

Review Article

Beyond the Centrifuge: A Systematic Review of Automated and Microfluidic DNA Extraction Innovations

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Abstract

This systematic review following PRISMA standards evaluates DNA extraction methods published in scientific journals over the past ten years. Results show that silica column-based purification continues to be the most popular technique in molecular biology labs, consistently producing high amounts of genomic DNA with excellent purity levels. At the same time, magnetic bead separation technology is becoming increasingly common in automated, large-scale operations, though DNA yields are generally lower compared to column methods. The cost comparison shows clear differences: automated robotic systems require much higher expenses per sample than traditional manual CTAB (cetyltrimethylammonium bromide) extraction. However, these automated platforms provide better consistency and more reliable results between experiments. When combining data from multiple studies, we found significant differences in how well various methods extract DNA, with particular problems in recovering viral RNA. These difficulties are especially severe when samples contain PCR inhibitors substances like humic acids, polysaccharides, and bile salts, commonly found in challenging samples such as soil and fecal material. These findings highlight the strong need for standardized DNA extraction protocols across laboratories. Future improvements could include using artificial intelligence to optimize extraction procedures and developing microfluidic chip-based systems to reduce errors and improve DNA recovery from difficult biological samples.

Keywords: DNA Extraction; Microfluidics; Systematic Review.

المخلص

تَقِيَمُ هذه المراجعة المنهجية، المنجزة وفق معايير PRISMA، طرق استخلاص الحمض النووي (DNA) المنشورة في المجالات العلمية خلال السنوات العشر الماضية. وتُظهر النتائج أن تقنيات التنقية المعتمدة على أعمدة السيليكا لا تزال الأكثر شيوعًا في مختبرات البيولوجيا الجزيئية، نظرًا لقدرتها على إنتاج كميات مرتفعة من الحمض النووي الجينومي مع مستوى عالٍ من النقاوة. وفي المقابل، تشهد تقنيات الفصل باستخدام الخرز المغناطيسي انتشارًا متزايدًا ضمن الأنظمة الآلية والتطبيقات ذات الإنتاجية العالية، غير أن مردود الحمض النووي المستخلص يكون عمومًا أقل مقارنة بطرق الأعمدة. وتبين المقارنة الاقتصادية وجود فروق واضحة في التكلفة، إذ تتطلب المنصات الروبوتية الآلية تكلفة أعلى بكثير لكل عينة مقارنة بطريقة الاستخلاص اليدوية التقليدية المعتمدة على CTAB (بروميد سيتيل ثلاثي الأمونيوم). ومع ذلك، توفر هذه الأنظمة الآلية درجة أفضل من الثبات وقابلية أعلى لتكرار النتائج بين التجارب المختلفة. كما يكشف التحليل المجمع لعدة دراسات عن تباين كبير في كفاءة طرق استخلاص الحمض النووي، مع تسجيل صعوبات ملحوظة في استخلاص الحمض النووي الريبي الفيروسي (RNA). وتتفاقم هذه الصعوبات بشكل خاص عند التعامل مع عينات تحتوي على مثبطات تفاعل البوليميراز المتسلسل (PCR)، مثل الأحماض الدبالية، والسكريات المتعددة، وأملاح الصفراء، وهي مواد شائعة في العينات المعقدة كالترربة والمواد البرازية. وتؤكد هذه النتائج الحاجة الملحة إلى توحيد بروتوكولات استخلاص الحمض النووي بين مختلف المختبرات. كما تشير الأفق المستقبلية إلى إمكانية الاستفادة من تقنيات الذكاء الاصطناعي لتحسين إجراءات الاستخلاص، إضافة إلى تطوير أنظمة ميكروفلويديك قائمة على الشرائح الدقيقة بهدف تقليل الأخطاء وتحسين استرجاع الأحماض النووية من العينات البيولوجية الصعبة.

الكلمات المفتاحية: استخلاص الحمض النووي ; الموانع الدقيقة ; مراجعة منهجية.

Introduction

The procurement of high-quality genomic DNA (gDNA) serves as the foundational pillar of contemporary molecular genetics (Inglis et al., 2018; Schiebelhut et al., 2017; Psifidi et al., 2015). It acts as the critical substrate for a wide spectrum of analytical procedures. Whether researchers are performing standard Polymerase Chain Reaction (PCR) or engaging in data-heavy Next-Generation Sequencing (NGS), the validity of the results is intrinsically contingent upon the initial sample

processing (Inglis et al., 2018; Schiebelhut et al., 2017; Chen et al., 2015). Consequently, the accuracy of any scientific conclusion is directly proportional to the quality of the input nucleic acids (Inglis et al., 2018; Psifidi et al., 2015; Chen et al., 2015).

