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NCI Biospecimen Evidence-Based Practices (BEBP) - Cell-free DNA: Biospecimen Collection and Processing

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This document contains guidance that is intended to facilitate the development of evidence-based standard operating procedures.

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Disclaimer

This document contains guidance that is intended to facilitate the development of evidence-based standard operating procedures.

Abstract

This evidence-based best practice document is applicable to the collection, processing, storage, and extraction of cfDNA from plasma for research and clinical analytical applications. Recommendations within this document pertain to the analysis of cfDNA of both tumor and fetal origin and detection of both somatic and germline mutations.

Attachments



[NCI BEBP_Expert-vett...](#)

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Guidelines

9.1.1 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings (CDC, 2007): <http://www.cdc.gov/hicpac/2007IP/2007isolationPrecautions.html>

9.1.2 Infection Prevention and Control Recommendations for Hospitalized Patients Under Investigation (PUIs) for Ebola Virus Disease (EVD) in U.S. Hospitals (CDC, 2014): <http://www.cdc.gov/vhf/ebola/healthcare-us/hospitals/infection-control.html>

9.1.3 Biorepositories and Biospecimen Research Branch (formerly Office of Biorepositories and Biospecimen Research), National Cancer Institute, National Institutes of Health. NCI Best Practices for Biospecimen Resources. 2016. <https://biospecimens.cancer.gov/bestpractices/2016-NCIBestPractices.pdf>

9.1.4 CLSI (formerly NCCLS): Procedures for the collection of diagnostic blood specimens by venipuncture; Approved Standard - Sixth Edition. CLSI document GP41-A6 (ISBN 1-56238-650-6). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007.

9.1.5 Clinical Proteomic Tumor Analysis Consortium, Office of Cancer Clinical Proteomics Research, National Cancer Institute. Prospective Biospecimen Collection Protocol, Blood Collection and Processing for Plasma and Whole Cell Components. v 2.0. 2013.

9.1.6 Qiagen. QIAamp Circulating Nucleic Acid Handbook. Second Edition, January 2011. <https://www.qiagen.com/us/resources/resourcedetail?id=0c4b31ab-f4fb-425f-99bf-10ab9538c061&lang=en>

9.1.7 International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Validation of Analytical Procedure: Text and Methodology Q2(R1) 2005 9.1.8 International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH guideline E18 on genomic sampling and management 13 of genomic data 2016.



Materials

- 5.1 Appropriate safety equipment as described in published guidelines (References 9.1.1, 9.1.2, 9.1.4 under Guidelines)
- 5.2 Plastic-backed absorbent bench paper
- 5.3 K₂ EDTA blood collection tubes (10 mL)(See Section 7.1 in the Attached PDF for Literature Evidence) or stabilizing cfDNA blood collection tube of choice (See Section 7.2 in the Attached PDF for Literature Evidence)
- 5.4 Antiseptic wipes
- 5.5 Vacutainer needle with hub or butterfly needle with Luer adapter
- 5.6 Tourniquet
- 5.7 Phlebotomy chair
- 5.8 Refrigerator (4°C)
- 5.9 Hi-speed centrifuge
- 5.10 Falcon tubes
- 5.11 Storage tubes suitable for centrifugation and storage at -80°C
- 5.12 Pipettes and sterile DNase-free tips for transfer
- 5.13 Freezer (-80°C or colder) for storage

Safety warnings

- ! The Guideline for Isolation Precautions (CDC-2007) should be used for all phases of blood collection and processing and cfDNA processing to prevent the transmission of infectious agents in a Healthcare setting (Reference 9.1.1).

Infection Prevention and Control Recommendations for Hospitalized Patients Under Investigation for Ebola Virus Disease (EVD) in U.S. Hospitals (CDC, 2014) should be consulted prior to biospecimen procurement from patients with suspected or confirmed EVD (Reference 9.1.2).

Ethics statement

Protocols developed using this Biospecimen Evidence-Based Practice may require approval by the user's institutional review board (IRB) or an equivalent ethics committee prior to implementation.

Before start

The purpose of this document is to provide evidence-based guidance for the proper collection and processing of cell-free DNA (cfDNA) from human plasma. This guidance is intended to support the development and execution of evidence-based Standard Operating Procedures (SOPs) for human biospecimen collection, processing, and storage to be used in conjunction with properly validated analytical assays.

