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# Establishment of a cytopathology unit and implementation of HPV genotyping for cervical cancer screening at the University Hospital Complex “Le Bon Samaritain”, N’Djamena, Chad

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## Abstract

Background: Cervical cancer remains one of the leading causes of deaths among women in sub-Saharan Africa, particularly in Chad, where access to screening and qualified personnel is limited.

Objective: To describe the implementation process of a cytopathology unit and HPV genotyping dedicated to the screening of cervical (pre)cancerous lesions at the Le Bon Samaritain University Hospital Complex (CHU-BS) in N’Djamena, Chad, as well as the challenges faced and the lessons learned.

Methods: The intervention followed four phases: needs assessment, procurement and installation of essential equipment, intensive training of local staff and a pilot screening campaign. Telecytology was integrated to support remote validation by external experts. In Parallel, a qualitative HPV genotyping protocol was implemented using the Bioperfectus Real-Time PCR Kit.

Results: A functional two-room cytology unit was established and local staff were trained to independently prepare and stain smears. Telecytology enables rapid validation and capacity building. During a 10-day campaign, 284 women were screened. Among 47 slides reviewed, 14 showed cytological abnormalities. HPV genotyping of six samples revealed two positives, identifying seven high-risk genotypes (HPV16, 35, 45, 52, 58, 66, 68), one low-risk genotype (HPV6), and multiple infections. The main challenges were limited equipment available and supply shortages, which were overcome through adapted solutions and collaboration.

Conclusion: This experience demonstrates that establishing a functional cytopathology service and implementing HPV genotyping is feasible in resource-limited settings through collaboration, tailored training, and adaptive use of available resources. The CHU-BS model provides a practical cancer screening services in Chad and similar Sahelian contexts.

# Introduction

Because of its incidence and mortality, cervical cancer stands as an important health issue in developing countries. Indeed, more than 661,000 new cases and nearly 350,000 deaths are recorded each year, and about 90% of deaths occur in Sub-Saharan Africa. [1] The disparity with northern countries can be explained by multiple challenges, including limited access to prevention, screening, early diagnosis, management, and case follow-up facilities, as well as a lack of knowledge among the population concerning pathology, strong sociocultural influences, and insufficient qualified human resources. [2] The World Health Organization (WHO) has set the goal of eliminating this cancer as a global health issue by 2030, focusing on vaccination, early screening, and treatment of women with precancerous lesions. [3]

Compared to other countries in the sub-region, in Chad, the available data on cancer remains limited, [4] but according to statistics from the Global Cancer Observatory, the country recorded approximately 1,111 new cases of cervical cancer and 841 deaths per year in 2022, making it the most frequent cancer among Chadian women and the most common cause of cancer-related deaths in this population. [5] The Ministry of Public Health and Prevention, through the National Cancer Control Program, is initiating actions, but organized screening activities remain opportunistic, and the lack of screening facilities and the scarcity of trained professionals limit these efforts for early detection of precancerous lesions. Access to cytopathology and human papillomavirus (HPV) genotyping remains restricted to a few rare hospital structures, and the majority of women have never undergone a Papanicolaou (Pap) smear or performed an HPV genotyping test.

It is with this in mind that we proceeded in October 2025 with the creation of a cytopathology unit and the implementation of the protocol for qualitative detection and genotyping of HPV in the laboratories of “Le Bon Samaritain” University Hospital Complex (CHU-BS) in N’Djamena, Chad. The objectives of this operation were to provide the hospital with a functional framework capable of ensuring cervical cancer screening operations.

This article therefore describes the process of setting up the cytology laboratory and the detection and genotypic differentiation of HPV, along with the challenges encountered and the lessons learned. In a Sahelian context with limited resources, this sharing of experience could inspire its reproduction or improvement in similar settings.

# Materials and Methods

## Period and site of the intervention

The idea of establishing a cytology unit at CHU-BS originated in 2023, with support from the Italian MAGIS Foundation (https://www.fondazionemagis.org/). However, the project only became operational in 2025. CHU-BS in N'Djamena (http://www.chu-lebonsamaritain.org/) is a healthcare complex located in the southern suburbs of the city, serving a population of approximately 100,000, primarily low-income residents from the capital, surrounding provinces, and even neighboring countries. The facility comprises a hospital, two health centers, a medical school, and a health school. It handles an average of 34,206 consultations annually and has a hospitalization capacity of 147 beds.

