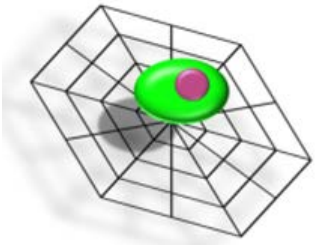


Circulating Tumor Cells TheRapeutic APheresis: a novel biotechnology enabling personalized therapy for all cancer patients

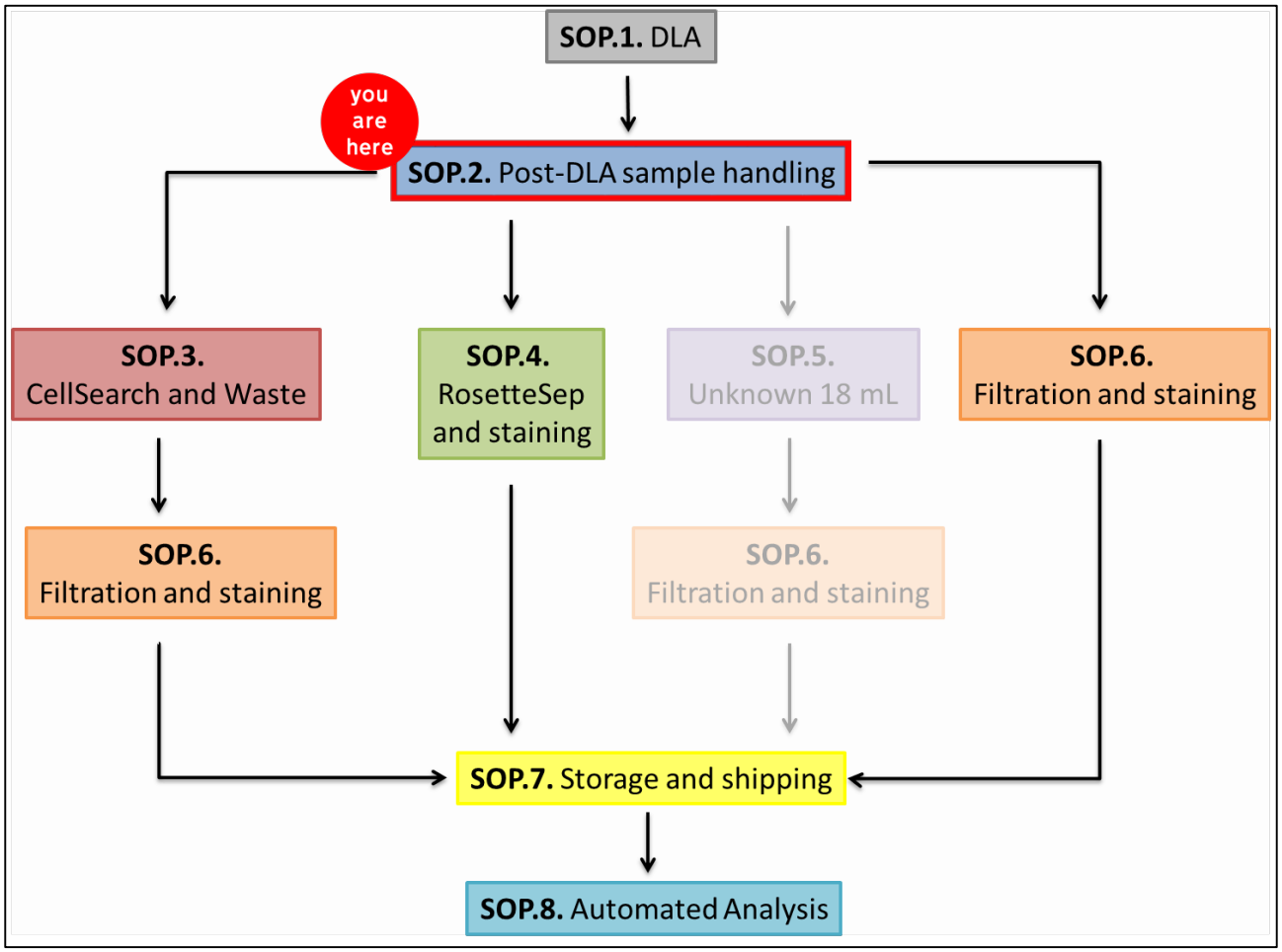
SOP.2 Post-DLA sample handling

Introduction

This Standard Operating Procedure (SOP) describes the splitting of the DLA product from the patient. These samples will then be used for CellSearch (**SOP.3.**), RosetteSep (**SOP.4.**), direct filtration (**SOP.2.**) and for shipment for CTC culturing.

