Blood Plasma Sample Collection and Handling for Proteomics Analysis
- A guide to obtain optimal plasma samples -

1 Introduction

Blood can be regarded as a complex liquid tissue that comprises cells and extra cellular fluid. The choice of a suitable specimen collection protocol is crucial to minimize artificial processes (e.g. cell lysis, proteolysis) occurring during specimen collection and preparation. Preanalytic procedures can alter the analysis of blood-derived samples. These procedures comprise the processes prior to the actual analysis of the sample and include steps needed to obtain the primary sample (e.g. blood), and to obtain the analytical specimen (e.g. plasma, serum, cells).

It has been reported that the most frequent faults in the preanalytical phase are the result of erroneous procedures for sample collection (e.g. blood drawing from an infusive line resulting in sample dilution). The design of blood collection devices may aid in correct sampling: evacuated containers sustain the draw of the accurate quantity of blood to ensure the correct concentration of additives or the correct dilution of the blood, such as in the case of citrated plasma. The speed of the blood draw is also controlled and restricts the mechanical stress.

The favoured site of collection is the median cubital vein, which is generally easily found and accessed. As such, it will be most comfortable for the patient and should not evoke additional stress. Preparation of the collection site includes proper cleaning of the skin with an alcohol (2-propanol). The alcohol must be allowed to evaporate, since commingling of remaining alcohol with the blood sample may result in hemolysis, raise the levels of analytes and cause interferences. The position of the patient (standing, lying, seated) can affect the hematocrit and hence change the concentration of analytes. The tourniquet should be applied 3-4 inches above the site of venipuncture and should be released as soon as blood begins flowing into the collection device. The duration of venous occlusion (> 1 min) can affect the sample composition. Prolonged occlusion may result in

hemoconcentration and subsequently resulting in an increase in miscellaneous analytes e.g. total protein levels. Blood should be collected from fasting patients in the morning between 7-9 am, because ingestion or circadian rhythms can alter the concentration of analytes considerably (e.g. total protein, hemoglobin, myoglobin).

1.1 Processing of blood samples

A quick separation of cells from the plasma is favourable, since cellular constituents may liberate substances that alter the composition of the sample. Generally, it is recommended that plasma and serum is centrifuged with 1300 to 2000 x g for 10 minutes within 30 min, after the collection of the sample. The temperature should generally be 15-24 ℃, unless recommended differently for distinct analytes like gastrin or A-type natriuretic peptide.

Processing at 4 ℃ appears to be attractive, because enzymatic degradation processes are reduced at low temperatures. However, platelets become activated at low temperatures and release intracellular proteins and enzymes, which affect the sample composition. Thus, processing at low temperatures should be performed only after thrombocytes have been removed. Since one centrifugation step may not be sufficient enough for depletion of platelets below 10 cells/nL a second centrifugation step (2500 x g for 15 min at room temperature) or filtration step may be required to obtain platelet poor plasma. This procedure is only applicable to plasma since platelets in serum are already activated.

2 Materials

- 20 gauge needles and the appropriate adapter (e.g. Sarstedt, Nümbrecht, Germany) or a Vacutainer system (BD bioscience, Franklin Lakes, USA)
- alcohol (2-propanol) in spray flask
- swabs
- examination gloves
- tourniquet or sphygmomanometer
- blood collection tubes (e.g. Sarstedt, Nümbrecht, Germany)
- centrifuge with a swinging bucket rotor (e.g. Sigma 4K15, Sigma Laborzentrifugen, Osterode, Harz)

- 10 mL syringe equipped with a cellulose acetate filter unit with 0.2 µm pore size and 5 cm^2 filtration area (e.g. Sartorius Minisart, Sarstedt)
- 2 mL cryo-vials
- pipette and tips

3 Methods

Venipuncture of a cubital vein is performed using a 20 gauge needle (diameter: 0.9 mm, e.g. butterfly system max. tubing length: 6 cm). If a tourniquet is applied, it should not remain in place for longer than 1 minute (risk of falsifying results due to hemoconcentration). As soon as the blood flows into the container, the tourniquet has to be released at least partially. If more time is required, the tourniquet has to be released so that circulation resumes and normal skin colour returns to the extremity.

