Dec 14, 2024

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

DOI
https://dx.doi.org/10.17504/protocols.io.kxygxy9rwl8j/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.kxygxy9rwl8j/v1

External link: https://biospecimens.cancer.gov/resources/bebp.asp

Protocol Citation: NCI Biorepositories and Biospecimen Research Branch 2024. NCI Biospecimen Evidence-Based Practices (BEBP) - Cell-free miRNA: Blood Collection and Processing. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygxy9rwl8j/v1

Manuscript citation:
Greytak SR, Engel KB, Hoon DSB, Elias K, Lockwood CM, Guan P, Moore HM (2023). Evidence-based procedures to improve the reliability of circulating miRNA biomarker assays. Clinical Chemistry and Laboratory Medicine. 62(1): 60-66. PMID 37129007.

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

Created: May 21, 2024

Last Modified: December 14, 2024

Protocol Integer ID: 100208

Keywords: microRNA, miRNA, plasma, serum, blood, preanalytic, cell-free miRNA, evidence-based, expert-vetted, cfmiRNA, nci biospecimen evidence, exosomal mirna, free mirna, blood collection, bebp

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, isolation, and storage of circulating cfmiRNA from plasma or serum that is intended for analysis in clinical and/or research settings; it does not include the requisite processing for analysis of exosomal miRNA. The ISO 21899.2020 standard (Reference 9.1.9) may be useful as a reference for validation and verification of SOPs produced using this BEBP.

Attachments

PDF

Cell-Free_miRNA_Bloo...

1.2MB

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/infectioncontrol.html

9.1.3 Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19) (CDC, 2020): https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html

9.1.4 Biorepositories and Biospecimen Research Branch, National Cancer Institute, National Institutes of Health. NCI Best Practices for Biospecimen Resources. 2016. https://biospecimens.cancer.gov/bestpractices/index.asp

9.1.5 CLSI (formerly NCCLS): Procedures for the collection of diagnostic blood specimens by venipuncture; Approved Standard - Sixth Edition. CLSI document H3-A6 (ISBN 1-56238-650-6). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007.

9.1.6 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.

9.1.7 HIPAA Authorization for Research
https://privacyruleandresearch.nih.gov/authorization.asp 9.1.8 Betsou, F. et al., Standard PREanalytical Code Version 3.0. Biopreservation and Biobanking 16, 9-12: 2018.

9.1.9 International Organization for Standardization. ISO 21899 Biotechnology- Biobanking- General requirements for the validation and verification of processing methods for biological material in biobanks. 2020.

Materials

5.1 Appropriate safety equipment as described in published guidelines (References 9.1.1, 9.1.2, and 9.1.5)
5.2 Plastic-backed absorbent bench paper
5.3 Blood collection tube of choice (See Sections 7.1 and 7.2 in the Attached PDF for LIterature Evidence)
5.4 Antiseptic wipes
5.5 Vacutainer needle (21-23 gauge) 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, such as cryotubes, suitable for centrifugation, storage at -80°C, and amenable to waterproof labeling using barcodes or with unique identifiers. RNase-free tubes are suggested.
5.12 LoBind tubes
5.13 Pipettes and sterile RNase-free tips for transfer
5.14 Freezer (.-80°C) for long-term storage. A -30°C freezer is acceptable if the anticipated duration of frozen storage is <1 year.

Safety warnings

Universal Precautions (CDC-2007) and guidelines associated with Coronavirus Disease 2019 (COVID-19) and Ebola should be used for all phases of blood collection and processing, and cfmiRNA processing (Reference 9.1.1, 9.1.2 and 9.1.3).

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 microRNA (cfmiRNA) from human plasma and serum. This guidance is intended to support the development and execution of evidence-based Standard Operating Procedures (SOPs) for human biospecimen collection, processing, and storage.

