Application of CytoPath®Easy Vials in Cervical Cancer Screening: Self-Sampling Approach

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Abstract

Context: *CytoPath®Easy* kit (DiaPath S.p.A.) offers a major advantage compared to other commercially available kits available for the screening of cervical cancer, as it does not require additional equipment for sample processing. Using this methodology, collected epithelial cells are immersed in a preservative liquid before setting as a thin layer on a slide via gravity sedimentation. **Aims:** To evaluate the suitability of the *CytoPath®Easy* kit for the processing of cervical samples, detection of pre-neoplastic lesions, and nucleic preservation and extraction for HR-HPV diagnosis. **Materials and Methods:** A total of 242 self-sampled cervical specimens were utilized, with 192 collected in *CytoPath®Easy* vials and 50 collected and processed using the *ThinPrep™* for comparative analysis. The samples underwent processing, Papanicolaou staining, and microscopic evaluation for morphological parameters. The extracted nucleic acids were assessed for purity and integrity, and the detection of high-risk human papillomavirus (HR-HPV) was carried out using the Alinity^{m} HR HPV system kit (Abbott Laboratórios Lda). **Results:** Both methods demonstrated effective performance, enabling the morphological assessment of the cervical epithelium. Statistical analysis indicated that *ThinPrep™* yielded significantly better results in terms of cellularity. Conversely, *CytoPath®Easy* exhibited superior performance in terms of the quantity of extracted DNA and its degree of purification. Concerning the time consumed during processing, both methods were comparable, with the *CytoPath®Easy* methodology standing out for its cost-effectiveness, as it does not necessitate additional instruments and consumables. **Conclusions:** The novel *CytoPath®Easy* methodology proves effective in preserving both nucleic acids and cell morphology characteristics, two crucial features for cervical cancer screening.

Keywords: Cervical cytology, HR-HPV, nucleic acids, pre-neoplastic lesions

Introduction

Cervical cancer ranks as the fourth most prevalent cancer in women globally, recording approximately 604,127 new cases and 341,831 deaths in 2020.[1] The cytology-based screening test for cervical cancer, often referred to as the Pap test, has significantly decreased both incidence and mortality rates.[2,3]

Currently, in line with European and international guidelines, the Pap test has been replaced by the molecular High-risk Human Papillomavirus (HR-HPV) test for the screening of women older than 30 years, whereas a cytology-based screening remains the recommended approach for women between 25 and 30 years old.[4-6] Used according to the guidelines, the HR-HPV test proves to be more sensitive than cytology in detecting HPV infection, displaying both a high negative predictive value (NPV) and reproducibility rate.[5,6] Moreover, self-sampling holds promise as an approach to enhance screening accessibility and adherence, particularly among underscreened women. Importantly, primary

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HPV testing opens avenues for innovative approaches in self-sampling collection.[7]

The *CytoPath®Easy* kit, developed by DiaPath S.p.A., became commercially available for the processing of both gynecological and non-gynecological samples. This method offers versatility and eliminates the need for new instrumentation and maintenance costs. The kit consists of a specially designed vial with a cap seal press, placed directly over the slide. In this process, epithelial cells are immersed in a preservative liquid, and a thin layer of cells on the slide is obtained through gravity sedimentation. Additionally, ancillary tests can be conducted on the remaining sample.[8] The primary objectives of this study were to assess the effectiveness of *CytoPath®Easy* vials in processing cervical samples for the detection of pre-neoplastic lesions and the preservation and extraction of nucleic acids for molecular analysis of HR-HPV.

Materials and Methods

Patients and samples

The study received approval from the Ethics Committee of the Escola Superior de Saúde do Instituto Politécnico do Porto and was conducted in adherence to the ethical principles outlined in the World Medical Association Declaration of Helsinki. Women who provided consent for participation completed a questionnaire at the time of collection, which included relevant data such as age, day of the last menstrual period, and previous cervix-related diseases. Strict measures were implemented to ensure data confidentiality.

A total of 242 volunteers residing in Portugal, aged between 18 and 60 years, were randomly selected for the study. Each participant received a collection vial, a cervix brush with a detachable top, and a flyer containing self-sampling instructions to facilitate proper self-sampling. To enhance the comparability of sample characteristics, women were evenly distributed based on age and the last menstrual period for different processing methodologies. Vials were stored at room temperature until processing, and women currently on their menstrual period were excluded from the study.

Processing and staining

A total of 242 cervical samples were obtained by self-sampling, with 192 samples placed in *CytoPath®Easy* vials and processed following *Diapath®* manufacturer instructions. Briefly, the flask was manually shaken for sample homogenization, the cap partially unscrewed, and the slide inserted into the flask slot with the cell imprint facing downward. After securely closing the flask, it was inverted and placed with the cap facing downward for 15 min (20 min for samples with low cellularity). Subsequently, the flask was inverted five times, placed with the lid upward, and the cap partially unscrewed to remove the slide.[8] As a control, 50 samples were collected for *ThinPrep™* vials with PreservCyt solution (Cytyc Corp., Boxborough, MA) and processed using the ThinPrep®2000 automatic equipment with gynecological samples program. The *ThinPrep™* methodology was chosen as a reference due to its global use and excellent results in preserving and processing cervical samples. Following processing, all slides were immediately placed in 96% ethanol for fixation for 15 min. The samples were then stained using the Papanicolaou technique, employing a regressive staining method with Harris hematoxylin.

