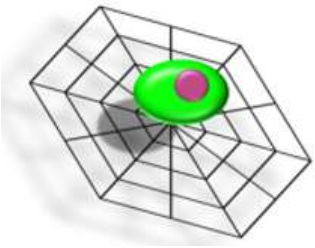



**Circulating Tumor Cells TheRapeutic APheresis: a novel biotechnology enabling personalized therapy for all cancer patients**

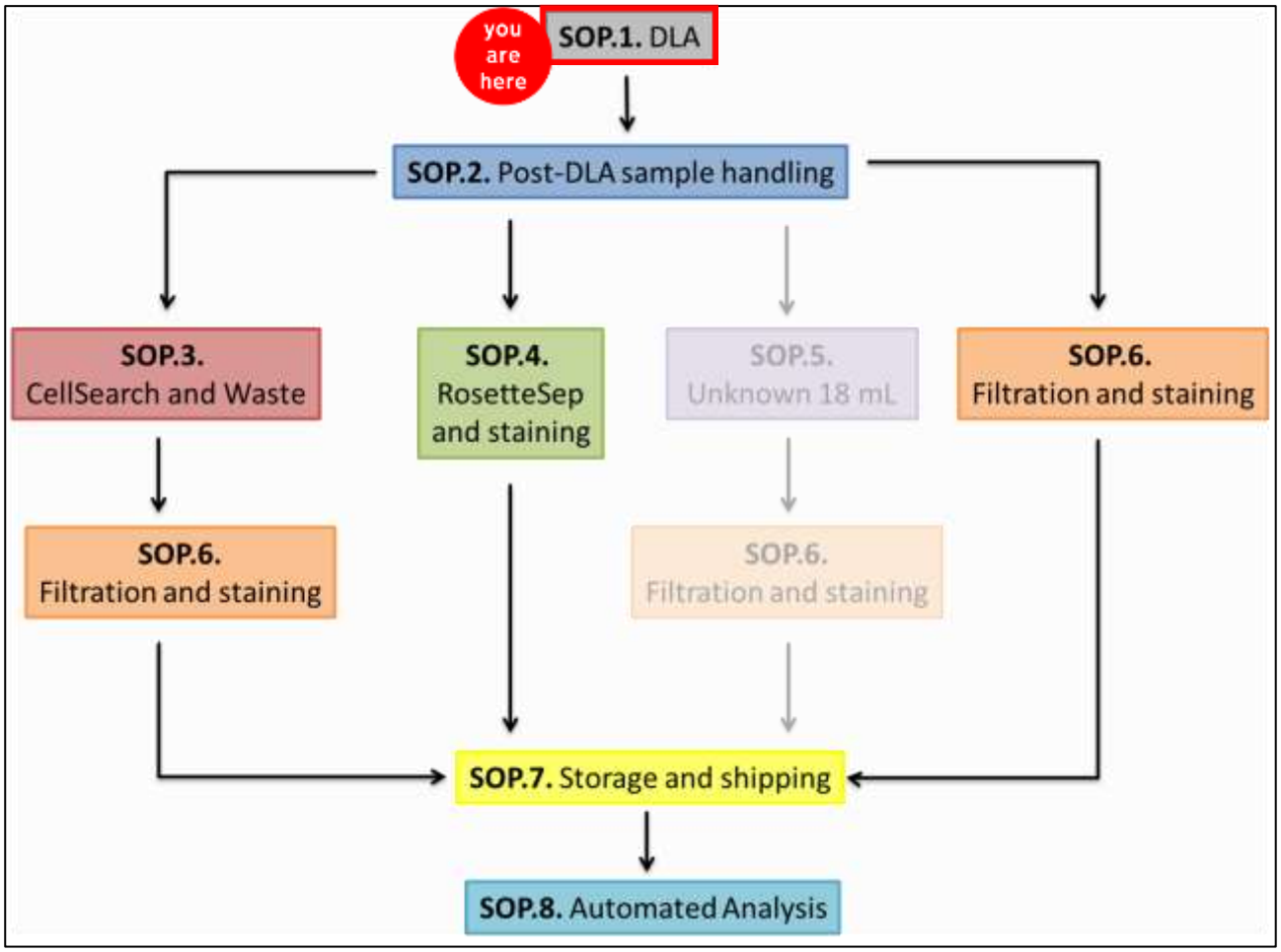
# SOP.1. Diagnostic LeukApheresis

# Introduction

This Standard Operating Procedure (SOP) describes the Diagnostic LeukApheresis (DLA) of patients in the CTCTrap Program. This SOP will be followed by **SOP.2.** in which the sample is split for different applications. This is the first protocol in the CTCTrap Program.

