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COMPARATIVE EVALUATION OF BLOOD COLLECTION TUBES AND EXTRACTION METHODS FOR CIRCULATING TUMOR DNA

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Abstract

Introduction. Circulating tumor DNA (ctDNA) has emerged as a valuable biomarker for non-invasive cancer diagnostics and treatment monitoring. However, pre-analytical variables, including blood collection and sample processing, significantly influence ctDNA yield and analytical reliability. Optimizing these conditions is crucial for ensuring reproducible and accurate ctDNA measurements.

Aim. To perform a comparative evaluation of blood collection tubes and DNA extraction methods for the isolation of circulating tumor DNA from the blood plasma of patients with oncological diseases.

Materials and Methods. Plasma samples were collected using two types of blood collection tubes: PaxGene and Streck. ctDNA was extracted using three different systems: Raissol, MagMax, and QiaAmp. Quantitative performance, reproducibility, and variability of ctDNA yield were assessed across all combinations of collection tubes and extraction methods. Statistical analysis was performed to evaluate differences among extraction systems.

Discussion. The results demonstrated that QiaAmp consistently provided higher ctDNA concentrations across both collection systems. Samples collected in PaxGene tubes exhibited lower variability and improved analytical stability compared with Streck tubes. Statistically significant differences among extraction methods were observed for PaxGene samples, while increased variability in Streck samples limited the ability to discriminate between methods. These findings emphasize the critical role of pre-analytical optimization in ctDNA workflows and support the implementation of standardized protocols to improve reliability in liquid biopsy analysis.

Conclusion. The results demonstrate that both blood collection tubes and the DNA extraction method significantly affect ctDNA recovery, highlighting the importance of optimizing and standardizing pre-analytical workflows for reliable liquid biopsy analysis.

Keywords. *circulating tumor DNA; liquid biopsy; DNA extraction; pre-analytical variables; blood collection tubes.*

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Резюме

СРАВНИТЕЛЬНАЯ ОЦЕНКА ПРОБИРОК ДЛЯ СБОРА КРОВИ И МЕТОДОВ ЭКСТРАКЦИИ ДЛЯ ЦИРКУЛИРУЮЩЕЙ ОПУХОЛЕВОЙ ДНК

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Введение. Циркулирующая опухолевая ДНК (ctDNA) стала ценным биомаркером для неинвазивной диагностики рака и мониторинга терапии. Однако преданалитические переменные, включая сбор крови и обработку образцов, существенно влияют на выход ctDNA и надежность анализа. Оптимизация этих условий необходима для обеспечения воспроизводимости и точности измерений ctDNA.

Цель. Целью данного исследования было проведение сравнительной оценки эффективности пробирок для сбора крови и методов экстракции ДНК при выделении циркулирующей опухолевой ДНК из плазмы крови пациентов с онкологическими заболеваниями.

Материалы и методы. Плазменные образцы были собраны с использованием двух типов пробирок: PaxGene и Streck. Экстракция ctDNA проводилась с использованием трех систем: Raissol, MagMax и QiaAmp. Оценивались количественные показатели, воспроизводимость и вариабельность выхода ctDNA для всех комбинаций пробирок и методов экстракции. Для оценки различий между методами экстракции проводился статистический анализ.

Обсуждение. Результаты показали, что система QiaAmp стабильно обеспечивала более высокие концентрации ctDNA при использовании обеих пробирок. Образцы, собранные в пробирки PaxGene, продемонстрировали меньшую вариабельность и улучшенную аналитическую стабильность по сравнению с пробирками Streck. Статистически значимые различия между методами экстракции наблюдались для образцов PaxGene, тогда как повышенная вариабельность в образцах Streck ограничивала возможность различия методов. Эти данные подчеркивают критическую роль оптимизации преданалитических условий и поддерживают внедрение стандартизированных протоколов для повышения надежности анализа жидкостной биопсии.

Заключение. Полученные результаты показывают, что как тип пробирок для сбора крови, так и используемые методы экстракции ДНК существенно влияют на выход циркулирующей опухолевой ДНК, что подчёркивает необходимость оптимизации и стандартизации преданалитических этапов для надёжного анализа жидкостной биопсии.

