Instructions for handling blood for ctDNA analysis
Regionernes Bio- og GenomBank

Purpose

The following instruction describes how to handle blood samples for later purification of circulating tumor DNA (ctDNA) collected in 'Bio- and GenomBank Denmark' (RBGB). The standard sample set taken for RBGB does not include enough plasma to secure a good yield of ctDNA. Thus, we offer a sample set with a larger volume of plasma. The handling procedure described here is a guideline and will be adjusted if new knowledge in the area emerges.

Background

The volume of blood taken from the patient will vary in consideration of other samples, medical treatment, and which types of analysis that have been planned. According to our current knowledge from collections through RBGB, a potential volume of up to 80 ml EDTA-blood may be required.

Based on the literature in the field it is recommended to centrifuge the plasma fractions after separation from the buffy coat. This second centrifugation step is to minimize contamination of DNA from blood cells, which due to the very small fraction ctDNA constitutes is important for optimal purification of ctDNA. If new studies contribute to the question of best practice within the field, the recommendation will be re-evaluated.

Consensus about centrifugation speed and temperature is not established. We recommend following spin settings used in the handling of blood in RBGB (described under "Handling of blood for ctDNA analysis" below) with focus on slow deceleration. If new studies contribute to the question of best practice in the field, the recommendation will be re-evaluated.

All samples should optimally be handled and frozen within a maximum of 3 hours.

Suggestions for blood samples for ctDNA analysis

There will be individual adjustments in blood collection and handling of blood for ctDNA analysis thus resulting in a fee paid by the project. Prices for collection can be obtained from the RBGB secretariat. Below are listed two examples of modified collections where ctDNA analysis is accommodated.

Example 1	Example 2

• 1x9/10 ml dry-glass or serum-gel tubes
  - 2x2 ml serum (for RBGB)
• 4x9/10 ml (or other fitting tube) EDTA tubes
  - 1x1,5 ml Whole blood (for RBGB)
  - 1xBuffycoat (for RBGB)
  - 8x2 ml plasma (for ctDNA)
	• 1x9/10 ml dry-glass or serum-gel tubes
	  - 2x2 ml serum (for RBGB)
	• 4x9/10 ml (or other fitting tube) EDTA tubes
	  - 1x1,5 ml Whole blood (for RBGB)
	  - 1xBuffycoat (for RBGB)
	  - 3x5,3 ml plasma in 9 ml tubes (for ctDNA)

Handling of blood for ctDNA analysis

1. It is important that the sample is received fresh, and centrifugation is done within 1½ hour, when possible, to allow the process time to be within 3 hours[1].
2. If a fraction of whole blood is to be saved, this is done before centrifugation of the EDTA tube following the standard guidelines in RBGB.
3. First round of centrifugation: Spin the EDTA tube at 2000xg[2] for 10 min at 4°C (recommend) or at room temperature. The centrifuge must have slow deceleration corresponding to 45 sec. This will ensure that the blood phases stay separated when the centrifuge brakes. The centrifugation should not be discontinued.
4. Second round of centrifugation: Transfer and collect plasma, until 0,5 cm above pellet from the centrifuged tubes, to a 50 ml tube and repeat spin at 2000xg[2] for 10 min at 4°C (recommend) or at room temperature. The centrifuge must have slow deceleration corresponding to 45 sec.
5. Pipette the plasma into microtubes (DNA low binding) marked EDTA-plasma for ctDNA. IMPORTANT: plasma until 0,5 cm above pellet/the bottom of the tube is transferred. The remaining 0,5 cm of plasma is discarded to avoid contamination of the plasma and secure a good preanalytical product.
6. This procedure results in approx. 4 ml plasma per 9 ml whole blood for extraction of circulating-free DNA.

Freezing of samples:

7. Buffy coat is stored at -20°C or -80°C, plasma and whole blood is stored at -80°C following the standard guidelines in RBGB.

Extraction of circulating-free DNA from plasma

There are several commercial kits for extracting cfDNA. At present, the literature indicates that the following kit from Qiagen gives the best yield. However, more studies of available kits are recommended so that the choice of kit can be based on results and direct comparison of kits.

Most frequently described used in the literature: QIAamp® Circulating Nucleic Acid kit from Qiagen for extraction of circulating-free DNA from human plasma (Cat. No. 55114)

Other kits described used in the literature:
QI-Aamp minElute ccfDNA mini kit (Qiagen Cat # 55284; Qiagen magnetic (QiaM))
Maxwell RSC ccfDNA plasma kit (Promega Cat # AS1480; Promega magnetic (ProM)

[1] Local conditions may differ from the recommended time and handling conditions. Agreement on separate transport may require payment from the project.

[2] g-value = 0.00001118*r*RPM^2, for example 4500 RPM at rotor radius 9 cm will correspond to 2038×g.

Literature

1. Markus H. et al. Evaluation of pre-analytical factors affecting plasma DNA analysis. Sci Rep. 2018.
2. Risberg B. et al. Effects of Collection and Processing Procedures on Plasma Circulating Cell-Free DNA from Cancer Patients. J Mol Diagn. 2018.
3. Diefenbach RJ, Lee JH, Kefford RF, Rizos H. Evaluation of commercial kits for purification of circulating free DNA. Cancer Genet. 2018.
4. Sorber L, et al. A Comparison of Cell-Free DNA Isolation Kits Isolation and Quantification of Cell-Free DNA in Plasma J Mol Diagn. 2017.

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