Prior to blood collection for proteomics analysis, blood is aspirated into a first container (e.g. 2.7 mL S-Monovette, Sarstedt, Nümbrecht, Germany). This is done to flush all surfaces and remove initial traces of contact induced coagulation. This sample is not useful for analysis. Afterwards, blood is drawn into a standard EDTA or citrate containing syringe (e. g. 9 mL EDTA-Monovette, Sarstedt, Nümbrecht, Germany). Depending on ease of blood flow, several samples can be collected. Free flow with mild aspiration has to be assured to avoid haemolysis.

After venipuncture, plasma is obtained by centrifugation for 10 min at 2000 x g at room temperature. This centrifugation has to be started in within 30 min after blood collection. The resulting plasma sample has now been separated from red and white blood cells in an efficient and gentle way. Nevertheless, a significant number of platelets (~25%) is still present in the sample. This requires an additional preparation step.

For a second centrifugation step to remove platelets the plasma sample is transferred into a second vial and centrifuged for 15 min at 2500 x g at room temperature. After this centrifugation, the supernatant is transferred in aliquots of 1.5 mL into cryo vials.

Samples are transferred to a –80°C freezer in within 30 min. Storage is at –80°C, transport of samples is done on dry ice.

4 Notes

4.1 Frequently made mistakes

4.1.1 Blood withdrawal

- The Patient was not fasting (i.e. had eaten prior to sampling).
- The blood was drawn from an infusive line.
- The blood was drawn in other position (e.g. supine, upright).
- The consumables used were different to those recommended.
- The expiry date of consumables was already reached.
- The tubes were not properly filled.
- The tubes were agitated vigorously (shaken instead of gentle movement to dissolve the anticoagulant).
- The blood sample tubes were not consistently kept at room temperature.
- The sample tubes were put on ice or in a refrigerator.

4.1.2 Lab handling

- The centrifugation was delayed more than 30 min after the blood withdrawal.
- A cooling centrifuge was adjusted below room temperature.
- The centrifugation speed was wrong (e.g. rounds per minute were set instead of g-force).
- The centrifugation time was wrong.
- The removal of blood plasma by pipetting was done without proper caution. Consequently the Buffy coat or the red blood cells were churned up.
- The second centrifugation of recovered plasma samples was delayed after end of first centrifugation.

4.1.3 Storage of samples

- The storage of samples was delayed.
- The storage temperatures were above -80℃.
- The labelling of sample containers is unreadable or confusable.
- The attachment of the labels to the sample containers was not sufficient during storage or handling resulting in lost of labels.

4.2 General recommendations

- A proper first centrifugation should produce a visible white blood cell layer (buffy coat) between red blood cells and plasma. If not, centrifugation speed or time may be wrong
- One should discard plasma that is icteric or exhibit signs of haemolysis. One should check with specialist if this may due to that particular disease.

5 References

* Omenn GS, States DJ, Adamski M, Blackwell TW, Menon R, Hermjakob H et al. (2005) Overview of the HUPO Plasma Proteome Project: results from the pilot phase with 35 collaborating laboratories and multiple analytical groups, generating a core dataset of 3020 proteins and a publicly-available database. Proteomics 5, 3226--45.

* Tammen H, Schulte I, Hess R, Menzel C, Kellmann M, Mohring T, Schulz-Knappe P. (2005) Peptidomic Analysis of Human Blood Specimens: Comparison between Plasma Specimens and Serum by Differential Peptide Display. Proteomics 13, 3414--3422

* Rai AJ, Vitzthum F. (2006) Effects of preanalytical variables on peptide and protein measurements in human serum and plasma: implications for clinical proteomics. Expert Rev. Proteomics 3, 409--26.