Recording of biospecimen pre-acquisition data

1 Whenever possible, extensive data relating to preacquisition conditions that may affect the integrity of the biospecimen should be recorded. Such data must include patient information (including age, gender, fasting status, diagnosis, physical activity level, and treatment type(s) and date(s)) 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 9.1.4). When possible, the complete blood count of the blood specimen should be recorded as it may be informative in identifying potential sources of bias. Appropriate authorization by HIPAA or another pertinent regulatory agency and informed patient consent must be obtained prior to the collection of patient blood and data for research purposes (Guideline 9.1.7).

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 9.1.4). Ensure that all labels are robust to all handling steps including but not limited to frozen storage, water, and commonly used solvents. The labeling scheme should accommodate the real-time documentation/recording of pre-analytical conditions (See 6.5.5 for additional details).

Collection tube considerations

3 Collection tubes containing ethylenediaminetetraacetic acid (EDTA), sodium citrate, acid citrate dextrose (ACD), citrate-theophylline-adenosine-dipyridamole (CTAD), or sodium-fluoride/ potassium-oxalate (NaF/KOx) are acceptable for blood collection as are select proprietary tubes containing a preservative such as Streck cfDNA BCT, Roche Cell-Free tubes, or Norgen cfDNA/cfRNA tubes)(See Section 7.3 in the Attached PDF for Literature Evidence). When specimen processing within 1-2 h of collection is not possible, then use of preservative-containing collection tubes is preferred. Use of serum tubes may also be acceptable if processing is well-controlled (See Sections 7.2 and 8.3.2 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively). Use of heparin, Streck RNA BCT, or PAXgene tubes is not advisable (See Section 7.3 in the Attached PDF for Literature Evidence). Optimally, tube choice should be validated during study design and remain consistent throughout the duration of the study. If changes to tube type are necessary, they must be validated in a pilot study as miRNA levels may differ between serum and plasma or among different anticoagulants (See Section 7.3 and 7.3 in the Attached PDF for Literature Evidence; See Section 8.3.2 in the Attached PDF for Expert Recommendations).

4 Blood collection tube choice may also be guided by specimen availability and the need to assess multiple analytes (cfmiRNA, cfDNA, etc.) from a single collection tube (See Section 8.3.1 in the Attached PDF for Expert Recommendations).

5 To ensure sufficient yield, ideally a minimum of 10 mL of blood per tube should be collected (Guideline 9.1.6). However, the total volume of blood required will depend on both the analytical method and the anticipated abundance of the cfmiRNA of interest. Consequently, smaller volumes may be sufficient. Care should be taken to avoid underfilling blood collection tubes containing an anticoagulant or preservative (See Section 8.3.3 in the Attached PDF for Expert Recommendations).

Blood Collection

6 Blood collection after the subject has fasted for >6 h is preferred, as eating prior to venipuncture may result in a diet-specific increase in fatty acids in plasma/serum (See Section 8.3.3 in the Attached PDF for Expert Recommendations).

7 Blood collection tubes should be stored at Room temperature (20-24°C) unless otherwise indicated by the manufacturer (See Section 8.3.2 in the Attached PDF for Expert Recommendations).

8 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 9.1.6).

9 Apply a tourniquet 3-4 inches above the venipuncture site (Guideline 9.1.5) 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 9.1.6). Collection from a port should be avoided (Guidelines 9.1.5 and 9.1.6). A vein imager should be used when available to improve venipuncture technique.

10 Clean the venipuncture site with an antiseptic wipe in a circular motion beginning at the insertion site (Guidelines 9.1.5 and 9.1.6) and allow to air dry. Once the skin is completely dried, anchor the vein by placing your thumb 2 inches below the site and pulling the skin taut to prevent the vein from moving (Guidelines 9.1.5 and 9.1.6).

11 Insert the 21-23 gauge butterfly needle (See Section 8.3.3 in the Attached PDF for Expert Recommendations) with Luer adapter into the vein at 30° angle and then push the evacuated tube into the hub or adapter (Guidelines 9.1.5 and 9.1.6).

12 Once blood flow is established, release the tourniquet (total elapsed tourniquet time should be < 00:01:00) (Guidelines 9.1.5 and 9.1.6) and ask the patient to open their hand.

