


STANDARD OPERATING PROCEDURE

 CEDARS-SINAI BOARD OF GOVERNORS REGENERATIVE MEDICINE INSTITUTE	INDUCED PLURIPOTENT STEM CELL CORE	PERIPHERAL BLOOD COLLECTION AND MINIMAL PROCESSING FOR REPROGRAMMING
	THE DAVID and JANET POLAK FOUNDATION STEM CELL CORE LABORATORY	

1. PURPOSE

To describe the procedure for minimal processing of whole blood samples in [BD Vacutainer® CPT™ tubes](#) and their shipment for reprogramming to Induced pluripotent Stem cells (iPSCs)

2. CPT TUBES

REF 362761 8 mL Draw Capacity (16 x 125mm tube Size)

3. SCOPE

We have established processes to isolate lymphocytes from freshly collected or commercial sources of human or mammalian peripheral blood (PB). The preferred format for the collection and shipment of such samples is the CPT tubes. This format allows the supplier to centrifuge the vacutainer(s) and separate the red blood cells from the plasma components prior to shipping. The protocol provides for the isolation of PBMCs from the plasma layer of the CPT tubes.

4. SAFETY PRECAUTIONS

This protocol involves handling human peripheral blood (PB) samples in a Biosafety Level 2 facility. The samples will be collected in Vacutainer by a trained phlebotomist, and received by the Cedars Sinai RMI iPSC Core.

Optional tests: Prior to opening the Vacutainer, several disease state tests can be performed, including testing for HIV, Hepatitis B and C, and syphilis. The latter test results should be made available within 1-3 working days from the collection date. If there is a positive result, the sample(s) will be properly discarded, and all laboratory equipment that was used to process the samples will be decontaminated. Individuals who will handle the samples should be trained on the guidelines for managing the risks of occupational exposure to bloodborne pathogens as described by OSHA in the document, [Standards-- C29 CFR; Bloodborne Pathogens 1910.1030](#).

5. PROCEDURE

COLLECTION

- 5.1 Enter the PB sample into the database of blood samples. Assign a number, using the coded sample received.
- 5.2 The BD Vacutainer® CPT™ Tube with Sodium Citrate should be at room temperature (18-25°C) and properly labeled for patient identification.
- 5.3 Spray the CPT Vacutainer lightly with 70% isopropanol/ethanol, wipe the alcohol off.
- 5.4 Collect blood into three (3) 8ml CPT tubes per sample using the standard technique for BD Vacutainer® Evacuated Blood Collection Tubes (see Venipuncture Technique & Sample Collection section and Prevention of Backflow section).
- 5.5 After collection, store tube upright at room temperature until centrifugation. **Blood samples should be centrifuged within two hours of blood collection for best results.**

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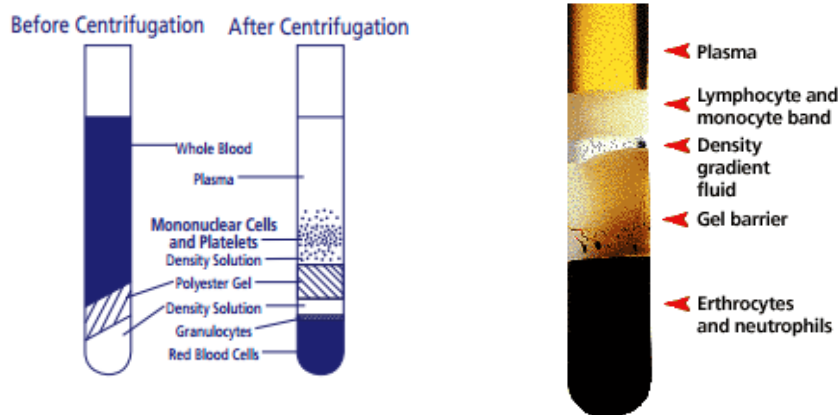
THE DAVID and JANET POLAK FOUNDATION
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PERIPHERAL BLOOD COLLECTION AND MINIMAL PROCESSING FOR REPROGRAMMING

SOP Number: SOP-WB-001

Version: B

- 5.6 Centrifuge tube/blood sample at room temperature (18-25°C) in a horizontal rotor (swing-out head) for a **minimum of 20 minutes (up to 30 minutes) at 1500 to 1800 RCF (Relative Centrifugal Force)**.



- 5.7 **For blood shipped in CPT tubes and reprogramming is performed within 24 hours:**

After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer (see Figure). An alternative procedure for recovering the separated mononuclear cells is to resuspend the cells into the plasma by inverting the unopened BD Vacutainer® CPT™ Tube gently 5 to 10 times. This is the preferred method for storing or transporting the separated sample for up to 24 hours after centrifugation.

- 5.8 After blood is collected the tubes are centrifuged and shipped to us at room temperature (**not frozen**). Use address below.