Microscopic evaluation

Three independent evaluators and an experienced cytotechnologist conducted the evaluation of samples by microscopic analysis. Cytologic scoring was performed according to the following parameters: adequacy parameters, cellular density, presence of transformation zone (TZ), presence of atypia or pre-malignant features of the lesion; morphological parameters (including the thickness of imprint/overlapping, background/debris, and preservation of cellular morphology), and staining properties, such as nuclear detail, hematoxylin color and nuclear differentiation, cytoplasmic staining, and differentiation. Samples were deemed unsatisfactory if fewer than 2,000 cells were observed, according to the Bethesda criteria.[9] The parameters and scores utilized for the microscopic assessment are outlined in Table 1.

Table 1: Detailed parameters and scores considered for the microscopic evaluation of cytological preparations processed by ThinPrepTM and CytoPath®Easy

Evaluation grid: criteria for sample microscopic evaluation	
Parameters	**Score**
Global characteristics	
Cellularity	
Sample with satisfactory cellularity	3
Sample or with borderline cellularity	2
Acellular or hypocellular sample	1
Imprint Thickness/Cell Overlap	
Absence of excessive overlap and adequate imprint thickness on virtually the entire slide	2
Intense cellular overlap and/or thick imprint in more than 30% of the slide	1
Presence of undesirable background	
Absence of undesirable background on virtually the entire slide	2
Presence of undesirable background in more than 30% of the slide	1
Preservation of cell morphology	
Preservation of cell morphology in virtually the entire slide	3
Preservation of cellular morphology in more than 50% of the slide, with the presence of some drying and fixation artifacts	2
Absence of cell preservation and/or presence of abundant drying and fixation artifacts	1
Nuclear staining	
Nuclear detail	
Well-defined chromatin pattern and detail in virtually all nuclei	3
Well-defined chromatin pattern and detail in most nuclei	2
Chromatin visible, but with loss of pattern and poorly defined details in most nuclei	1
Haematoxylin colour and nuclear differentiation	
Blue/purple staining and optimal nuclear staining intensity in virtually all nuclei	4
Blue/purple staining in most nuclei and of acceptable intensity	3
Nuclear staining present but poorly represented (pale) or with overstained nuclei	2
Pink/red/green coloration in more than 50% of nuclei or virtually all nuclei without nuclear differentiation	1
Cytoplasmic staining/differentiation	
The three colours are equally represented, the pink, orange and blue tones being visible, thus making it possible to differentiate between basophil cells and eosinophil cells	3
The three colours are present, but at least one of them is underrepresented, thus making it difficult to differentiate between basophil and eosinophil cells	2
Inappropriate or missing colour spectrum across most of the slide	1

Genomic detection of HR-HPV

The genomic detection of HR-HPV was performed using an automated technology using Alinity^{m} HR HPV AMP Kit (Abbott, Laboratórios Lda). The automated nucleic acid extraction was performed in the automated system followed by quantitative polymerase chain reaction (qPCR). To perform the analysis, a 2 mL aliquot of the cell suspension from each sample was transferred to an Eppendorf tube and sent to Pathology Service of the Unidade Local de Saúde de Matosinhos. The HR-HPV test was conducted on 242 samples following the manufacturer instructions.[10] The automated Alinity^{m} methodology enables the *in vitro* detection of 14 different HR-HPV genotypes in clinical specimens. The assay specifically identifies HPV genotypes 16, 18, and 45, whereas concurrently detecting other HR genotypes, classified as group A (31/33/52/58) and group B (35/39/51/56/59/66/68). Alinity^{m} HR-HPV methodology uses the human beta-globin sequence as a housekeeping gene internal control.[10]

Nucleic acids quality evaluation

For the assessment of nucleic acids, a 2 mL aliquot of the cell suspension of each sample was transferred to an Eppendorf tube. Samples were then centrifuged (1300 rpm, 7 min), washed twice in distilled water, and stored at -20°C. Based on comparable cellularity levels observed under the microscope, 19 *ThinPrep™* samples and 33 *CytoPath®Easy* samples were chosen for nucleic acid extraction. DNA extraction was performed using the ExtractMe® DNA Tissue Kit, following the manufacturer's instructions (BLIRT S.A.).[11] DNA abundance and purity were assessed using the NanoDrop 1000 spectrophotometer (Thermo Scientific®, Massachusetts). The spectrophotometric reading of each sample was carried out at 230 nm, 260 nm, and 280 nm. DNA integrity was evaluated via electrophoresis in a 1.5% agarose gel containing GreenSafe Premium (NZYTech) for the visualization of DNA under UV light on a transilluminator (VILBER Lourmat 312-365).

