

DNA Extraction from Cervical Tumors for Molecular Diagnostics and FFPE Quality Control

Dr. Bernard Kafor^{1*} and Professor Charles Okolie²

¹Department of Medical Laboratory Science, Faculty of Health Sciences, Maduka University Ekwegbe, Nigeria.

²Department of Medical Laboratory Science, Faculty of Allied Health Sciences, State University of Medical and Applied Science, Igbo-Eno, Nigeria.

*Correspondence:

Dr. Bernard Kafor, Department of Medical Laboratory Science, Faculty of Health Sciences, Maduka University Ekwegbe, Nigeria.

Received: 04 Sep 2026; Accepted: 06 Oct 2026; Published: 17 Oct 2026

Citation: Bernard Kafor, Charles Okolie. DNA Extraction from Cervical Tumors for Molecular Diagnostics and FFPE Quality Control. Cancer Sci Res. 2026; 9(4): 1-5.

ABSTRACT

Cervical cancer molecular testing increasingly depends on the recovery of DNA that is not merely abundant, but analytically fit for the intended assay. This requirement is particularly important for formalin-fixed paraffin-embedded (FFPE) cervical specimens, which constitute the dominant archival material in histopathology laboratories but are affected by fixation-induced fragmentation, cross-linking, base modification, and variable tumor content. This narrative review examines the specimen, pre-analytical, extraction, and quality-control factors that determine the performance of genomic DNA from cervical tumors in human papillomavirus (HPV) genotyping, somatic variant analysis, DNA methylation assays, and next-generation sequencing (NGS). Fresh/frozen tissue provides the highest molecular integrity but is less available in routine practice, whereas FFPE tissue enables retrospective and clinically annotated studies when fixation, tumor enrichment, extraction chemistry, and DNA qualification are controlled. Silica membrane and magnetic bead workflows offer the greatest standardization for routine molecular laboratories; classical organic and salting-out methods remain useful in selected research or resource-constrained settings but require stronger control of purity, reproducibility, and hazardous reagents. Importantly, DNA concentration alone is an inadequate acceptance criterion for degraded specimens. A fit-for-purpose workflow should integrate pathological assessment of tumor fraction, fluorometric DNA quantification, purity assessment, and an amplifiability or fragment-quality metric matched to the downstream platform. For cervical cancer, this approach links histopathology to reliable detection of HPV-related and host genomic alterations while reducing false-negative results and sequencing artifacts. Implementation in low-resource settings should prioritize standardized fixation, tumor enrichment, validated extraction protocols, staged quality control, and targeted molecular assays before expansion to broader sequencing. Such an integrated workflow can improve the reproducibility and clinical interpretability of cervical cancer molecular diagnostics across diverse laboratory environments.

Keywords

Cervical cancer, DNA extraction, Formalin-fixed paraffin-embedded tissue, Molecular diagnostics, Quality control.

Introduction

Cervical cancer remains a major global health problem, with a disproportionate burden in low- and middle-income countries. Persistent infection with oncogenic human papillomavirus (HPV) is the central etiological event, but progression from infection to invasive malignancy reflects additional viral, host-genomic, epigenetic, and microenvironmental changes [1-4]. Large-scale

genomic studies have demonstrated recurrent alterations in pathways involving PIK3CA, ERBB family members, immune-regulatory loci, chromatin modifiers, and other cancer-associated genes, reinforcing the role of molecular characterization in translational research and, increasingly, precision oncology [1-5].

The analytical validity of these molecular findings begins before PCR or sequencing. Tissue selection, ischemic interval, fixation, paraffin processing, tumor fraction, storage, DNA extraction chemistry, and post-extraction quality control can each alter the amount and molecular integrity of recoverable DNA [6-12]. In

cervical pathology, these variables are especially relevant because diagnostic material may consist of small biopsies, curettings, cytology specimens, or archival FFPE blocks with variable tumor cellularity.

Accordingly, the practical question is not which extraction method yields the largest numerical DNA concentration, but which workflow produces DNA that is fit for the intended molecular application. This review therefore integrates specimen selection, pre-analytical control, extraction technology, DNA qualification, and downstream assay requirements into a cervical-cancer-specific diagnostic framework. Particular emphasis is placed on FFPE tissue because it is the most accessible substrate for retrospective molecular studies and routine histopathology-linked testing.