## Methodological approach

The intervention included four main and complementary stages:

i) Needs analysis: the purpose of this phase was to identify the strengths and limitations of the existing technical platform, the availability of human resources, and the current practices in the prevention and management of cervical cancer. Using a survey form, several aspects were examined (functional activities in the fight against cancers; existence of a referral service for training, procurement, validation, intervention; human resources and training background in cancer prevention). This highlighted the importance of acquiring necessary equipment, identifying potential partners, and confirming the need for laboratory staff to receive practical training in basic cytology techniques.

ii) Purchasing furniture, supplies, and equipment: to meet the minimal standards of cytological practice, a dedicated area was selected, thoroughly cleaned, and properly prepared. Orders for essential materials and equipment were placed with both domestic and international suppliers, prioritizing items according to immediate laboratory needs. The microscope employed is a Euromex model equipped with an adapted Euromex camera (Euromex Microscopen BV, Wholesaler in Arnhem, Netherlands). For optical micrography and telecytology, the setup includes the microscope, a capture module, and a laptop with the associated imaging software. Cytological staining was performed using the standard Pap method.

iii) Employee training and capacity building: an intensive training session was organized for the benefit of laboratory technicians, biologists, and medical students. The modules included the preparation and fixation of Pap smears; the Pap staining technique; the morphological recognition of normal, inflammatory, and atypical cells; the analysis and interpretation of samples according to the Bethesda 2014 system; quality assurance and sample traceability; biosafety and laboratory waste management. Some reference books were used as training materials. [6] [7] [8]

iv) Pilot screening campaign: along with the setting up of the unit, we conducted a cytological screening campaign among the mobilized and willing women. The cervical smears were collected by trained personnel (gynecologists, midwives, medical students, biologists). The staining was performed by medical students and biologists. The analysis was conducted by a cytologist, and the suspected cases were confirmed by the pathologist.

## Validation of an HPV detection protocol

The protocol for qualitative detection and genotyping of HPV was implemented using the BioPerfectus real-time polymerase chain reaction (PCR) kit, which enables the detection and genotypic differentiation of 21 types of HPV, divided into high-risk oncogenic genotypes and low-risk genotypes.

## HPV DNA extraction

HPV DNA was extracted using the QIAamp® Viral DNA Mini Kit (Qiagen, Hilden, Germany; cat. no. 51104), following the manufacturer's instructions with adaptations for cervical swab samples. Briefly, the cotton tip of each swab was hydrated with 700 µL of 0.9% NaCl and vortexed for 30 s. For each sample, 1.5 mL microcentrifuge tubes were prepared for DNA extraction. Twenty microliters (20 µL) of proteinase K were added to each tube, followed by the addition of 300 µL of the corresponding sample. Subsequently, 200 µL of buffer AL were added, and the mixture was vortexed for 15 s and incubated at 56°C for 10 minutes without shaking (temperature 56°C, speed 0). Then, 200 µL of absolute ethanol were added to each tube and mixed by vortexing for 15 s. The lysate was applied to a QIAamp® DNA Mini Spin Column and centrifuged at 8,000×g for 1 minute. The column was sequentially washed with 500 µL of Buffer AW1 (8,000×g for 1 minute) and 500 µL of Buffer AW2 (14,000×g for 1 minute), followed by a final centrifugation at 14,000×g for 3 minutes to remove residual wash buffer. DNA was eluted in 30 µL of Buffer AVE, incubated at room temperature for 1 minute, and centrifuged at 8,000×g for 1 minute. Eluates were stored at -20°C until downstream amplification. A negative extraction control was included in each batch to monitor potential contamination and the presence of PCR inhibitors. Although the BioPerfectus kit (SDK60102/104) specifies certain extraction systems, the QIAamp® DNA Mini Kit was used due to its local availability and prior validation for viral DNA extraction. [9]

## HPV genotyping by real-time PCR

HPV amplification and genotyping were performed strictly following the protocol of the BioPerfectus real-time PCR kit (version 1.4, November 2022). [10] Master mixes (A-H) were prepared according to the manufacturer's instructions, and 2 µL of extracted DNA was added to each reaction. Amplification was performed on a Rotor-Gene Q thermocycler (Qiagen) using the following cycling conditions: uracil-N-glycosylase (UNG) treatment at 50°C for 5 min, initial denaturation at 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 60°C for 40 s.