***This is SOP.1. Diagnostic Leukapheresis; version 1.1-102015***

## Workflow of procedures in the CTCTrap program



# SOP.1. Diagnostic LeukApheresis

# 1. Pre-DLA procedures

1. Diagnostic Leukapheresis (DLA) should be conducted in the morning to allow for same-day processing or same-day shipping, as required by protocol instructions. Schedule coordination must include the processing laboratory.
2. Check informed consent before starting the procedure and check blood count data.
3. For CTCTrap: draw one 10 mL CellSave tube (for **SOP.3.**) and draw 20 mL EDTA tubes (for **SOP.4.**).

## 1.1. Optimization of the DLA procedure

1. Instruct subjects to hydrate for 72 hours prior to the scheduled procedure, and to avoid strenuous exercise, alcohol, or caffeinated drinks for 48 hours prior to the procedure.
2. On the morning of the procedure, subjects may eat breakfast but are asked to limit intake of fluids (especially caffeinated drinks), in order to minimize bathroom needs during the procedure. In addition, subjects are also instructed to not take blood pressure medicines but to bring these with them to the apheresis center. If protocol requires a fasting collection, that sample should be obtained and time allowed for a breakfast prior to initiating the DLA procedure.
3. Subjects should wear loose-fitting clothing, especially clothing with sleeves that can be raised above the elbow.

# 2. Procedure description

- The DLA procedure will take approximately 70 min.
- Subjects will be seated in a reclining position for the duration of the procedure, with both arms resting comfortably on cushioned armrests.
- Vital signs (temperature, heart rate, blood pressure) will be obtained prior to initiation of the procedure.
- A sterile needle will be placed into a vein in one arm and remain in the arm throughout the procedure.
- A sterile plastic intravenous catheter will be placed into a vein in the other arm and remain in the arm throughout the procedure.
- Note: Any extra blood vacutainer tube collections that are to be obtained should be collected prior to the initiation of the DLA (10 mL blood in CellSave tube + 20 mL blood in EDTA tubes).
- Important: A full blood count including a differential white blood cell (WBC) count must be obtained from the subject's peripheral blood before starting DLA.

- The leukapheresis staff will then begin the DLA procedure under constant nursing supervision according to the instruction provided in 4. of this guideline. Blood will flow from the subject through the needle in one arm, through the sterilized apheresis machine, and return back to the subject through the catheter in the other arm. Blood flow will be adjusted according to blood pressure during the procedure.
- A small amount of anticoagulant (ACD-A) is used as blood is pumped out to prevent clotting. Citric acid/sodium citrate removes free calcium. If the subject experiences symptoms of numbness suggesting low calcium levels the subject should be provided with oral calcium supplementation. The collection unit may be rocked gently during the procedure to prevent clumping.
- Note: The use of heparin as an anticoagulant should be avoided if the cells will be used for molecular testing. Approval must be obtained from the protocol team if a site's blood center only uses heparin for this procedure.
- At the completion of the procedure, the needle and catheter will be removed, pressure-dressing bandages will be applied, and the subject will be checked for bleeding and stable vital signs prior to discharge.

# 3. Post- DLA procedures for patients

- Instruct subjects to rest, drink extra fluids, and eat well.
- Instruct subjects to keep the pressure dressing bandages in place for at least 3-4 hours after the procedure, to keep the sites dry and to avoid exercise or heavy lifting for the rest of the day.