Recording of biospecimen preacquisition data

- 1 Whenever possible, extensive data should be recorded relating to preacquisition conditions that may affect the integrity of the biospecimen. Such data may include patient information (including age, gender, fasting status, diagnosis, treatment, and date of last treatment received) as well as details relating to biospecimen acquisition (including number of venipuncture attempts, patient position, tourniquet usage, and date and time of blood collection) (Guideline 9.1.3). Variability in all preacquisition and acquisition variables (including but not limited to sample collection, labeling, transport, and storage) should be minimized via strict accordance to a validated SOP (Guideline 9.1.8) and all deviations from the SOP should be recorded for each sample.
- 2 Label each collection tube with unique unambiguous identifiers, such that the tube can be readily matched to all relevant patient and specimen handling data (Guideline 9.1.3). Ensure that all labels are robust to all handling steps including but not limited to frozen storage, water, and commonly used solvents.

Collection tube considerations

- 3 Optimally, collection tubes containing EDTA should be used to prevent coagulation. Alternatively, acid citrate dextrose is also an acceptable anticoagulant. Heparin and citrate should be avoided (See Section 7.1 for Literature Evidence and Section 8.3.3 for Expert Recommendations in the Attached PDF).
- 4 Streck Blood Collection Tubes (BCT) or other cfDNA stabilizing tubes are recommended when processing delays longer than 2 h are anticipated (See Section 7.2 for Literature Evidence and Section 8.3.3 for Expert Recommendations in the Attached PDF).
- 5 The required volume of blood collection is dependent on both the downstream analytical platform and endpoint measured, but generally one to two  10 mL tubes will ensure sufficient cfDNA yield (See Section 8.3.4 in the Attached PDF for Expert Recommendations); however, use of collection tubes between 2.7 and 10 mL is acceptable, depending on the downstream application (See Section 7.3 in the Attached PDF for Literature Evidence).

Blood Collection

- 6 If using K₂ EDTA tubes, optimally tubes should be pre-chilled on  On ice for a minimum of  00:05:00 to collection, but this may not be necessary (Guideline 9.1.4). If using a cfDNA stabilizing tube, tubes must remain at room temperature.

- 7 The patient must be seated for at least  00:05:00 before venipuncture with the arm positioned on a slanting armrest such that there is a straight line from the shoulder to the wrist (Guideline 9.1.4).
- 8 Apply a tourniquet 3-4 inches above the venipuncture site (Guideline 9.1.3) with enough pressure to provide adequate vein visibility. Have the patient form a fist. Select the median, cubital, basilic, or cephalic veins for venipuncture (Guidelines 9.1.4 and 9.1.5). Collection from a port should be avoided (Guideline 9.1.5).
- 9 Clean the venipuncture site with an antiseptic wipe in a circular motion beginning at the insertion site (Guidelines 9.1.4 and 9.1.5). Once dry, anchor the vein by placing your thumb 2 inches below the site and pulling the skin taut to prevent the vein from moving (Guidelines 9.1.4 and 9.1.5).
- 10 Insert the 21-23 gauge butterfly needle (See Section 8.3.2 in the Attached PDF for Expert Recommendations) with Luer adapter into the vein at a 30° angle and then push the evacuated tube into the hub or adapter (Guidelines 9.1.4 and 9.1.5). Alternatively, a vacutainer needle (with hub attached) may be used (Guidelines 9.1.4 and 9.1.5).
- 11 Once blood flow is established, release the tourniquet (total elapsed tourniquet time should be < 1 min) (Guidelines 9.1.4 and 9.1.5) and ask the patient to open their hand.
- 12 Make sure that tube additives do not touch the stopper or the end of the needle during venipuncture (Guideline 9.1.5).
- 13 Optimally the first 0.5-3 mL of blood should be discarded prior to collecting the EDTA plasma (Guideline 9.1.5). It may be acceptable to collect blood without discarding blood.
- 14 Immediately after completely filling the tube (See Section 8.3.4 in the Attached PDF for Expert Recommendations), remove the tube leaving the needle inserted until all tubes have been filled. Place gauze over the puncture site and remove the needle (Guideline 9.1.4). Slowly and gently invert each tube 8-10 times (Guideline 9.1.5).
- 15 If using K₂ EDTA tubes, tubes containing blood specimens should be stored vertically  On ice (Guideline 9.1.5, See Section 8.3.5 in the Attached PDF for Expert Recommendations). If using Streck BCT tubes or other cfDNA stabilizing tubes, tubes containing blood specimens should be stored vertically at room temperature until processing (See Section 8.3.5 in the Attached PDF for Expert Recommendations).