### Collaboration and support

The setting up of the cytopathology unit was based on a collaborative and multisectoral approach. On the strategic level, the project was initiated and coordinated by the CHU-BS, with technical and financial support from the Italian MAGIS Foundation. At the operational level, the intervention relied on both external and internal resources. External support included a cytologist and a pathologist who contributed to examination validation and capacity-building activities. Internal resources comprised two laboratory technicians, one biologist, one medical student, three gynecologists, and several midwives. In addition, an institutional partnership with Slem Medical (N’Djamena, Chad) was established to ensure the supply of specific reagents and consumables, thereby mitigating stockout-related challenges.

# Results

## Establishment of the unit

The hospital management allocated a dedicated space consisting of two separate areas (Figure 1). The first area includes a reception and patient registration desk, a workstation for staining and slide mounting, and designated storage spaces for equipment. These areas were organized to follow the logical workflow of a cytology unit. The second area is equipped with a gynecologic examination table for colposcopy, visual inspection, and the collection of cytological and biopsy specimens (Figure 2). Due to the need for a quiet environment for slide reading and analysis, the microscopic analysis station was established in the training room of the Laboratoire des Grandes Épidémies Tropicales (LAGET) [11] of CHU-BS, separate from the technical processing area. [12]

## Staff capacity building

An intensive training, both theoretical and practical, was provided on-site over 5 days to a multidisciplinary team. At the end of this phase, the technicians were able to independently prepare and stain the cytological slides. However, the section on analyzing and interpreting was subject to a separate schedule due to time constraints. To detect and validate suspicious features, a screening system incorporating telecytology (capturing microscopic images, uploading to a shared space, and a pathologist's review) was developed (Figure 2E); a pathologist was involved to confirm all suspicious cases, validate reports, and regularly review cases deemed negative.

## Pilot screening campaign

Taking advantage of the Pink October Campaign, committed to raising awareness and preventing gynecological cancers, we opened the doors of the unit for free to women eligible for cytological screening for cervical cancer.

During the two weeks of the campaign, 284 women were tested, each undergoing two smears and two swab samples for genotyping. The smears were analyzed by the cytologist and confirmed by the pathologist. Genotyping was performed by biologists. Clinical, virological results, and cytopathological aspects will be the subject of a subsequent publication.

Figure 3 shows a photo sent via telemedicine for confirmation, and Table 1 presents the preliminary results of 47 slides, including 14 cases with suspicious lesions.

Preliminary HPV genotyping of six tested samples revealed two positive cases, with the genotypic results summarized in Table 2.

## Difficulties encountered and suggested solutions

Several logistical and human constraints have been faced when establishing the cytology unit within the CHU-BS laboratory. One of the most significant was the limited availability of cytology-specific equipment in Chad. This obstacle forced us, for instance, to use tongue depressors as substitutes for Ayre spatulas and cosmetic spray in place of cytofixative. Similarly, due to a limited number of staining containers, we resorted to using storage boxes for micropipette tips, whose volume allowed complete immersion of cytological preparations in the solutions. Finally, given the supply challenges that are common in Chad, a transitional strategy involved beginning with a large initial stock of uncommon supplies such as dyes, reagents, and cytobrushes.

In terms of human resources, the lack of prior cytology training and the demanding schedules posed significant challenges. To address this, a customized, practice-focused on-site program was organized, taking service planning into account. This enabled rapid skills development, although the module dedicated to analysis and interpretation had to be specially designed in a hybrid format, with theoretical activities online and practical sessions conducted in person with the pathologist.

Additionally, limited commitment from some stakeholders represented a major challenge, as it threatened the service's continuity in case of personnel unavailability. This prompted us to request that the hospital administration encourage formal commitment, particularly by establishing a structured framework for the laboratory's operations.

The current dependence on external expertise for validation further underscores the need for a complete transfer of skills in the short to medium term, especially since treatment initiation requires prior histologic confirmation and continuous involvement of a pathologist.

From an organizational and functional perspective, the unit's space is shared with other activities and stakeholders, necessitating a revision of laboratory workflow to integrate it into existing processes. Access to technical areas should be restricted to authorized personnel to prevent

uncontrolled exposure and ensure traceability.

