Journal of the American Society of Cytopathology (2023) 12, 170–180

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jascyto.org/

Telecytologic diagnosis of cervical smears for triage of self-sampled human papillomavirus–positive women in a resource-limited setting: concept development before implementation

Pierre Vassilakos, MD[a,b], Holly Clarke, MBBS[a,*], Micol Murtas, MD[a], Thomas Stegmüller, MSc[c], Ania Wisniak, MD[a], Farida Akhoundova, MD[a], Zacharie Sando, MD[d], George Enow Orock, MD[e], Jessica Sormani, PhD[a], Jean-Philippe Thiran, PhD[c], Patrick Petignat, MD[a]

[a] *Gynecology Division, Geneva University Hospital, Geneva, Switzerland*
[b] *Geneva Foundation for Medical Education and Research, Geneva, Switzerland*
[c] *Swiss Federal Institute of Technology Lausanne, Lausanne, Switzerland*
[d] *Gyneco-Obstetrics and Paediatric Hospital, Yaoundé, Cameroon*
[e] *Buea Regional Hospital, Buea, Cameroon*

Received 12 December 2022; received in revised form 12 January 2023; accepted 8 February 2023

KEYWORDS
Cytology;
Telepathology;
Diagnostic imaging;
Cervical cancer;
Prevention

Introduction Cytology is an option for triaging human papillomavirus (HPV)-positive women. The interpretation of cytologic slides requires expertise and financial resources that are not always available in resource-limited settings. A solution could be offered by manual preparation and digitization of slides on site for real-time remote cytologic diagnosis by specialists. In the present study, we evaluated the operational feasibility and cost of manual preparation and digitization of thin-layer slides and the diagnostic accuracy of screening with virtual microscopy.

Materials and methods Operational feasibility was evaluated on 30 cervical samples obtained during colposcopy. The simplicity of the process and cellularity and quality of digitized thin-layer slides were evaluated. The diagnostic accuracy of digital versus glass slides to detect cervical intraepithelial neoplasia grade 2

*Corresponding author: Holly Clarke, MBBS, Gynecology Division, Geneva University Hospital, Boulevard de la Cluse 30, Geneva CH-1205, Switzerland; Tel.: +41 766 383 226.
E-mail address: Holly.clarke@hcuge.ch (H. Clarke).

https://doi.org/10.1016/j.jasc.2023.02.001

or worse was assessed using a cohort of 264 HPV-positive Cameroonian women aged 30 to 49 years. The histologic results served as the reference standard.

Results Manual preparation was found to be feasible and economically viable. The quality characteristics of the digital slides were satisfactory, and the mean cellularity was 6078 squamous cells per slide. When using the atypical squamous cells of undetermined significance or worse threshold for positivity, the diagnostic performance of screening digital slides was not significantly different statistically compared with the same set of slides screened using a light microscope ($P = 0.26$).

Conclusions We have developed an innovative triage concept for HPV-positive women. A quality-ensured telecytologic diagnosis could be an effective solution in areas with a shortage of specialists, applying a same day "test-triage-treat" approach. Our results warrant further on-site clinical validation in a large prospective screening trial.

Introduction

Cervical cancer remains the fourth most common cancer diagnosed worldwide, with >300,000 deaths in 2018.[1] The distribution of cervical cancer deaths varies substantially across the world, with 90% occurring in low- and middle-income countries (LMICs), reflecting unequal access to screening.[2] The World Health Organization (WHO) has emphasized the importance of acting immediately to eliminate cervical cancer in LMICs by the end of the century through a comprehensive approach, including evidence-based prevention interventions such as vaccination and screening, in addition to timely treatment of precancerous and cancerous lesions.[2] While awaiting comprehensive vaccine rollouts in LMICs, effective screening and treatment programs could prevent one half of the predicted cervical cancer deaths during the next 50 years.[3]

Primary screening with human papillomavirus (HPV) testing is recommended for women aged >30 years in LMICs by the WHO and as the preferred primary screening method by the American Cancer Society.[4,5] Its high sensitivity and negative predictive value allow for longer durations between screening intervals.[6] Additionally, some devices provide rapid point-of-care HPV testing through analysis of self-obtained vaginal samples. These advantages have improved the efficiency of screening programs and expanded screening coverage, leading to increased referrals of eligible women for treatment. One notable disadvantage is that HPV testing alone has limited specificity and can lead to unnecessary investigations and overtreatment. Therefore, a triage strategy is necessary for HPV-positive women. Reflex cytology has been proposed in Western countries as the appropriate triage method. However, this is unfeasible in settings lacking adequate infrastructure, trained cytotechnologists, and reliable follow-up.

A solution for countries with limited resources could be affordable digital imaging technology for real-time remote cytologic diagnosis by specialists. Using this method, the preparation and digitization of cervical smears from HPV-positive women would be performed on site during the same visit using a "test-triage-and-treat" approach. This process eliminates the need for in-house cytopathologists and might allow for reliable, cost-effective triage of HPV-positive women.

Whole slide imaging, a subset of digital imaging, encompasses the process of scanning entire glass slides and converting the data into high-resolution digital images transferred to a computer monitor. Whole slide imaging has been adopted in surgical pathology,[7] and evidence has shown that the diagnostic performance of digital microscopy is equivalent to that of light microscopy.[8-10] The emergence of affordable portable scanners equipped with software that can convert microscope images of slides into whole slide images (WSIs) stored and accessed at a distance in real time, offers innovative solutions for cervical cancer screening in resource-limited settings.[11]

Telecytologic diagnosis of cervical smears using whole slide imaging technology could, therefore, be integrated as a triage test using a same day test-triage-and-treat approach to identify HPV-positive women who require immediate treatment or follow-up. For this, the preparation and digitization of Papanicolaou (Pap) smears must be conducted on site. Although in high-income countries, liquid-based cytology is the preferred method of preparing samples, liquid-based cytology requires advanced robotic technologies that are resource intensive and potentially unfeasible for LMICs. However, affordable manual liquid-based cytology methods suited to resource-limited settings are available.[12,13]

Before introducing this new triage concept to cervical cancer screening programs in low-resource contexts, the feasibility, cost, and diagnostic accuracy of the telecytology process must be assessed. Thus, we designed a two-part study. First, we evaluated the operational feasibility and cost of manual preparation and digitization of thin-layer slides. Second, we compared the diagnostic performance between virtual cytology and glass slide cytology.