# 4. Performing the DLA procedure using different Collection Equipment and Tubing Sets

Use an automated cell separator device with applicable software/firmware that is licensed and approved by the regulatory authorities (e.g. CE Mark) approval to collect mononuclear cells. A whole blood (inlet) to anticoagulant ratio (inlet: anticoagulant) of 10:1 to 12:1 is used. Follow specific guidelines in accordance with the device manufacturer's recommendation and apheresis center procedures.

| | | | |
|---|---|---|---|
| **Terumo BCT** | COBE® Spectra | Mononuclear Cell | 70600 or 777006-00: White Blood Cell set<br>70620 or 70629: Functionally Closed White Blood Cell set |
| **Fenwal Inc.** | Amicus™ | Mononuclear Cell | R4R2326: Mononuclear Cell Kit – Functionally Closed |
| **Terumo BCT** | Spectra Optia® | Mononuclear Cell | Mononuclear Cell 10110: Spectra Optia® Collection Set<br>IDL Set 10310 cMNC collection protocol |

## 4.1. COBE Spectra™ Apheresis System manual MNC Collection Procedure:

- Program the apheresis device for mononuclear cells (MNC) collection procedure.
- Set the collection flow rate to 1.0 mL per minute to avoid exceeding the required final volume of 40-50 mL.
- Set the concurrent plasma collection volume to 0 mL.

## 4.2. COBE Spectra AutoPBSC® Collection Procedure:

- Program the apheresis device to collect a minimum of 4 harvests with the following parameters:
    - Harvest volume of 4 mL
    - Chase volume of 6 mL
    - Concurrent plasma collection volume of 0 mL.
- Refer to the user manual to program the device for AutoPBSC procedure and collect the required autologous plasma and total MNC component volume
- Depending on the patient's complete blood count (CBC), the number of harvests may exceed the required minimum harvest value.

## 4.3. Amicus™ Separator:

- Program the apheresis device to collect MONONUCLEAR CELL with the following parameters:
    - 1000-1400 mL cycle volume
    - 40 mL storage plasma
    - Whole blood volume (WBV) to process (0,5 – 1 X patient TBV).
- Calculate the number of full cycles (2-3) per Amicus™ Separation MNC Collection Chart, using patient height, weight, and TBV.

# 5. Spectra Optia® Apheresis System (step-by-step):

## 5.1. Program the apheresis device for mononuclear cell (MNC) collection procedure.

- Enter a value of 0 mL for collection of plasma into the product bag.
- Enter values of 16 mL for the chamber flush and 4 mL for the chamber chase.
- Process 0.5 to 1 times the patient's total blood volume (excluding anticoagulant)

## 5.2. Performing the MNC collection

- If applicable, prior to starting the collection remove any excess priming fluid in the product bag per the manufacturer's recommendations or apheresis center procedure.
- Use Anticoagulant Citrate Dextrose Solution, formula A (ACDA) for all diagnostic cell collections

## 5.3. Selecting the Procedure

**1.** Touch Select Procedure. The procedure selection screen appears.

**2.** Touch Mononuclear Cell (MNC) Collection.

**3.** Touch Confirm. The system loads the procedure software.

## 5.4. Loading and Priming the Tubing Set

Follow the instructions in the Spectra Optia Apheresis System Essentials Guide to load, test, and prime the tubing set.

## 5.5. Entering and Confirming Patient and Procedure Data

### *5.5.1. Entering patient data*

**1.** Touch the buttons on the screen to enter the following patient information:

- Sex
- Height
- Weight
- Hematocrit (Hct)
- White Blood Cell (WBC) count
- Platelet count

The Spectra Optia system uses sex, height, and weight to calculate the patient's total blood volume (TBV).

**2.** Touch Confirm. The run values screen appears.

### *5.5.2. Reviewing and confirming run values*

1. Review the run values that appear on the screen. A black border appears around the button of the primary run target.
2. To change a value, perform the following steps:
    a. Touch the button on the screen that corresponds to the value you want to change. The data entry pad appears.
    b. Enter a new value.

If you change a value, the color of the value on the button changes from white to yellow. Values that were affected by a change appear with a yellow arrow. The arrow points up or down to indicate an increase or decrease in the value as a result of the change.

3. Enter a value for TBV processed between 0.5 and 1.
4. Enter a value for plasma volume (mL) of zero (0).
5. Touch Confirm.