This is SOP.2. Post-DLA sample handling; version 1.0-102015

Workflow of procedures in the CTCTrap program



SOP.2. Post-DLA sample handling

This **SOP.2.** describes how to proceed with the DLA product immediately after leukapheresis has finished. DLA product should be divided and treated as described below before proceeding to the different detection systems.

1. Sterile conditions

After DLA, the samples should be divided immediately into aliquots for subsequent analysis.

All Post-DLA sample handling procedures to divide the DLA product should be carried out under sterile conditions, preferable in a flow hood.

2. Cell count

Determine the white blood cell (WBC) counts and mononuclear cell (MNC) counts (differential WBC) using an automated flow-cytometric based hematology analyzer, e.g. CellDyn 3500 or Ruby, Abbott Diagnostics, Santa Clara, USA.

3. For CellSearch analysis

1. Dilute an aliquot of the DLA product containing 2 x 10^8 WBC (around 2 mL) to a final volume of 8 mL with CellSearch® Circulating Tumor Cell Kit Dilution Buffer (stored at room temperature).
2. Transfer this to a CellSave tube (containing the CellSave reagent).
3. Keep CellSave tubes with DLA sample at room temperature at least overnight, until analysis.
4. Process this sample according to **SOP.3. CellSearch and Waste**.

4. For RosetteSep

1. Transfer 18mL of the DLA product to a 50 mL tube.
2. Add CellSave reagent from two CellSave tubes for fixation and mix well (use 200 µL CellSave per 10 mL sample) .
3. Keep at room temperature at least overnight, until analysis.
4. Process this 18mL of DLA along with 2 EDTA tubes containing whole blood (20mL total) according to **SOP.4. RosetteSep and Staining**.

5. For direct filtration

1. Take 50 x 10^6 WBCs and dilute to a final volume of 7.5 mL with CellSearch® Circulating Tumor Cell Kit Dilution Buffer.
2. Transfer this to a CellSave tube (containing the CellSave reagent).
3. Keep this CellSave tube with DLA sample at room temperature at least overnight, until analysis.
4. Process this sample according to **SOP.6. Filtration staining and scanning**.

6. For CTC culturing

1. Take 2 mL DLA product and spin down at 300g for 5 minutes.
2. Remove the supernatant and add 5 mL RPMI-1640 (supplemented with 10% FBS or 10% FCS) for washing.
3. Mix well and spin down at 300g for 5 minutes.
4. Resuspend the pellet in 2.5 mL RPMI-1640, supplemented with 10% FBS or 10% FCS.
5. Transfer this to a 2.5 mL cryotube. If necessary, add medium to fill the tube completely; do not leave any air above the cells.
6. Insulate the tube by wrapping it in cotton wool or similar material.
7. Ship the cells at room temperature to the following address:

   Csaba Vizler
   Biological Research Centre, Hungarian Academy of Sciences Temesvári krt. 62.
   Szeged
   Hungary-6726

   Use one of the following identifiers:

   FEDEX: 2905-4386-0
   TNT: 507
   DHL: 412 018 761

7. Storage

Freeze the surplus of DLA material for cryopreservation as 1 mL aliquots according to local SOPs.

8. Checklist SOP.2.

Sample name	
Operator name	
Draw date DLA	Clinical site
Prep date	Clinical site

Total sample volume	
After SOP.1., was the DLA immediately processed (SOP.2.)?	☐ Yes ☐ No
During division of the sample, were sterile conditions taken into account?	☐ Yes ☐ No
WBC count	
MNC count	
For CellSearch, were 2 x 10^8 WBC used for dilution?	☐ Yes ☐ No
For CellSearch, was CellSave added?	☐ Yes ☐ No
For RosetteSep, was 18 mL used?	☐ Yes ☐ No
For RosetteSep, was CellSave added?	☐ Yes ☐ No
For direct filtration, was 50 x 10^6 WBCs used for dilution?	☐ Yes ☐ No
For direct filtration, was CellSave added?	☐ Yes ☐ No
For CTC culturing, was 2 mL processed?	
Notes:	