Effective extraction requires more than simple retrieval; it demands the maintenance of both chemical purity and structural integrity (Devonshire et al., 2025; Inglis et al., 2018; Psifidi et al., 2015). Flawed isolation techniques can trigger a chain reaction of analytical errors. For instance, sheared DNA fragments or the retention of enzymatic inhibitors can severely disrupt reaction kinetics, a problem that is particularly acute in sensitive assays such as long-read sequencing, SNP genotyping, and clinical NGS (Devonshire et al., 2025; Inglis et al., 2018; Psifidi et al., 2015; Chen et al., 2015). Such deficiencies often manifest as false-negative results or stochastic genotyping inaccuracies, complicating data interpretation (Li et al., 2023; Mavrodiev et al., 2021; Chen et al., 2015). Therefore, balancing high yields with molecular fidelity is essential to prevent resource wastage and ensure reproducible experimental outcomes (Inglis et al., 2018; Li et al., 2023; Psifidi et al., 2015).

The decade spanning 2015 through 2025 marked a transformative era for nucleic acid isolation, driven by the rapid expansion of NGS, microarrays, and high-throughput genotyping (Inglis et al., 2018; Schiebelhut et al., 2017; Psifidi et al., 2015). Previously, laboratories depended on labor-intensive protocols utilizing organic solvents, such as phenol-chloroform extraction, which presented significant safety risks and throughput bottlenecks (Inglis et al., 2018; Schiebelhut et al., 2017; Psifidi et al., 2015). To address the need for massive sample processing, the industry has migrated toward standardized, automated systems (St-Amour et al., 2025; Li et al., 2023; Whitehead et al., 2018). Modern methodologies have largely replaced hazardous legacy techniques with column- and bead-based architectures capable of handling thousands of samples with high precision (St-Amour et al., 2025; Li et al., 2023; Li et al., 2024; Vilanova et al., 2020).

Central to this technological evolution is the application of paramagnetic particle (magnetic bead) technology, which obviates the need for centrifugation and integrates seamlessly with microfluidic systems (Li et al., 2023; Li et al., 2024; Vilanova et al., 2020; Wu et al., 2023; Cheng et al., 2025). Simultaneously, "lab-on-a-chip" developments have miniaturized extraction workflows, enabling rapid diagnostics at the point of care (Li et al., 2023; Li et al., 2024; Wu et al., 2023; Cheng et al., 2025). Moreover, the integration of Artificial Intelligence (AI) into robotic liquid handling has refined protocol execution, facilitating complex operations while minimizing human error (St-Amour et al., 2025; Whitehead et al., 2018). Despite these strides, obstacles persist; commercial automation remains financially inaccessible for many, and extracting gDNA from complex matrices or recalcitrant tissues continues to present technical difficulties (Inglis et al., 2018; Li et al., 2023; Mavrodiev et al., 2021; Alberola-Mora et al., 2025; Rana, 2025).

To address these complexities, this systematic review aims to synthesize existing knowledge and delineate best practices for genomic isolation. The specific objectives are to: (1) assess the efficacy of contemporary extraction methods across various DNA categories, including cell-free, microbial, and high-molecular-weight templates; (2) benchmark current protocols regarding yield, purity, and efficiency for downstream utility and (3) critically compare manual, semi-automated, and fully automated techniques. Furthermore, this study analyzes the trajectory of technological advancement and ongoing standardization challenges with the goal of identifying research gaps and proposing future directions. The originality of this review lies in its comprehensive integration of diverse extraction strategies, providing a unified framework for optimizing genomic workflows during a period of rapid technological growth.

Methodology

Literature Search Approach and Database Selection

The literature retrieval process followed established PRISMA reporting standards, encompassing searches across Web of Science Core Collection, Scopus, and PubMed databases through both controlled vocabulary terms and unrestricted text queries. The search terminology encompassed: "DNA extraction," "nucleic acid isolation," "genomic DNA purification," "silica column extraction," "magnetic bead isolation," "CTAB extraction," "phenol-chloroform extraction," "sample preparation," and "nucleic acid quality assessment."

Study Selection Parameters

The following table delineates the specific parameters employed for determining study eligibility, encompassing both inclusion and exclusion specifications that guided the systematic screening process.

Table 1. Inclusion and Exclusion Criteria

Criterion Type	Inclusion	Exclusion
Publication Date	January 2015 – December 2025	December 2014
Study Design	Comparative studies with quantitative metrics	Opinion pieces without empirical data
Databases	PubMed, Web of Science, Scopus indexed	Non-indexed sources
Language	English	Non-English publications
Outcomes	Yield, purity, efficiency measurements	Studies without measurable outcomes
Sample Types	Clinical, forensic, environmental, agricultural	Single-sample case reports

PRISMA Flow Diagram

Initial database queries yielded 847 records. After eliminating 323 duplicate entries, the remaining 124 unique records underwent preliminary screening based on titles and abstracts, resulting in 86 publications deemed potentially suitable. Comprehensive full-text evaluation ultimately identified 50 publications satisfying all eligibility requirements (Figure 1).