Material and methods

Setting

The present study was a part of a wider partnership between the University Hospitals in Geneva, the University Hospital of Yaoundé, Cameroon, and the University of Dschang, Cameroon, with the aim of improving cervical cancer screening in Cameroon. A total of 1582 study participants were recruited in Dschang between 2018 and 2019 as a part of this collaboration (ClinicalTrials.gov identifier, NCT03757299; promoting comprehensive cervical cancer prevention and better women health in Cameroon). The cytologic samples from 264 HPV-positive participants were considered in the diagnostic agreement section of the present study. To assess the feasibility of telecytologic diagnoses, 30 cervical smear tests were taken from consenting women in the Geneva University Hospitals gynecology department in 2022 (ClinicalTrials.gov identifier, NCT05474404; telecytology as a triage tool in LMICs). The National Ethics Committee of Cameroon (approval no. 2018/07/1083/CE/CNERSH/SP) and the Ethical Cantonal Board of Geneva, Switzerland (approval no. CCER 2022-00314) granted ethical approval for the present study.

Operational feasibility and incremental cost

Thin-layer pap-stained slides

To test the feasibility of preparation and digitization of thin-layer slides, we used SurePath slides (Quest Diagnostics) for 2 reasons: (1) the area of the cell deposit is small (circle, 13 mmØ), resulting in a faster slide scanning time and smaller file size; and (2) a low-cost manual version of the SurePath Pap test is available targeted to resource-limited settings.

Preparation and digitization of liquid-based slides

A total of 30 cervical samples were taken from the Maternity Hospital, Geneva, after patient consent. The transformation zone was sampled using a spatula. The spatula head was then detached and immediately immersed in a vial with 10 mL of PreservCyte fluid (Cytyc Corp). The sample was prepared using the manual SurePath liquid-based preparation method (BD SurePath Direct to Slide Kit). Preparation involves cell randomization, pipetting, and enrichment of cervical cells through centrifugation using a settling chamber to create a cellular slide preparation (Fig. 1). Pap staining was then applied within the chamber (Supplementary Information 1).

All 30 glass slides were digitized using a compact portable scanner (Ocus40; Grundium Oy). The scanner creates a whole slide image using a digital microscope system with robotics. It features a 12-megapixel image sensor with a 40× objective (numerical aperture, 0.75) and can be connected wirelessly to a laptop computer. To optimize the focus, the Z-stack modality of acquisition at 3 focal plane levels at 1-µm intervals was used. In Europe, the Ocus40 is certified for diagnostic use. All digitized SurePath glass slides are referred to as WSIs.

The ease of the manual preparation method and digitization of the slides was assessed by asking 3 operators to rate the difficulty using a 5-point rating scale (score 1, very difficult; to score 5, very easy). The mean time for manual preparation and digitization of the glass slides was calculated.

The cellularity of the glass slide was estimated using a 40× objective and an eyepiece with a field number of 20. The total number of cells was calculated using the following formula: $N = n\,(acd/amf)$, where N is the total cell count, n is the mean cell count of 10 adjacent fields of view along the horizontal diameter in the center of the circle, acd is the area

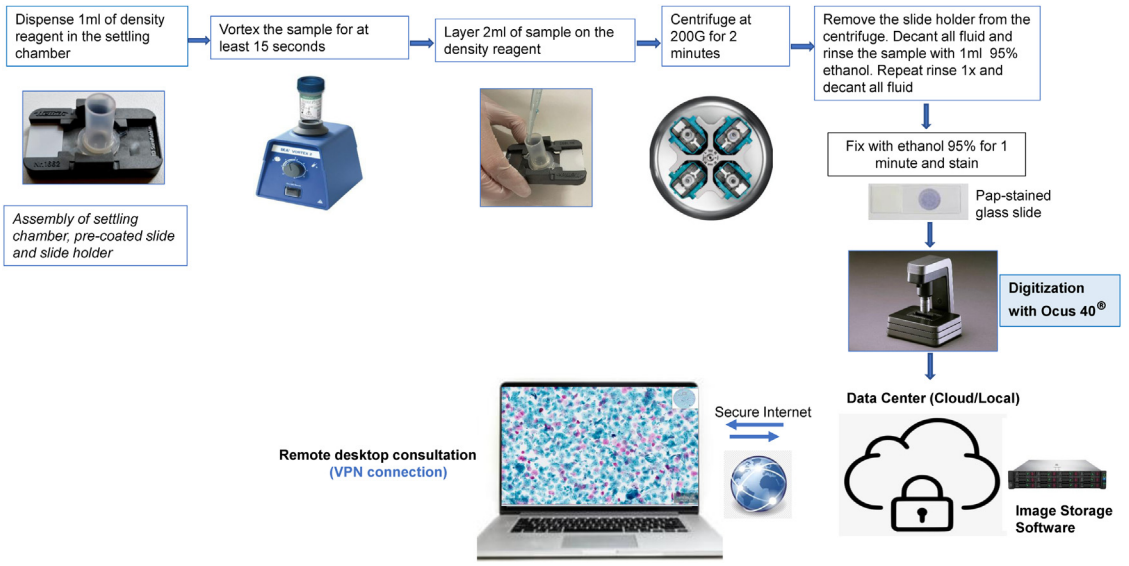

Figure 1 Process of manual preparation of liquid-based slides and digitalization for virtual microscopy.

of cell deposit, and amf is the area of the microscopic field. A cellularity of ≥5000 squamous cells was considered satisfactory.

