1. **General**

This study aims to evaluate the correlation and bridging potential between different plasma collection tubes and protocols using blood samples from healthy subjects

1. **Objectives** 
   1. Objective 1: To confirm the feasibility of using the room-temperature collection method for PROphetâ
   2. Objective 2: To evaluate the correlation of each protein between the eight experimental protocols and the standard protocol
   3. Objective 3: To evaluate the PROphet score correlation between the eight experimental protocols and the standard protocol
2. **Statistical Measures**

The statistical tests used are:

* + - Spearman and Pearson correlation coefficients for log (RFU) between different protocols
    - Goodness of fit (R2), slope, and intercept for best linear fit between PROphet prediction on standard protocol, to those extracted on experimental protocols after bridging.
    - Reproducibility of PROphet result (POSITIVE / NEGATIVE) between the standard protocol and the experimental protocols.

1. **Acronyms**
   1. BCT – Blood Collection Tube
   2. CPT - Cell Preparation Tube
   3. BCD - Blood Collection Device
   4. RT – Room Temperature
2. **Materials and Equipment**
   1. Blood samples from healthy donors over the age of 18 who have previously provided informed consent for study procedures provided by BioIVT.
   2. K2-EDTA BCT, 4ml (BD, cat# 367861)
   3. Cell-Free DNA BCT tube, 10ml (Streck, cat # 230470)
   4. Protein Plus BCT tube, 5ml (Streck, cat # 230628)
   5. Mononuclear CPT, 8ml (BD Vacutainer™, cat # 362761)
   6. Centrifuge with swinging bucket rotor
   7. 5 ml polypropylene conical tubes (Fisher Scientific, cat# 14-282-304)
   8. Sterile cryovials with writing surface (Biologix, cat# 81-8204)
   9. Sterile Pasteur pipettes
   10. Ice bucket
   11. Bar-Code sticker labels
   12. Cryogenic specimen storage box (for example Eppendorf cat # 0030140516)
   13. VAMS 4-sampler Mitra Device in a Specimen bag and a desiccant (Trajan, cat# VM-304SNLR-E05)
   14. Zeba™ Spin Desalting Columns 7K MWCO (Thermo Scientific # 89890)
   15. SomaScan Assay Buffer (AB) (651-00125)
   16. Ziplock Biohazard bag (Alexred, cat# NH 2156)
3. **Appendices**
   1. Appendix A - On-site blood collection and plasma preparation protocol
   2. Appendix B - Blood collection and plasma preparation checklist
   3. Appendix C - OncoHost Laboratory plasma preparation protocol
   4. Appendix D - Tubes and labels
   5. Appendix E - Donor Follow Up Form
   6. Appendix F - User\_Manual\_for Streck\_Plasma
4. **Experimental Procedure**
   1. OncoHost will supply BioIVT with kits, each containing the components listed in Table 1, one kit per blood donor.
   2. Items 1-7 will be prelabelled with the appropriate barcode sticker label comprising the donor’s number and the specific type of tube as follows: 1x EDTA blood collection tube, 3x Protein Plus BCT tubes, 3x Cell-Free DNA BCT tube, 1x Mononuclear CPT tube, 1x Mitra Device, and 5-ml polypropylene conical tubes.

**Table 1**: blood collection kits content.

|  |  |  |
| --- | --- | --- |
| # | Description | Quantity |
|  | K2-EDTA BCT, 4ml | 1 |
|  | Cell-Free DNA BCT, 10ml | 3 |
|  | Protein Plus BCT, 5ml | 3 |
|  | BD Vacutainer™ Mononuclear CPT, 8ml | 1 |
|  | 30ul Mitra Device (4-sampler) in a Specimen bag and a desiccant (VAMS) | 1 |
|  | 5-ml polypropylene conical tubes | 2 |
|  | Sterile cryovials with writing surface | 6 |
|  | Spare Barcode sticker labels with a donor number | 4 |
|  | Ziplock Bio-hazard Bag | 1 |

* 1. The study will examine different blood isolation, processing, and shipment protocols with respect to 5 potential parameters:
     + Tube used for blood collection:
       1. EDTA tube
       2. CPT tube
       3. Streck cfDNA tube
       4. Streck Protein Plus tube
     + Location of blood processing into plasma:
       1. Point of collection
       2. OncoHost CLIA lab
     + Shipment temperature:
       1. Ambient temperature
       2. -80ºc
     + Time passed from blood sample collection to plasma isolation:
       1. Up to 4 hours
       2. 24 hours
       3. 72 hours
       4. 120 hours
     + Physical means to stabilize proteins
       1. Freezing of plasma samples.
       2. Desiccating plasma on a solid phase carrier (VAMS).