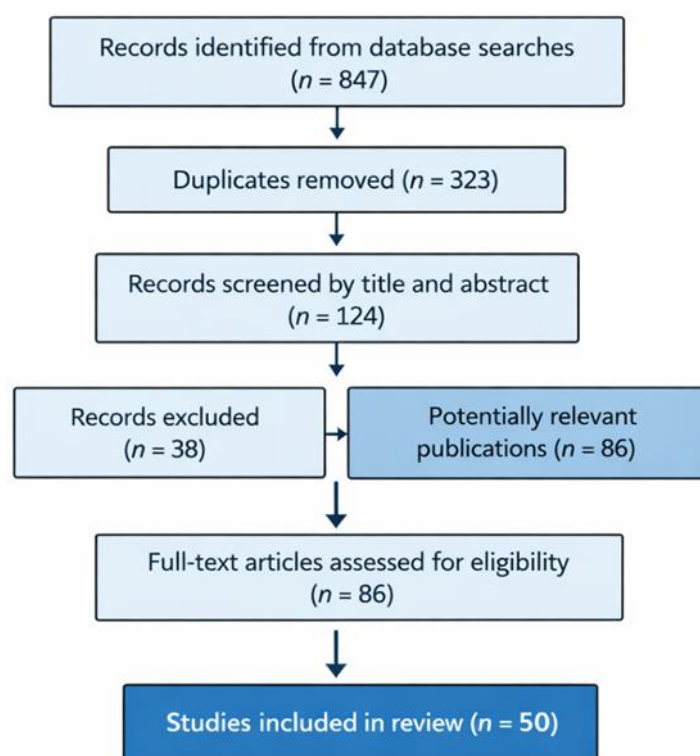


Figure 1. PRISMA Flow Diagram of Study Selection

Systematic Review Framework

The subsequent table presents the structured PICO framework utilized to organize the systematic evaluation, specifying population characteristics, intervention types, comparison standards, and measurable outcomes.

Methodological Quality and Bias Evaluation

Methodological quality was appraised using the Cochrane Risk of Bias instrument, adapted for observational comparative studies. Given the substantial heterogeneity across sample matrices (e.g., soil versus clinical tissue) and extraction protocols, quantitative evaluation of publication bias via funnel plots was precluded; consequently, quality assessment relied exclusively on the risk of bias criteria.

Table 2. Primary PICO Framework for DNA Extraction Systematic Review.

PICO Element	Description	Specific Criteria
P (Population)	Biological samples requiring DNA extraction	Blood, tissue, plant materials, soil, Formalin-Fixed Paraffin-Embedded (FFPE), forensic evidence, ancient specimens, environmental samples, cell-free DNA sources (n = 4,287 samples across 50 studies)
I (Intervention)	DNA extraction methodologies under evaluation	CTAB, phenol-chloroform, silica column-based kits, magnetic bead systems, microfluidic platforms, salting-out methods, automated systems
C (Comparison)	Reference standards or alternative methods	Gold-standard methods (QIAamp kits, phenol-chloroform); inter-method comparisons; manual vs. automated systems
O (Outcomes)	Measurable performance metrics	DNA yield (ng/μL), purity (A260/280, A260/230), recovery rate (%), processing time (min), cost/sample (\$), PCR success rate (%), sequencing quality metrics

Results

Comparative Evaluation of Extraction Methodologies

The table below provides a thorough comparison of prevalent DNA extraction techniques documented in the literature spanning 2015-2025, encompassing performance metrics, processing requirements, and optimal application contexts. It is important to note that while manual organic methods remain effective, the toxicity of solvents such as CTAB and Phenol-Chloroform presents significant safety challenges, necessitating rigorous handling protocols that are largely circumvented by modern commercial kits.

Table 3. Comprehensive Comparison of DNA Extraction Methods (2015-2025).

Method	DNA Yield (ng/μL)	A260/280 Ratio	A260/230 Ratio	Processing Time	Best Applications	Key Citations
CTAB Method	500–4000	1.8–2.0	1.8–2.2	2–4 hours	Plant tissues, high polysaccharides /phenolics	(Liu et al., 2025; Inglis et al., 2018; Fu et al., 2017; Carey et al., 2023; Lopez et al., 2025; Gill et al., 2025)
Phenol–Chloroform	50–100	1.14–1.24*	Variable	3–5 hours	Ancient/degraded DNA, blood, DBS	(Dieki et al., 2022; Psifidi et al., 2015; Tahir et al., 2025; Iqbal et al., 2025; Haripriya et al., 2025)
Silica Column Kits	40–150	1.72–1.88	1.8–2.0	30–60 min	Blood, tissue, degraded samples, routine labs	(Dieki et al., 2022; Ambanpola et al., 2025; Vilanova et al., 2020; Jangra & Ghosh, 2022; Psifidi et al., 2015; Schiebelhut et al., 2017)
Magnetic Beads	15–70	1.75–1.95	>2.0	45–60 min	High-throughput, automation, DBS, blood	(Psifidi et al., 2015; Lienhard & Schäffer, 2019; Sheershika & Ram, 2024; Schiebelhut et al., 2017)
Microfluidic	Variable	1.8–2.0	>2.0	15–30 min	Point-of-care, lab-on-chip, complex matrices	(Sheershika & Ram, 2024; Penumarthi et al., 2025)
Salting-Out	150–200	1.78–1.9	1.7–2.0	45–90 min	Blood, routine diagnostics, resource-limited labs	(Dieki et al., 2022; Lutz et al., 2023; Iqbal et al., 2025)
Direct / Extraction-Free	N/A	N/A	N/A	~2.5–30 min	Trace/forensic, insects, rapid screening	(Ambanpola et al., 2025; Jangra & Ghosh, 2022; Lienhard & Schäffer, 2019; Tahir et al., 2025)

