Clinical Application of ISO and CEN/TS Standards for Liquid Biopsies—Information Everybody Wants but Nobody Wants to Pay For

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BACKGROUND: Liquid biopsies are emerging as valuable clinical biomarkers for cancer monitoring. Although International Organization for Standards (ISO) and Technical Specifications from the European Committee for Standardization (CEN/TS) standardized workflows exist, their implementation in clinical practice is underdeveloped. We aimed to assess the applicability of ISO and CEN/TS standards in a real-world clinical setting, with a particular focus on evaluating the impact of preanalytical parameters and hemolysis on liquid biopsy analysis.

METHODS: We evaluated 659 peripheral blood samples from advanced prostate cancer patients against ISO and CEN/TS standards and documented all essential criteria, including tube draw order, filling level, temperature, and time tracking from blood draw to storage. We assessed hemolysis and its effect on circulating tumor DNA (ctDNA) and circulating tumor cell (CTC) analysis.

RESULTS: Our results demonstrated a high compliance rate, with 96.2% (634/659) of samples meeting essential ISO and CEN/TS criteria. We did not observe a significant impact on ctDNA or CTC detection rates between hemolytic and nonhemolytic samples. Hemolysis was identified in 12.9% (40/311) of plasma samples from our advanced prostate cancer cohort, and within the draw order of 5 blood collection tubes, hemolysis did not significantly increase from tube 1 to 5. In total, 83.8% (552/659) of blood collection tubes had high fill levels above 80% of nominal filling level.

CONCLUSIONS: Our study demonstrates the feasibility and benefits of adhering to ISO and CEN/TS standards in a clinical liquid biopsy study. The standards revealed that hemolysis occurred frequently but did not impair downstream ctDNA and CTC analysis in our cohort of advanced prostate cancer patients.

Introduction

Liquid biopsies play a crucial role as potentially prognostic and predictive biomarkers and for disease monitoring in cancer (1). While their clinical usage is increasing, standardization remains a challenging task (2, 3). The quality of a blood sample is particularly critical when isolating and analyzing low-abundance circulating tumor DNA (ctDNA) fragments or delicate circulating tumor cells (CTCs) (4–6). In routine clinical practice, blood sample collection is prone to various preanalytical errors, such as inadequate blood collection tubes, underfilling of blood tubes, improper tube inversion, extended transport times, suboptimal temperature conditions during transport, and others (7, 8). To overcome these challenges, large consortia and societies have developed standards ensuring the highest quality of blood samples for subsequent liquid biopsy analysis. These include the European Liquid Biopsy Society (9), CancerID (10), Standardization of generic Pre-analytical procedures for In-vitro DIAgnostics for Personalized Medicine (11, 12), BLOODPAC (13), and the International Liquid Biopsy Standardization Alliance (14). These efforts have resulted in liquid biopsy preanalytical standards provided by the International Organization for Standards (ISO) and Technical Specifications from the

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Received December 14, 2023; accepted May 9, 2024.
https://doi.org/10.1093/clinchem/hvae079

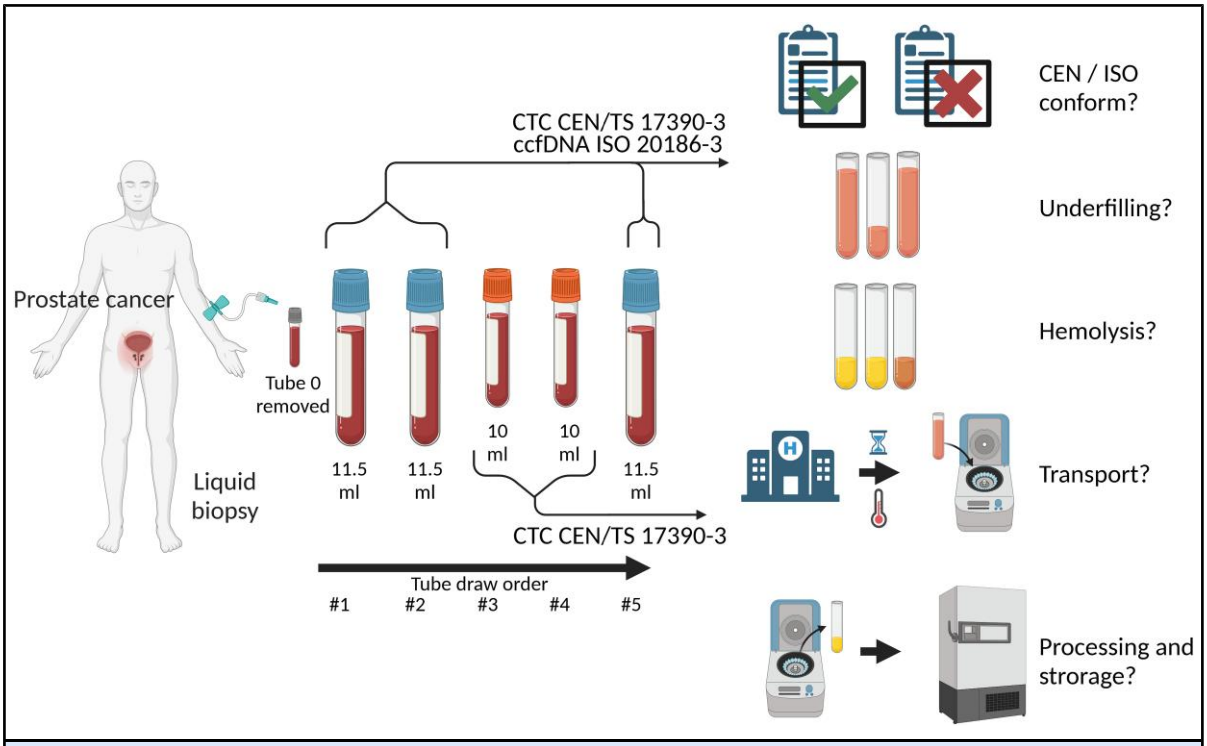

Fig. 1. Overview of blood collection tube order, applied ISO CEN/TS standards, and parameters to be evaluated. Created with BioRender.com.