## Discussion

The WHO aims to eliminate cervical cancer by the end of the century through its global strategy, which sets 2030 targets: 90% of girls vaccinated against HPV, 70% of women screened at ages 35 and 45, and 90% of women with detected lesions receiving treatment. Without intervention, mortality could increase by 30% (to over 400,000 deaths per year) in low-income countries by 2030, with an increased risk of mortality in the African region. [3] From the initial infection with a high-risk oncogenic HPV to the development of cancerous lesions, cervical cancer progresses through curable pre-neoplastic stages with a high potential for regression, which evolve over a relatively long period of time. [13] This therefore offers an opportunity where early detection is possible by various methods (including cytology) and where actions can be taken to prevent progression to an irreversible stage, much more difficult and costly to manage.

Cervical cytology is a test used to detect abnormalities in cervical cells. Cells are collected during a gynecological examination and then stained and examined under a microscope. [14] Despite its technical demands, this examination remains a useful tool for identifying at-risk women and directing them to appropriate treatment. The choice of cytological technique is driven by its simplicity, its ability to detect precancerous lesions early as well as infections (Candida, Trichomonas vaginalis, Gardnerella vaginalis, and Actinomyces) [15] that may require treatment, its affordability, and its potential for automation (telepathology and integration of artificial intelligence). Most importantly, it is highly effective in reducing cervical cancer incidence when implemented systematically.

There are several screening strategies available: visual inspection with acetic acid or Lugol’s iodine, for example, is a simple, low-cost method suitable for low-resource areas, but its sensitivity remains moderate, and its specificity is heavily dependent on the operator’s experience and judgment. [16] HPV detection tests, on the other hand, are recommended as first-line tests because they offer high sensitivity for detecting high-risk infections, but their cost, equipment requirements, and logistical challenges limit their large-scale accessibility. [17] Despite its moderate sensitivity, cytology occupies an intermediate position because it enables the detection of precancerous lesions and the assessment of their severity, which is essential for subsequent management. [18] In the Chadian economic context, it is therefore a balanced compromise between diagnostic performance, operational feasibility, and cost.

The cytopathology unit of the CHU-BS is now operational, with staff able to independently handle sample collection, staining, and preliminary slide reviewing. This operation represents a significant step forward in the fight against cervical cancer in N'Djamena. The integration of telecytology, even in its embryonic stage, offers an advantage compared to an environment without screening, as well as the possibility of ensuring remote supervision and the gradual skill enhancement of local staff; this constitutes a significant option for strengthening health systems where qualified human resources and screening facilities are lacking. [19]

### Lessons learned

This experience has allowed us to highlight the importance of an integrated and participatory approach in project implementation. From planning to mission implementation, shared skills were required for progress at each project milestone. Among the most important lessons is also the need for planning of material and human needs, coupled with a realistic assessment of already available resources. Collaboration between institutions and among clinicians, technicians, and external experts has also proven crucial, as external partners provide additional skills and resources and will remain essential in the future, particularly for overcoming supply-related challenges.

On the technical front, despite the difficulties associated with Internet access and computer tool integration, the establishment of a system of double examination and remote validation, combined with the digitalization of cytological micrographs, opens up new avenues for diagnosis, teaching, and research in a resource-limited environment. Finally, there is the possibility of expanding the laboratory to include histology and molecular markers in the medium term.

Similar experiences of establishing cytology laboratories have been documented in other low-income countries. [20, 21] These initiatives not only recognize the value of implementing such projects as an effective means of strengthening national screening programs but, above all, rely on very comparable pillars: institutional commitment, the involvement of external expertise and networking, the identification of suppliers to address supplies shortages, intensive practical training, the contribution of various professional profiles (medical and paramedical), and once again, the use of digital microscopy. [22]

The experience at CHU-BS clearly shows that cervical screening services can be successfully implemented even in resource-limited settings by combining three essential elements: targeted staff training, innovative adaptation to local constraints, and the integration of telecytology. This approach provides a practical and replicable model for other health facilities in Chad.

The preliminary cytology results, with nearly 9% high-grade abnormalities, confirm that many women remain undiagnosed until advanced stages due to the lack of systematic screening. The identification of highly oncogenic HPV (16, 35, 45, 52, 58, 68), which are common in Central Africa, [23] [24] [25] highlights the urgency of establishing a structured, national screening strategy, combining cytology, HPV testing, and follow-up.