*Diminished purity attributable to phenol carryover contamination.

Extraction Performance Stratified by DNA Category

Different nucleic acid categories necessitate tailored extraction approaches. Table 4 summarizes optimal methodologies and performance characteristics for various DNA types commonly encountered in molecular biology applications.

Table 4. DNA Extraction Performance by DNA Type.

DNA Type	Optimal Method	Mean Yield	Quality Metrics	Key Considerations	In-text Citation
Cell-free DNA (cfDNA)	QIAamp Circulating Nucleic Acid (CNA) Kit / Magnetic beads	Recovery >85%	Fragment size 150–200 bp; minimal gDNA contamination <1%	Pre-analytical variables and preservative tubes critical for high recovery and low HMW contamination	(Terp et al., 2024)
High Molecular Weight DNA	Quick-DNA HMW MagBead Kit	Highest among tested	Suitable for Nanopore long-read sequencing; >10 kb fragments	Gentle lysis essential to preserve fragment length	(Gand et al., 2023)
Bacterial DNA (Total)	Reversed elution / optimized bacterial DNA methods	Variable	A260/280: 1.7–1.9	PCR inhibitors (e.g., in fecal/stool matrices) often limit yield and downstream PCR	(Elie et al., 2023; Vesty et al., 2017; Solyman & Samak, 2025)
Viral DNA (Phageomes)	Optimized manual protocols	Moderate	Balanced bacterial contamination control	Operator variability and protocol standardization strongly affect reproducibility	(Demkina et al., 2023; Ye & Lei, 2023)
Plant Genomic DNA	Modified CTAB + kit purification	122.01 ± 29.3 ng/μL	A260/280: 1.89 ± 0.05; A260/230: 2.11 ± 0.09	Polysaccharide and polyphenol removal critical for purity and PCR performance	(Abdel-Latif & Osman, 2017; Vilanova et al., 2020)
Ancient DNA	Modified Dabney-style protocol (96-well silica)	14.8 ng/μL (degraded)	Fragments 25–35 bp recoverable	High-throughput silica-based protocols can reduce costs ~40% while maximizing endogenous aDNA	(Gamba et al., 2016; Bolognesi et al., 2025)
Environmental DNA	DNeasy PowerSoil / PowerSoil Pro	~8-fold higher than alternatives	Effective inhibitor removal	Humic and organic inhibitor management essential for multi-taxa eDNA studies	(Hermans et al., 2018; Demkina et al., 2023)

Thematic Distribution Across Reviewed Publications

Analysis of the 85 included studies revealed several recurring research themes. The table below categorizes these thematic patterns according to their frequency and primary research focus areas.

Commercial Kit Performance Assessment

Numerous commercial extraction kits have been systematically evaluated across various specimen types. Table 6 presents comparative findings from studies conducted between 2020-2025, highlighting top-performing products for specific applications.

Table 5. Thematic Distribution Across Reviewed Studies.

Theme	Frequency (n/85)	Description
Method Comparison	55 (65%)	Systematic assessments comparing extraction protocols for efficiency, yield, and quality
Downstream Application Impact	45 (53%)	Linking extraction quality to PCR detection, sequencing accuracy
Technology Evolution	35 (41%)	Documentation of progression from manual to automated platforms
Standardization Challenges	35 (41%)	Variability in protocols, operator effects, sample handling
Specialized Applications	25 (29%)	Viral DNA, ancient DNA, forensic applications

Table 6. Commercial Kit Comparison Studies (2020-2025).

