Advice for clinicians on the handling of blood samples and details of separation of plasma for samples requiring circulating tumour DNA (ctDNA) analysis

Due to the unstable nature of ctDNA, there are specific sample requirements needed to ensure the quality of testing.

Note: Do not send blood or plasma samples to the laboratory on Fridays.

1. For ctDNA testing from blood the following sample is required:

   1x 8-10ml blood (whole) in Streck Cell-Free DNA BCT® tube or Janssen CellSave Preservative Tubes (at room temperature)

   When taking blood in a Streck or CellSave tube, ensure the tube is inverted at least 10 times to ensure full mixing of the blood and preservative.

   Note: EDTA tubes can be used but do not preserve the quality of the ctDNA as efficiently as Streck or CellSave (see step 2 for details).

   The blood sample should not be frozen or refrigerated

2. Blood samples need to be spun down to separate and extract plasma (see point 3 for details). This needs to be performed:

   - Within 96 hours of the sample being taken if Streck or CellSave tubes are used for sample collection (see additional information below regarding the time critical nature of samples).

     Therefore, if Streck or CellSave blood samples are being sent to Laboratory Genetics, they should be dispatched as soon as possible to reach the lab within 24 hours. Royal Mail Blue Guaranteed Delivery boxes are recommended.

   - Within 24 hours of the sample being taken if EDTA tubes are used for sample collection.

     Note: Owing to this time pressure, EDTA tubes are not recommended for collection of blood samples for ctDNA analysis unless the plasma separation step can be performed in the collecting hospital.

3. Details of plasma separation:

    - Handle blood tubes and plasma transfer in a safety cabinet.
    - Prepare six labeled 2 ml Eppendorf tubes per CellSave/Streck tube for plasma collection
    - Centrifuge the CellSave/Streck tube for 10 mins at 1000 x g at 4^{o}C
    - Remove the plasma supernatant into the first two 2 ml Eppendorf tubes, leaving 3-5 mm of plasma above the buffy coat layer
    - If keeping the buffy coat layer, transfer this to a new labeled tube now
    - Centrifuge the 2 ml Eppendorf tubes to remove any remaining red or white cells at 2000 x g for 10 mins.
    - Transfer the new supernatant into the remaining 2 ml tubes, 1 ml in each to ensure no bursting when the plasma is frozen

4. Plasma samples should be frozen at -80^{o}C immediately. Samples must remain frozen until ctDNA extraction can be performed.

    If plasma samples are being sent shipment to Laboratory Genetics needs to be on dry ice to maintain the condition of the plasma.

5. ctDNA is extracted from plasma samples within Laboratory Genetics using in-house SOPs.

Time critical nature of samples

Bloods sent for circulating tumour DNA (ctDNA) analysis should be taken in Streck of CellSave preservative tubes, which allow the stable shipment of samples from remote locations for analysis. CellSave tubes were originally developed to stabilise circulating tumour cells (CTCs), however, also serve to preserve the ctDNA fraction within the cell free DNA (cfDNA) population by preventing the degradation of the ctDNA as well as preventing white blood cells from lysing and releasing genomic DNA. This additional genomic cfDNA could effectively mask the ctDNA signal in the sample and hinder the ability to detect clinically relevant tumour associated mutations.

The current laboratory policy is that blood samples in Streck or CellSave preservative tubes received for ctDNA testing must be received within 96 hours from the time of sampling. After this point, samples received in the laboratory will be destroyed and a resample requested. This policy is in place to maximise the ctDNA yield from the sample and to maintain the integrity of any ctDNA in the blood sample.

The 96 hour limit is based on published information:

- ctDNA has a very short half life so the longer it is kept unprocessed, the more likely it is to degrade[4], thus affecting the yield of ctDNA from the sample.
- Rothwell et al. (2016)[1] at the CRUK Manchester Institute found that cfDNA levels were consistent between CellSave bloods processed at 4 hours and 96 hours post collection; this finding is consistent with the preservation of white blood cells and thus the maintenance of the ctDNA fraction in the blood. CTCs have also been shown to be stabilised for up to 96 hours in CellSave tubes[1-3].
- Unfortunately, the Rothwell et al (2016) paper does not measure levels of cfDNA after 96 hours and there is little published regarding ctDNA stability after 96 hours in CellSave tubes.

References:

1. Rothwell et al., Genetic profiling of tumours using both circulating free DNA and circulating tumour cells isolated from the same preserved whole blood sample, *Molecular Oncology*, 2016, 10:566-574
2. Qin et al., Stabilization of circulating tumour cells in blood using a collection device with a preservative reagent, *Cancer Cell International*, 2014, 14:23
3. Swennenhuis et al., Efficiency of whole genome amplification of single circulating tumour cells enriched by CellSearch and sorted by FACS, *Genomic Medicine*, 2013, 5:11
4. Diaz et al., Liquid Biopsies: Genotyping Circulating Tumour DNA, *Journal of Clinical Oncology*, 2014, 32:6:579-586

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