13 Make sure that tube additives do not touch the stopper or the end of the needle during venipuncture (Guideline 9.1.6).

14 Non-cfmiRNA clinical specimens should be collected first (See Section 8.3.3 in the Attached PDF for Expert Recommendations). If no other blood specimens are being collected, discard the first 2-3 mL of blood prior to collecting blood specimens for cfmiRNA analysis (Guideline 9.1.6; See Section 8.3.3 in the Attached PDF for Expert Recommendations).

15 After completely filling the tube, immediately remove the tube leaving the needle inserted and slowly and gently invert the tube as recommended by the manufacturer for the tube type of choice (Guideline 9.1.6).

16 After filling the last collection tube, place gauze over the puncture site and remove the needle (Guideline 9.1.5).

Store anticoagulant and serum tubes containing blood specimens upright (Guideline 9.1.6) at Room temperature (20-25°C) (See Sections 7.4 and 7.5 in the Attached PDF for Literature Evidence; See Sections 8.3.4 and 8.3.5 in the Attached PDF for Expert Recommendations).

Processing Delay

17 In most instances, blood specimens should be centrifuged as soon as possible, optimally within 02:00:00 of venipuncture (See Sections 7.4 and 7.5 in the Attached PDF for Literature Evidence; See Sections 8.3.4 and 8.3.5 in the Attached PDF for Expert Recommendations). However, if proprietary tubes containing a preservative are used, then a pre-centrifugation delay of 24-48 h at room temperature is acceptable (See Sections 7.3 and 8.3.4 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively). Serum separator tubes should be processed immediately, but serum tubes relying on natural clot formation should be processed after 1 h at room temperature (See Section 8.3.5 in the Attached PDF for Expert Recommendations).

Regardless of tube type, agitation of blood should be minimized during a processing delay as hemolysis alters cfmiRNA levels (See Section 7.6 in the Attached PDF for Literature Evidence).

Blood Processing

18 Centrifuge blood collection tubes using a protocol validated for the tube type (See Section 8.3.6 in the Attached PDF for Expert Recommendations). Acceptable

centrifugation speeds and durations include the following ranges, 820-3500 x g for 1-20 min at 4°C or room temperature (See Section 7.7 in the Attached PDF for Literature Evidence).

19 If a two-step centrifugation is desired transfer plasma or serum to a new Falcon tube (or an equivalent container), carefully leaving the buffy coat behind.

20 Further removal of platelets, white blood cells, and cellular debris can be achieved through a second centrifugation at 10,000-16,000 x g for 00:15:00 (See Section 7.8 in the Attached PDF for Literature Evidence) or filtration (See Sections 7.9 and 8.3.6 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively). While the suitability of including a filtration or second centrifugation step will depend on the abundance of the miRNA of interest, a secondary processing step is generally recommended with the notable exceptions of miRNAs with low levels of expression and discovery-based studies (See Sections 7.9 and 7.8 in the Attached PDF for Literature Evidence; See Section 8.3.6 in the Attached PDF for Expert Recommendations).

21 Serum and plasma specimens with evidence of hemolysis must be excluded from cfmiRNA analysis (See Section 7.6 in the Attached PDF for Literature Evidence). Hemolysis should be quantified by measurement of hemoglobin concentration or when possible, using the ratio of miR-23a to miR-452 or miR-451a (See Section 7.6 in the Attached PDF for Literature Evidence). Use of a spectrophotometer or other visual method is not recommended due to low detection sensitivity (See Section 7.6 in the Attached PDF for Literature Evidence). Residual platelet count should be enumerated by flow cytometry or an impedance-based method (See Section 7.7 in the Attached PDF for Literature Evidence; See Section 10.2 in the Attached PDF for Table 2).