Statistical analysis

The microscopic evaluation resulted in scores, as well as the nucleic acid quality and amplification parameters were statistically analyzed using the GraphPad Prism® 8.0 software (GraphPad Software Inc., San Diego). A significance level of 0.05 (α) was applied to all conducted statistical tests. The *t*-student test for independent samples was utilized to assess the total scores obtained from different methodologies and individual parameters. The distribution of TZ, HR-HPV infection, genotyping, and atypical cells among samples processed by both methodologies was compared using the Chi-square test. Results are presented as mean ± standard deviation.

Results

Microscopic evaluation

Cytological preparation and staining characteristics

For the cytological assessment, samples were collected into vials, and a macroscopic evaluation was performed to register the presence or absence of mucus and/or other components that could compromise the imprinting of the cells into the slide for *CytoPath®Easy*. Samples lacking cellularity were omitted, including three samples processed using *CytoPath®Easy*. Following processing by each methodology, we analyzed cytological features, at low microscopic magnification, and observed the global characteristics of the cell imprint [Figure 1a and d]. Both methodologies enabled a thin-layer imprint, without excessive non-diagnostic elements, and with good cellularity and preservation of cell morphology. At high magnification, we could discern nuclear details and hematoxylin color, along with cytoplasmic differentiation. There was no significant difference observed between the methods, as depicted in Figure 1b, c, e and f.

Concerning the evaluation of overall characteristics, including cellularity, imprint thickness/cell overlap, presence

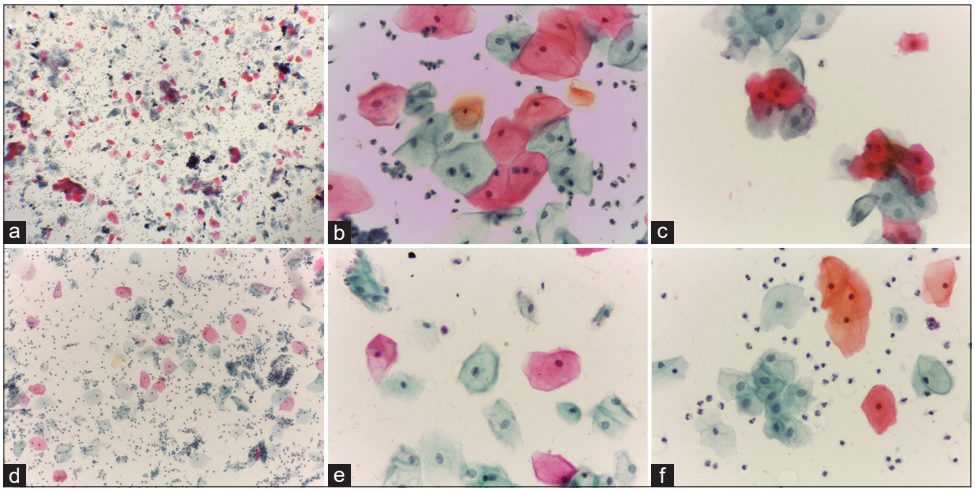

Figure 1: Microscopic evaluation of cell features analyzed after sample processing using each methodology, Papanicolaou staining, and mounting. Global characteristics of the cell imprint are observed in (a) (*ThinPrep™*) and (d) (*CytoPath®Easy*) at a low magnification field (100x); background, cellularity, and cell morphology preservation characteristics, as well as nuclear detail, hematoxylin color and cytoplasmic differentiation are observed at high magnification–400x (b, c (*ThinPrep™*), e and f (*CytoPath®Easy*))

of undesirable background, and preservation of cell morphology, both methods yielded comparable and satisfactory mean results (*CytoPath®Easy*: 9.2 vs. *ThinPrep™*: 9.6, $P = 0.168$). Notably, a significant difference in cellularity was observed between the two methods (*CytoPath®Easy*: 2.5 vs *ThinPrep™*: 2.8, $P = 0.048$). However, other parameters such as imprint thickness/overlapping (CytoPath®Easy: 1.9 vs. *ThinPrep™*: 1.8, $P = 0.243$), presence of background/debris (*CytoPath®Easy*: 1.9 vs. *ThinPrep™*: 2.0, $P = 0.135$), and morphological preservation (*CytoPath®Easy*: 2.9 vs. *ThinPrep™*: 3.0, $P = 0.487$) showed no statistically significant differences, as indicated in Figure 2 and Table 2.

Detailed observation and scoring of staining properties revealed similar and high scores for both methods. Microscopic evaluation based on the main criteria for detecting atypia and abnormalities showed no statistically significant differences between the two groups. Comparable mean values were found for nuclear details (*CytoPath®Easy*: 2.9 vs. *ThinPrep™*: 3.0, $P = 0.263$), hematoxylin color, and nuclear differentiation (*CytoPath®Easy*: 3.7 vs. *ThinPrep™*: 3.7, $P = 0.766$), and cytoplasmic staining and differentiation (*CytoPath®Easy*: 2.5 vs. *ThinPrepTM*: 2.5, $P = 0.855$), as depicted in Figure 3 and Table 2.