The quality characteristics of the 30 WSIs were assessed by an experienced cytopathologist in virtual cytology and scored as excellent, good, fair, or poor according to the quality of the staining, sharpness, and visualization through the cell clusters. A high-resolution laptop monitor and QuPath, version 0.3.0 (open source software for digital pathology and WSI analysis), were used for reading and annotation of the WSIs. The mean time for screening the WSIs was calculated and compared with the corresponding screening time for the glass slides.

Incremental cost

We determined the extra costs incurred if we added telecytology as a triage test for HPV-positive women using a test-triage-and-treat approach. We based our cost assessment on the actual screening program used in Dschang, Cameroon, where ∼2000 women are screened annually. We have found that ∼20% were HPV positive.[14]

Diagnostic accuracy of screening with virtual microscopy

Training

Six cytotechnologists were trained to read digitized slides by a pathologist proficient in digital cytology. A reference atlas was created to demonstrate known negative and positive illustrative cases. The cytomorphologic features that distinguish the Bethesda squamous and glandular categories were illustrated in the WSIs and compared with the glass slides. Additionally, cytotechnologists performed side-by-side screening of the SurePath glass slides and matched WSIs to gain experience in reading digitized slides. On termination of the training period, an anonymous survey in the form of a Likert scale questionnaire was distributed to assess the cytotechnologists' impression regarding the quality of virtual microscopy and their confidence in their ability to screen WSIs.

Diagnostic performance

To assess the diagnostic performance of screening WSIs, we used a cohort of 294 HPV-positive Cameroonian women (age, 30-49 years), who had been screened with cervical cytology (SurePath) between September 2018 and July 2019. These women had been previously included in a cross-sectional study exploring different triage methods to detect cervical intraepithelial neoplasia grade ≥2 (≥CIN2).[15]

Histologic assessment of cervical biopsies and endocervical brushings served as the reference standard. The biopsies were performed at the 6-o'clock position within the transformation zone near the squamocolumnar junction when no lesion was seen or at the site of the lesion or lesions, if identified.

The slides were retrieved from the archives of the original study and digitized using the Ocus40 scanner. Eventually, 264 slides were satisfactory for evaluation. A total of 30 cases were excluded because of missing data, unsatisfactory samples for analysis, or nonvisualization of the cervix. The WSIs were split randomly into equal parts and distributed to the same cytotechnologists who had screened the 264 cases between September 2018 and July 2019 using light microscopy.[15]

The cytotechnologists were unaware of the original cytologic and histologic diagnoses but were aware of the HPV-positive status. The routine diagnostic procedure followed was the same as that for conventional light microscopy. The following classification based on the Bethesda system (2014) was used[16]: (1) negative for intraepithelial lesion or malignancy (NILM); (2) atypical squamous cells of undetermined significance (ASC-US); (3) low-grade squamous intraepithelial lesion (LSIL); (4) atypical glandular cells (AGC); (5) atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H); (6) high-grade squamous intraepithelial lesion (HSIL); and (7) cancer. All positive results classified as ASC-US or worse (≥ASC-US) were forwarded to a cytopathologist for the final diagnosis. Cases deemed negative by cytology were assessed by a single cytotechnologist and were not forwarded for a second opinion unless the patient had a history of CIN or cancer.

Statistical analysis

Statistical analyses were performed using R, version 4.1.2 (R Foundation for Statistical Computing). The histopathologic results served as the reference standard when measuring the diagnostic accuracy of WSIs versus glass slides for the detection of ≥CIN2 and ≥CIN3 lesions. We used the McNemar test to compare sensitivity and specificity, the generalized score statistic to compare the positive and negative predictive values, and a regression model approach to compare the positive and negative diagnostic likelihood ratios. The interrater reliability was calculated between the 2 techniques using Cohen's kappa and percentage agreement scores. Receiver operating characteristic (ROC) curves (sensitivity against 1 − specificity) were generated for both diagnostic methods and the associated areas under the ROC curve calculated. The Venkatraman test was used to test the statistical equality of the ROC curves. A 2-tailed P value of 0.05 was considered statistically significant.

The estimated Cohen's kappa precision for our sample of 264 patients was calculated assuming a moderate agreement of kappa of 0.5 between the glass slide and WSI diagnosis for 3 cytologic categories (NILM, ASC-US or LSIL, and AGC, ASC-H, HSIL, or cancer). Assuming a rate of 80% NILM, 10% ASC-US or LSIL, and 10% AGC, ASC-H, HSIL, or cancer based on prior experience of cervical cancer screening in this population and considering a type I error

rate alpha of 0.05, a sample of 264 patients provided a ±0.1 marginal error.

Results

Preparation and digitization of liquid-based slides and quality assessment

The preparation of the 30 samples was judged very easy (score 5) by 3 operators. The quality of the corresponding WSIs was scored as good for 18 of 30 slides and excellent for 12 of 30 slides, considering the parameters of stain quality, visualization through cell clusters, and sharpness. The identification of abnormal cells was easier for isolated cells than for cellular groups (Supplementary Information 2 and 3).

The mean estimated cellularity was 6078 squamous cells per slide. Of the 30 samples, 22 had sufficient cellularity (5000-12,100 cells) and 8 had unsatisfactory cellularity (<5000 cells). However, in 6 of these 8 cases, we found abnormal cells despite the unsatisfactory cellularity; therefore, we considered these slides to be adequate.

The mean time for manual preparation of the slides was 20 minutes (range, 18-25 minutes), and the mean time for digitization was 6 minutes (range, 5-6 minutes). The file size of the WSIs was an average of 1 GB. The mean screening time was 21 minutes (range, 15-31 minutes) for WSIs versus 7 minutes (range, 4−8 minutes) for the corresponding glass slides.