Processing Delay

- 16 Optimally, EDTA blood should be centrifuged within  02:00:00 of venipuncture, although a delay of up to 4 h at room temperature or 24 h at 4°C is acceptable (See Sections 7.4 and 8.3.5 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively). If blood is collected in a Streck BCT tube, then room temperature or ambient storage or transport for up to 3 days before processing is acceptable (See Sections 7.2 and 8.3.5 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively).
- 17 Regardless of tube type, agitation of blood after initial tube inversion should be minimized during a processing delay (See Section 7.5 in the Attached PDF for Literature Evidence).

Blood Processing

- 18 To separate cells from the remaining plasma, centrifuge blood collection tubes at 800-1600 g for 20 min at 4°C or room temperature (See Section 8.3.6 in the Attached PDF for Expert Recommendations). See 7.6 in the Attached PDF for Literature Evidence and for alternative speeds and durations. 
- 19 Transfer plasma to a new Lo-Bind (or an equivalent container)(See Section 8.3.6 in the Attached PDF for Expert Recommendations), carefully leaving the buffy coat behind. [Plasma may be stored in liquid nitrogen between centrifugations (See Section 7.7 in the Attached PDF for Literature Evidence).]
- 20 To separate cell debris and organelles as well as to ensure cell removal from plasma, a second centrifugation at 14000-16000 g for 10-20 min at room temperature or 4°C is preferred (See Sections 7.8 and 8.3.6 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively), but filtration is an acceptable alternative (See Section 7.8 in the Attached PDF for Literature Evidence). 
- 21 Plasma should be aliquoted into new tubes suitable for -80°C storage (See Sections 7.8 and 8.3.7 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively). The required volume ranges from  400 µL to more than  10 mL of plasma, depending on the extraction method and analytical platform. 

Interim Plasma Storage

- 22 Optimally, storage of plasma post-centrifugation but prior to cfDNA extraction should be limited to 3 h or less at 4°C, up to 3 months at -20°C, or 9 months at -80°C (See Section 7.9 in the Attached PDF for Literature Evidence). However, the expert panel has found that long-term storage at  -80 °C is acceptable for most analyses (See Section 8.3.7 in the Attached PDF for Expert Recommendations). For noninvasive prenatal testing 



(NIPT), refrigerated storage of Streck plasma for 3-4 days is acceptable (See Section 8.3.7 in the Attached PDF for Expert Recommendations).

- 23 cfDNA should be extracted from frozen plasma immediately after thawing at  Room temperature (See Section 8.3.7 in the Attached PDF for Expert Recommendations). DNA should be extracted after the first thaw (See Sections 7.10 and 8.3.7 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively).

cfDNA Extraction and Quantification

- 24 Optimally, cfDNA should be extracted using a circulating nucleic acid kit or an equivalent kit (See Section 8.3.8 in the Attached PDF for Expert Recommendations). In the attached PDF, see Sections 7.11 and 8.3.8 for acceptable alternatives and for Literature Evidence and Expert Recommendations, respectively.
- 25 cfDNA should be quantified by real-time or digital PCR using multiple amplicons but use of fluorometry is also acceptable (See Sections 7.12 and 8.3.10 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively). PCR-based methods are preferred when evaluating fragment size, but electrophoretic methods are also acceptable (See Sections 7.12 and 8.3.10 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively).
- 26 cfDNA suitability for subsequent analysis should be evaluated by real-time PCR (See Section 7.13 in the Attached PDF for Literature Evidence).
- 27 Extracted cfDNA may be stored as aliquots at  -20 °C (See Section 7.14 in the Attached PDF for Literature Evidence). Optimally, cfDNA should only be used after the first thaw (See Section 8.3.9 in the Attached PDF for Expert Recommendations).
- 28 Each analytical assay should be validated for accuracy, precision, specificity, and sensitivity using suitable reference material (Guideline 9.1.7) (See Section 7.15 in the Attached PDF for Literature Evidence).

Protocol references

References considered during the development of this NCI BEBP document are listed below (also See Section 9.2 in the Attached PDF) and include hyperlinks to the PubMed abstract and NCI Biospecimen Research Database curation where applicable. References are cited within the Summaries of Literature Evidence (See Section 7.0) in the Attached PDF.

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