European Committee for Standardization (CEN/TS). In particular, ISO 20186-3:2019 constitutes a standard for isolation of circulating cell-free DNA (ccfDNA) from plasma, while CEN/TS 17390-3:2020 focuses on specifications for analytical staining of CTCs (15–17). These standards encompass the entire workflow, including documentation, blood sample collection, and processing and storage of samples in a controlled environment. Despite their undeniable importance, widespread implementation of these standards remains limited (18, 19).

Our objective was to evaluate the implementation of ISO and CEN/TS standardized workflows in a real-world clinical liquid biopsy study involving patients with advanced prostate cancer. We aimed to assess the clinical applicability of the standards, identify relevant preanalytical parameters, and determine common factors leading to noncompliance with ISO or CEN/TS guidelines. The parameters investigated included hemolysis, the filling level and inversion of blood collection tubes, the order of tube draws, transport time, and transport conditions. Moreover, we addressed the question of whether hemolysis influences ccfDNA and CTC analysis.

Here, we present a comprehensive preanalytical dataset obtained through the systematic application of ISO and CEN/TS standards for ccfDNA and CTCs, which, to our knowledge, has not been reported before. The resulting preanalytical parameters provide reassurance that samples obtained following these standards meet the quality requirements to justify elaborate and costly liquid biopsy analyses.

Materials and Methods

PATIENT COHORT

A total of 659 peripheral blood samples were collected longitudinally from 25 patients of a castration resistant prostate cancer cohort. Patients were enrolled when undergoing change in systemic therapy due to progressive disease. Blood samples for longitudinal liquid biopsy monitoring were collected at the Division of Oncology, Medical University of Graz, Austria, before changing therapy (visit 1) and at approximately 12-week intervals thereafter, with an average number of 5.4 (±2.8) visits per patient. At each visit a minimum of 2 and a maximum of 6 blood collection tubes were obtained (Fig. 1).

ETHIC APPROVAL

The ethics committee of the Medical University of Graz gave ethical approval for this study protocol and patient information (31-353 ex 18/19) following the

Declaration of Helsinki and good clinical practice, and written informed consent was obtained from all patients.

BLOOD SAMPLE COLLECTION AND PROCESSING

Blood samples were collected in approximately 12-week intervals. At each visit a minimum of 2 and a maximum of 6 blood collection tubes were collected. The first tube (= tube 0) was not used for ctDNA or CTC detection in this study due to possible contamination with epithelial cells of the skin puncture. The 5 additional blood samples were drawn in the following order: 2 × 10 mL PAXgene Blood ccfDNA tubes (PreAnalytiX), followed by 2 × 8.5 mL Acid Citrate Dextrose Solution A (ACD-A) tubes (BD), and lastly, an additional 1 × 10 mL PAXgene Blood ccfDNA Tube (PreAnalytiX) (Fig. 1). All samples were collected following the respective ISO and CEN/TS standards. Blood filling levels were assessed for all blood samples. Blood samples were transported at room temperature in an isolated transport box, which kept the temperature during transport at approximately room temperature ± 2°C. This was measured with a temperature data logger for establishing the optimal transport conditions.

ISO AND CEN/TS STANDARDS

The ISO and CEN/TS standards were followed for processing of PAXgene Blood ccfDNA tubes (PreAnalytiX) intended for ctDNA isolation, as outlined in the international standard ISO 20186-3:2019 (16). Similarly, ACD-A tubes intended for CTC isolation and staining were processed according to the CEN/TS 17390-3 standards (17). CTCs were enriched from ACD-A tubes (BD) using the Smart Biopsy Cell Isolator (CytoGen) (20) and the AdnaTest ProstateCancerPanel AR-V7 in accordance with the manufacturer's instructions (QIAGEN). An overview of the procedures can be found in Fig. 2. The verbal form "shall" in the ISO and CEN/TS standards are "must have" requirements, and we assessed the following parameters in detail: patient sample pseudonym ID; date and time of blood drawing; identity of the person who drew blood; identity of the person who processed the blood samples; temperature and storage condition at the blood collection site; verification of tube inversion; temperature conditions during transport; time of call for sample pick-up; time required from blood draw until call; date and time of sample pick-up; date and time of sample arrival at the laboratory; volume of tube fill level; additional notes regarding sample tampering; tube type with catalog number, lot number, and expiration date; proper labeling of tubes according to standard operating procedures; centrifugation procedures for tubes; duration from blood draw to processing and storage; timing and storage temperature and location of the sample. The reporting form used for documentation is included in the Supplemental Data 1.

CTC ISOLATION AND ANALYSIS

CTC were isolated with 2 approaches: AdnaTest ProstateCancerPanel AR-V7 (QIAGEN) (tube 1) and Smart Biopsy Cell Isolator (CytoGen) (tubes 3–4). The AdnaTest was used as described elsewhere (21) and was adapted according to the recommendation of the manufacturer (QIAGEN). This included validation of PAXgene Blood ccfDNA tubes for the ADNATest, which perform equally well as ACD-A tubes (data not shown). Moreover, all complementary DNA (cDNA) products of enriched CTC mRNA fractions were analyzed for tumor-specific and control genes as recommended by the manufacturer (QIAGEN). AdnaTest samples were processed directly without long-term storage, and CytoGen-enriched CTC cytospins were stored according to the manufacturer at −80°C for long-term storage.