Note: samples collected into the cfDNA tubes will require further processing according to Standard Bio “SomaScan® Assay: Streck Cell-Free DNA BCT® Plasma Pre-Processing –

User Manual” (Appendix F).

* 1. The different protocols are described in Table 2

**Table 2**:Alternative blood collection, isolation and shipment protocols

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Protocol # | Tube type | Location of centrifugation and plasma isolation | Shipment temperature | Time from collection to centrifugation |
| 1\* | EDTA | Point of collection | -800C | Up to 4 hours |
| 2 | VAMS | Point of collection | RT | Up to 4 hours |
| 3 | CPT | Point of collection | -800C | Up to 4 hours |
| 4 | Streck cfDNA | OncoHost lab | RT | 24 hours |
| 5 | Streck Protein Plus | OncoHost lab | RT | 24 hours |
| 6 | Streck cfDNA | OncoHost lab | RT | 72 hours |
| 7 | Streck Protein Plus | OncoHost lab | RT | 72 hours |
| 8 | Streck cfDNA | OncoHost lab | RT | 120 hours |
| 9 | Streck Protein Plus | OncoHost lab | RT | 120 hours |

\*Standard protocol

* 1. In brief, BioIVT will collect blood samples from 25 healthy donors over three separate days, with 8–10 donors per session.
  2. Blood samples from each healthy donor will be collected as outlined in Appendix A: On-site Blood Collection and Plasma Preparation Protocol, and Table 1 above, following the list below:
* 1 x EDTA tube
* 3 x Cell-Free DNA BCT tubes
* 3 x Protein Plus BCT tubes
* 1 x BD Vacutainer™ Mononuclear CPT tube
  1. The time and date of blood sample collection and processing will be documented in the “Blood collection and plasma preparation checklist” (Appendix B).
  2. After blood collection, the experiment will be divided into 2 arms:

1. Arm 1 – includes the 3x Cell-Free DNA BCT tubes and 3x Protein Plus BCT tubes.
   * 1. The tubes will be shipped at room temperature to the OncoHost lab at 1110 SE Cary Parkway, Suite 205, Cary, North Carolina, 27518, immediately after blood collection. BioIVT will coordinate and perform the Shipment using their FedEx account.
     2. Samples of protocols 4, and 5 will be processed to plasma 20-28 hours after blood collection, as described in Appendix A.
     3. Samples of protocols 6 and 7 will be processed to plasma 68-76 hours after blood collection, as described in Appendix A.
     4. Samples of protocols 8 and 9 will be processed to plasma 116-124 hours after blood collection, as described in Appendix A.
     5. Plasma samples will be stored at -80ºC until analysis.
     6. Desalting will be conducted on frozen "Plasma Cryovial\_3" from protocols 6 and 7, following the procedure outlined in Appendix F.
   1. Arm 2 – includes the 1 x EDTA and 1 x BD Vacutainer™ Mononuclear CPT tubes.
      1. The blood in the 1 x EDTA and 1 x BD Vacutainer™ Mononuclear CPT tube will be further used for plasma isolation as described in Appendix A. Processing will be performed at the point of collection and frozen until shipment to the OncoHost lab.
      2. Plasma samples in the cryovials will be shipped on dry ice and VAMS will be shipped at ambient temperature. Shipment will be coordinated and performed by BioIVT, using their FedEx account.
2. Upon arrival of the samples to the lab, all samples will be documented in the LabGuru.
3. Cryovials containing the plasma samples will be stored at -80°C until analysis.
4. Further extraction of the plasma from the VAMS will be performed as described in “On-site blood collection and plasma preparation protocol” (Appendix A).
5. Proteomic analysis will be performed \according to SOP ONC-100 Oncohost PROphet® Proteomic Assay.
6. **Plates plan**
   1. **Phase I**
      * **EDTA plasma x25**
      * **CPT x25**
      * **STRECK ctDNA 72hr + desalination x25**
      * **STRECK Protein+ 72hr + desalination x25**
      * **STRECK ctDNA 72hr - desalination x25**
      * **STRECK Protein+ 72hr - desalination x25**
   2. **Phase II (pending phase I)**
      * **STRECK ctDNA/Protein+ 120hr ± desalination x25**
      * **VAMS x25**