22 Plasma or serum should be aliquoted into multiple tubes suitable for cryostorage at -80 °C. Generally, a plasma or serum aliquot of 100-1,000 µL is adequate for a single extraction using a proprietary kit; however, the volume of plasma or serum required will depend on several factors that include extraction method, assay, and anticipated cfmiRNA abundance. Aliquot volume should be chosen to optimize cfmiRNA detection and avoid multiple freeze-thaw cycles. Optimally tubes should be RNase-free. While a barcode labeling system is recommended, any clear and robust labeling system that can withstand frozen storage and common solvents is acceptable (See Section 8.3.7 in the Attached PDF for Expert Recommendations). The labeling system should accommodate annotation of the preanalytical conditions experienced by each aliquot; please consult the International Society for Biological and Environmental Repositories (ISBER)'s Sample PREanalytical Code (SPREC) as a comprehensive example of biospecimen documentation (Guideline 9.1.8).

Interim Plasma/Serum Storage (Note: not applicable for exosome analysis)

23 Optimally, cfmiRNA analysis should be conducted immediately, whether after extraction or by direct analysis of plasma or serum (See Sections 7.10 and 7.11 in the Attached PDF for Literature Evidence; See Section 8.3.7 in the Attached PDF for Expert Recommendations). However, if immediate analysis is not possible, storage of plasma at room temperature or 4°C for up to 3 h, at -20°C for several months, or -80°C for years is acceptable for most cfmiRNA endpoints. Potential effects of each storage temperature should be carefully considered for the targeted miRNA(s); for example, effects associated with freeze-thaw cycling may be more severe than a pre-extraction delay at 4°C. Freeze-thaw cycling should be avoided (See Sections 7.12 and 8.3.8 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively). Interim storage of serum should be avoided (See Sections 7.10 and 7.11 in the Attached PDF for Literature Evidence).

24 Frozen serum and plasma aliquots should be thawed on wet ice with occasional gentle mixing for the minimum time necessary (See Section 8.3.8 in the Attached PDF for Expert Recommendations).

25 Thawed serum and plasma should be thoroughly mixed and centrifuged at 1000-1600 x g for 1-2 min to pellet cryoglobulins immediately prior to miRNA extraction or analysis (See Sections 7.12 and 8.3.8 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively).

6.7 miRNA Extraction and Quantification

26 For analytical methods requiring extraction (See 8.3.9), a miRNA-specific commercial extraction kit is preferred, but phenol-chloroform based methods are acceptable (See Section 7.13 in the Attached PDF for Literature Evidence). The expert panel advises against the use of column-based extraction kits for miRNA discovery studies (See Section 8.3.9 in the Attached PDF for Expert Recommendations).

27 Optimally, analysis should be performed immediately following extraction (See Sections 7.14 and 8.3.10 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively). Based on reports in the literature, interim storage of extracted miRNA or cDNA in low bind tubes at or below -80 °C may be acceptable for several months (See Section 7.14 in the Attached PDF for Literature Evidence). However, the expert panel advises that long-term storage should be limited to plasma/serum specimens, and if short-term storage of extracted miRNA is required then Tris buffer or ethanol should be used as the medium (See Section 8.3.10 in the Attached PDF for Expert Recommendations).

28 cfmiRNA levels quantified by real-time PCR should be expressed as an average quantification cycle (Cq) value relative to the Cq values of two or more constitutively expressed miRNA transcripts that display stable expression across the experimental conditions and disease states anticipated during the study (See Sections 7.15 and 8.3.11 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively). Additionally, miRNA/RNA used in any assay can be quantified using capillary electrophoresis and/or fluorometric methods, but these methods are unacceptable if extraction included carrier RNA.

29 All analytical methods used should be evaluated for reproducibility and standardized to minimize platform-specific effects (See Sections 7.15 and 8.3.11 in the Attached PDF for Literature Evidence and Expert Recommendations, respectively). The ISO 21899.2020 standard (Guideline 9.1.9) may be useful as a reference for further validation and verification.

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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Acknowledgements

We thank Dr. Kevin Elias (Brigham And Women's Hospital, MA), Dr. Dave Hoon (Saint Johns' Cancer Institute, CA), and Dr. Tina Lockwood (University of Washington, WA) for their participation on the expert panel and their insightful recommendations.