SHALLOW WHOLE-GENOME SEQUENCING

ccfDNA isolated from plasma samples was subjected to shallow whole-genome sequencing on Illumina's NovaSeq platform as described previously (6, 22), and the tumor fraction was determined using the ichorCNA algorithm (23).

EVALUATION OF HEMOLYSIS IN PLASMA SAMPLES

Plasma samples underwent visual and NanoDrop spectrophotometric (Thermo Fisher Scientific) evaluation to assess erythrocyte lysis and lipemia. Hemolysis was assessed using a lipemia-independent hemolysis score, as described by Appierto (24). Absorbance was measured at 385 nm to identify lipemic samples and 414 nm for free hemoglobin. The hemolysis score was calculated using the following formula: Hemolysis score = (Absorbance 414nm–Absorbance 385 nm) + 0.1× Absorbance 385 nm (24). Samples with a hemolysis score >0.25 were classified as hemolytic as described previously (25).

Results

COMPLIANCE WITH ISO AND CEN/TS STANDARDS

Of the total 659 blood samples in our clinical cohort, 96.2% (634/659) met the essential criteria to be considered ISO and CEN/TS compliant. The remaining 3.8% (25/659) of samples did not meet ISO and CEN/TS compliance due to missing information regarding the identity of the person who collected the blood specimen (3.0%, 20/659) and lack of tube inversion after blood collection (1.5%, 10/659).

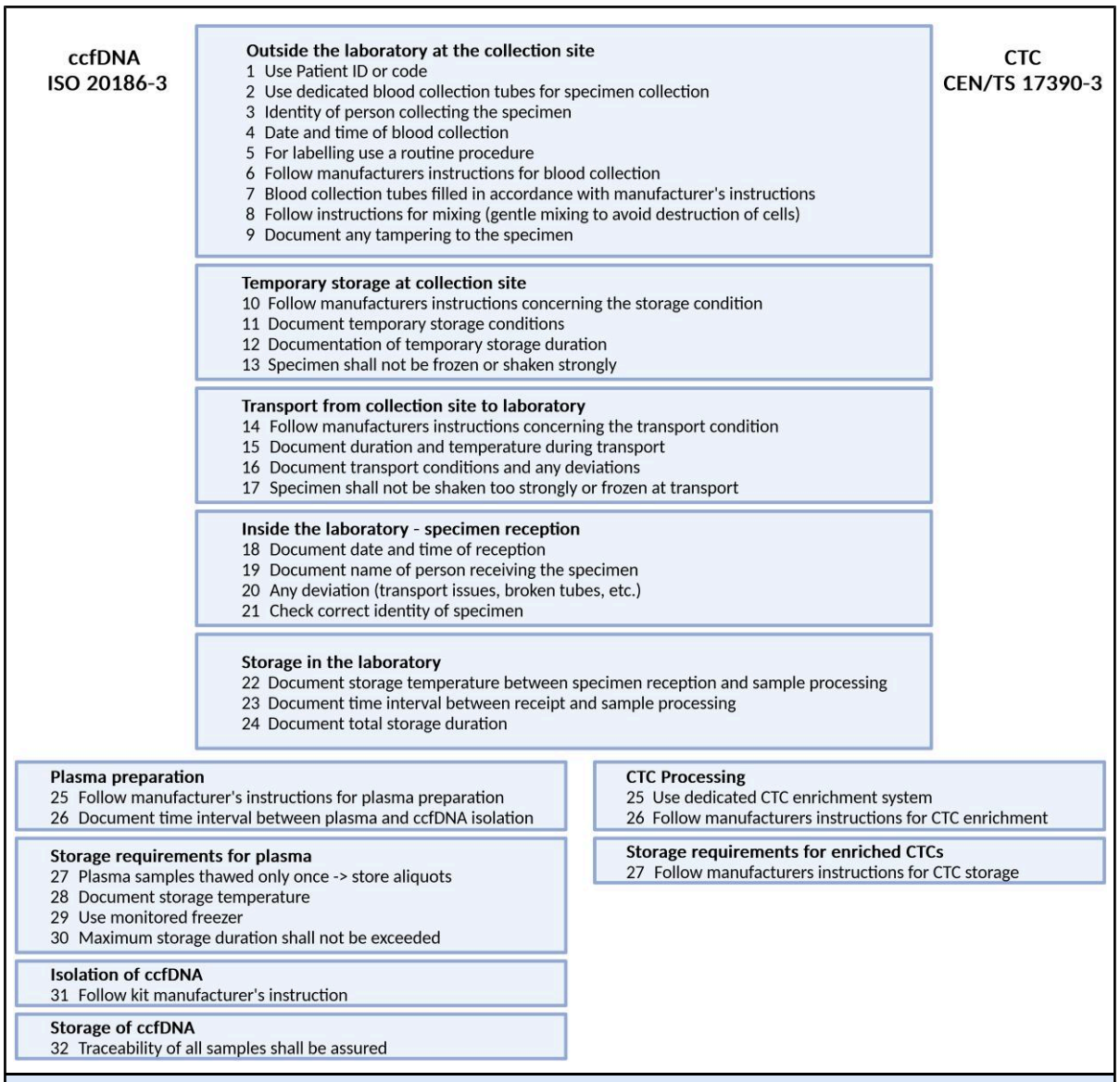

Fig. 2. Overview of the criteria that must be met to assign blood samples as ISO or CEN/TS compliant in our study. Each single step described here is mandatory; omitting or not fulfilling a step will lead to a non-compliant blood sample. The complete ISO and CEN/TS standards are available elsewhere (16, 17). Created with BioRender.com.