Scope and Review Approach

This article is a focused narrative review. This review prioritizes peer-reviewed literature on cervical cancer genomics, HPV-related molecular testing, FFPE pre-analytics, DNA extraction, tumor enrichment, and DNA quality assessment, with additional emphasis on recent literature relevant to NGS and cervical cancer biomarkers. It is not presented as a systematic review or meta-analysis; therefore, no claim of exhaustive study capture or formal risk-of-bias assessment is made. Evidence is synthesized to support a practical, fit-for-purpose workflow for diagnostic and translational molecular pathology.

Cervical Specimen Types and Their Molecular Consequences **Fresh and fresh-frozen tumor tissue**

Fresh or rapidly frozen cervical tumor tissue generally provides high-molecular-weight DNA with minimal fixation-related chemical modification. When collection and cryopreservation are tightly controlled, such material is well suited to whole-genome sequencing, whole-exome sequencing, copy-number analysis, methylation profiling, and other applications that benefit from long, intact DNA fragments. Its principal limitation is operational rather than analytical: prospective collection, rapid transport, reliable ultra-low-temperature storage, and biobanking infrastructure are required. These constraints reduce routine availability in many pathology services and are particularly important where electricity and cold-chain continuity cannot be guaranteed.

Formalin-fixed paraffin-embedded tissue

FFPE tissue is the most important practical source of cervical tumor DNA because it preserves morphology, supports long-term storage, and links molecular data to the original histopathological diagnosis. The same formalin chemistry that preserves tissue architecture, however, creates DNA-protein cross-links, fragmentation, and base damage. Cytosine deamination can generate C>T/G>A artifacts, while prolonged fixation and older block age can reduce amplifiable template [6-11]. Despite these limitations, modern extraction and library-preparation strategies permit robust targeted sequencing and other molecular assays from appropriately qualified FFPE DNA [9-13].

Cytology specimens and small biopsies

Residual liquid-based cytology material, cervical swabs, and small biopsies can provide DNA for HPV detection, genotyping, methylation assays, and selected host-genomic tests. Their major analytical limitation is often cellular composition: low tumor fraction, inflammatory cells, blood, necrosis, and normal epithelium can dilute the molecular signal. This is critical when the assay is intended to detect low-variant-allele-frequency somatic mutations. Cervical and self-collected specimens are also being investigated for HPV and methylation-based screening or triage, where the biological target and required analytical sensitivity differ from tumor sequencing [14,15].

Pre-analytical Determinants of DNA Fitness

Tissue handling and fixation

Pre-analytical standardization is the first quality-control step. Delayed fixation permits enzymatic degradation, whereas excessive formalin exposure increases cross-linking and fragmentation. Ten percent neutral buffered formalin is the routine histopathology standard, but fixation time, specimen thickness, temperature, and processing conditions should be documented because they influence downstream molecular performance [6,7]. Molecular laboratories should avoid treating all FFPE blocks as analytically equivalent simply because histomorphology is acceptable.

Tumor cellularity, necrosis, and pathological review

An H&E-stained section should be reviewed before extraction to confirm the diagnosis, estimate viable tumor content, identify necrosis or hemorrhage, and mark a tumor-rich region. The minimum acceptable tumor fraction is assay-dependent; it should be derived from the validated analytical sensitivity of the downstream method rather than from a universal threshold. Macrodissection is usually sufficient when tumor-rich areas can be clearly separated, whereas laser-capture microdissection offers higher precision when malignant cells are sparse or intimately admixed with non-neoplastic tissue [16,17].

Block age and storage

Archived FFPE blocks remain valuable for molecular studies, although storage can alter DNA quality and block age alone does not predict assay success. Some older material has performed well in NGS studies, whereas highly fragmented DNA can compromise downstream analyses [9,10]. Therefore, archival age should prompt assay-specific qualification rather than automatic exclusion.

DNA Extraction Technologies

Organic extraction

Phenol-chloroform extraction can recover relatively high-molecular-weight DNA and remains useful in research settings. Proteinase K digestion and detergent-mediated lysis are followed by phase separation and alcohol precipitation. The method is inexpensive in reagent terms and flexible across tissue types, but it is labor-intensive, uses hazardous organic solvents, is difficult to automate, and is vulnerable to carry-over contaminants that inhibit PCR. For routine clinical molecular diagnostics, these limitations

reduce its attractiveness compared with standardized solid-phase systems.