Incremental cost

Our calculation was based on the telecytologic screening of 400 HPV-positive women annually. The startup and operational costs for our particular program based in Cameroon were calculated as $32,063 and $2498, respectively. We anticipated the incremental annual running cost per HPV-positive woman to be $6.24 (2498 divided by 400; Supplementary Information 4).

WSI quality and diagnostic confidence

The trained cytopathologists unanimously agreed that the visualization, screening, and annotations of the cells on the monitor was easy and that identification of benign and atypical cells on WSIs was easy (Table 1). The participants unanimously reported that they preferred screening with glass slides because it was faster. Mixed responses were given regarding the quality of focus through the clusters on the WSIs and the cytotechnologist's comfort rendering a diagnosis using WSIs.

Table 1 Participant responses to survey statements regarding screening virtual thin-layer Pap slides.

Participant No.	Visualization, screening, and annotations of cells on monitor was easy	Focus through clusters on WSIs was as good as on glass slides	Identification of benign and atypical cells with WSIs was easy	I was comfortable rendering a diagnosis with WSIs	I prefer screening with virtual microscopy in my daily routine activity
1	Agree	Neutral	Agree	Agree	Disagree
2	Agree	Disagree	Agree	Neutral	Disagree
3	Agree	Disagree	Agree	Agree	Disagree
4	Agree	Disagree	Agree	Agree	Disagree
5	Agree	Disagree	Agree	Neutral	Disagree
6	Agree	Disagree	Agree	Agree	Disagree

Abbreviations: Pap, Papanicolaou; WSI, whole slide image.

Diagnostic agreement and accuracy of screening with virtual and light microscopy

Study population

Among the 1582 eligible women recruited as study participants, 294 (18.6%) were HPV-positive and eligible for triage. After the exclusion of 30 cases, the final analysis included 264 HPV-positive women. The results obtained from the histologic and cytologic examinations are shown in Fig. 2.

Diagnostic agreement

The comparison between the WSI and glass slide diagnoses is presented in Table 2. The overall agreement was 82.6% (95% confidence interval, 78.0-87.1), and the kappa coefficient showed moderate agreement (0.51; Table 3). When stratified into 3 diagnostic categories (NILM, ASC-US/LSIL, AGC/ASC-H/HSIL/cancer), a greater observer agreement (86.0%) and kappa coefficient (0.60) were obtained. The highest diagnostic concordance was found in the grouped diagnoses stratified by ≥CIN2 and ≥CIN3, with an agreement of 79.4% (kappa, 0.58) and 90.5% (kappa, 0.74) respectively (Table 3).

Diagnostic accuracy

Of the 48 women triaged as positive at the ≥ASC-US threshold using light microscopy, 27 (56%) were diagnosed with ≥CIN2 by histologic examination (Table 4). The corresponding rate was 48.3% (29 of 60) for virtual microscopy. Five CIN2 and two CIN3 lesions were missed by light microscopy and two CIN2 and three CIN3 lesions were missed by virtual microscopy.

No statistically significant difference was found in the diagnostic accuracy of WSIs compared with glass slides for ≥CIN2 (Table 5). However, the specificity (82.7%), positive predictive value (30.0%), and positive likelihood ratio (495.9) for the WSIs were significantly lower at the ≥CIN3 threshold when compared with those for the glass slides (88.1%, 39.6%, and 758.1, respectively). The ROC curve analysis of the cytologic cutoff values for WSI compared with glass slide cytology did not show a statistically significant difference in the areas under the ROC curve at both the ≥CIN2 (0.90 versus 0.87; $P = 0.260$) and ≥CIN3 (0.90 versus 0.92; $P = 0.497$) thresholds of detection (Fig. 3).

Discussion

Although the potential value of telepathology in health systems is promising, its introduction into LMICs for diagnostic use necessitates careful preclinical evaluations of the feasibility and diagnostic safety. The aim of the present study was to evaluate an innovative triage modality for HPV-positive women to detect cervical precancerous lesions in a resource-poor context. The present study is, to the best of our knowledge, the first conducted regarding this concept within a same day test-triage-and-treat approach.

Overall, the preparation and digitization of slides was operationally feasible based on the procedure's acceptability and estimated costs and quality of the digital images obtained. The centrifugation-based method described is similar