TUBE FILL LEVEL ASSESSMENT

We assessed the fill level of both PAXgene Blood ccfDNA tubes (11.5 mL total volume, including 1.5 mL tube supplements) and ACD-A tubes (10 mL total volume, including 1.5 mL tube supplements). The mean fill level of all 659 blood tubes was 92.4% ± 15.8 (Fig. 3A). The mean fill level for tube 1 was 88.9% ± 16.7 (N = 133), tube 2: 87.8% ± 18.2 (N = 133), tube 3: 97.6% ± 12.8 (N = 132), tube 4: 98.4% ± 10.0 (N = 130), and tube 5: 89.6% ± 16.5 (N = 131). Notably, we observed differences between the 2 tube types, with the 11.5 mL PAXgene Blood ccfDNA tubes exhibiting a lower mean fullness level compared to the 10 mL ACD-A tubes, measuring 88.8% ± 17.1 (N = 397) vs 98.0% ± 11.5 (N = 262), respectively ($P < 0.0001$, 1-way ANOVA). There was no statistically significant difference between the first and the last tube drawn from each patient ($P = 0.6917$, 1-way ANOVA). To further categorize the filling level performance, we divided it into 10% steps. We

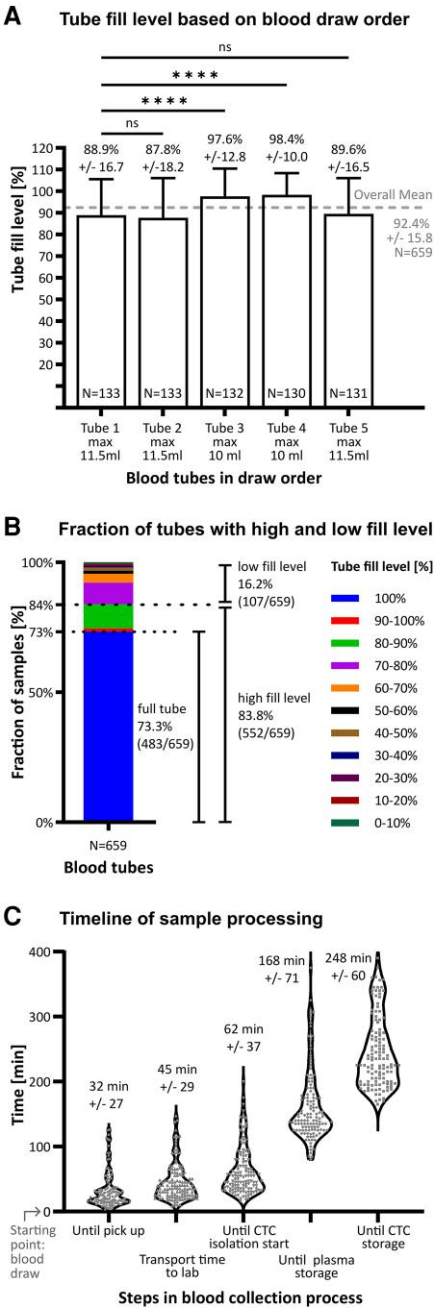

Fig. 3. Tube fill level assessment and timeline of sample processing. (A), Evaluation of the level of tube fullness based on draw order. **** $P < 0.0001$, 1-way ANOVA; (B), Fraction of blood collection tubes with high and low fill level; (C), Documented timeline for each sample following the whole ISO and CEN/TS workflows. Timeline represented in minutes; values above the violin plot are mean values with SD. Abbreviation: ns, not significant.

defined fill levels >80% as high and from 1% to 79% as low. Of the total 659 blood collection tubes evaluated, 83.8% (552/659) had a high fill level, while the remaining 16.2% (107/659) had a low fill level (Fig. 3B). Of all tubes, 73.3% (483/659) were completely filled.

TIMING OF SAMPLES

Thorough documentation facilitated a detailed timeline for each blood sample, tracking its condition. In our clinical setting, the mean time from blood sampling to transport was 32 ± 27 min (Fig. 3). The duration from blood draw to laboratory arrival was 45 ± 29 min. Subsequently, samples were forwarded to CTC or plasma isolation. The mean time from blood draw to start of CTC isolation was 62 ± 37 min. Adhering to ISO 20186-3:2019 standards for plasma isolation and storage, the entire process averaged 168 ± 71 min. For CEN/TS 17390-3:2020 compliant CTC isolation and storage for staining procedures, the process averaged 248 ± 60 min.

HEMOLYSIS SCORE

Of the 659 blood samples, 311 samples were forwarded to plasma isolation including assessment of the hemolysis score for this study. Among the 311 plasma samples, the majority, 87.1% (271/311), were classified as non-hemolytic, with a hemolysis score <0.25. In contrast, the remaining 12.9% (40/311) exhibited hemolysis, with a hemolysis score >0.25 (Fig. 4A). When examining if there were statistically more or less hemolytic samples in the first, second, or last blood draw, we did not observe a significant difference related to the blood draw order ($P = 0.4626$ 1-way ANOVA). We discovered that 17.3% (22/127) of samples from tube 1, 7.4% (7/94) of samples from tube 2, and 12.2% (11/90) of samples from tube 5 displayed hemolysis (hemolysis score >0.25).