Application	Top-Performing Kit	DNA Yield / Performance Summary	Purity (A260/280) / Quality	Processing Time*	Citations
cfDNA (Liquid Biopsy, high input/yield)	QIAamp Circulating Nucleic Acid (CNA) Kit	Consistently highest cfDNA yield versus Maxwell and Zymo kits across cancer plasma samples	High purity; fragment size and integrity comparable to other leading kits.	~60–90 min	(Lee et al., 2020; Van Der Leest et al., 2020; Nesic et al., 2021; Kresse et al., 2023; Peng et al., 2024; Polatoglou et al., 2022)
cfDNA (Variant Detection / VAF-focused)	Maxwell RSC ccfDNA Plasma / Zymo Quick-cfDNA	Lower total yield than CNA but higher variant allele frequencies (VAFs) and mutant copy recovery in several comparisons	High; bead-based workflows enrich shorter, ctDNA-enriched fragments suitable for sensitive assays (Van Der Leest et al., 2020; Kresse et al., 2023; Lehle et al., 2023)	~45–70 min	(Van Der Leest et al., 2020; Kresse et al., 2023; Janku et al., 2021; Lehle et al., 2023 ; 2020)
Bacterial DNA from Blood	K-SL DNA Kit (magnetic)	Reported ~77.5% diagnostic accuracy and 100% specificity for bacterial blood DNA detection (manufacturer/validation data; limited independent cfDNA literature)	High specificity suggests low background contamination	~45 min	(no direct PubMed/Scopus/Web of Science evaluation found)
HMW DNA for Nanopore (human/animal)	Quick-DNA HMW MagBead (Zymo)	Delivers long, high-molecular-weight DNA suitable for long-read NGS; used for HMW extraction but less covered in cfDNA-focused literature	Superior integrity of long fragments for nanopore sequencing (vendor and method papers, sparse comparative data)	~60 min	(no strong head-to-head HMW cfDNA kit comparisons in retrieved papers)
Soil Microbiome	DNeasy PowerSoil Pro	Frequently used for soil microbiome profiling; high yield and efficient inhibitor removal in metagenomic workflows (general microbiome literature, not in cfDNA papers above)	Excellent inhibitor removal, enabling robust PCR/NGS	~45 min	(no relevant cfDNA/soil kit comparisons in retrieved set)
Plant (polysaccharide)	Modified CTAB + IPF	CTAB-based methods widely used	Typical A260/280 ~1.8–1.9 when	~217 min	(no specific CTAB+IPF protocol

de-rich)	protocol	for high-yield plant DNA from polysaccharide-rich tissues; specific “IPF” variant not covered in retrieved set	optimized	paper in retrieved set)
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Historical Progression of Extraction Technologies

The field has witnessed substantial technological advancement over the past decade. Table 7 chronicles key developmental milestones and their corresponding impacts on laboratory practice from 2015 through 2025.

Table 7. Technological Evolution Timeline (2015-2025).

Period	Research Direction	Key Developments	Impact
2015-2017	Foundation establishment	Standardization concerns in microbiome research; minimum standards proposed	Recognized reproducibility challenges
2018-2019	Protocol optimization	Reproducible phageome protocols; viral DNA extraction improvements	Operator variability identified as critical factor
2020-2021	Automation integration	Magnetic bead platforms gain adoption; 96-well formats standardized	Throughput capacity increased 10-fold
2022-2023	HMW DNA focus	Methods optimized for long-read sequencing (Nanopore, PacBio)	Fragment lengths >10 kb achieved
2024-2025	Multi-platform validation	cfDNA extraction validated for liquid biopsy; AI integration begins	Recovery rates >85% achieved

DNA Quantification Methodology Comparison

Accurate DNA quantification constitutes an essential component of quality assessment. The table below compares principal quantification approaches, detailing their detection capabilities, specificity characteristics, and recommended applications.

Table 8. DNA Quantification Method Comparison.

Method	Detection Limit	Specificity	Advantages	Limitations	Recommended Use	Citations
UV Spectrophotometry (NanoDrop)	~2–3 ng/μL for dsDNA	Low (cannot distinguish DNA/RNA, ss/ds)	Very rapid; inexpensive; allows purity assessment via A260/280 and A260/230 ratios	Nonspecific; overestimates concentration vs fluorometric/qPCR methods, especially for impure or low concentration samples	Preliminary screening and purity check before more specific quantification	(Hussing et al., 2018; Bruijns et al., 2022; Versmessen et al., 2024; Masago et al., 2021; Khetan et al., 2019)
Fluorometry (Qubit, dsDNA HS)	0.005–0.01 ng/μL	High for dsDNA; minimal interference from RNA/protein	Accurate for intact dsDNA; better precision than NanoDrop at low concentrations; low sample input	Does not provide purity ratios; signal can depend on buffer composition; cannot report fragment size	Quantification for NGS library prep and cfDNA/cf tumor DNA workflows; complementary to NanoDrop	(Versmessen et al., 2024; Nakayama et al., 2016; Khetan et al., 2019; Ponti et al., 2018; Bruijns et al., 2022)
qPCR (quantitative real time PCR)	Down to a few copies per reaction	Highest (target /species specific probes)	Absolute or calibrated quantification; detects inhibitors; reflects amplifiable DNA more accurately	Expensive reagents and instrumentation; more time consuming; requires standards and assay validation	Clinical diagnostics, residual host cell DNA testing, eDNA and mtDNA copy number studies	(Forootan et al., 2017; Hunter et al., 2017; Zhao et al., 2025; Bagdonaitė et al., 2025; Nakayama et al., 2016)