Ключевые слова. *циркулирующая опухолевая ДНК (ctDNA); жидкостная биопсия; экстракция ДНК; преданалитические переменные; пробирки для сбора крови.*

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Түйіндеме

ҚАН ЖИНАУ ТҮТІКТЕРІН ЖӘНЕ ҚАН АЙНАЛЫМДАҒЫ ҚАТЕРЛІ ІСІК ДНҚ-сын БӨЛУ ҮШІН ӘДІСТЕРІН САЛЫСТЫРМАЛЫ БАҚЫЛАУЫ

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Кіріспе. Циркуляциялық қатерлі ісік ДНҚ (ctDNA) ракты инвазивті емес диагностикалау және емдеу мониторингі үшін маңызды биомаркерге айналды. Алайда, қан жинау және үлгілерді өңдеу сияқты алдын ала аналитикалық факторлар ctDNA шығымына және талдау сенімділігіне елеулі әсер етеді. Бұл жағдайларды оңтайландыру ctDNA өлшеулерінің дәлдігі мен қайта өндірілетіндігін қамтамасыз ету үшін өте маңызды.

Мақсат. Зерттеудің мақсаты – Онкологиялық аурулары бар науқастардың қан плазмасынан қан айналымдағы ісік ДНҚ-сын бөліп алу үшін қолданылатын қан жинау пробиркалары мен ДНҚ экстракция әдістерінің тиімділігін салыстырмалы түрде бағалау.

Материалдар мен әдістер. Плазма үлгілері екі типтегі қан жинау түтіктерінде жиналды: PaxGene және Streck. ctDNA Raissol, MagMax және QiaAmp жүйелерін қолдану арқылы шығарылды. Барлық түтік пен экстракция әдістерінің комбинациялары бойынша ctDNA шығымы, қайта өндірілушілігі және вариабельдігі бағаланды. Экстракция әдістері арасындағы айырмашылықтарды бағалау үшін статистикалық талдау жүргізілді.

Талқылау. Нәтижелер QiaAmp жүйесінің екі түтік үшін де жоғары ctDNA концентрациясын тұрақты түрде қамтамасыз ететінін көрсетті. PaxGene түтіктерінде жиналған үлгілер Streck түтіктерімен салыстырғанда төмен вариабельдік пен жоғары аналитикалық тұрақтылық көрсетті. PaxGene үлгілерінде экстракция әдістері арасындағы статистикалық маңызды айырмашылықтар байқалды, ал Streck үлгілеріндегі жоғары вариабельдік әдістер арасындағы айырмашылықты анықтауды шектеді. Бұл нәтижелер алдын ала аналитикалық шарттарды оңтайландырудың маңызды рөлін көрсетеді және сенімді сұйықтық биопсиясы талдауын қамтамасыз ету үшін стандартталған протоколдарды енгізуді қолдайды.

Қорытынды. Алынған нәтижелер қан жинау пробиркаларының түрі мен ДНҚ экстракция әдістері қан айналымдағы ісік ДНҚ-сының шығымына елеулі әсер ететінін көрсетті, бұл сұйық биопсия талдауының сенімділігін арттыру үшін алдын ала аналитикалық кезеңдерді оңтайландыру мен стандарттаудың маңыздылығын көрсетеді.

Түйінді сөздер. *қан айналымдағы қатерлі ісік ДНҚ (ctDNA); сұйықтық биопсиясы; ДНҚ экстракциясы; алдын ала аналитикалық факторлар; қан жинау түтіктері.*

Дәйексөз үшін:

Айткулова А.М., Каденова Т.Б., Габдулкайюм А., Ережепов Д.А., Рахимова С.Е., Кожамкулов У.А., Каиров У.Е., Ермекбаева Б.А., Акильжанова А.Р., Сарбасов Д.Д. Қан жинау түтіктерін және қан айналымдағы қатерлі ісік ДНҚ-сын бөлу үшін әдістерін салыстырмалы бақылауы // Ғылым және Денсаулық сақтау. 2025. Vol.27 (6), Б. 7-13. *doi 10.34689/SH.2025.27.6.001*

Introduction

Molecular genetic methods play a key role in modern oncology by enabling personalized treatment strategies, improving patient stratification, and allowing dynamic monitoring of therapeutic effectiveness. In recent years, liquid biopsy approaches based on the analysis of circulating tumor DNA (ctDNA) have gained increasing clinical and research relevance due to their minimally invasive nature and their ability to capture the molecular landscape of tumors in real time [2,14]. Unlike conventional tissue biopsies, liquid biopsy offers the possibility of repeated sampling, facilitating longitudinal assessment of tumor evolution and treatment response.