BLOOD TUBE ORDER AND HEMOLYSIS

We aimed to investigate whether hemolysis levels differed among multiple blood tubes collected at the same sampling time point. For instance, we sought to determine if the presence of hemolysis in the first blood collection tube would indicate consistent hemolysis in subsequent tubes. Therefore, we assessed 121 blood draw time points, where a minimum of 2 and a maximum of 6 blood collection tubes were obtained at each time point. We assessed the hemolysis score only for tubes where plasma was isolated (tubes 1, 2, and 5). The remaining tubes were directly forwarded to CTC isolation (tubes 3 and 4) without plasma isolation or were removed due to possible skin cell contamination (tube 0). Our findings revealed that in 77.7% (94/121) of a blood draw series, the plasma

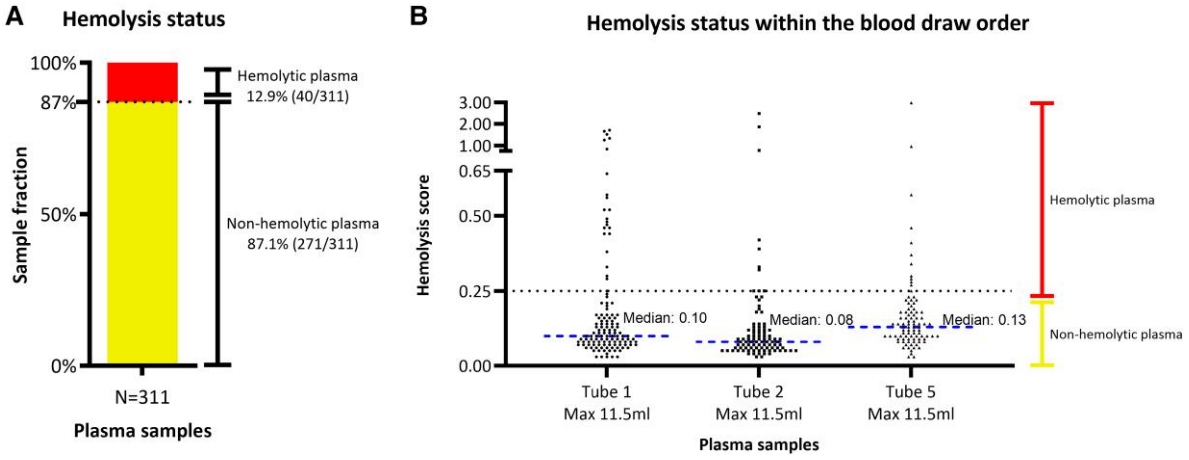

Fig. 4. Hemolysis status of plasma samples based on the spectroscopically measured hemolysis score. (A), Fraction of hemolytic plasma samples; (B), Hemolysis assessment within the tube order, with no statistically significant difference ($P = 0.4626$ 1-way ANOVA). Dashed line indicates hemolysis score threshold of 0.25.

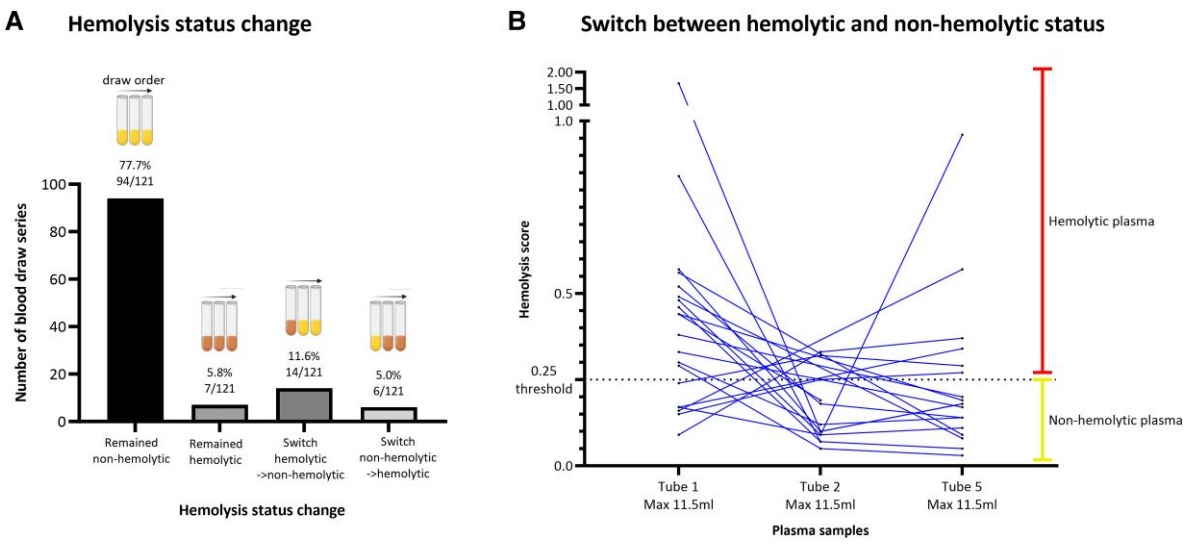

Fig. 5. Hemolysis status on sequentially drawn blood samples. (A), Hemolysis changes during the draw order; (B), Detailed overview of the hemolysis score of plasma samples, which switched from hemolytic to nonhemolytic status and vice versa. Samples that did not switch their hemolysis status are not shown for better clarity. Created with BioRender.com.

remained nonhemolytic for all tubes (Fig. 5). In 5.8% (7/121) of the blood draw series, all blood tubes showed hemolysis. Interestingly, in 11.6% (14/121) of the blood draw series, only the first tube showed hemolysis, but subsequent tubes were not hemolytic. In contrast, 5.0% (6/121) of the blood draw series exhibited initial tubes without hemolysis, but subsequent tubes showed detectable hemolysis (Fig. 5). Notably, we did not observe any instances of a transition from hemolytic to nonhemolytic and then back to hemolytic, or vice versa, within any of the blood draw series.