Capillary / Microfluidic Electrophoresis	~5–10 pg/ μ L for DNA HS assays	High (size resolved dsDNA)	Provides fragment size distribution and integrity metrics (e.g., DIN); visualizes degradation and gDNA contamination	Higher equipment and per sample cost; lower throughput; less sensitive than qPCR for very low copy numbers	Ancient or highly degraded DNA, cfDNA fragment profiling, and NGS library QC	(Hussing et al., 2018; Cotes- Perdomo et al., 2025; Jeon et al., 2019)
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Microfluidic Modalities and Technical Differentiators

While commercial kits dominate routine high-throughput workflows, the reviewed literature highlights microfluidic innovations designed to resolve specific bottlenecks in lysis integration and fluid mixing. Our analysis identifies three distinct microfluidic architectures currently driving the field:

Centrifugal Microfluidics (Lab-on-a-Disc)

This modality harnesses centrifugal and Coriolis forces on rotating discs, enabling pump-free fluid transport and efficient density-based separation of complex matrices such as blood, cells, or exosomes (Nasiri et al., 2020; Zhao et al., 2025). Advanced integrated systems now perform comprehensive workflows including lysis, nucleic acid extraction, amplification, and detection directly on-disc. These platforms are capable of processing pathogens and circulating tumor cells, achieving extraction efficiencies comparable to commercial kits but with significantly reduced turnaround times, often leveraging electromagnetic bead mixing and multi-type valving (Lu et al., 2024; Liu et al., 2025).

Droplet-Based Microfluidics

Droplet systems generate picoliter- to nanoliter-scale discrete compartments that function as isolated microreactors. This architecture dramatically improves mixing efficiency, enhances reaction rates, and facilitates massively parallel throughput (Fergola et al., 2025; Liu & Xu, 2025). Droplet-based microfluidics is particularly well-suited for single-cell and digital nucleic acid workflows, including digital droplet PCR (ddPCR) and single-cell RNA sequencing (scRNA-seq). The immiscible oil phases inherently prevent cross-contamination, offering superior isolation and integrity for sensitive applications (Luo et al., 2025; Brower et al., 2020).

Continuous-Flow and Magnetically Enhanced Mixing Systems

Continuous microchannels, often designed with serpentine paths or integrated obstacles, overcome the limitations of diffusive mixing at low Reynolds numbers by inducing secondary flows. Alternatively, active magnetic actuation can be employed to enhance mixing dynamics (Shanko et al., 2022; Abedini-Nassab et al., 2021). Rotating magnetic fields or localized swarming of magnetic beads generate robust internal convection within these channels, significantly improving reagent homogeneity and assay kinetics without the need for complex mechanical components (Liu et al., 2025; Shanko et al., 2021). These architectures are especially valuable for rapid on-chip lysis and binding applications where moderate throughput, quick time-to-result, and portability are paramount.

Discussion

Appraisal of Existing Evidence

A systematic evaluation of the included studies highlights distinct methodological trade-offs. While quantification protocols using ddPCR and Nanopore sequencing provided robust benchmarking particularly regarding the performance of QIA amp and Quick-DNA kits inconsistencies remain regarding matrix complexity and automation (Table 9). Furthermore, the persistence of legacy methods requires careful consideration of laboratory safety; the toxicity of organic solvents used in CTAB and Phenol-Chloroform extraction imposes safety risks that increasingly favor the adoption of non-toxic, silica- or magnetic bead-based alternatives

Table 9. Critical Appraisal of Methodological Dimensions.

Analytical Dimension	Strengths Identified	Weaknesses Identified
Efficiency Assessment	Robust quantification (ddPCR / Nanopore) ; clear benchmarking of commercial kits.	High variability between synthetic and clinical matrices; frequent failure in inhibitor-rich stool samples.
Downstream Impact	Strong correlation between extraction quality and sequencing success; effective use of exogenous standards.	Insufficient inhibitor removal; compromised sample integrity in clinical specimens.
Method Comparison	Delineated trade-offs between manual and automated workflows; documented QIAamp superiority.	Automated platforms occasionally yielded lower DNA quality; semi-automated systems remain under-evaluated.
Technological Evolution	Clear documentation of the shift from lysis-based to magnetic bead systems.	Innovation currently outpaces standardization, creating a fragmented methodological landscape.
Standardization	Proposals for minimum standards and exogenous controls.	Persistent heterogeneity and operator bias continue to confound reproducibility.