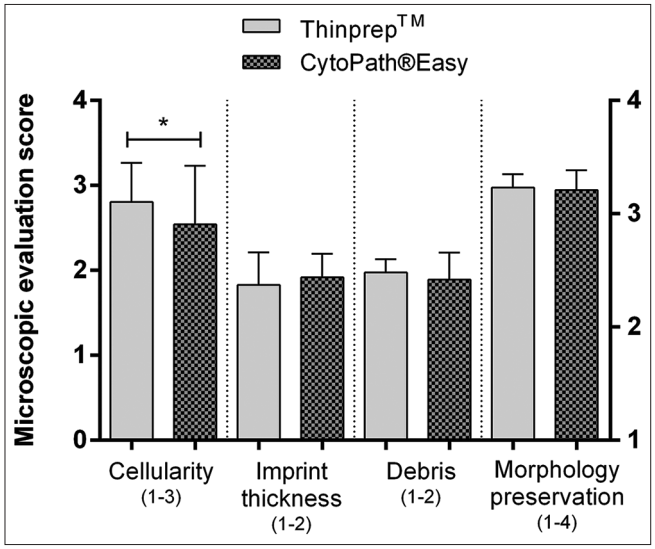

Figure 2: Graphic representation of global characteristics scores-cellularity, imprint thickness/cell overlap, presence of undesirable background, and preservation of cell morphology. Results showed both *CytoPath®Easy* and *ThinPrep™* provide similar results, although significant statistical differences were observed in cellularity

Atypia, pre-neoplastic lesions, and other cytological findings

The presence of the TZ component was assessed by identifying at least two groups of five well-preserved endocervical and/or metaplastic cells. The TZ was noted in 40 samples processed with *CytoPath®Easy* (21%) and in 11 samples processed with *ThinPrep™* (27%), with no statistically significant differences ($P = 0.483$), as indicated in Table 3.

Microscopic analysis revealed atypical/abnormal cells in 39 cases. Among these, 31 cases were from *CytoPath®Easy* processed samples (16%): 6 were classified as low-grade squamous intraepithelial lesions (LSIL), and 25 as atypical squamous cells of undetermined significance (ASC-US). In *ThinPrep™* samples, eight cases (20%) with atypical cells were identified, and classified as ASC-US. No statistical differences

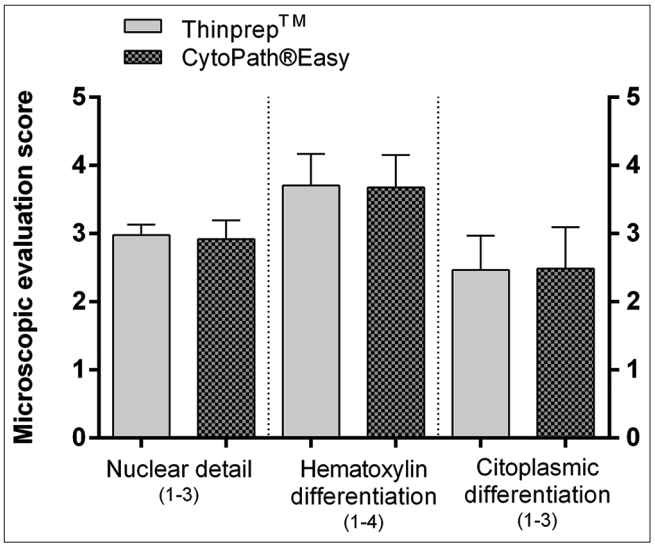

Figure 3: Graphic representation of staining properties. Similar mean values were found with regard to nuclear detail, hematoxylin color, nuclear differentiation, cytoplasmic staining, and differentiation

Table 2: Summary of findings on cellular morphology and staining characteristics

	Parameters	CytoPath®Easy *n*=192	ThinPrep™ *n*=50	*P*
Morphological characteristics	Cellularity (score 1-3)	2.5	2.8	0.048
	Thickness of imprint/Overlapping (1-2)	1.9	1.8	0.243
	Background/debris (1-2)	1.9	2	0.135
	Preservation of cellular morphology (1-3)	2.9	3	0.487
	Total (1-10)	9.2	9.6	
Staining properties	Nuclear detail (1-3)	2.9	3	0.263
	Hematoxylin color and nuclear differentiation (1-4)	3.7	3.7	0.766
	Cytoplasmic staining and differentiation (1-3)	2.5	2.5	0.855
	Total (1-10)	9.1	9.2	

were observed when comparing methods in terms of the proportion of samples with atypical cells and/or pre-malignant intraepithelial lesions ($P = 0.600$).

Additionally, other infections/agents were detected through cytological evaluation, including a shift in flora suggestive of bacterial vaginosis (two cases), fungal organisms morphologically consistent with *Candida* species (four cases), and *Trichomonas vaginalis* (one case), irrespective of the collection/processing methodology. A concise summary of the results is presented in Table 3.