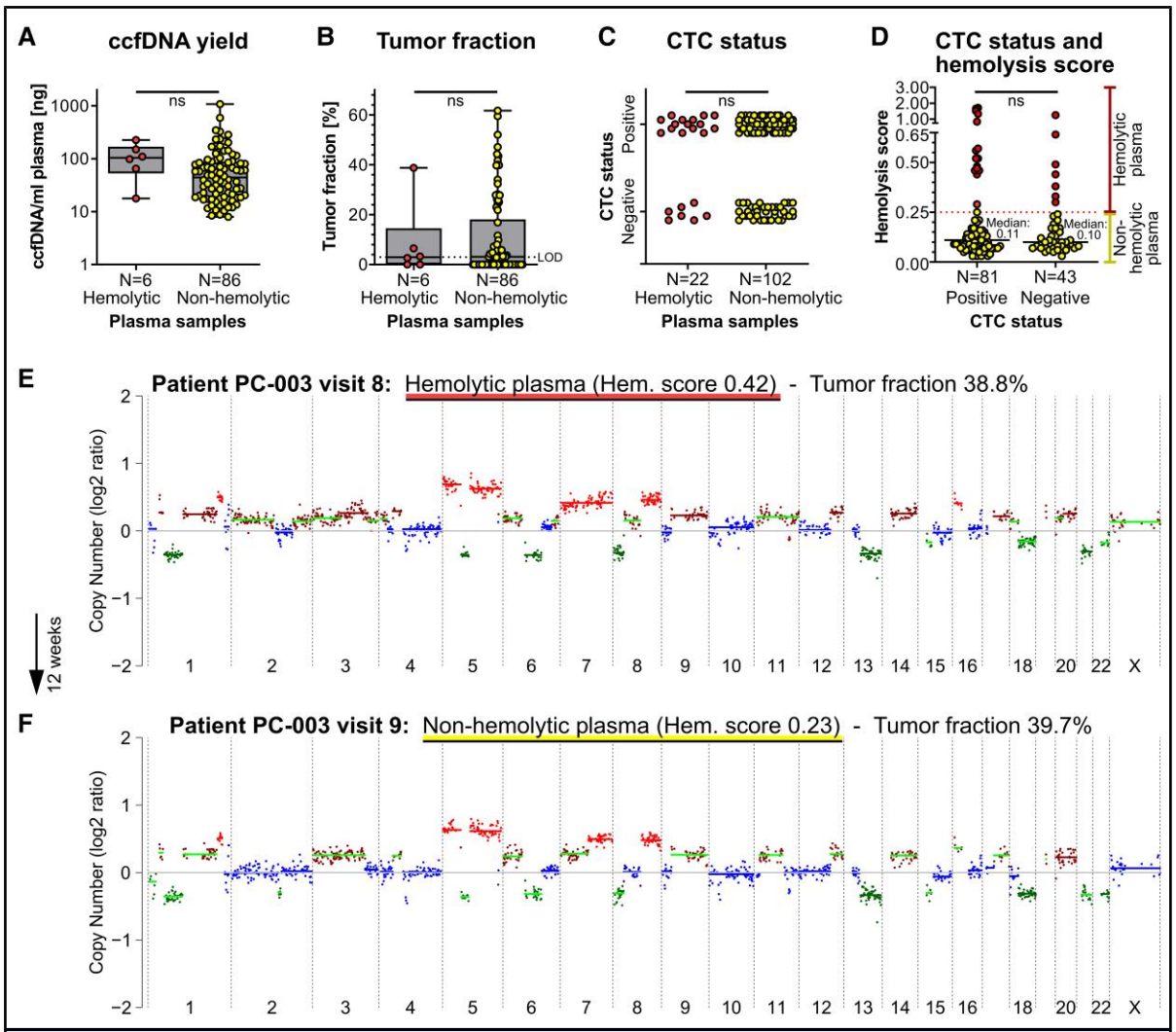

Fig. 6. ctDNA and CTC analysis of hemolytic and nonhemolytic samples. No statistically significant difference in (A) ccfDNA yield, (B) tumor fraction, and (C) CTC status between hemolytic and nonhemolytic samples. (D), Spectroscopically measured hemolysis score of CTC positive and CTC negative samples, with no statistically significant difference. Dashed line indicates hemolysis score threshold of 0.25; (E), Plasma sequencing copy number profile of a hemolytic plasma sample derived from patient PC-003, with a tumor fraction of 38.8%. (F) Corresponding copy number profile of a nonhemolytic plasma sample from the same patient after 12 weeks, with a tumor fraction of 39.7%. Abbreviation: ns, not significant.

CORRELATION BETWEEN SAMPLE PROCESSING, HEMOLYSIS AND CTDNA

A total of 132 samples underwent analysis through shallow whole-genome sequencing, and matched hemolysis scores were available for 92 of these samples (Fig. 6). Tumor fractions exceeding the limit of detection (>3%) were identified in 50.0% (3/6) of hemolytic samples and 51.2% (44/86) of nonhemolytic samples (Fig. 6). There was no statistical difference observed between hemolytic and nonhemolytic plasma samples concerning tumor fraction ($P = 0.9896$, Mann–Whitney test), and patient-specific copy number alteration profiles were detected in hemolytic and nonhemolytic samples (Fig. 6E and F). Exploring additional associations, a weak positive correlation emerged between the time from blood draw until lab arrival and tumor fraction ($rs = 0.2$, $P = 0.039$). However, no statistically significant correlations were identified between tumor fraction and the other documented preanalytical parameters, compliance with ISO and CEN

standards, hemolysis, or duration of plasma storage at −80°C until DNA isolation (Supplemental Table 1).