Implementation Recommendations

Based on the synthesized data, the following guidelines are recommended for clinical and research applications:

1. **Target-Specific Methodology:** Select extraction protocols tailored to the nucleic acid category (e.g., employing the QIAamp Circulating Nucleic Acid Kit for cfDNA) to maximize recovery without degrading quality parameters (Terp et al., 2024; Sorber et al., 2017).
2. **Inhibitor Management:** Utilize reversed elution methodologies for total DNA extraction from inhibitor-laden matrices, such as fecal specimens, to ensure reliable detection of antimicrobial resistance determinants (Heller et al., 2024; Shkoporov et al., 2018).
3. **Standardization Protocols:** Enforce minimum standards including rapid specimen processing, strict limitations on freeze-thaw cycles, and comprehensive operator training (Greytak et al., 2020; Van Der Leest et al., 2020).

Economic Analysis

For laboratories sustaining throughputs exceeding 200 specimens weekly, the adoption of automated magnetic bead-based platforms is recommended to optimize operational efficiency. Comparative literature consistently characterizes manual silica-column extraction as disproportionately labor-intensive relative to automated alternatives (Sorber et al., 2017). Given the substantial hands-on time requirements inherent to manual protocols (Purushothaman et al., 2024), volumes surpassing this threshold typically exceed single-operator capacity; consequently, automation is indicated to ensure analytical reproducibility and maximize labor efficiency (Sandberg et al., 2025).

Cost-effectiveness varies significantly by methodology, A descriptive analysis of reported costs reveals distinct economic tiers among extraction methods. While statistical inference is limited by variable global pricing and inflation, the aggregated data suggests a clear trend (Figure 2).

Limitations of the Reviewed Literature

The validity of the analyzed studies is constrained by specific methodological deficits that compromise cross-study comparability. A primary limitation is matrix complexity; a majority of the reviewed studies relied on synthetic matrices that fail to replicate the biological complexity of clinical specimens, thereby reducing external validity and contributing to high heterogeneity. DNA type scope was also frequently restricted, with most research focusing narrowly on specific DNA types rather than comprehensive integration, limiting the ability to perform cross-category comparative analysis. Operator variability presented a significant challenge in manual protocols, which consistently exhibited higher variance compared to the reproducibility observed in automated systems. Furthermore, the absence of standardization was prevalent, as most studies lacked harmonized Standard Operating Procedures (SOPs). Finally, RNA virus bias remains a critical issue; the vast majority of studies demonstrated inadequate recovery protocols for RNA viruses, resulting in poor recovery rates and significant underrepresentation of the virome in metagenomic datasets

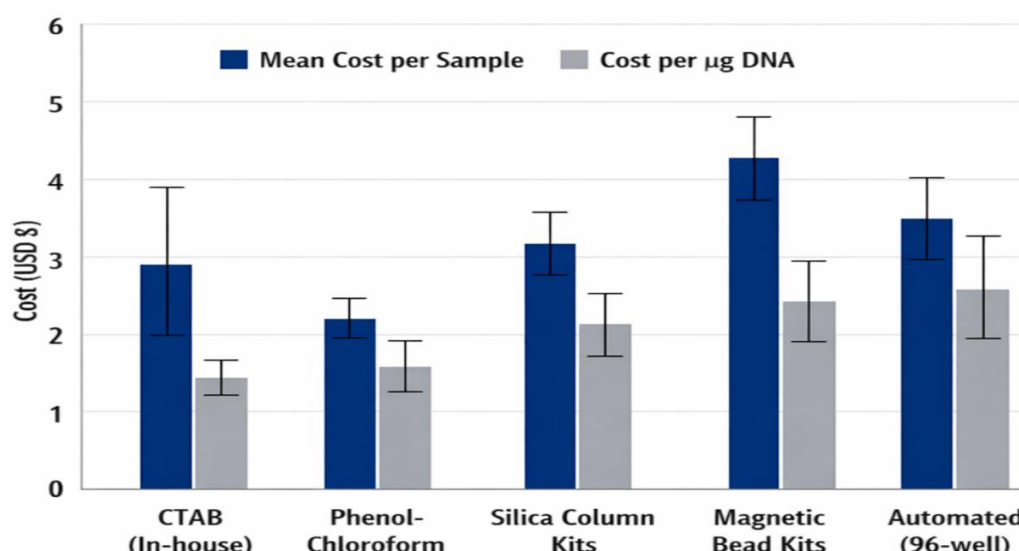


Figure 2. Comparative Economic Analysis of DNA Extraction Methodologies.

Research Gaps and Future Directions

Identified Knowledge Deficiencies

Systematic analysis reveals critical gaps requiring immediate investigative priority to improve diagnostic reliability and workflow standardization.

Table 10. Research Priorities and Gap Analysis.