Induced Pluripotent Stem Cell (iPSC) Core

ATTN: LOREN ORNELAS

Cedars-Sinai Medical Center

8700 Beverly Boulevard

Advanced Health Sciences Pavilion, Suite A8500

Los Angeles, CA 90048


Telephone: (310) 423-7074

Email: iPSCCore@cshs.org

Director: Dhruv Sareen, PhD

Note: CPT tubes containing separated blood cells must be placed in a red biohazard bag containing an absorbent material, such as thick paper towels. All glass has the potential for breakage; therefore, precautionary measures should be taken during handling. Please follow the instructions listed under “General Packaging Requirements” below.

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Important Parameters

Temperature: Since the principle of separation depends on a density gradient, and the density of the components varies with temperature, the temperature of the system should be controlled between 18-25°C during separation.

Centrifugation: Since the principle of separation depends on the movement of formed elements in the blood through the separation media, the "RCF" should be controlled at 1500 RCF to 1800 RCF. The time of centrifugation should be a minimum of 20 minutes. (As noted in the trouble shooting section, some specimens may require up to 30 minutes for optimal separation.) Centrifugation of the BD Vacutainer® CPT™ Tube up to 30 minutes has the effect of reducing red blood cell contamination of the mononuclear cell fraction. Centrifugation beyond 30 minutes has little additional effect. The BD Vacutainer® CPT™ Tube may be re-centrifuged if the mononuclear "band" or layer is not disturbed.

Time: Blood samples should be **centrifuged or separated within two hours of blood drawing**. Red blood cell contamination in the separated mononuclear cell fraction increases with longer delays in sample separation. Mononuclear cell recovery decreases with increased time delay before centrifugation, falling to approximately 40% mononuclear cell recovery at 24 hours.

General Packaging Requirements: Regulations require that shipments containing Biological substance, Category B materials are triple packaged. The triple packaging consists of the following:

- A leak proof primary receptacle – CTP Vacutainers are an acceptable primary receptacle
- A leak proof secondary packaging – A sealable biohazard bag is an acceptable secondary receptacle
- An outer rigid packaging of adequate strength for its capacity, mass and intended use

For liquids, absorbent material in sufficient quantity to absorb the entire contents must be placed between the primary receptacle(s) and the secondary packaging.

Marking Requirements: Packages containing UN3373 materials must be clearly marked with the proper shipping name of "Biological substance, Category B" with the characters being at least 6 mm high. The UN3373 mark must be in a square on point configuration (diamond shaped) with each side being a minimum of 50 mm (or 2 inches) in length with the UN3373 characters being at least 6 mm in height.



Biological substance, Category B

Always check with your shipping company for their Biological substance, Category B shipping requirements.

FedEx Packaging Guidelines for UN3373 Shipments:

http://www.fedex.com/us/packaging/guides/UN3373_fxcom.pdf

UPS Packaging Guidelines for UN3373 Shipments:

http://www.ups.com/content/us/en/resources/ship/hazardous/biological_substances.html

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TROUBLESHOOTING

PROBLEM	POSSIBLE	CAUSE SOLUTION
Granulocyte Contamination Greater than 10%.	Centrifuge not at proper speed.	Adjust centrifuge speed to produce 1500-1800 RCF.
	Centrifuge or BD Vacutainer® CPT™ Tube not at room temperature (18-25°C).	Allow centrifuge and BD Vacutainer® CPT™ Tube to come to room temperature (18-25°C).
	Delay in centrifugation.	Centrifuge as soon as possible after obtaining blood specimen.
	Abnormal sample with high ratio of granulocytes to mononuclear cells.	Subsequent separation step using standard FICOLL™ Hypaque™ method.
Red blood cell contamination.	BD Vacutainer® CPT™ Tube or centrifuge not at room temperature (18-25°C).	Allow centrifuge or BD Vacutainer® CPT™ Tube to come to room temperature (18-25°C).
	Centrifugation time too short.	Increase time of centrifugation (up to 30 minutes).
	MCHC below normal ⁽⁸⁾ .	Increase time of centrifugation (up to 30 minutes).
Too few cells.	Leucopenia.	Collect additional BD Vacutainer® CPT™ specimens as required.
Platelet excess.	High platelet count.	Wash separated cells two times for 15 minutes at 100 RCF.
No defined or distinct mononuclear layer.	Adapter incorrect size.	Use 16 x 125mm centrifuge tube adapter.
	Centrifuge not calibrated correctly.	Have centrifuge calibrated.
	Centrifuge speed too low.	Increase centrifuge speed to produce 1500-1800 RCF.
	Centrifuge time too short.	Increase time of centrifugation (up to 30 minutes).
	Hyperlipemic Sample.	Obtain fasting blood specimen.
No gel movement.	Centrifuge speed too low.	Increase centrifuge speed to produce 1500-1800 RCF.
	Centrifuge temperature less than 18°C.	Increase centrifuge setting to 18-25°C.