We evaluated the ccfDNA yield of 92 samples within the context of hemolysis. There was no statistical difference in ccfDNA yield between hemolytic (N = 6) and nonhemolytic plasma samples (N = 86) ($P = 0.0960$, Mann–Whitney test) (Fig 6).

We found no statistically significant correlation between hemolysis and various preanalytical parameters such as ISO and CEN/TS compliance, tube inversion, tube fill level, or processing time (Supplemental Table 1). However, exploring potential associations between clinical parameters and hemolysis, we identified weak positive correlations with a number of patient-specific factors. These include the administration of chemotherapy before (rs = 0.1, $P = 0.036$) or after blood collection (rs = 0.2, $P = 0.001$); the presence of lung (rs = 0.2, $P = 0.003$), liver (rs = 0.1, $P = 0.009$), or multiorgan (rs = 0.1, $P = 0.019$) metastases; and elevated levels of C-reactive protein (rs = 0.2, $P = 0.002$) or lactate dehydrogenase (rs = 0.1, $P = 0.022$) (Supplemental Table 2). Importantly, the sample size in several groups (e.g., hemolytic samples, samples without ISO and CEN compliance) was very small, which is reported in detail in Supplemental Tables 1 and 2.

CORRELATION BETWEEN SAMPLE PROCESSING, HEMOLYSIS AND CTCS

Tubes 3 and 4 (ACD-A) of each blood series, where available, were forwarded to CTC enrichment using the Smart Biopsy Cell Isolator. In 9.1% (23/252) of these samples, we encountered issues with blood coagulation. We observed no correlation between coagulation issues (tubes 3–4; ACD-A) and frequency of hemolysis of matched plasma samples (tubes 1, 2, 5; PAXgene Blood ccfDNA) within a blood series. While a weak negative correlation was identified between CEN/TS compliance and coagulation issues (rs = −0.2, $P = 0.022$), this correlation lost significance when considering the 2 sources of noncompliance independently (i.e., missing information regarding the identity of the person who collected the blood specimen and absence of tube inversion after blood collection).

A total of 129 blood samples (tube 1, PAXgene Blood ccfDNA) were analyzed using the AdnaTest. AR-V7 was detected in 34.9% (45/129), AR-FL in 56.6% (73/129), PSA in 51.2% (66/129), and PSMA in 62.8% (81/129) of the samples. 65.9% (85/129) were positive for at least one of these markers and were thus considered CTC-positive. For 124 of these samples, matched hemolysis scores were available (Fig. 6). Positive AdnaTest results were observed both in hemolytic and nonhemolytic samples (Fig. 6). We detected CTCs in 68.2% (15/22) of hemolytic and 64.7% (66/102) of nonhemolytic samples (Fig. 6C). No statistical difference was found between hemolytic (N = 22) and nonhemolytic (N = 102) samples regarding CTC AdnaTest results ($P = 0.8370$, Mann–Whitney test) or hemolysis score vs CTC AdnaTest results ($P = 0.8989$, Mann–Whitney test). No correlation between hemolysis and CTC detection by AdnaTest was observed, suggesting that hemolysis had no impact on the AdnaTest. Similarly, no significant correlation was found between CTC detection and documented preanalytical parameters or compliance with CEN/TS standards (Supplemental Table 1).

Discussion

Our study demonstrates the feasibility and benefits of adhering to ISO and CEN/TS standards in a clinical liquid biopsy study. We found that over 96% of blood samples in our study met the criteria for ISO and CEN/TS compliance. The most frequently missed criterion was the documentation of the identity of the person who collected the blood specimen. Only a small fraction of samples failed to comply with the standards due to missing tube inversion. Notably, our results indicate that hemolysis is a common phenomenon in advanced prostate cancer patients, affecting 13% of our samples. This is likely associated with patient-specific factors such as therapy or disease progression, as we have minimalized preanalytical errors by following ISO and CEN/TS, and we found no correlation with preanalytical parameters but rather with several clinical parameters. An important finding is the absence of a significant impact on ctDNA or CTC detection rates between hemolytic and nonhemolytic samples. However, due to the sample size in several groups, limited robustness of statistical conclusions must be acknowledged. Notably, we successfully identified ctDNA copy number alterations and CTCs in hemolytic blood samples. These results offer reassurance that even hemolytic samples can provide tumor-relevant information. It is crucial to emphasize that all samples subjected to our ctDNA/CTC-hemolysis analysis originated from stabilization tubes (PAXgene Blood ccfDNA tube), and our investigations did not include other blood collection tubes. In our study, with a maximum of 6 blood tubes collected sequentially and the first tube removed due to possible contamination with skin cells, we found that blood tubes drawn later in the collection process had no significant difference in the hemolysis score (Fig. 4). Interestingly, we showed that a switch from nonhemolytic to hemolytic status (11.6%) could happen, with the initial tubes being nonhemolytic and later ones hemolytic. Conversely, in 5% of the draw

series, the first tubes were hemolytic and subsequent tubes nonhemolytic. We assume this can be explained by prolonged tourniquet application and stress on venous vessels and blood cells (26).

We investigated consecutively drawn tube fill levels, noting no significant difference between tubes 1 and 5, contrary to expectations of decreasing fill levels due to prolonged venous stasis by long tourniquet placement (27). Additionally, over 83% of blood tubes displayed high fill levels. Special attention is needed when filling high-volume tubes, like PAXgene Blood ccfDNA tubes. While their caps are equipped with spill-over protection to ensure secure opening, this safety feature poses a challenge for visually assessing the fill level, especially when drawing the last milliliter of blood into an almost full tube. As a result, the mean fill level of these larger tubes was lower (approximately 89%) compared to smaller tubes like ACD-A (approximately 98%). Despite this, both tube types provided sufficient material for follow-up analyses.