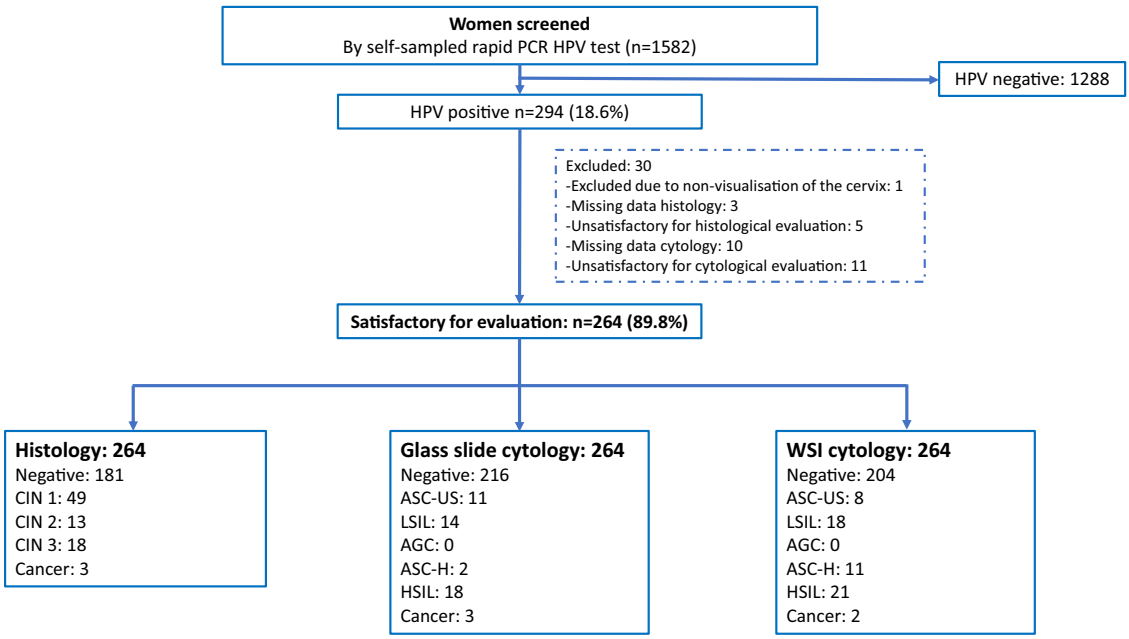

Figure 2 Flow chart showing study population, whole slide image (WSI) cytology, glass cytology, and histologic results. AGC, atypical glandular cells; ASC-H, atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; PCR, polymerase chain reaction.

Table 2 Comparison of cytologic diagnosis between WSI cytology and glass slide cytology.

WSI diagnosis	Glass slide diagnosis (n)							
	NILM	ASC-US	LSIL	AGC	ASC-H	HSIL	Cancer	Total
NILM	194	5	5	0	0	0	0	204
ASC-US	11	2	5	0	0	0	0	18
LSIL	5	0	3	0	0	0	0	8
AGC	0	0	0	0	0	0	0	0
ASC-H	5	4	1	0	0	1	0	11
HSIL	1	0	0	0	2	17	1	21
Cancer	0	0	0	0	0	0	2	2
Total	216	11	14	0	2	18	3	264

Abbreviations: AGC, atypical glandular cells; ASC-H, atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy; WSI, whole slide image.

to other manual methods successfully used in the past.[13,17] It is most appropriate for low-volume laboratories and, therefore, well suited to our program in Cameroon, considering we anticipate 400 HPV-positive women annually.

Using the SurePath manual technique, we usually found satisfactory cellularity, complying with the 2014 Bethesda System requirements update, which includes a minimum of 5000 squamous cells on liquid-based preparations.[18] The quality characteristics of all WSIs were rated as good or excellent by a cytopathologist and were adequate for diagnosis. Visualization and annotations were found to be ergonomically friendly. The mean time for diagnosis with the WSIs was longer than that for the glass slides screened using a light microscope. Similar findings have been reported in other studies, with interpretation of thin-layer slides requiring a longer time for WSIs than for glass slides.[19-21] The longer screening time of WSIs is a barrier to using digital cytology in a large-volume laboratory. The average screening time of 21 minutes we observed in the present study would allow for screening of ~3 slides per hour, which would equate to many fewer than the 100 slides per 8-hour working day limit set by the Clinical Laboratory Improvement Amendments of 1988. However, in Cameroon, we do not expect to enroll >2 or >3 HPV-positive women per day. Thus, the proposed triage modality would be appropriate in this context and for other peripheral healthcare centers. Furthermore, the screening time could be reduced using computer-aided diagnostic tools. Thus, we are evaluating an artificial intelligence-based algorithm, which might allow for the automatic classification of negative cases and locating and highlighting the areas in the WSIs most likely to contain abnormal cells.

We found that using Z-stack improved viewing of the WSIs. Like others,[22] we used 3 z-planes at 1 μm for the SurePath slides. Despite this, occasionally fine nuclear details were not well distinguished, and the ability to focus when examining 3-dimensional cell clusters was sometimes limited. Other Z-stack settings might allow for better focusing and should be investigated.

A successful introduction of WSI-based diagnosis requires proper training of the cytotechnologists to acquire the skills and confidence needed to interpret digital images.[20]

Table 3 Interobserver agreement for cytologic diagnosis between WSI cytology and glass slide cytology.

Histologic diagnosis	WSIs versus glass slides			
	Individual diagnosis[a]		Grouped diagnosis[b]	
	Observed agreement	Kappa	Observed agreement	Kappa
Negative (n = 181)	87.8 (83.0-92.6)	0.31 (0.13-0.49)	89.0 (84.3-93.5)	0.36 (0.16-0.57)
CIN1 (n = 49)	75.5 (63.4-87.5)	0.26 (0.00-0.51)	79.6 (68.3-90.8)	0.35 (0.02-0.68)
≥CIN2 (n = 34)	64.7 (48.6-80.7)	0.49 (0.30-0.69)	79.4 (65.8-93.0)	0.58 (0.35-0.82)
≥CIN3 (n = 21)	66.7 (46.5-86.8)	0.48 (0.20-0.75)	90.5 (77.9-100)	0.74 (0.43-1.00)
Overall (n = 264)	82.6 (78.0-87.1)	0.51 (0.41-0.62)	86.0 (81.7-90.1)	0.60 (0.48-0.71)

Abbreviations: CIN1, cervical intraepithelial neoplasia grade 1; CIN2, cervical intraepithelial neoplasia grade 2; CIN3, cervical intraepithelial neoplasia grade 3; WSI, whole slide image.

Data presented as percentages (95% confidence intervals).

[a]Each cytologic diagnosis was compared separately.

[b]Diagnoses were grouped into 3 categories: negative for intraepithelial lesion or malignancy; low-grade squamous intraepithelial lesion/atypical squamous cells of undetermined significance; and atypical glandular cells/atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion/high-grade squamous intraepithelial lesion/cancer.

Table 4 Histopathologic outcomes stratified by WSI and glass slide results.