Circulating tumor DNA consists of short fragments of tumor-derived DNA released into various biological fluids, including blood, cerebrospinal fluid, urine, and pleural or ascitic effusions; however, blood plasma remains the most widely used and clinically relevant source for ctDNA analysis [8,15]. In patients with malignant neoplasms, ctDNA is released into the bloodstream primarily because of tumor cell apoptosis and necrosis, as well as active secretion mechanisms associated with tumor progression [9,16]. Due to its short half-life in circulation, estimated to range from several minutes to a few hours, ctDNA is highly susceptible to degradation and dilution by background genomic DNA released from lysed leukocytes, particularly under suboptimal pre-analytical conditions [1,13]. These characteristics necessitate careful control of sample handling to preserve ctDNA integrity and analytical sensitivity.

The low concentration and pronounced interindividual variability of ctDNA represent major methodological challenges that limit its broader implementation in routine clinical diagnostics. Even in patients with advanced-stage malignancies, ctDNA levels may fluctuate considerably depending on tumor burden, biological activity, and sample processing conditions. Accumulating evidence indicates that ctDNA yield is strongly influenced by pre-analytical variables, including the type of blood collection tubes used, time to plasma separation, stabilization chemistry, storage conditions, and DNA extraction methodology [4,12,17]. Variability introduced during these early stages can significantly affect downstream analytical performance, leading to reduced reproducibility and compromised quantitative accuracy.

Consequently, optimization and standardization of sample preparation workflows are considered essential prerequisites for reliable ctDNA analysis and for the successful translation of liquid biopsy technologies into routine clinical practice. Comparative evaluation of established blood collection systems and DNA extraction methods under standardized laboratory conditions may provide critical insights into sources of analytical variability and support the development of robust, reproducible protocols. Therefore, **the aim of the present study** was to optimize sample preparation conditions for ctDNA isolation from blood plasma by comparatively evaluating commonly used blood collection tubes and DNA extraction methods.

Materials and Methods

Venous blood samples were collected from 15 patients with confirmed malignant neoplasms at the Kazakh National Research Institute of Oncology and Radiology (KazIOR, Almaty, Kazakhstan) using standard venipuncture procedures in accordance with institutional protocols. All

enrolled patients met the inclusion criteria, which comprised metastatic involvement of regional lymph nodes or distant organ metastases corresponding to advanced disease stages (TNM stage III–IV), as well as ongoing targeted therapy for at least one week prior to blood collection. These criteria were selected to ensure a clinically relevant cohort with detectable levels of circulating tumor DNA.

Blood was drawn into two types of commercially available blood collection systems: PAXgene Blood DNA Tubes (Qiagen, Germany) and Cell-Free DNA BCTs (Streck, USA). For each patient, blood was collected in duplicate tubes of each type (9 mL per tube) to minimize sampling bias and to enable parallel processing. Following collection, blood tubes were stored at room temperature for up to 7 days prior to plasma separation, in accordance with manufacturers' recommendations and to simulate delayed sample processing conditions commonly encountered in clinical practice and multicenter studies.

Plasma separation was performed using a standardized two-step centrifugation protocol designed to minimize cellular contamination. Initially, whole blood samples were centrifuged at 1,600 × g for 30 min at room temperature to remove cellular components. The plasma supernatant was carefully transferred to sterile 2 mL polypropylene tubes without disturbing the buffy coat and subjected to a second high-speed centrifugation at 14,000 × g for 10 min at 4 °C to eliminate residual cellular debris. The resulting cell-free plasma was aliquoted and frozen using a gradual cooling procedure (−20 °C for 24 h followed by storage at −80 °C) until further analysis.

Circulating tumor DNA was isolated from 2 mL of plasma using one of three extraction methods: QIAamp MinElute ccfDNA Kit (Qiagen, Germany), MagMAX™ Cell-Free DNA Isolation Kit (Thermo Fisher Scientific, USA), or CF Extra Raissol™ Reagent Kit (Raissol Bio, Russia). All extractions were performed strictly according to the manufacturers' protocols by the same operator to reduce inter-operator variability. Eluted ctDNA samples were stored at −20 °C until downstream quantitative and qualitative analyses.