We meticulously tracked the timing of every step involved in blood collection, transport, and processing, in accordance with the demands of the ISO and CEN/TS standards. Our laboratory implemented a fast turnaround time of about 2.5 hours for ISO-compliant plasma storage for ccfDNA extraction and about 4 hours for CEN/TS-compliant CTC enrichment and storage. These processing times comprise the entire workflow, from the moment of blood draw to final storage. Important to mention is that all samples for this study were obtained from one clinical center. To extend these findings to a multicenter study or remote hospitals, the use of stabilization tubes becomes crucial to ensure the high quality of liquid biopsy samples. Additionally, it is essential for the medical staff to undergo specialized training, ensuring collection of all specified parameters outlined in the standards. In our study, the personnel resources required for creating standard operating procedures, conducting training of medical staff, and sample processing involved 2 full-time equivalents of biomedical scientists serving as liquid biopsy managers. Notably, we successfully addressed a pivotal challenge—the elapsed time between blood draw and sample pick-up. This interval is often an unknown variable and is difficult to control in many liquid biopsy studies where blood samples are collected during clinical routine and forwarded for research purposes. Factors like temperature variations, tube inversion, and the need for pseudonymization further complicate these challenges. Our observations revealed that the presence of dedicated liquid biopsy managers greatly enhanced our ability to adhere to ISO and CEN/TS standards in a clinical setting.

The application of ISO and CEN/TS standards in liquid biopsy samples offers a significant advantage to biobanks, leveraging their liquid specimens for future projects. This has also been identified as an important factor by European research infrastructure for biobanking (https://www.bbmri-eric.eu) (28). The preanalytical data collected becomes pivotal for development of novel liquid biopsy assays, seeking to access previously collected liquid biopsy cohorts within biobanks. ISO and CEN/TS conform biobanked liquid biopsy samples may reduce the necessity for new prospective clinical studies, allowing a more time-efficient and cost-effective development of liquid biopsy assays. Similarly, within the in vitro diagnostic regulation in the EU and the US FDA, preanalytical parameters are becoming crucial for liquid biopsy workflows (29). A key step for the seamless integration of ISO and CEN/TS standards into liquid biopsy practices involves obtaining ISO 15189 accreditation for laboratories or Clinical Laboratory Improvement Amendments accreditation in the US. ISO 15189 is a globally recognized standard that establishes criteria for personnel competencies and quality management within medical laboratories (30). Crucially, this standard comprises entire workflows from blood collection to the final report. Therefore, it also incorporates preanalytical guidelines, enabling ISO 15189-certified laboratories to align their operations with the specific requirements outlined in ISO and CEN/TS standards for liquid biopsies (31).

In conclusion, our study underscores the invaluable insights that are obtained by adhering to the available ISO and CEN standards for liquid biopsies. Indeed, this is "information everybody wants but nobody wants to pay for" to quote Dr. Howard I Scher from the Memorial Sloan Kettering Cancer Center, New York, NY. Although implementation of ISO and CEN/TS standards may require substantial investment in terms of personnel and resources, it provides information on critical preanalytical parameters and ensures that costly and elaborate liquid biopsy analyses are built on a solid foundation.

Data Availability

All data produced in the present study are contained in the manuscript.

Author Declaration

A version of this paper was previously posted as a preprint on medRxiv as https://doi.org/10.1101/2023.12.04.23299422.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: ctDNA, circulating tumor DNA; CTC, circulating tumor cell; ISO, International Standards Organisation; CEN/TS, Technical Specifications from the European Committee for Standardization; ccfDNA, circulating cell free DNA; ACD-A, Acid Citrate Dextrose Solution A.

Author Contributions: *The corresponding author takes full responsibility that all authors on this publication have met the following required criteria of eligibility for authorship: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved. Nobody who qualifies for authorship has been omitted from the list.*

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Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form.*

Research Funding: L. Bonstingl was supported by the PhD Program AMBRA (Advanced Medical Biomarker Research) of the Medical University of Graz together with the FFG K1 center Center for Biomarker Research in Medicine (CBmed). K. Sallinger was supported by the doctoral school Translational Molecular and Cellular Biosciences of the Medical University of Graz and CBmed. This work was supported by the K1 COMET Competence Center CBmed, which is funded by the Federal Ministry of Transport, Innovation and Technology (BMVIT); the Federal Ministry of Science, Research and Economy (BMWFW); Land Steiermark (Department 12, Business and Innovation); the Styrian Business Promotion Agency (SFG); and the Vienna Business Agency. The COMET program is executed by the Austrian Research Promotion Agency (FFG). The research was supported by a grant of the Verein für Krebskranke of the Medical University of Graz (Austria) and by MEFO Graz, the Medical Research Funding Society of the Medical University of Graz (Austria).

Disclosures: None declared.

Role of Sponsor: The funding organizations played no role in the design of the study, choice of enrolled patients, review and interpretation of data, preparation of the manuscript, or final approval of the manuscript.

Acknowledgments: The authors thank Daniel Kummer for technical support and Gerlinde Gornicec, Carina Kreuter, Sylvia Tripolt, Karin Groller, and Lisa Jaritz from the study coordination team of the Division of Oncology, Medical University of Graz for their excellent support in patient enrollment. Figures were created with BioRender.com and Prism GraphPad.

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