Histologic diagnosis	NILM	ASC-US	LSIL	AGC	ASC-H	HSIL	Cancer	Total
Glass slide diagnosis (n)								
Negative	168	6	7	0	0	0	0	181
CIN1	41	1	5	0	0	2	0	49
CIN2	5	2	1	0	0	5	0	13
CIN3	2	2	1	0	2	11	0	18
Cancer	0	0	0	0	0	0	3	3
Total	216	11	14	0	2	18	3	264
WSI diagnosis (n)								
Negative	160	8	6	0	6	1	0	181
CIN1	39	8	0	0	0	2	0	49
CIN2	2	1	2	0	3	5	0	13
CIN3	3	1	0	0	2	12	0	18
Cancer	0	0	0	0	0	1	2	3
Total	204	18	8	0	11	21	2	264

Abbreviations: AGC, atypical glandular cells; ASC-H, atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; CIN1, cervical intraepithelial neoplasia grade 1; CIN2, cervical intraepithelial neoplasia grade 2; CIN3, cervical intraepithelial neoplasia grade 3; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy; WSI, whole slide image.

Consistent with previous studies,[23] we found that reticence to render a diagnosis with digital slides diminished as the training session progressed. At the end of the session, all the participants described themselves as confident in reading digital images. The main limitation identified was the longer time required to interpret WSIs, notably in the context of high volumes of slides.

We found that the annual running cost of consumables is affordable for our local facility's needs. However, initial start-up funding for the scanner will be needed, in addition to the modest projected operational costs for telecytologic screening.

The second part of the study aimed to ensure that patient safety would not be compromised by the introduction of WSI telecytology as a triage test. The results demonstrated that the diagnostic performance at the CIN2 threshold of screening with WSIs was not significantly different statistically compared with the same slides screened using a light microscope.

We did not find studies on the diagnostic accuracy and agreement that had used our exact method. Most studies had evaluated static images,[24] and very few studies examining platforms that permit visualization of entire slides have been reported. One report of whole slides showed that the

Table 5 Performance parameters of WSI and glass slide cytology at a threshold of ≥ASC-US.

Performance metrics and histologic thresholds	Glass slide	WSI	*P* value
≥CIN2 (n = 34; 12.9%)			
Sensitivity	79.4 (62.1-91.3)	85.3 (68.9-95.0)	0.317
Specificity	90.9 (86.4-94.3)	86.5 (81.4-90.7)	0.059
PPV	56.3 (41.2-70.5)	48.3 (35.2-61.6)	0.143
NPV	96.8 (93.4-98.7)	97.5 (94.4-99.2)	0.389
PLR	869.7 (558.9-1353.0)	632.8 (443.3-903.4)	0.147
NLR	22.7 (11.7-43.9)	17.0 (7.6-38.2)	0.397
≥CIN3 (n = 21; 7.9%)			
Sensitivity	90.5 (69.6-98.8)	85.7 (63.7-97)	0.317
Specificity	88.1 (83.3-91.9)	82.7 (77.4-87.3)	0.020[a]
PPV	39.6 (25.8-54.7)	30.0 (18.8-43.2)	0.015[a]
NPV	99.1 (96.7-99.9)	98.5 (95.8-99.7)	0.263
PLR	758.1 (524.4-1096.0)	495.9 (358.0-686.9)	0.012[a]
NLR	10.8 (2.9-40.4)	17.3 (6.0-49.3)	0.253

Abbreviations: ≥ASC-US, atypical squamous cells of undetermined significance or worse; CIN2, cervical intraepithelial lesion grade 2; CIN3, cervical intraepithelial neoplasia grade 3; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, predictive positive value; WSI, whole slide image.
Data presented as percentages (95% confidence intervals).
[a]Statistically significant difference.

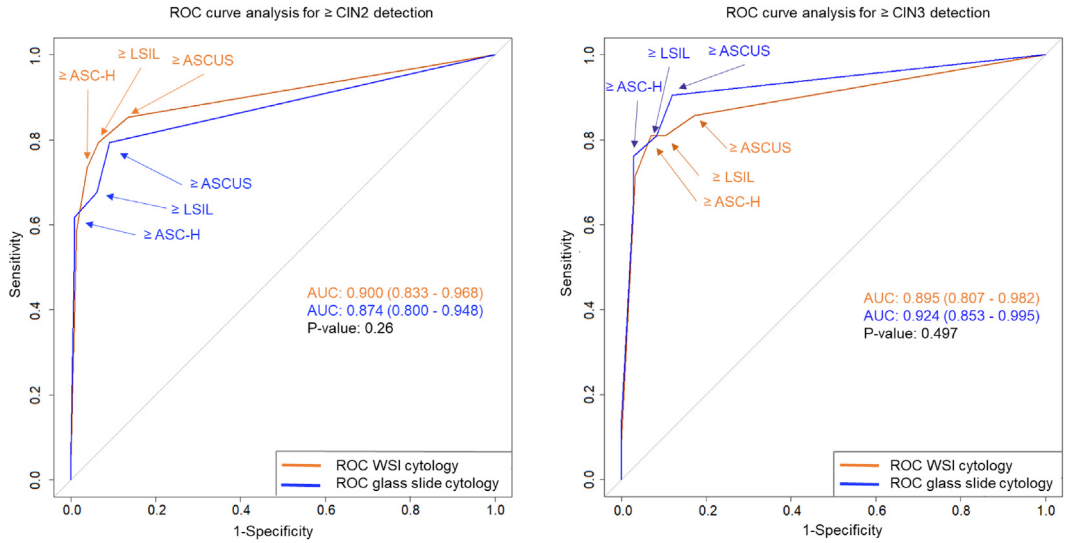

Figure 3 Receiver operating characteristic (ROC) curve analysis for cervical intraepithelial neoplasia grade 2 or worse (≥CIN2) and ≥CIN3 detection by whole slide image (WSI) and glass slide cytology. Although the performance parameters at all cutoff values were statistically indistinguishable between the 2 diagnostic methods of screening, the atypical squamous cells of undetermined significance or worse (≥ASC-US) cutoff obtained the highest sensitivity for both methods. ASC-H, atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion; ASCUS, atypical squamous cells of undetermined significance; AUC, area under the receiver operating characteristic curve; LSIL, low-grade squamous intraepithelial lesion.