## 5.6. Emptying the Saline Drip Chamber

1. Follow the instructions on the screen to perform the following steps:
    a. Empty the saline drip chamber.
    b. Rehang the saline container.
2. Touch Confirm.

## 5.7. Priming the Inlet Line and the Return Line

Be sure to perform the steps in the order indicated below and on the screen.

1. Prime the inlet line. If you are using the diversion bag to collect a blood sample, prime the inlet line to the inlet line manifold only. If you prime the line to the needle, you will dilute the sample with saline.
2. Prime the return line. If you are using a blood warmer on the return line, prime the blood warmer tubing set.
3. Clamp the inlet line and the return line.
4. Close the inlet saline line.
5. If you are not using the diversion bag, clamp and seal the line to the bag, and then remove the bag.
6. Touch Confirm. The screen appears instructing you to connect the patient.

## 5.8. Connecting the Patient and Starting the Run

1. Follow the instructions on the screen to perform the following steps:
    a. Connect the patient lines. If you are performing a peripheral venipuncture, perform the following steps to use the needle protector on the inlet needle:
        i. Prepare the venipuncture site, according to your standard operating procedure.
        ii. Position the needle protector away from the wings of the needle so it does not interfere with the venipuncture.
        iii. Grasp the wings, remove the tip protector from the needle, and perform the venipuncture.
        iv. Secure the needle tubing, according to your standard operating procedure.
    b. Unclamp the inlet line.
2. If you are performing a peripheral venipuncture and want to use the diversion bag on the inlet line, perform steps a. through e. before you proceed to step 3:
    a. Perform the venipuncture with the inlet needle.
    b. Unclamp the inlet line.
    c. Unclamp the line to the diversion bag.
    d. Allow the desired volume of blood to flow into the diversion bag.
    e. Clamp and then seal the line to the diversion bag. You may also remove the bag.
3. Touch Start Run. The system diverts the saline used to prime the tubing set to the saline container.
4. Follow the instructions on the screen to close the return saline line.
5. Touch Continue.
6. Follow the instruction on the screen to unclamp the return line.
7. Touch Continue.
8. Follow the instructions on the screen to empty the saline drip chamber and rehang the saline container.
9. Touch Continue. The system begins drawing the patient's blood into the tubing set, and the main run screen appears.

## 5.9. Monitoring the Run

1. View the information about the run that appears on the main run screen. If you want to return to the main run screen after viewing a different screen, touch the go back button, the active menu button, or the tab for the current screen.
2. From the main run screen, touch the Run menu button and perform the following steps to access additional screens:
    a. To access the collection status screen, touch the Collection Status tab.

b. To access the advanced control screen, touch the Advanced Control tab.

## 5.10. Monitoring the Patient and Adjusting the Procedure

### *5.10.1. Managing Chamber flush*

At least two cycles (two chamber flushes) should be processed. If after the first 1500 mL no flushing accorded, release chamber content to sampling bag manually (see advanced screen) after 1800 mL . The second cycle /flush should be obtained after 900 mL to 1200 mL.

### *5.10.2. Managing citrate toxicity*

1. Touch the pause button to pause the pumps. An alarm occurs, indicating the procedure was paused and the pumps were stopped.
2. Notify the attending physician of the patient's condition, according to your facility's standard operating procedures.
3. Touch the go back button to go to the main run screen, and decrease the AC infusion rate to the desired value.
4. Touch the active alarm button to return to the alarm screen, and touch Continue to resume the procedure.
5. If decreasing the AC infusion rate does not alleviate the symptoms, touch the pause button to pause the pumps again and notify the attending physician.

### *5.10.3. Changing the patient data*

1. Touch the Data menu button. The data tabs appear.
2. Touch the Patient Data tab. The patient data screen appears.
3. Adjust the data, as necessary:
   a. To adjust the Hct, touch Hct and enter the new hematocrit.
   b. To adjust the WBC count, touch WBC and enter the new count.
   c. To adjust the Platelet count, touch Platelet and enter the new count.
4. Touch Confirm to save the change.

