

Circulating Tumor Cells Therapeutic Apheresis: a novel biotechnology enabling personalized therapy for all cancer patients

SOP.3. CellSearch and Waste collection Of DLA and whole blood samples

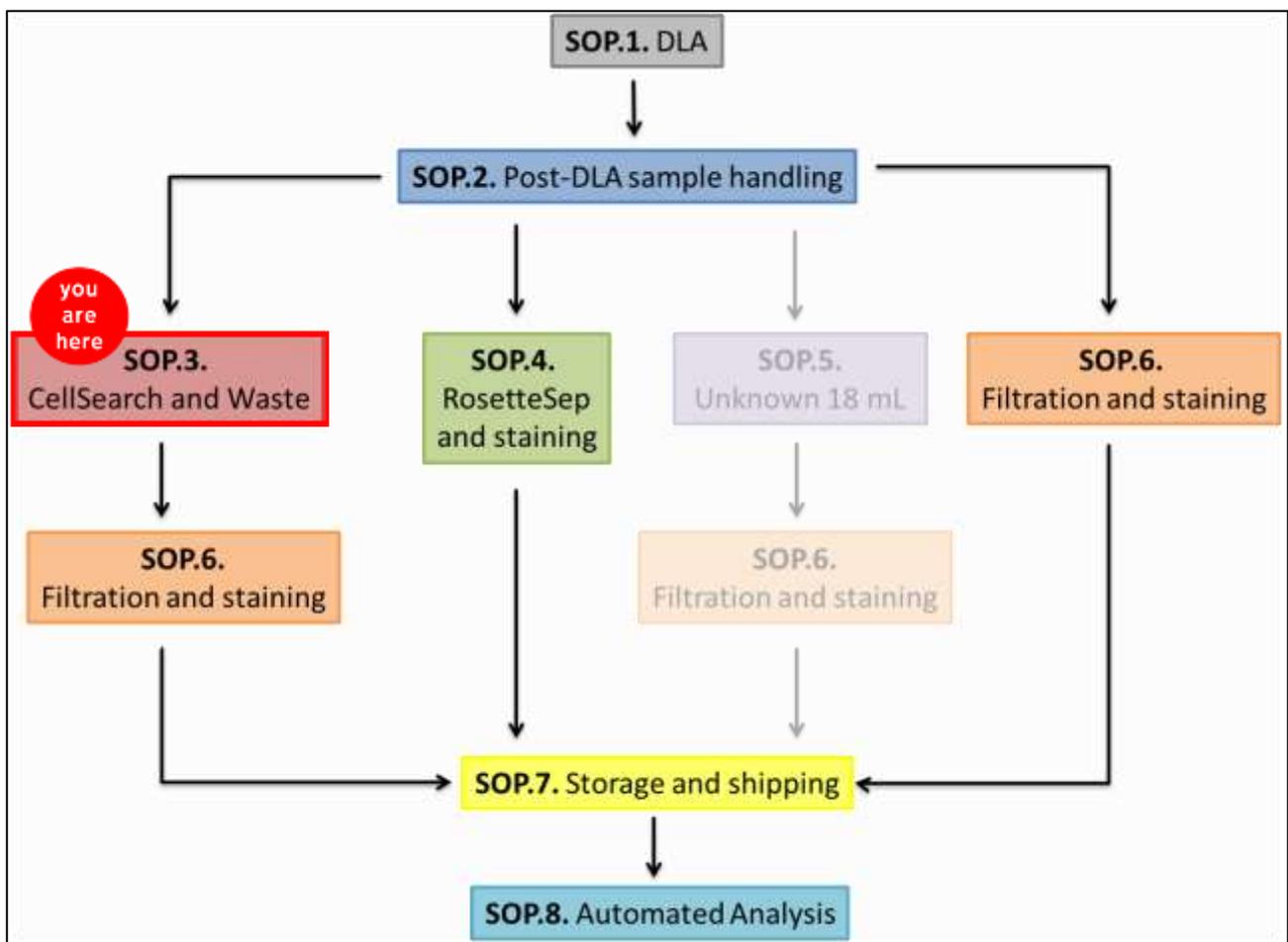


Introduction

This Standard Operating Procedure (SOP) describes the processing of samples with the CellSearch and collection of CellSearch Waste. Sample input can be a whole blood CellSave sample or DLA product (SOP.2.). This SOP will be followed by filtration of the waste (SOP.6.) and storage of cartridges (SOP.7).