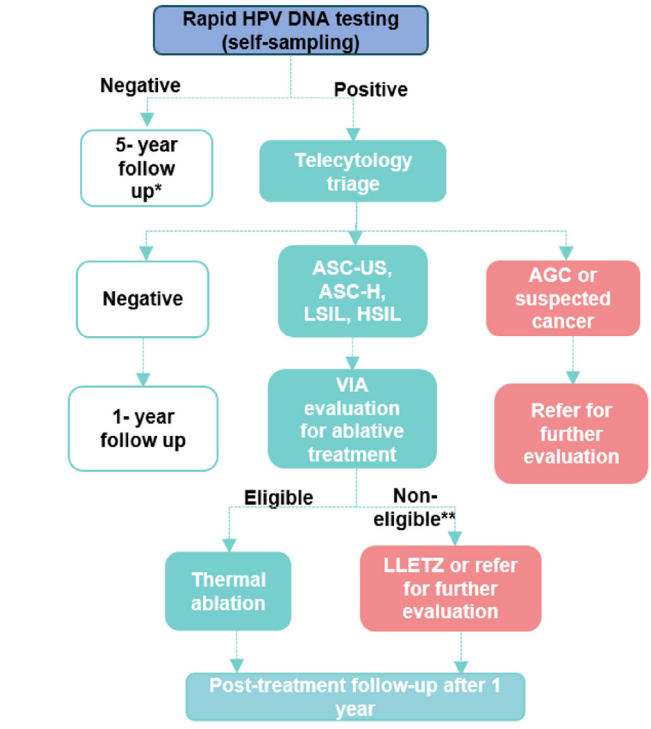

Figure 4 Proposed algorithm for a same day test-triage-and-treat approach using telecytology as a triage tool. AGC, atypical glandular cells; ASC-H, atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; HIV, human immunodeficiency virus; HSIL, high-grade squamous intraepithelial lesion; HPV, human papillomavirus; LLETZ, large loop excision of transformation zone; LSIL, low-grade squamous intraepithelial lesion; VIA, visual inspection with acetic acid.

diagnostic accuracy for precancerous lesions on SurePath slides was equal for virtual and conventional microscopy.[19] Other studies also reported diagnostic agreement. In 1 case, the agreement between glass slides and WSIs using the Aperio system ranged from 90% to 100% among the reviewers.[20] Another study comparing 2 different data sets (light microscopy versus light microscopy and virtual microscopy versus light microscopy) demonstrated similar overall concordance rates of 97.8% and 95.3%, respectively.[25]

We found interpretative variability in our cytologic diagnoses between virtual and light microscopy (Table 3). The interpretative variability of cervical cytology among well-trained observers has been acknowledged in the literature.[26,27] However, the discrepancies noted in our study did not affect the performance of screening with WSIs when applying the threshold of ≥ASC-US for the detection of ≥CIN2. Therefore, the virtual diagnosis of Pap smears does not appear to confer a greater risk of missing clinically significant lesions compared with light microscopy. It appears sufficiently sensitive, with acceptable specificity, and could, therefore, be beneficial for triage.

A screening and triage model could be designed as follows (Fig. 4): (1) self-collected rapid HPV testing (GeneXpert system); (2) triage by telecytologic diagnosis (Pap smear); (3) treatment of HPV-positive women; and (4) follow-up at 1 year. For samples with low cellularity, we propose repeating the Pap smear and digitization on the same day of the visit. In the case of obscuring inflammation, we would recall the patient after local treatment.

Visual inspection with acetic acid (VIA) would be used exclusively to assess a patient's eligibility for thermal ablation or the need for referral for further evaluation. This might allow for better care than solely relying on VIA, which despite its poor sensitivity and specificity, is the recommended triage method by the WHO for a screen-and-treat approach.[28] Before its implementation, a prospective on-site validation study is required to compare the performance of a telecytologic diagnosis versus VIA. The performance of combined telecytologic diagnosis and HPV genotyping should also be explored.

Our study had several strengths. First, operational feasibility was explored rigorously to identify and analyze practical barriers. Second, the diagnostic accuracy of WSIs and glass slides was verified by histologic examination for all participants. Finally, the cytotechnologists followed identical screening procedures for virtual and light microscopy, and the same cytotechnologists assessed the glass slides and WSIs. The main limitation of the present study was that we performed an off-site validation that did not investigate the operational time, strength of the internet connection, or complexity and cost of sending digital slides from Cameroon to remote consultants. Furthermore, the study design did not permit an intraobserver evaluation. Finally, the number of ≥CIN2 or ≥CIN3 lesions was relatively small. Therefore, a future study with a larger number of cases should be considered for further research to detect a potential difference in diagnostic accuracy.

Conclusions

We developed an innovative triage concept for HPV-positive women offering a quality-ensured telecytologic diagnosis in areas that lack specialists. The expected small number of HPV-positive women who would need to be screened each day in peripheral healthcare settings suggests that telecytology could be an effective triage tool to implement with a same day "test-triage-and-treat" approach. The results of the present study have demonstrated that the manual preparation of slides and that their digitization is feasible. The diagnostic performance of virtual microscopy was equivalent to light microscopy using ≥ASC-US as the threshold of positivity. Our results warrant further on-site clinical validation in a large prospective screening trial.

Funding sources

The present study was supported by the Private Foundation of University Hospitals Geneva and Tech4Dev (available at: https://tech4dev.com/).

Conflict of interest disclosures

The authors made no disclosures.

Acknowledgments

We thank the cytotechnologists of Unilabs, Geneva, for screening slides and the team in Dschang, Cameroon, and the Gynecology Division, Geneva, for supporting the project.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jasc.2022.11.003.