Salting-out and proteinase K methods

Salting-out avoids phenol and chloroform by precipitating proteins with concentrated salts while retaining DNA in the aqueous phase, followed by alcohol precipitation [18-21]. The method has been applied to FFPE tissue as well as other clinical specimens, although the performance demonstrated in one specimen type should not be assumed for cervical tumors without validation [19,21]. It is technically accessible in resource-constrained laboratories, but manual handling, residual salts, and potential PCR inhibition require appropriate controls and local validation for sensitive molecular assays [20,21].

Silica membrane-based extraction

Silica membrane systems are widely used because they combine standardized chemistry, relatively rapid processing, and compatibility with fresh, frozen, and FFPE material. Under chaotropic conditions DNA binds to silica, contaminants are removed by wash steps, and purified DNA is eluted in low-salt buffer. For FFPE tissue, successful workflows usually incorporate efficient deparaffinization, prolonged proteinase K digestion when necessary, and heat-assisted reversal of formalin cross-links. Their reproducibility and ease of validation make silica systems a practical default for many diagnostic laboratories.

Magnetic bead-based extraction

Magnetic bead platforms use paramagnetic particles with nucleic-acid-binding surfaces to capture DNA, permitting washing and elution without column centrifugation. Their major advantages are automation, scalability, closed or semi-closed workflows, reduced hands-on time, and suitability for high-throughput laboratories. Performance nevertheless depends on bead chemistry, lysis efficiency, DNA input, and the degree of FFPE fragmentation. Automated extraction should therefore be validated with representative cervical specimen types rather than assumed to be equivalent across platforms.

Quality Assessment: From DNA Yield to Fitness for Purpose

A central weakness in many extraction studies is the use of DNA concentration as the principal outcome. In FFPE material, a high mass concentration can coexist with severe fragmentation or poor amplifiability. Quality control should therefore answer four separate questions: how much DNA is present, how pure it is, how intact or amplifiable it is, and whether the amount of usable template is sufficient for the intended assay [12,22].

Quantification and purity

Fluorometric assays that selectively measure double-stranded DNA are generally preferable for determining usable DNA concentration in degraded histopathology samples. UV spectrophotometry remains useful for identifying protein, phenol, or other contaminants through absorbance ratios, but it can overestimate DNA concentration because it measures total UV-absorbing

material. A combined workflow in which spectrophotometry assesses purity and fluorometry estimates double-stranded DNA provides more informative qualification than either method alone [12].

Integrity and amplifiability

Gel or automated electrophoretic methods can characterize fragment distribution, while PCR- or qPCR-based quality assays estimate the fraction of template capable of amplification. Multiplex PCR has also been used to predict whether FFPE DNA is likely to perform adequately in array-based genomic analysis [22]. For NGS, fragment size, library complexity, duplicate rate, coverage uniformity, and assay-specific quality metrics should be considered together. Laboratories should define acceptance criteria during validation and apply them consistently to clinical or research samples.

Downstream Molecular Applications in Cervical Cancer HPV detection, genotyping, and viral-host analysis

Persistent high-risk HPV infection is fundamental to cervical carcinogenesis [3,23]. DNA extracted from cervical specimens supports HPV detection and genotype assignment, and sequencing-based approaches can additionally characterize viral integration and viral-host genomic relationships. Because HPV assays may target short genomic regions, they can tolerate more fragmented DNA than some broad host-genome applications; nevertheless, poor extraction or inhibition can still cause false-negative results.

Somatic mutation and copy-number profiling

Genomic studies have established a heterogeneous landscape of somatic alterations in cervical cancer [1,2]. Contemporary clinical sequencing has further demonstrated potentially actionable alterations in a subset of tumors, supporting the translational relevance of high-quality tumor DNA [5]. Targeted panels are often more compatible with FFPE material than whole-genome approaches because they require less input and can be designed around shorter fragments. However, fixation artifacts, low tumor fraction, and insufficient unique template can still distort variant calling and must be addressed bioinformatically and analytically.

DNA methylation and emerging biomarkers

Host and viral DNA methylation are being investigated as biomarkers for cervical cancer detection and triage, while broader molecular biomarkers are under study for prognosis and treatment selection [14-25]. Bisulfite conversion can further damage or reduce available DNA, so extraction and qualification should be selected with the planned epigenetic assay in mind.

A Fit-for-Purpose Workflow for Cervical Tumor DNA

A practical cervical molecular pathology workflow should begin with the clinical question and work backward to the specimen and extraction method. For a small FFPE biopsy intended for a focused mutation panel, tumor enrichment and amplifiable DNA may be more important than maximal molecular weight. For discovery WGS, fresh/frozen tissue and high-integrity DNA are preferable.

