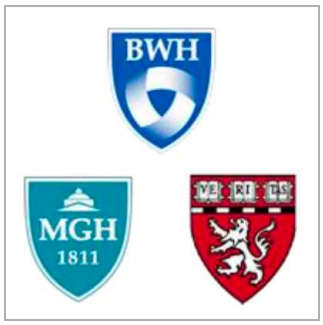

NOV 21, 2022

WORKS FOR ME 1

CPT Processing

In 1 collection

COMMENTS 0

DOI
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Daniel's workspace

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ABSTRACT

This protocol explains the Standard Operating Protocol for processing CPT.

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PROTOCOL CITATION

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COLLECTIONS

BIOSPECIMENS SOPs

KEYWORDS

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OWNERSHIP HISTORY

Feb 18, 2021	Liz Brydon	Protocols.io
May 03, 2021	Yuliya Kuras	
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Oct 03, 2022	Daniel El Kodsi	

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PARENT PROTOCOLS

Part of collection
BIOSPECIMENS SOPs

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GUIDELINES

FREEZER STORAGE

Freezers are divided into 4 shelves, with 6 racks per shelf, and 24 boxes that can be held in each shelf. In total, 576 boxes, approximately 2,160 sample sets, can be stored in one -80°C freezer. The first three shelves are designated by visit number: Shelves A1-6 (top shelf) house samples from enrollment visits, shelves B1-6 (2nd shelf) house samples from the 1st year follow-up, and shelves C1-6 (3rd shelf) house samples from the 2nd year follow-up. Shelves D1-6 contain packed red blood cell tubes (PRBC), DNA, and RNA, extracted from blood as described in the protocols above. CSF is designated between two freezers in selected racks. Freezer storage and transactions of samples are recorded in the Freezerworks Inventory software.

MATERIALS TEXT

MATERIALS:

1. DPBS Solution
2. DMSO
3. FBS (thaw in 4C before processing)
4. Nalgene CryoPreservation Tubes
5. Falcon 15 mL Conicals
6. 10 mL serological pipettes

SAFETY WARNINGS

Please refer to Safety Data Sheets (SDS) for health and environmental hazards. Gain all required consent and experimental approvals before beginning any procedures.

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Processing Protocol

1 ***Prep Steps:**
Label the appropriate number of cryopreservation vials with program and sample name, cell type, and date.
Put On ice .
Prepare before processing CPTs:

- FBS (heat shocked/heat inactivated) and put On ice to pre-cool.
- Create solution to equal [M] 10 % (v/v) DMSO in [M] 90 % (v/v) FBS (heat shocked/heat inactivated) and put On ice to precool.
- FBS is usually stored at 9 mL in -20C. The 10% DMSO/90% FBS mixture is referred to as Freeze Media.

2 The BD Vacutainer CPT Tube with Sodium Citrate should be at Room temperature (18-25°C) and properly labeled for patient identification.

3 Collect blood into the tube using the standard procedure.

4 After collection, store tube upright at room temperature until centrifugation. Blood samples should be centrifuged **within 2 hours** of blood collection for best results.

5 Centrifuge tube/blood sample at
1500 rcf, Room temperature, 00:30:00 , in a horizontal rotor (swing-out head) .

Note

1. Note: **Remix** the blood sample immediately prior to centrifugation by gently inverting the tube **8 to 10 times**. Also, check to see that the tube is in the proper centrifuge carrier/adaptor.
2. **WARNING:** Excessive centrifuge speed (over 2000 RCF) may cause tube breakage and exposure to blood and possible injury.

$$\text{RPM Speed Setting} = \sqrt{\frac{(\text{RCF}) \times (100{,}000)}{(1.12) \times (r)}}$$