The quality and concentration of isolated ctDNA were assessed using complementary analytical approaches. DNA concentration was measured fluorometrically using a Qubit 2.0 fluorometer with the Qubit™ dsDNA High Sensitivity Assay Kit (Invitrogen, USA). Spectrophotometric assessment of nucleic acid purity was performed using a NanoDrop 2000 (Thermo Fisher Scientific, USA). Fragment size distribution and integrity of ctDNA were evaluated using an Agilent 2100 Bioanalyzer with High Sensitivity DNA reagents (Agilent Technologies, USA). DNA fragments within the size range of 120–250 bp were considered optimal and representative of circulating tumor-derived DNA.

Statistical analysis was performed using GraphPad Prism version 10. Prior to comparative analysis, data distribution was assessed for normality. Comparisons between two independent groups were conducted using the Student's t-test for normally distributed data. For small sample sizes (n = 5 per group) and non-normal distributions, non-parametric statistical methods were applied. Differences among DNA extraction methods within each blood collection system were evaluated using the Kruskal–Wallis test, followed by Dunn's multiple comparisons post-hoc test with Holm correction to account for multiple testing. Quantitative data are presented as mean ± standard deviation (SD), median, and range. A p-value of less than 0.05 was considered statistically significant.

Results

Between October 2024 and February 2025, venous blood samples were collected at the Kazakh National Research Institute of Oncology and Radiology (Almaty, Kazakhstan) from patients with various malignant neoplasms. All enrolled patients (100%) presented with advanced disease (clinical stages III–IV). A total of 30 plasma samples were obtained from 15 patients. The study cohort consisted of 9 females and 6 males, with a mean age of 56 years. These samples were used for subsequent comparative analyses of blood collection systems and ctDNA extraction methods.

Analysis of ctDNA concentration across individual patients revealed no statistically significant difference between PAXgene and Streck blood collection tubes (Mann–Whitney U test, p = 0.34). The median ctDNA concentration was 0.352 for PAXgene samples and 0.385 for Streck samples. In the PAXgene group, ctDNA values ranged from 0.128 to 1.230, whereas Streck samples demonstrated a wider range from 0.098 to 2.380. Accordingly, the interquartile range was narrower for PAXgene samples, reflecting a more compact distribution of individual measurements, while Streck samples exhibited a broader interquartile span and higher upper values (Figure 1). The increased dispersion in the Streck group was evident from the wider spread of individual data points and the presence of higher maximum concentrations, whereas ctDNA measurements obtained from PAXgene tubes clustered more closely around the median, indicating greater analytical consistency across the study cohort.

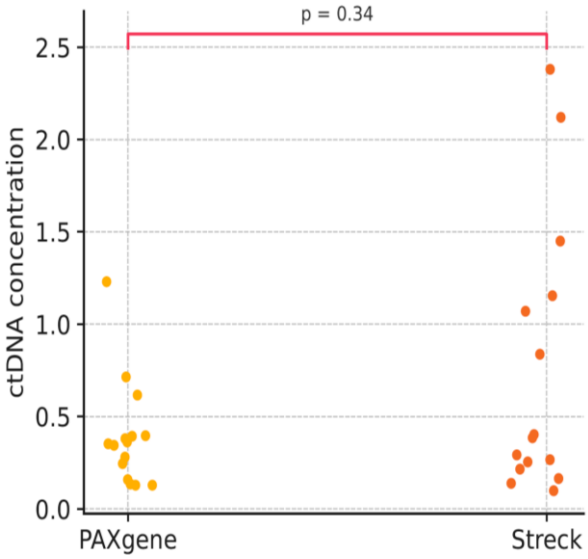

Figure 1. ctDNA concentration per patient in PAXgene and Streck tubes

Scatter plot showing individual ctDNA concentrations measured in plasma samples collected in PAXgene and Streck blood collection tubes. Each dot represents one patient. Statistical comparison was performed using the Mann–Whitney U test (p = 0.34).