Alinity^{m} HR-HPV molecular test

A total of 242 samples were examined to identify genomic HR-HPV using Alinity^{m} technology. The HR-HPV test yielded positive results in 24 samples, with an equal proportion observed between the two cell collection methods: *CytoPath®Easy*, 20 cases (10%) vs. *ThinPrep™*, 4 cases (10%), yielding a non-significant difference ($P = 0.892$). Positivity for HR-HPV group B was identified in 19 cases (80%), with co-infections involving genotypes from groups A and B found in 2 cases. One case tested positive for genotype (s) from group A (4%), and other co-infections were observed in one case (4%), such as with genotype 45 and group A genotype (s). These findings were independent of the collection/processing methodology ($P > 0.05$), as outlined in Table 4. Nevertheless, a larger sample size is required to confirm this trend, especially regarding the case positive for genotype 18 (4%), which belonged to the *ThinPrep™* group.

The amplification of viral DNA sequences occurred within a similar cycle threshold (CT) range for the different methodologies (22.36 for *CytoPath®Easy* and 22.94 for *ThinPrep™*). Additionally, Alinity^{m} HR-HPV CTs for the human beta-globin sequence were recorded and compared between the two methodologies. Both preservative fluids facilitated the amplification of the sample control within a low number of cycles: 19.91 for *CytoPath®Easy* and 20.74 for *ThinPrep™*.

Concurrent atypia/lesions in HR-HPV positive cases were observed in 14 cases (70%) for *CytoPath®Easy* and in 2 cases (50%) for *ThinPrep™* processed samples. Despite this difference, statistical significance was not attained ($P = 0.525$). A summary of these results is provided in Table 4.

Nucleic acid assessment

The assessment of DNA purity was calculated via spectrophotometric analysis of the ratio of absorbance readings at 260 nm/280 nm (A260/A280), whereby a range of 1.7–1.9 is considered of optimum purity. The average A260/A280 values for DNA extracted from both methods fall within this range (*CytoPath®Easy* (1.84 ± 0.2) and *ThinPrep™* (1.75 ± 0.5)), indicating minimal contamination by proteins or other molecules absorbing near 280 nm [Figure 4]. In contrast, the A260/A230 ratio calculated for nucleic acids extracted was below the reference values for both methods-*CytoPath®Easy* samples (1.57 ± 0.5) and *ThinPrep™* samples (0.77 ± 0.4), signifying contamination by reagents such as phenol and/or salts. Nevertheless, no statistically significant differences between methods were observed for A260/A280 ($P = 0.6435$), but significant differences were shown for A260/A230 ($P < 0.0001$), demonstrating *CytoPath®Easy* method performed better regarding this parameter.

Notably, although the number of cells used for nucleic acids extraction was not measured, significant differences in DNA yield were observed on the NanoDrop 1000 spectrophotometer

Table 3: Summary of findings on adequacy and atypia/pre-malignant lesions

	Parameters	CytoPath®Easy *n*=192	ThinPrep™ *n*=50	*P*
Adequacy and atypia	Unsatisfactory samples (*n*,%)	3 (1.6%)	0 (0%)	0.374
	Presence of transformation zone (*n*,%)	40 (21%)	11 (27%)	0.399
	Presence of atypia/pre-malignant lesion (*n*,%)	31 (16%)	8 (20%)	0.600
	Low-grade squamous Intraepithelial Lesion (*n*)	6	0	0.206
	Atypical Squamous cells of undetermined significance (*n*)	25	8	0.585
	Microorganisms (*n*,%)	4 (2.1%)	3 (6%)	0.141

Table 4: Summary of results of HR-HPV molecular test

	Parameters	CytoPath®Easy *n*=192	ThinPrep™ *n*=50	*P*
HR-HPV molecular test	HR-HPV positive cases (*n*,%)	20 (10%)	4 (10%)	0.892
	Group B positive cases (*n*,%)	16 (8.3%)	3 (8.0%)	0.585
	Group A positive cases (*n*,%)	1 (0.5%)	0	0.610
	Co-infection with genotypes from groups A and B (*n*,%)	2 (1.0%)	0	0.469
	Genotype 18 positive cases (*n*,%)	0	1 (2.0%)	0.050
	Other co-infections (*n*,%)	1 (0.5%)	0	0.610
	Atypia/pre-malignant lesion on HR-HPV positive cases (*n*,%)	14 (67%)	2 (50%)	0.525

reader. DNA samples obtained from *CytoPath®Easy* exhibited a higher concentration of extracted DNA compared to *ThinPrep™* samples, with a 2.1-fold change difference. An average value of 29.55 µg/µL was obtained from samples collected using *CytoPath®Easy* vials, whereas *ThinPrep™* samples yielded 14.12 µg/µL. Both methodologies enabled the extraction of high molecular weight DNA, characterized by clear and intense bands on an electrophoresis gel, indicating overall excellent integrity of the extracted nucleic acids, as observed in Figure 5.

Processing time, materials, and equipment

Regarding processing time, both methodologies were comparable, with an average of 1.5 min per sample. Although the total processing time per sample was greater for *CytoPath®Easy*, the methodology allowed manual and consecutive sample processing, meaning that for about 15 samples processed, the average time per sample remained similar. It is important to highlight that the *CytoPath®Easy* kit is administered entirely manually, eliminating the need for additional equipment, filters, or other consumables. These features render this new methodology highly cost-effective.