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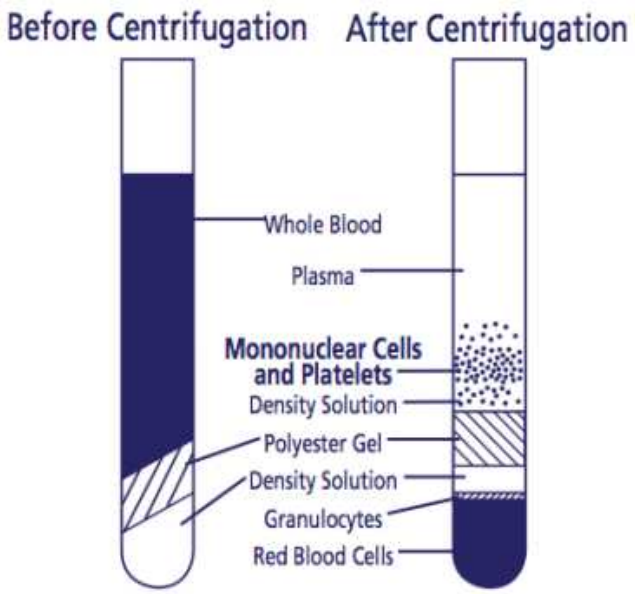
Where ***r*** (expressed in centimeters) is the radial distance from the centrifuge center post to the tube bottom, when the tube is in the horizontal position and RCF is the desired centrifugal force, 1500-1800 in this case.



6 After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer (see figure). Transfer all the plasma and cell layer into a 15 mL Falcon tube.

Note

$$\text{RPM Speed Setting} = \sqrt{\frac{(\text{RCF}) \times (100{,}000)}{(1.12) \times (r)}}$$

Where ***r*** (expressed in centimeters) is the radial distance from the centrifuge center post to the tube bottom, when the tube is in the horizontal position and RCF is the desired centrifugal force, 1500-1800 in this case.

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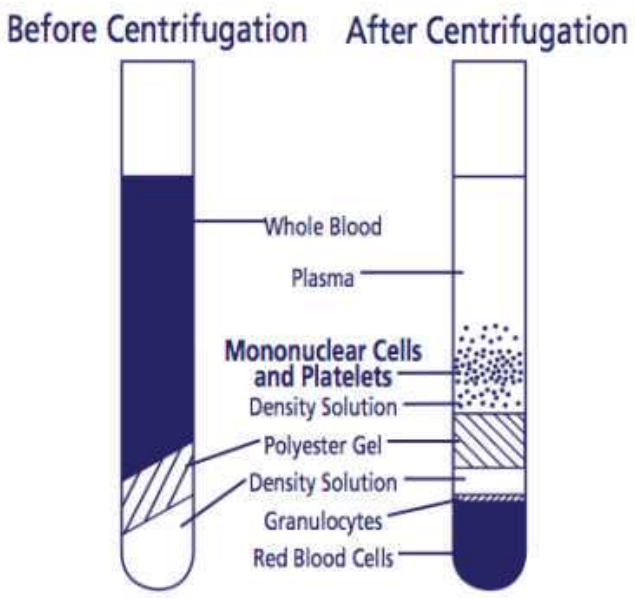

7 **Alternatively, if processing tubes within 24 hours,** resuspend cells into plasma by inverting **unopened** tube gently 5 to 10 times. The sample can be stored upright in room temperature for up to 24 hours after centrifugation. **Before collecting cells, remix tubes by gently inverting 5-8 times.** To collect the cells, pipette entire contents of tube above the gel into a 15 mL size conical centrifuge tube with a cap

Washing Protocol

8 Slowly add DPBS to bring volume to 14-15 mL (15 mL if processing within 24 hours) by tilting serological pipette tip to wall of tube. Cap tube. Mix cells by gently pipetting using serological pipette.

9 Centrifuge at 300 rcf, Room temperature, 00:15:00 . Gently decant as much supernatant as possible without disturbing cell pellet.

10 Resuspend cell pellet by gently pipetting.

11 Slowly add DPBS to bring volume to 9-10 mL (10 mL if processing within 24 hours). Cap tube. Mix cells thoroughly using serological pipette. Be careful of bubbles.

Citation: Clemens Scherzer, Bradley Hyman, Charles Jennings CPT Processing
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12 Remove 30 µL cell suspension for counting (see following section after Cryopreservation of PBMC's Protocol for Counting Cells protocol).