The CHU-BS model also demonstrates that a reliable service can function even without an on-site pathologist, thanks to remote expert review and progressive capacity building. This reduces dependence on external institutions and improves diagnostic quality.

In the medium term, this initiative could serve as a national reference platform for: training technicians and clinicians, expanding screening to provincial hospitals, introducing digital and artificial intelligence-assisted cytology, and preparing for future histopathology services.

Overall, this experience offers a realistic, scalable, and sustainable model to strengthen cervical cancer control in Chad and support progress toward WHO elimination targets.

## Conclusions

The establishment of the cytopathology unit at the CHU-BS in N'Djamena demonstrates that it is possible, even in a resource-limited context, to establish a functional cervical cancer cytological screening service by using a collaborative approach, local staff training, and adaptation of available resources. This experience provides a solid foundation for the future development of integrated pathology services in Chad and other similar Sahelian contexts. Moreover, the implementation of the HPV genotyping protocol demonstrates that the entire HPV diagnostic process can now be performed locally in Chad.

**Figure 1.** Spatial organization of the cytology unit

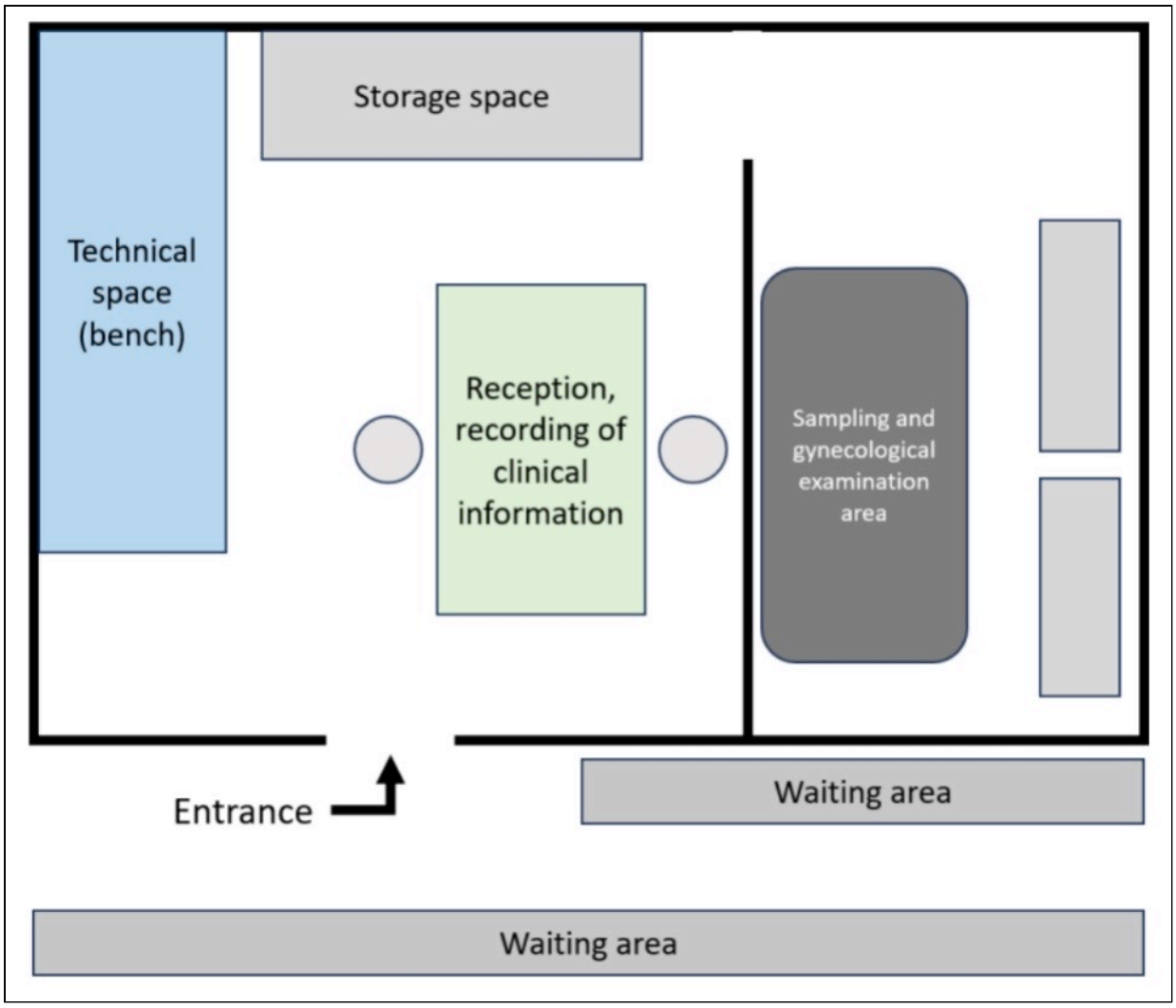


*Figure description...*