### *5.10.4. Discontinuing the Run*

1. Touch the End Run menu button.
2. Do one of the following:
   - To discontinue the run and perform rinseback, touch the Rinseback tab and follow the instructions in Step below, starting with Step A.2.
   - To discontinue the run and skip rinseback, touch the Disconnect tab and follow the instructions in Step 10 below, starting with Step B.2.

## 5.11. Ending the Run

### *A. Extending the run*

1. Touch the button for the run target that you want to increase, and use the data entry pad to enter a new value for the target. The run values screen appears.
2. Review the run values.
3. Touch Confirm. The system will continue the run. When the new target is attained, the run targets screen appears and the system sounds a tone.

### *B. Ending the run with rinseback*

1. Touch rinseback. The screen appears instructing you to confirm your selection to perform rinseback.
2. Touch Proceed to Rinseback, and then touch Confirm.
3. Follow the instructions on the screen to clamp the inlet line.
4. Touch Continue. The system tests the pressure in the inlet line.
5. Follow the instructions on the screen to open the inlet saline line, and to clamp and then seal the lines to the plasma and collection bags.
6. Touch Continue. The screen appears that shows the status of the rinseback.
7. When rinseback is complete, follow the instructions in Step 11 below.

## 5.12. Ending the run without rinseback

1. Touch the End Run menu button.
2. Touch the Disconnect tab. The screen appears asking you to confirm your selection to disconnect the patient.
3. Touch Proceed to Disconnect, and then touch Confirm.
4. Follow the instructions in Step 11 below.

## 5.13. Completing the Procedure

1. For disconnecting the patient, follow the instructions on the screen to perform the following steps:
    a. Close the inlet saline line.
    b. Clamp the inlet line and the return line.
    c. Disconnect the patient lines. If you performed a peripheral venipuncture, perform the following steps to remove the needle with the needle protector:
        i. Release the needle tubing, according to your standard operating procedure.

ii. Prepare the dressing and place it over the venipuncture site, according to your standard operating procedure.

iii. Ensure the finger hook on the needle protector points up. Slide the needle protector forward into position under the wings of the needle.

iv. Place the index finger of one hand inside the finger hook. While maintaining appropriate pressure on the venipuncture site, pull the tubing with the other hand so that the needle slides into the needle protector.

v. Continue pulling the tubing until you hear a "click," indicating that the needle protector is locked in place. Once the needle is locked in the needle protector, release the finger hook while maintaining pressure on the venipuncture site.

vi. Dispose of the needle, according to your standard operating procedure.

d. Seal the AC line, the saline line, and the lines to the bags.

**2.** Touch Unload. The system confirms that the saline lines are closed, and the inlet and return lines are clamped. Then it raises the cassette. The procedure summary screen appears.

### 5.14. Reviewing the procedure summary data

**1.** Review the data on page 1 of the procedure summary.

**2.** Touch Next Page.

**3.** Review the data on page 2 of the procedure summary.

### 5.15. Reviewing the report

**1.** Touch the Data menu button. The data tabs appear.

**2.** Touch the Report tab. The report screen appears.

**3.** Touch Current.

**4.** Touch the arrow button to scroll to the detail pages.

**5.** Confirm that the chamber flush volume was changed to 91 mL.

**6.** Confirm that the chamber flush volume was changed to 16 mL after two collection phases occurred.

### 5.16. Removing the Tubing Set

Follow the instructions in the Spectra Optia® Apheresis System Essentials Guide to remove the tubing set.

## 6. Continue in SOP.2.

IMPORTANT: Immediately proceed to **SOP.2.** for the processing of the DLA sample for further applications.

# 7. Checklist SOP.1.

| | |
|---|---|
| Sample name | |
| Patient number | |
| Operator name | |
| Draw date | Clinical site |
| Was 10 mL blood for a CellSave tube drawn? | ☐ Yes ☐ No |
| Was 20 mL blood for EDTA tubes drawn? | ☐ Yes ☐ No |
| Volume DLA-product | |
| Concentration WBC | |
| Concentrations lymphocytes | |
| Concentrations monocytes | |
| Concentration basophils | |
| Calculation MNC | |
| Notes: | |