Comparison of extraction methods revealed pronounced method-dependent differences in ctDNA recovery (Figure 2). In PAXgene samples, a statistically significant overall difference among extraction methods was detected (Kruskal–Wallis test, p = 0.029). Among the evaluated approaches, QIAamp consistently demonstrated higher ctDNA concentrations compared with both Raissol and MagMax. Post-hoc analysis using Dunn's multiple comparisons test confirmed a statistically significant difference between QIAamp and Raissol (p < 0.05), whereas differences between Raissol and MagMax as well as between MagMax and QIAamp did not reach statistical significance. In addition to higher absolute values, QIAamp in PAXgene samples showed a narrower dispersion, indicating more consistent analytical performance.

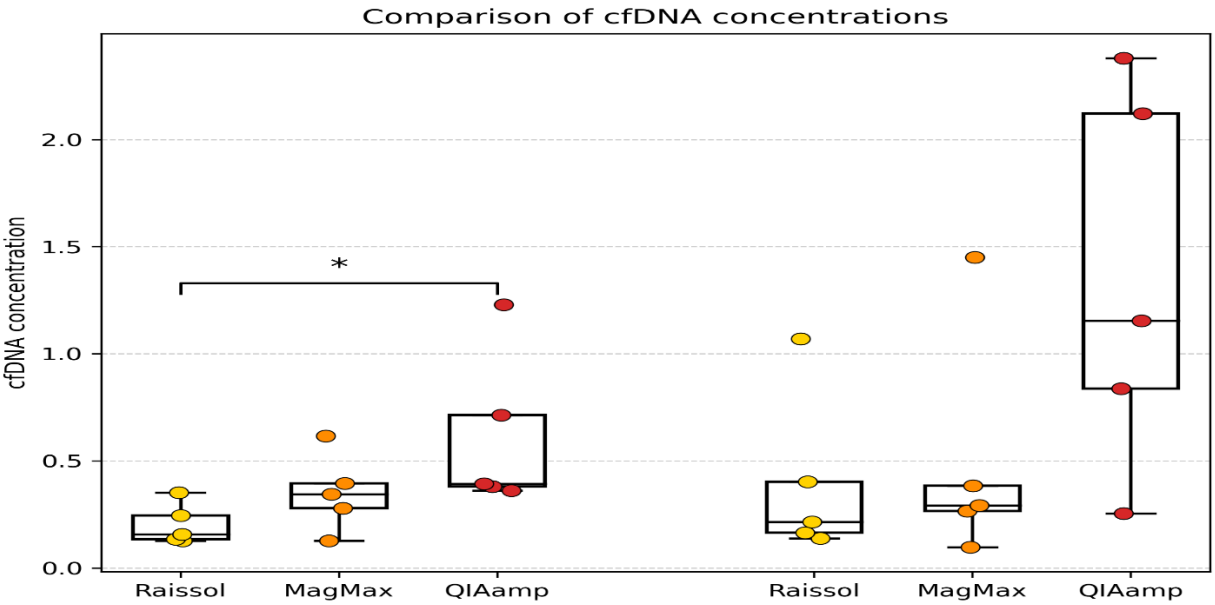

Figure 2 Comparison of ctDNA yield by extraction method

Box-and-whisker plots showing ctDNA yield obtained using Raissol, MagMax, and QIAamp extraction methods for PAXgene and Streck samples. Boxes represent the interquartile range with the median indicated by a horizontal line; whiskers show minimum and maximum values. Individual data points are displayed. Statistical analysis was performed using the Kruskal–Wallis test followed by Dunn's post-hoc test. *p < 0.05.

In Streck samples, QIAamp also yielded the highest absolute ctDNA concentrations across extraction methods (Figure 2); however, the overall comparison did not reach statistical significance (Kruskal–Wallis test, p = 0.134). This lack of significance was accompanied by substantially increased variability, particularly for Raissol and MagMax, as reflected by wider ranges and interquartile distributions (Table 1). Despite this variability, the consistent trend toward higher ctDNA recovery with QIAamp across both blood collection systems underscores its superior extraction efficiency.

Table 1.

ctDNA concentration according to blood collection tube and extraction method

Comparison	Median ctDNA	Range	Statistical test	p-value
PAXgene vs Streck	0.352 vs 0.385	0.128–1.230 vs 0.098–2.380	Mann–Whitney	0.34
PAXgene – Raissol	0.158	0.128–0.352	—	—
PAXgene – MagMax	0.344	0.128–0.616	—	—
PAXgene – QIAamp	**0.393**	**0.362–1.230**	**Kruskal–Wallis**	**0.029***
Streck – Raissol	0.215	0.138–1.070	—	—
Streck – MagMax	0.292	0.098–1.450	—	—
Streck – QIAamp	1.154	0.254–2.380	Kruskal–Wallis	0.134

* Post-hoc Dunn test (Holm-adjusted): QIAamp vs Raissol.