References

1. Arbyn M, Weiderpass E, Bruni L, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. *Lancet Glob Health*. 2020;8:e191–e203.
2. Global strategy to accelerate the elimination of cervical cancer as a public health problem. Available at: https://www.who.int/publications-detail-redirect/9789240014107. Accessed April 8, 2022.
3. Simms K, Steinberg J, Caruana M, et al. Impact of scaled up human papillomavirus vaccination and cervical screening and the potential for global elimination of cervical cancer in 181 countries, 2020–99: a modelling study. *Lancet Oncol*. 2019;20:394–407.
4. *WHO guideline for screening and treatment of cervical precancerous lesions for cervical cancer prevention*. 2nd ed. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.

5. Fontham ET, Wolf AM, Church TR. Cervical cancer screening for individuals at average risk: 2020 guideline update from the American Cancer Society. *CA Cancer J Clin*. 2020;70:321−346.
6. Gage J, Schiffman M, Katki H, et al. Reassurance against future risk of precancer and cancer conferred by a negative human papillomavirus test. *J Natl Cancer Inst*. 2014;106:dju153.
7. Pantanowitz L, Sharma A, Carter A, Kurc T, Sussman A, Saltz J. Twenty years of digital pathology: an overview of the road travelled, what is on the horizon, and the emergence of vendor-neutral archives. *J Pathol Inform*. 2018;9:40.
8. Azam A, Miligy I, Kimani P, et al. Diagnostic concordance and discordance in digital pathology: a systematic review and meta-analysis. *J Clin Pathol*. 2020;74:448−455.
9. Mukhopadhyay S, Feldman M, Abels E, et al. Whole slide imaging versus microscopy for primary diagnosis in surgical pathology. *Am J Surg Pathol*. 2018;42:39−52.
10. Araújo A, Arboleda L, Palmier N. The performance of digital microscopy for primary diagnosis in human pathology: a systematic review. *Virchows Arch*. 2019;474:269−287.
11. Holmström O, Linder N, Kaingu H, et al. Point-of-care digital cytology with artificial intelligence for cervical cancer screening in a resource-limited setting. *JAMA Netw Open*. 2021;4:e211740.
12. Khalbuss W, Rudomina D, Kauff N, Chuang L, Melamed M. SpinThin, a simple, inexpensive technique for preparation of thin-layer cervical cytology from liquid-based specimens. *Cancer*. 2000;90:135−142.
13. Bergeron C, Fagnani F. Performance of a new, liquid-based cervical screening technique in the clinical setting of a large French laboratory. *Acta Cytol*. 2003;47:753−761.
14. Levy J, Preux M, Kenfack B, et al. Implementing the 3T-approach for cervical cancer screening in Cameroon: preliminary results on program performance. *Cancer Med*. 2020;9:7293−7300.
15. Vassilakos P, Wisniak A, Catarino R, et al. A cross-sectional study exploring triage of human papillomavirus (HPV)-positive women by visual assessment, manual and computer-interpreted cytology, and HPV-16/18−45 genotyping in Cameroon. *Int J Gynecol Cancer*. 2021;31:808−816.
16. Nayar R, Wilbur D. *The Bethesda System for Reporting Cervical Cytology*. Cham, Switzerland: Springer International Publishing; 2015.
17. van Hemel B, Buikema H, Groen H, Suurmeijer A. Accuracy of a low priced liquid-based method for cervical cytology in 632 women referred for colposcopy after a positive Pap smear. *Diagn Cytopathol*. 2009;37:579−583.
18. Davey D, Souers R, Goodrich K, Mody D, Tabbara S, Booth C. Bethesda 2014 implementation and human papillomavirus primary screening: practices of laboratories participating in the College of American Pathologists PAP education program. *Arch Pathol Lab Med*. 2019;143:1196−1202.
19. Evered A, Dudding N. Accuracy and perceptions of virtual microscopy compared with glass slide microscopy in cervical cytology. *Cytopathology*. 2011;22:82−87.
20. Hanna M, Monaco S, Cuda J, Xing J, Ahmed I, Pantanowitz L. Comparison of glass slides and various digital-slide modalities for cytopathology screening and interpretation. *Cancer Cytopathol*. 2017;125:701−709.
21. Wright A, Smith D, Dhurandhar B, et al. Digital slide imaging in cervicovaginal cytology: a pilot study. *Arch Pathol Lab Med*. 2012;137:618−624.
22. Donnelly A, Mukherjee M, Lyden E, et al. Optimal z-axis scanning parameters for gynecologic cytology specimens. *J Pathol Inform*. 2013;4:38.
23. Bongaerts O, Diest P, Pieters M, Nap M. Working toward consensus among professionals in the identification of classical cervical cytomorphological characteristics in whole slide images. *J Pathol Inform*. 2015;6:52.
24. Lee E, Kim I, Choi J, et al. Accuracy and reproducibility of telecytology diagnosis of cervical smears. *Am J Clin Pathol*. 2003;119:356−360.
25. Bongaerts O, Clevers C, Debets M, et al. Conventional microscopical versus digital whole-slide imaging-based diagnosis of thin-layer cervical specimens: a validation study. *J Pathol Inform*. 2018;9:29.
26. Stoler M, Schiffman M. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL triage study. *JAMA*. 2001;285:1500.
27. Bigras G, Wilson J, Russell L, Johnson G, Morel D, Saddik M. Interobserver concordance in the assessment of features used for the diagnosis of cervical atypical squamous cells and squamous intraepithelial lesions (ASC-US, ASC-H, LSIL and HSIL). *Cytopathology*. 2013;24:44−51.
28. WHO guidelines for the use of thermal ablation for cervical pre-cancer lesions. Available at: https://www.who.int/publications-detail-redirect/9789241550598. Accessed April 8, 2022.