Discussion

On a global scale, the outcomes from this study indicate that the *CytoPath®Easy* kit demonstrates effective performance in both morphological assessment of cervical epithelium and molecular detection of HR-HPV in samples. Statistical analysis of the morphological evaluation reveals differences only in terms of cellularity. Samples lacking cellularity, which are part of the *CytoPath®Easy* kit, were subjected to macroscopic analysis for the presence of mucus or other components that could hinder imprint formation. The confirmation of cell absence occurred during subsequent processing through a dispersion and filtration system. Consequently, it can be inferred that the lack of cells is likely a result of inadequate self-sampling. Despite emerging recommendations for the implementation of self-sampling, significant barriers persist.[12] One aspect involves the confidence of women in self-sampling, coupled with the essential requirement for a thorough education on the procedure to ensure satisfactory outcomes.[7,12] Research indicates the reliability of HPV self-sampling in comparison to cervical samples collected by clinicians. Overall, there is widespread acceptance and positive attitudes toward self-sampling, particularly among women who are difficult to reach or who do not regularly attend screenings.[12-15] Drawing from this information, several European nations have already incorporated the utilization of self-sampling kits into their strategies to enhance the coverage of screening programs.[13,16]

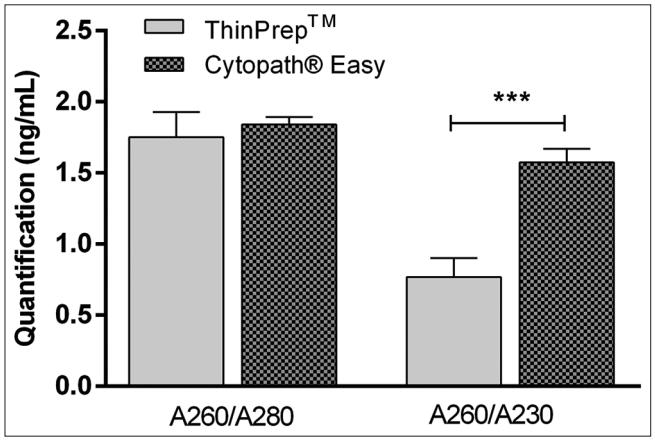

Figure 4: Graphic representation of results obtained by a spectrophotometric reading of DNA extracted from *ThinPrep™* and *CytoPath®Easy* samples. A260/280, A260/A230, and DNA yield were quantified and compared. Significant differences were observed on A260/A230 ($P < 0.0001$). NanoDrop 1000 spectrophotometer reader reveals a bigger concentration of extracted DNA from *CytoPath®Easy* vials

Our findings concerning the cytology-based microscopic analysis indicate that the processing of samples using the *CytoPath®Easy* kit maintains overall characteristics effectively. This includes achieving a thin-layer imprint devoid of excessive non-diagnostic elements, with good cellularity and preservation of cell morphology. When observed at higher magnification, nuclear detail, hematoxylin color, and cytoplasmic differentiation exhibit no significant differences between methods. Additionally, a comparison between the *ThinPrep™* filtration methodology and the *CytoPath®Easy* kit reveals similar proportions of cases with TZ representation and atypical cells in the slides. Furthermore, regardless of the collection/processing methodology, only a minimal number of samples exhibited microorganisms. Although our preliminary results suggest promising accuracy for *CytoPath®Easy*, further studies would benefit from a larger number of cases with atypia and premalignant lesions to effectively draw conclusive remarks about its morphologic diagnostic capacity. Importantly, our study demonstrated that the *CytoPath®Easy* kit facilitates the extraction of cervical sample DNA with a high yield and quality. Although it is recognized that the optimal DNA quality is typically achieved with fresh-frozen tissue and/or cytological specimens, the practicality of systematic freezing in pathology laboratories on a daily basis is often limited. Therefore, numerous studies have been conducted to evaluate the performance of various fixatives in DNA extraction, catering to both research and diagnostic requirements,[17-19] including for cervical cancer screening purposes.[20-24] Although the *ThinPrep™* fixative liquid is methanol-based[25] and *CytoPath®Easy* primarily

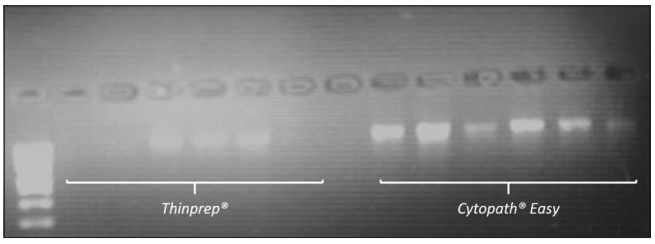

Figure 5: Representative image of electrophoresis gel (1.5% agarose) running samples of both methodologies- *ThinPrep™* and *CytoPath®Easy*, captured on a UV light reader

relies on an ethanol-based solution,[8] both solutions have demonstrated satisfactory outcomes in DNA extraction from cervical cells and are both currently used for cervical cancer screening worldwide.[21-24]