This is SOP.3. CellSearch and Waste; version 1.0-092015

Workflow of procedures in the CTCTrap program



SOP.3. CellSearch and Waste collection Of DLA and whole blood samples

The CellSearch is as an FDA approved machine standard operated. The Automatic Sample Collection Device (ASCD) can be connected to the CellSearch Autoprep to collect waste, or waste can be collected manually. Both procedures are described in this SOP. Additionally, plasma will be collected from whole blood samples before loading it in the Autoprep.

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1. Plasma collection

Plasma is collected from whole blood CellSave tubes only. Not from DLA product.

1. Gently invert the blood sample stored into CellSave tubes at RT.
2. Transfer 7.5 mL blood sample from the CellSave tube to a CellSearch conical tube (preferably 24h after blood draw).
3. Spin down at 800xg for 10 minutes without using the brake.
4. Carefully collect 1-2 ml plasma without disturbing the buffy coat in a sterile 2 mL eppendorf tube. Collect the plasma up to 1 cm above the layer of red blood cells; you will leave behind approximately 1 mL plasma above the buffy coat (see Figure 1).
5. Label the eppendorf tube accordingly and store at -80°C.
6. Add the same volume as the collected plasma of CellSearch Dilution Buffer to the patient sample.
7. Continue the regular protocol of preparing the sample by adding 6.5 mL CellSearch Dilution Buffer and spinning down at 800xg for 10 minutes without using brake.

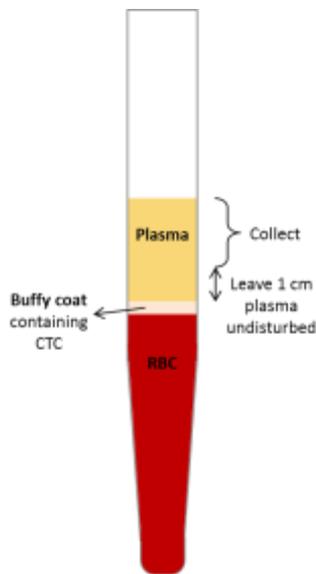


Figure 1 Plasma collection from conical tubes after centrifuging the whole blood sample.

2. CellSearch

For CellSave blood the CellSearch Autoprep can be run at the normal protocol.

For DLA product, containing $2 \cdot 10^8$ WBC, process the sample as “control” within the CellTrack Autoprep System. Proceed with CellSearch as instructed by the manufacturer.

IMPORTANT: When running automatic collection of the waste, there **always has to be placed a whole blood sample at the first sample station** in the Autoprep. The ASCD uses the red colour of the first blood sample to start collection. Based on this, it will determine the schedule of collection of all other samples, not using the red blood cells anymore for detection. When the first sample is a DLA sample, devoid of red

blood cells, the ASCD will be unable to detect the sample and will time out, thereby failing to collect all other samples in the same Autoprep run as well.

3. Waste collection

3.1 ASCD procedure

To start the ASCD, start the program on the laptop. Follow the instructions on the screen. Be careful to name the correct amount of samples when starting the ASCD, to ensure complete collection of all samples and prevent flooding of the system. If you run the CellSearch Control Cells, load these at the last position of the Autoprep to prevent cross contamination with the other collected samples.

Add 1 mL 0.2M EDTA (pH 7.6) to the 50 mL Greiner tubes that will collect samples to prevent coagulation.

Start the Autoprep first, and start the ASCD collection within 3 minutes after this. It is normal that the total volume of the samples differ from each other. This is dependent on the amount of samples loaded into the Autoprep. The waste tubes can contain some red colour, these are highly diluted red blood cells and is normal.

3.2 Manual procedure



Figure 2 Manual waste collection from the CellSearch Autoprep by unscrewing the top from the waste container (left) and collecting the sample in a 50 mL Greiner tube.

Add 1 mL 0.2M EDTA (pH 7.6) to the 50 mL Greiner tubes that will collect samples to prevent coagulation.