**Figure 2.** Workstations in the cytology laboratory.A)Patient reception and data recording; B)staining area; C)area for mounting and drying cytological preparations;D)sampling room; E)Optical micrography and telecytology device (microscope, capture module, laptop with the software associated with the micrography module).


*Figure description...*

**Figure 3.** Optical micrographs of GX100 and GX400 cervicovaginal smears captured by our telemedicine device and sent for confirmation.

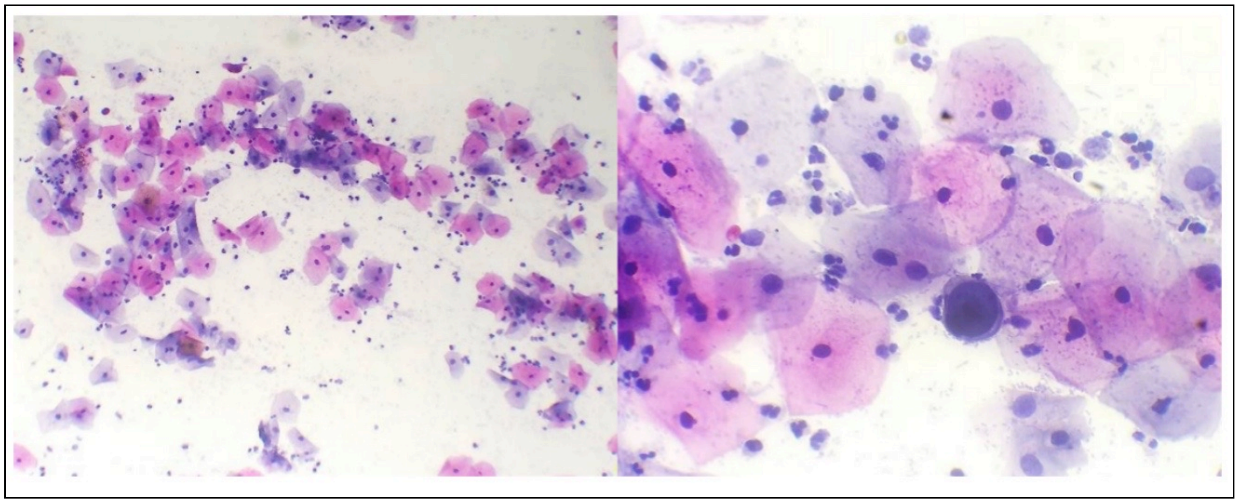

*Figure description...*

**Table 1.** Preliminary results from the first 47 participants.

| Results | Frequency (%) | |
|---|---|---|
| Negative for intraepithelial lesion or malignancy | 33 (70.22) | |
| Atypical squamous cells of undetermined significance | 02 (4.25) | |
| Atypical squamous cells – cannot exclude high-grade squamous intraepithelial lesion | 01 (2.13) | |
| Low-grade squamous intraepithelial lesion | 06 (12.76) | |
| High-grade squamous intraepithelial lesion | 04 (8.51) | |
| Adenosquamous carcinoma | 01 (2.13) | |

*Table note...*

**Table 2.** Preliminary HPV genotyping results.

| Sample ID | Result | Genotypes detected |
|---|---|---|
| 003 | Positive | HPV 16, 66, 58, 35, 6 |
| 016 | Negative | - |
| 018 | Negative | - |
| 019 | Negative | - |
| 020 | Positive | HPV 58, 45, 52, 68 |
| 026 | Negative | - |

*HPV, human papillomavirus; HR, high-risk; LR, low-risk.*

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