Overall, QIAamp consistently demonstrated superior ctDNA recovery across both blood collection systems, while PAXgene tubes provided improved analytical stability and reproducibility.

Discussion

The method-dependent differences in ctDNA recovery observed in the present study (Figure 2, Table 2) are consistent with a growing body of evidence highlighting the critical role of extraction chemistry in the efficient isolation of fragmented circulating DNA. Multiple comparative investigations have demonstrated that silica membrane–based extraction systems provide superior recovery of short DNA fragments characteristic of ctDNA compared with magnetic bead–based or precipitation-based approaches [3,5,6,9]. This advantage is particularly relevant for downstream applications requiring high analytical sensitivity, such as digital PCR or low-frequency variant detection.

In PAXgene samples, QIAamp yielded significantly higher ctDNA concentrations compared with Raissol, as confirmed by post-hoc statistical analysis. Similar results have been reported by El Messaoudi et al., who showed that silica-based extraction methods improve both yield and reproducibility of ctDNA in plasma samples from patients with solid tumors [6]. Devonshire et al. further demonstrated that differences in DNA extraction workflows can introduce substantial quantitative bias in digital PCR measurements, underscoring the importance of method standardization for reliable ctDNA analysis [3]. Comparable findings have been reported in more recent technical evaluations comparing commercial extraction platforms under controlled experimental conditions [5,7,10].

Although QIAamp also produced higher absolute ctDNA values in Streck samples, the absence of significance in this group likely reflects increased pre-analytical variability. Blood stabilization systems are known to differ in their ability to prevent leukocyte lysis and subsequent release of background genomic DNA during storage and transport, which can dilute the ctDNA signal and increase variability [11,18,20]. Meddeb et al. emphasized that even minor deviations in pre-analytical handling may significantly affect ctDNA integrity and concentration, particularly in sample processed after prolonged storage [12]. This phenomenon becomes especially pronounced in studies involving heterogeneous clinical cohorts or delayed plasma separation [18,19].

Importantly, the integrated analysis of patient-level ctDNA variability (Figure 1), extraction-dependent performance (Figure 2), and summary statistics (Table 2) reinforces the concept that reliable ctDNA measurement depends on the combined optimization of blood collection systems and extraction methods. Even in cohorts dominated by advanced-stage malignancies, where ctDNA levels are generally elevated, methodological variability remains a major determinant of analytical robustness. These findings align with current recommendations advocating the implementation of standardized, validated workflows across all stages of liquid biopsy analysis to ensure reproducibility and facilitate clinical translation [16,17].

Study limitations

A limitation of this study is the relatively small sample size and the inclusion of patients exclusively with advanced-stage (III–IV) malignancies, which may limit the generalizability of the findings to earlier disease stages.

Conclusions

The findings of this study confirm that both blood collection systems and DNA extraction methods play a crucial role in determining the efficiency, variability, and reproducibility of circulating tumor DNA (ctDNA) isolation from blood plasma. Although no statistically significant differences in overall patient-level ctDNA concentrations were observed between PAXgene and Streck blood collection tubes, PAXgene samples exhibited lower variability and greater analytical stability, indicating more consistent preservation of ctDNA under the applied pre-analytical conditions.

Comparative evaluation of DNA extraction methods revealed method-dependent differences in ctDNA recovery. Among the tested approaches, the QIAamp extraction system consistently demonstrated higher ctDNA yields and significantly outperformed the Raissol method in PAXgene samples, highlighting the advantages of silica membrane–based extraction technologies for the isolation of fragmented circulating DNA. While QIAamp also produced higher absolute ctDNA concentrations in Streck samples, increased variability limited the statistical significance of these differences.

Overall, the combined assessment of blood collection tubes and extraction methods underscores the necessity of integrated optimization of pre-analytical and analytical workflows for ctDNA analysis. Implementation of standardized blood collection systems together with high-efficiency DNA extraction methods may substantially improve the robustness and reproducibility of ctDNA-based assays, thereby enhancing the clinical utility of liquid biopsy approaches in oncology.

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