For manual collection of the CellSearch Autoprep Waste, unscrew the top of the waste container (see Figure 2, left). When you see the blood coming out of the Autoprep into the tubing, place a 50 mL Greiner tube under the outlet (see Figure 2, right). The waste can exit the tube at a high flow rate and therefore it is

advised to start with a larger container to get some practice. Take proper precautions. Stop collecting the sample when you see red blood cells are gone from the waste tubing and Instrument Buffer is discarded. Usually, the amount of volume collected is between 25 mL and 35 to 40 mL. Sometimes very diluted blood comes through, be careful not to keep collecting for too long and overflow the tube, or to start collecting the next sample in the previous sample tube.

3.3 Time line for sample collection

This table lists the approximate time (in minutes) of CellSearch Autoprep Waste sample exiting the waste tube and arriving at the Waste Collection Device.

Total samples in run \ Sample number	1	2	3	4	5	6	7	8
1	60.7							
2	63.6	73.9						
3	66.1	76.4	88.7					
4	68.6	78.7	91.1	102.8				
5	71.0	81.2	93.5	105.2	118.2			
6	73.4	83.7	96.1	107.8	120.8	133.7		
7	76.0	86.3	98.7	110.4	123.4	136.3	149.3	
8	78.6	88.9	101.3	113.0	126.1	139	151.9	164.9

4. Cartridges

Scan and score the cartridge according to CellSearch protocol.

For the DLA samples, scan the corresponding cartridge as “sample” on the CellTracks Analyzer by formatting the used Magnest and manually entering the sample information.

Directly after scanning the cartridge in the CellTracks, remove the cartridge from the Magnest and store this in the refrigerator (4°C) for a maximum of 4 weeks (continue in **SOP.8.**). Use careful labelling to be able to locate samples after a long period, also at different sites in the consortium. Note the CTC count and lot number of the used CTC kit on the label as well.

5. Checklist SOP.3.

Sample name	
1	5
2	6
3	7
4	8
Operator name	
Draw date	Clinical site
Prep date	Clinical site
Cartridge scan date	Clinical site
Was plasma collected?	<input type="checkbox"/> Yes <input type="checkbox"/> No
Did the CellSearch run without errors?	<input type="checkbox"/> Yes <input type="checkbox"/> No
Kit and lot number	
Was Waste collected without errors?	<input type="checkbox"/> Yes <input type="checkbox"/> No
Was EDTA added to all Waste collection containers?	<input type="checkbox"/> Yes <input type="checkbox"/> No
Volume Waste Collection	
1	5
2	6
3	7
4	8

Were the cartridges scanned and scored?	<input type="checkbox"/> Yes <input type="checkbox"/> No																				
<table border="1"> <thead> <tr> <th data-bbox="143 297 296 331">Cartridge ID</th> <th data-bbox="539 331 563 365"></th> </tr> </thead> <tbody> <tr> <td data-bbox="143 331 167 365">1</td> <td data-bbox="539 331 563 365">5</td> </tr> <tr> <td data-bbox="143 405 167 439">2</td> <td data-bbox="539 405 563 439">6</td> </tr> <tr> <td data-bbox="143 477 167 510">3</td> <td data-bbox="539 477 563 510">7</td> </tr> <tr> <td data-bbox="143 548 167 582">4</td> <td data-bbox="539 548 563 582">8</td> </tr> </tbody> </table>	Cartridge ID		1	5	2	6	3	7	4	8	<table border="1"> <thead> <tr> <th data-bbox="960 297 1086 331">CTC count</th> <th data-bbox="1217 331 1241 365"></th> </tr> </thead> <tbody> <tr> <td data-bbox="960 331 984 365">1</td> <td data-bbox="1217 331 1241 365">5</td> </tr> <tr> <td data-bbox="960 405 984 439">2</td> <td data-bbox="1217 405 1241 439">6</td> </tr> <tr> <td data-bbox="960 477 984 510">3</td> <td data-bbox="1217 477 1241 510">7</td> </tr> <tr> <td data-bbox="960 548 984 582">4</td> <td data-bbox="1217 548 1241 582">8</td> </tr> </tbody> </table>	CTC count		1	5	2	6	3	7	4	8
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Were cartridges stored at 4°C for max 4 weeks?	<input type="checkbox"/> Yes <input type="checkbox"/> No																				
Notes:																					