*	*CCS sample positive and SCS samples negative*	*CCS sample positive and SCS samples positive*	*Invalid test*	*Untreated (time limit exceeded)*	*Agreement rate (%)*	*Kappa (95% CI)*	*Sensitivity (95% CI)*	*Specificity (95% CI)*
Placed immediately in the cell medium (D0)	122	2	3	30	0	0	96.8	0.90 (0.81-0.99)	0.91 (0.81-1.00)	0.98 (0.96-1.00)
Storage for 7 days at room temperature (D7)	103	4	1	28	10	11	96.3	0.89 (0.80-0.99)	0.97 (0.90-1.00)	0.96 (0.93-1.00)

agreement (Kappa =0.89) and high sensitivity (0.97) and specificity (0.93) (*Table 3*).

DISCUSSION

According to Bruni *et al*. (2010) an average of 12% of women worldwide had a detectable cervical HPV infection varying by geography and age. Our study showed a high prevalence of HPV positive tests (27%). This prevalence peaked between the ages of 20 and 30 (43%) and declined thereafter. It has been shown that levels of positive HPV can vary by geography and age (Bruni *et al*., 2010). Some epidemiological studies have reported a significant reduction in HPV prevalence throughout the third decade of life (Sellors *et al.,* 2000).

Another important aspect of this study's findings referred to type-specific HPV prevalence in women with normal cytology (11,5%; 18/157). Bruni *et al.* (2010) reported that the prevalence of HPV in women with normal cytology results was 11.7% worldwide and 9% in Western Europe. In our study, HPV was positive in 94% of the cases with cytological abnormalities. This very good specificity confirms that the HR-HPV test can be positioned as a reliable and rapid diagnostic tool for the early identification and prevention of cervical cancer.

Our results showed a high prevalence of Other HPV type in the population studied (67% for normal cytology and 53% for abnormal cytology). The second most prevalent one was HPV-16. If physicians are aware that HPV-16 is the most prevalent type in the world, the pool composition of 12 oncogenic HPV types could be an important requirement for good screening.

We observed that HPV detection by self-collected vaginal sample was comparable to clinician cervical sample. There was a high degree of concordance in HPV results (Kappa =0.90) and good sensitivity (0.91) and specificity (0.98). Although there were variations in tools, instructions, transport media, storage and HPV tests used, several studies have reported that self-collected HPV DNA testing is comparable to physician-collected HPV DNA testing (Agorastos *et al*., 2005; Anhang *et al*., 2005; Ørnskov *et al*., 2021).

In France, HPV sampling is generally done by a gynaecologist, but also by other healthcare professionals (general practitioner, midwife, etc.). Cells are scraped from the cervix, washed in a liquid solution (storage medium), then sent to the laboratory. Our study showed that self-collected and dry-stored vaginal samples, which were stored at room temperature for 7 days, had equal overall performance for the detection of HR-HPVs. These results are encouraging for HPV screening using self-collected samples, as logistical and time-sensitivity issues do not appear to be an immediate concern within a reasonable time after sample collection. The use of self-collected cervical samples could be a simple and inexpensive approach in cervical cancer screening programs due to their high pre analytical stability.

CONCLUSIONS

Our data showed that the HPV assay performed in the self-collected vaginal samples have shown high consistency of results with the clinician cervical samples. These results demonstrated the technical feasibility of using self-collected swab material.

Conflicts of interest

No potential conflicts of interest were reported by the authors.

References

Agorastos T., Dinas K., Lloveras B., et al. (2005). Self-sampling versus physician-sampling for human papillomavirus testing. *Int J STD AIDS.* **16**, 727-729.

Anhang R., Nelson J.A., Telerant R., et al. (2005). Acceptability of self-collection of specimens for HPV DNA testing in an urban population. *J Womens Health.* **14**, 721-728.

Apgar B.S., Zoschnick L., Wright T.C. (2003). The 2001 Bethesda system terminology. *Am Fam Physician.* **68**, 1992-1998.

Bosch F.X., Lorincz A., Munoz N., Meijer C.J., Shah K.V. (2002). The causal relation between human papillomavirus and cervical cancer. *J Clin Pathol.* **55**, 244-265.

Bruni L., Diaz M., Castellsagué X., Ferrer E., Bosch F.X., de Sanjosé S. (2010). Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *J Infect Dis.* **202**, 1789-1799.

Cohen J. (1960). A coefficient of agreement for nominal scales. *Educ Psychol Meas.* **20**, 37-46.

Landis J.R., Koch G.G. (1977). The Measurement of Observer Agreement for Categorical Data. *Biometrics.* **33**, 159-174.

Ørnskov D., Jochumsen K., Steiner P.H., et al. (2021). Clinical performance and acceptability of self-collected vaginal and urine samples compared with clinician-taken cervical samples for HPV testing among women referred for colposcopy. A cross-sectional study. *BMJ Open.* **11**, e041512.

Reques L., Rolland C., Lallemand A., et al. (2021). Comparison of cervical cancer screening by self-sampling papillomavirus test versus pap-smear in underprivileged women in France. *BMC Women's Health.* **21**, 221.

Schiffman M., Doorbar J., Wentzensen N., de Sanjose S., Fakhry C., Monk B.J., Stanley M.A., Francheschi S. (2016). Carcinogenic human papillomavirus infection. *Nat Rev Dis Primers.* **2**, 16086.

Sellors J.W., Mahony J.B., Kaczorowski J., et al. (2000). Prevalence and predictors of human papilloma virus infection in women in Ontario, Canada. *CMAJ.* **163**, 503-508.

Verdoodt F., Jentschke M., Hillemanns P., Racey C., Snijders P., Arbyn M. (2015). Reaching women who do not participate in the regular cervical cancer screening programme by offering self-sampling kits: a systematic review and meta-analysis of randomised trials. *Eur J Cancer.* **51**, 2375-2385.

Walboomers J.M., Jacobs M.V., Manos M.M., Bosch F.X., Kummer J.A., Shah K.V., Snijders P.J., Peto J., Meijer C.J., Munoz N. (1999). Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol.* **189**, 12-19.