Table 1: Proposed fit-for-purpose workflow linking cervical tumor pathology, DNA extraction, quality control, and downstream molecular testing.

Step	Key decision	Preferred practice	Failure risk	Corrective action
1. Pathology review	Is viable tumor present?	Review H&E; annotate tumor-rich region	Low tumor fraction; necrosis	Macrodissect or select another block
2. Pre-analytics	Was fixation/processing acceptable?	Document fixative and duration when available	Cross-linking; fragmentation	Use short-amplicon/FFPE-validated assay
3. Extraction	Which chemistry matches specimen and throughput?	Validated silica or magnetic-bead workflow for routine FFPE	Low yield; inhibitors; variability	Repeat digestion/extraction or alternative method
4. Quantification	How much dsDNA is available?	Fluorometric measurement	Spectrophotometric overestimation	Re-quantify with dsDNA-specific assay
5. Purity	Are inhibitors likely?	UV spectrum/ratios as supportive QC	PCR inhibition; library failure	Additional cleanup or re-extraction
6. Integrity/amplifiability	Is DNA usable for intended assay?	Fragment or qPCR/PCR-based QC	False negatives; poor NGS complexity	Reduce assay breadth or obtain new specimen
7. Molecular testing	Does assay match DNA fitness?	Use validated input and QC thresholds	Artifacts; incomplete profiling	Repeat with orthogonal/alternative method

For HPV genotyping or methylation triage, assay-specific sensitivity and target length become dominant considerations.

Considerations for Resource-Constrained Laboratories

Resource limitations should not be equated with an absence of molecular quality assurance. The highest-yield interventions are often procedural: prompt fixation, avoidance of over-fixation, careful block selection, pathologist-guided tumor enrichment, standardized extraction, and staged DNA qualification. A laboratory may obtain greater diagnostic value by implementing a well-validated targeted PCR or small NGS panel with robust quality control than by adopting broad sequencing without adequate pre-analytics. Salting-out or manual silica workflows may be appropriate where automation is unaffordable, but reproducibility, contamination control, positive and negative controls, and assay validation remain essential.

For sub-Saharan African cervical cancer research, archival FFPE collections represent an important molecular resource because they can connect genomic data with histopathology and clinical outcomes. The 2025 cervical-cancer FFPE workflow literature further supports the feasibility of integrating molecular testing with routinely processed material [13]. Multi-institutional harmonization of fixation, tissue annotation, DNA qualification, and minimal metadata would substantially improve comparability across studies.

Challenges, Evidence Gaps, and Priorities

Three evidence gaps remain important. First, extraction methods are frequently compared using yield and purity without measuring downstream diagnostic accuracy. Second, universal DNA acceptance thresholds are inappropriate because assay chemistry, amplicon length, sequencing platform, and variant allele-frequency requirements differ. Third, cervical-cancer-specific validation studies from low-resource settings remain limited relative to the disease burden. Future studies should compare workflows using paired specimens, defined tumor fractions, orthogonal reference methods, and clinically meaningful endpoints such as successful

library generation, sensitivity for known variants, reproducibility, and failure rate.

Conclusion

Reliable molecular characterization of cervical cancer depends on a chain of quality that begins with the tissue specimen and ends with an assay-specific interpretation. DNA extraction should therefore be regarded as an integrated diagnostic process rather than an isolated laboratory step. Fresh/frozen tissue remains optimal when high molecular integrity is required, but FFPE tissue is the most clinically accessible substrate and can support robust HPV, genomic, and epigenetic testing when pre-analytical damage and tumor composition are actively managed. For routine FFPE cervical specimens, pathologist-guided tumor selection, validated silica membrane or magnetic bead extraction, fluorometric quantification, purity assessment, and an amplifiability or fragment-quality metric provide a defensible core workflow. The critical endpoint is not maximum DNA yield but sufficient analyzable template for the intended molecular platform. This fit-for-purpose principle is particularly relevant to laboratories expanding molecular pathology in resource-constrained settings: standardization and quality control should precede technological complexity. By aligning specimen handling, extraction chemistry, DNA qualification, and assay design, cervical cancer molecular testing can become more reproducible, clinically interpretable, and transferable across institutions. Future cervical cancer studies should report these pre-analytical and DNA-quality variables explicitly so that molecular findings can be compared, validated, and translated into diagnostic and precision-oncology practice.

References

1. Ojesina AI, Lichtenstein L, Freeman SS, et al. Landscape of genomic alterations in cervical carcinomas. *Nature*. 2014; 506: 371-375.
2. Cancer Genome Atlas Research Network. Integrated genomic and molecular characterization of cervical cancer. *Nature*. 2017; 543: 378-384.