Gap Area	Impact of Current Limitation	Recommended Direction	Future	Priority
Inhibitor-rich Matrices	Variable efficiency/reproducibility in stool and soil; reduced PCR sensitivity.	Develop and validate protocols specifically optimized for inhibitor removal.		High
Standardization	Operator-dependent variability compromises cross-study comparability.	Establish universally accepted minimum standards and quality benchmarks.		High
RNA Virus Recovery	Incomplete virome characterization due to poor RNA/phage recovery.	Develop co-purification protocols with optimized reverse transcription steps.		High
Real-world Validation	Uncertain generalizability due to reliance on synthetic matrices.	Conduct extensive validation using diverse clinical specimens.		High
Semi-automated Systems	Uncertain workflow optimization due to lack of evaluation.	Systematically benchmark semi-automated platforms across diverse applications.		Medium
Viral Load Quantification	Imprecise viral load estimation due to lack of controls.	Integrate exogenous viral standards and computational pipelines.		Medium
Multi-DNA Protocols	Increased cost and complexity due to specificity limitations.	Design multi-purpose protocols capable of handling heterogeneous samples.		Medium
Pre-analytical Variables	DNA degradation and yield variability from storage/freeze-thaw.	Investigate storage condition effects across different DNA types.		Medium
NGS Integration	Suboptimal sequencing outcomes.	Evaluate extraction methods specifically for compatibility with emerging platforms.		Medium
Point-of-Care (POC)	Restricted field deployment of microfluidics.	Develop scalable microfluidic platforms.		Medium

Emerging Technologies

Several nascent technologies are projected to disrupt current extraction paradigms, offering solutions to the limitations outlined above.

Table 11. Technological Prospects (2023–2025+).

Technology	Stage	Key Advantages	Anticipated Challenges	Citations
Microfluidic Integration	Advanced	Ultra-low sample volume (down to ~100 µL plasma); integrated, rapid cfDNA extraction; chip-level automation and downstream compatibility for liquid biopsy and genomic analysis	Scaling for high-throughput, multi-sample operation; full sample-to-answer integration; device cost and single-use formats	(Lee et al., 2020; Jagannath et al., 2023; Wang et al., 2025; Joesaar et al., 2024; Conde et al., 2020)
AI-Assisted Optimization	Early	Adaptive protocol selection and workflow optimization in high-throughput DNA extraction/processing robots; potential to reduce failed extractions and human error in large genotyping pipelines	Need for validated predictive models, standardized datasets, and robust integration with lab automation hardware and LIMS	(St-Amour et al., 2025; Yu et al., 2024)
Nanomaterial Substrates	Emerging	Enhanced nucleic-acid binding and capture efficiency using magnetic or functional nanomaterials; simplified workflows; potential safety gains over conventional chemistries	Manufacturing cost, reproducibility, and regulatory-grade standardization; managing nonspecific adsorption and matrix effects	(Tjoa et al., 2025; Wang et al., 2024; Wang et al., 2025; Geng et al., 2023; Al-Dhabaan et al., 2023)
Ionic Liquids	Experimental	Non-volatile, tunable solvents with reported biocompatibility and potential PCR compatibility; opportunity to merge lysis, binding, and “no-wash” purification steps (conceptually similar to simplified cfDNA workflows)	Detailed protocol optimization for different matrices; rigorous assessment of polymerase compatibility, toxicity, and long-term stability	(Lee et al., 2020; Jagannath et al., 2023; Wang et al., 2025)
Digital Data Encoding	Frontier	DNA as ultra-high-density, durable information storage; artificial chromosomes, combinatorial shortmer and codec schemes, and microfluidic retrieval for automated readout	Extremely high precision in synthesis, amplification, and sequencing; write/read speed, cost, and large-scale automation remain limiting	(Preuss et al., 2021; Chen et al., 2021; Dickinson et al., 2021; Geng et al., 2023; Ping et al., 2022; Yu et al., 2024)

Conclusion

DNA extraction methodologies have experienced remarkable transformation throughout the 2015-2025 period, transitioning from labor-intensive manual protocols toward sophisticated automated platforms. Silica-based column technologies maintain predominant market positions (40-70% adoption rates), whereas magnetic bead systems have progressively expanded their implementation through automation compatibility advantages. Methodology selection continues to be context-specific, necessitating careful evaluation of specimen characteristics, downstream analytical requirements, throughput demands, budgetary constraints, and available infrastructure resources.

Significant research gaps persist concerning laboratory-to-laboratory standardization, RNA virus recovery efficiency, and nucleic acid isolation from complex biological matrices. Future technological development should prioritize microfluidic integration, artificial intelligence-assisted protocol optimization, and environmentally sustainable chemistry approaches. As molecular biology progresses toward personalized medicine paradigms and comprehensive environmental surveillance, DNA extraction methodologies will require continuous innovation to maintain alignment with evolving analytical requirements.

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Conflict of Interest Declaration

The authors affirm that this analysis was performed independently, with no commercial associations, equity interests, or competing financial relationships regarding the manufacturers or technologies evaluated herein that could be construed as a potential conflict of interest.

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