In this study, the average A260/A280 ratio of DNA extracted from *CytoPath®Easy* and *ThinPrep™* samples fell within the expected range (1.8–2.0), indicating the absence of protein contamination. However, the A260/A230 ratios for both methods were lower than the reference values, suggesting contamination by reagents such as phenol, carbohydrates, and salts. Although there were no statistically significant differences between the methods in terms of A260/A280, they were statistically significant in relation to A260/A230. The diminished A260/A230 ratios could be attributed to contamination by substances with the absorbance at lower wavelengths, possibly, linked to the composition of the preservative solution and/or contaminants and intrinsic substances in the samples. For instance, certain preservative solutions may contain ethylenediaminetetraacetic acid (EDTA), whose absorbance occurs near 230 nm.[25-27] In this study, this phenomenon did not disrupt the amplification process in the Alinity^{m} HR-HPV test, as comparable results were achieved for internal controls in both methods. Previous studies have also reported data showing low A260/A230 values after preserving nucleic acids in *PreservCyt*.[28] However, further investigation would be necessary to its impact on more sensitive methodologies involving nucleic acids for amplification and sequencing.

An important aspect to be explored in subsequent studies is the DNA yield capacity associated with various collection and preservative fluids used in clinical settings. In this study, *CytoPath®Easy* demonstrated a higher DNA extraction yield (2.1 times higher) and exhibited minimal reagent contamination compared to samples collected using the *ThinPrep™* method. The consistently high quality and quantity of nucleic acids obtained from cells preserved with *CytoPath®Easy* vials were corroborated by the low cycle threshold (CT) of the sample control amplification. In terms of this molecular parameter, both preservative fluids enabled the amplification of the housekeeping control gene within a low number of cycles, indicating cellular adequacy, sample extraction, and amplification efficiency.[29]

Regarding processing time, both methodologies exhibited an average of 1.5 min per sample. Additionally, the application of *CytoPath®Easy* is highly cost-effective as it does not require additional equipment, filters, or other consumables for sample processing.

Although only explored for gynecological samples, *CytoPath®Easy* was designed to provide comparable results for non-gynecological samples.[8] Further studies are warranted to comprehensively investigate *CytoPath®Easy*'s characteristics across various parameters, including its application in immunocytochemistry ancillary tests. Additionally, extending its use to other sample types, such as urological samples, could contribute to the implementation of screening strategies, particularly in countries with lower income levels, where incidence and mortality rates are elevated in related diseases.[30]

Altogether our results showed that, despite certain variances observed between methods, the innovative *CytoPath®Easy* demonstrates the effectiveness in preserving nucleic acids and cell morphology characteristics—both crucial factors in cervical cancer screening. Furthermore, this novel methodology does not demand expensive equipment, presenting particular promise for Pathology Laboratories dealing with a limited number of liquid-based samples and for screening and diagnostic purposes in low-income countries.

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Conflicts of interest

There are no conflicts of interest.

References

1. Human papillomavirus and Related Disease Report - HPV center. Available from: https://hpvcentre.net/statistics/reports/XWX.pdf. [Last accessed on 2023 Mar 29].
2. Eun TJ, Perkins RB. Screening for cervical cancer. Med Clin North Am 2020;104:1063.
3. Bedell SL, Goldstein LS, Goldstein AR, Goldstein AT. Cervical cancer screening: Past, present, and future. Sex Med Rev 2020;8:28–37.
4. Bhatla N, Aoki D, Sharma DN, Sankaranarayanan R. Cancer of the cervix uteri: 2021 update. Int J Gynecol Obst 2021;155:28–44.
5. Maver PJ, Poljak M. Primary HPV-based cervical cancer screening in Europe: Implementation status, challenges, and future plans. Clin Microbiol Infect 2020;26:579-83.
6. Armaroli P, Villain P, Suonio E, Almonte M, Anttila A, Atkin WS, *et al*. European code against cancer: Cancer screening. Cancer Epidemiol 2015,39:S139-52.
7. Tatar O, Haward B, Zhu P, Griffin-Mathieu G, Perez S, McBride E, *et al*. Understanding the challenges of HPV-based cervical screening: Development and validation of HPV testing and self-sampling attitudes and beliefs scales. Curr Oncol 2023;30:1206-19.
8. DiaPath S.p.A. CytoPath®Easy: Complete solutions for liquid-based cytology. Available from: Available from: https://www.diapath.com/product/cytopath-easy--cp350-2609. [Last accessed on 2023 Mar 29].
9. Nayar R, Wilbur DC. The Bethesda System for Reporting Cervical Cytology: Definitions, Criteria, and Explanatory Notes. 3rd ed. Springer; 2015.
10. Abbott. Alinity mHR HPV Assay. Available from: https://www.molecular.abbott/int/en/products/infectious-disease/alinity-m-hr-hpv-