3. Schiffman M, Castle PE, Jeronimo J, et al. Human papillomavirus and cervical cancer. *Lancet*. 2007; 370: 890-907.
4. 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.
5. Friedman CF, Ravichandran V, Miller K, et al. Assessing the genomic landscape of cervical cancers clinical opportunities and therapeutic targets. *Clin Cancer Res*. 2023; 29: 4660-4668.
6. Srinivasan M, Sedmak D, Jewell S. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. *Am J Pathol*. 2002; 161: 1961-1971.
7. Bass BP, Engel KB, Greytak SR, et al. A review of preanalytical factors affecting molecular protein and morphological analysis of formalin-fixed paraffin-embedded tissue. *Arch Pathol Lab Med*. 2014; 138: 1520-1530.
8. Do H, Dobrovic A. Sequence artifacts in DNA from formalin-fixed tissues causes and strategies for minimization. *Clin Chem*. 2015; 61: 64-71.
9. Carrick DM, Mehaffey MG, Sachs MC, et al. Robustness of next generation sequencing on older formalin-fixed paraffin-embedded tissue. *PLoS One*. 2015; 10: e0127353.
10. Cazzato G, Caporusso C, Arezzo F, et al. Formalin-fixed and paraffin-embedded samples for next generation sequencing problems and solutions. *Genes Basel*. 2021; 12: 1472.
11. Einaga N, Yoshida A, Noda H, et al. Assessment of the quality of DNA from various formalin-fixed paraffin-embedded tissues and the use of this DNA for next-generation sequencing with no artifactual mutation. *PLoS One*. 2017; 12: e0176280.
12. Simbolo M, Gottardi M, Corbo V, et al. DNA qualification workflow for next generation sequencing of histopathological samples. *PLoS One*. 2013; 8: e62692.
13. Varesano S, Ciccarese G, Parente P, et al. Integrating molecular diagnostics into cervical cancer screening a workflow using FFPE tissue samples. *Curr Issues Mol Biol*. 2025; 47: 679.
14. Wentzensen N, Sherman ME, Schiffman M, et al. Utility of methylation markers in cervical cancer early detection appraisal of the state-of-the-science. *Gynecol Oncol*. 2009; 112: 293-299.
15. Sumiec EG, Yim ZY, Mohy-Eldin H, et al. The current state of DNA methylation biomarkers in self-collected liquid biopsies for the early detection of cervical cancer a literature review. *Infect Agent Cancer*. 2024; 19: 62.
16. Espina V, Wulfschle JD, Calvert VS, et al. Laser-capture microdissection. *Nat Protoc*. 2006; 1: 586-603.
17. Bonner RF, Emmert-Buck M, Cole K, et al. Laser capture microdissection molecular analysis of tissue. *Science*. 1997; 278: 1481-1483.
18. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988; 16: 1215.
19. Rivero ERC, Neves AC, Silva-Valenzuela MG, et al. Simple salting-out method for DNA extraction from formalin-fixed paraffin-embedded tissues. *Pathol Res Pract*. 2006; 202: 523-529.
20. Ali N, Rampazzo RCP, Costa ADT, et al. Current nucleic acid extraction methods and their implications to point-of-care diagnostics. *Biomed Res Int*. 2017; 2017: 9306564.
21. Javadi A, Shamaei M, Mohammadi Ziazi L, et al. Qualification study of two genomic DNA extraction methods in different clinical samples. *Tanaffos*. 2014; 13: 41-47.
22. van Beers EH, Joosse SA, Ligtenberg MJ, et al. A multiplex PCR predictor for aCGH success of FFPE samples. *Br J Cancer*. 2006; 94: 333-337.
23. Wentzensen N, Vinokurova S, von Knebel Doeberitz M. Systematic review of genomic integration sites of human papillomavirus genomes in epithelial dysplasia and invasive cancer of the female lower genital tract. *Cancer Res*. 2004; 64: 3878-3884.
24. Lizano M, Carrillo-Garcia A, De La Cruz-Hernandez E, et al. Promising predictive molecular biomarkers for cervical cancer. *Int J Mol Med*. 2024; 53: 50.
25. Garg P, Krishna M, Subbalakshmi AR, et al. Emerging biomarkers and molecular targets for precision medicine in cervical cancer. *Biochim Biophys Acta Rev Cancer*. 2024; 1879: 189106.