assay. [Last accessed on 2022 Sep 02].
11. BLIRT S.A. Kit for DNA isolation from animal tissue and cell culture. Available from: https://blirt.eu/wp-content/uploads/2018/05/DNA_TISSUE_en_08062018-min.pdf. [Last accessed on 2023 Mar 27].
12. Madzima TR, Vahabi M, Lofters A. Emerging role of HPV self-sampling in cervical cancer screening for hard-to-reach women: Focused literature review. Can Fam Physician 2017;63:597-601.
13. World Health Organization. HPV self-sampling in Sweden leading to faster elimination of cervical cancer. Available from: https://www.who.int/europe/news/item/08-09-2022-hpv-self-sampling-in-sweden-leading-to-faster-elimination-of-cervical-cancer. [Last accessd on 2023 Mar 27].
14. Fujita M, Nagashima K, Shimazu M, Suzuki M, Tauchi I, Sakuma M, *et al*. Implementation of a self-sampling HPV test for non-responders to cervical cancer screening in Japan: Secondary analysis of the ACCESS trial. Sci Rep 2022;12:14531.
15. Nuttchote P, Oranratanaphan S, Termrungruanglert W, Triratanachat S, Chaiwongkot A, Baedyananda F, *et al*. Comparison of detection rate of high risk HPV infection between self-collected HPV testing and clinician-collected HPV testing in cervical cancer screening. Taiwan J Obstet Gynecol 2019;58:477-81.
16. Aasbø G, Tropè A, Nygård M, Christiansen IK, Baasland I, Iversen GA, *et al*. HPV self-sampling among long-term non-attenders to cervical cancer screening in Norway: A pragmatic randomised controlled trial. Br J Cancer 2022;127:1816-26.
17. Berrino E, Annaratone L, Miglio U, Maldi E, Piccinelli C, Peano E, *et al*. Cold formalin fixation guarantees DNA integrity in formalin fixed paraffin embedded tissues: Premises for a better quality of diagnostic and experimental pathology with a specific impact on breast cancer. Front Oncol 2020;10,173.
18. Bressan EA, Rossi ML, Gerald LT, Figueira A. Extraction of high-quality DNA from ethanol-preserved tropical plant tissues. BMC Res Notes 2014;7:1-6.
19. Piskorz AM, Ennis D, Macintyre G, Goranova TE, Eldridge M, Segui-Gracia N, *et al*. Methanol-based fixation is superior to buffered formalin for next-generation sequencing of DNA from clinical cancer samples. Ann Oncol 2016;27:532-9.
20. Silva A, Salta S, Henrique R, Jerónimo C. Comparison and optimization of DNA extraction methods from cervical cells collected in ThinPrep™ PreservCyt. Citotech Online Case Rev 2020;5,1–10. doi: 10.26537/citotech.vi5.3778.
21. Zhao FH, Hu SY, Bian JJ, Liu B, Peck RB, Bao YP, *et al*. Comparison of ThinPrepTMand SurePath liquid-based cytology and subsequent human papillomavirus DNA testing in China. Cancer Cytopathol 2011;119:387-94.
22. Dunn ST, Allen RA, Wang S, Walker J, Schiffman M. DNA extraction: An understudied and important aspect of HPV genotyping using PCR-based methods. J Virol Methods 2007;143:45-54.
23. Yeo SJ, Kang MK, Nam KH, Kim JS, Kim TH, Lee KH. Comparison of the solution of ThinPrepTM (TM) Pap test with the Cervical Sampler (TM) of Hybrid Capture (HC) II for detecting Human papillomavirus (HPV) DNA. Korean J Gynecol Oncol 2005;16:70-6.
24. Kartiki AJ, Shaikhali MB, Ashwini P, Kunjal L, Pooja S, Rakhi BD, *et al.* Comparative analysis of ThinPrep™ and CellSolutions liquid-based cervical cytology along with human papillomavirus DNA testing: A study of 412 cases. Int J Health Sci Res 2022;12. doi: 10.52403/ijhsr.20220724.
25. Hologic. ThinPrep™ PreservCyt Solution - Material safety data sheet. 2013. p. 1–5. Available from: https://www.rmlonline.com/images/data/attachments/0000/1967/Hologic_Thinprep_SDS.pdf. [Last accessed on 2023 Mar 29].
26. Mathot L, Wallin M, Sjöblom T. Automated serial extraction of DNA and RNA from biobanked tissue specimens. BMC Biotechnol 2013;13:1-6.
27. Thermo Scientific. T042-Technical Bulletin. NanoDrop Spectrophotometers, 260/280 and 260/230 Ratios. 2010. Available from: https://assets.thermofisher.com/TFS-Assets/CAD/Product-Bulletins/TN52646-E-0215M-NucleicAcid.pdf. [Last accessed on 2023 Mar 27].
28. Reynolds JP, Zhou Y, Jakubowski MA, Wang Z, Brainard JA, Klein RD, *et al*. Next-generation sequencing of liquid-based cytology non–small cell lung cancer samples. Cancer Cytopathol 2017;125:178-87.
29. Oštrbenk VA, Šterbenc A, Seme K, Poljak M. Alinity m HR HPV assay fulfills criteria for human papillomavirus test requirements in cervical cancer screening settings. J Clin Microbiol 2020;58:e01120-19. doi: 10.1128/JCM.01120-19.
30. Shi J, Tarkiainen L, Martikainen P, van Raalte A. The impact of income definitions on mortality inequalities. SSM-Population Health 2021;15:100915.