13 Centrifuge at 300 rcf, Room temperature, 00:15:00. Gently decant as much supernatant as possible without disturbing cell pellet. Resuspend the pellet using finger until no clumps are visible. Pelleted cells will start dying if not promptly resuspended.

Cryopreservation of PBMC's Protocol

14

Note

- *In general, 1 CPT blood tube to 2mL freezing medium. 1mL freezing medium per cryovial*
- *1 cryovial generally stores a cell concentration of 6x10^6 cells/mL.*

15 Following Step 13 under Washing Protocol, resuspend cells in 1 mL Freeze Media and gently mix to resuspend cells:
Centrifuge at 300 rcf, Room temperature, 00:15:00. Gently decant as much supernatant as possible without disturbing cell pellet. Resuspend the pellet using finger until no clumps are visible. Pelleted cells will start dying if not promptly resuspended.

16 Gently swirling tube, add drop-wise another 1 mL Freeze Media (per tube of blood) and immediately place On ice.

17 **Immediately** dispense 1 mL cell suspension per vial.

18 Place vial in Room temperature freezing container previously equilibrated to 4 °C and place **immediately** into -80 °C.

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Note

Do not snap freeze cells. Do not keep vials containing cells and freezing solution on ice for too long before they are placed in -80°C freezer. DMSO is toxic to cells so their viability will suffer if they are not frozen quickly enough. Don't freeze too many simultaneously if you lack experience.

19 After 24 hours, remove vial from both -80°C and freezing container and place in separate box. Transfer into liquid nitrogen (LN2) for long-term storage.

Note

Do not place recently removed freezing container containing vial from -80°C directly into liquid nitrogen. Ensure that freezing container equilibrates to 4°C before reuse for future storage of vials. Do this by placing freezing container in 4°C fridge for 3-4 days and set out at room temperature (18-25°C) for use. **Never reuse freezing container for storage of filled vials after it has just been removed from -80°C*

20 In designated excel file, note the date and time the blood draw was performed, the time the sample underwent the first centrifugation, the time the sample went to the -80°C freezer, and the time the sample was transferred to liquid nitrogen.

5m

Counting Cells Protocol

21 To get an equal cell distribution, mix cell suspension prior to adding stain and again just before loading hemacytometer.

22 To prepare hemacytometer, first clean hemacytometer with H_2O and then with 70% ETOH. Dry off with Kimwipe.

5m

23 *Staining cells with Trypan Blue:* In microcentrifuge tube, combine 30 µL cell suspension with 6 µL 0.4% Trypan Blue (5:1). Mix and allow dilution to incubate for 00:05:00 at Room temperature (15-30°C).

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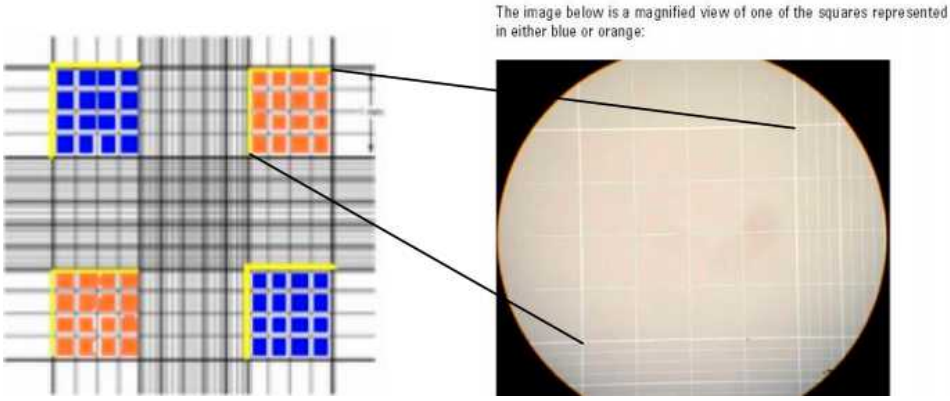
24 *Loading Hemacytometer:* Place hemacytometer on counter. Center a cover glass over the hemacytometer chambers.

25 Inject 10 µL cell dilution into one chamber. Be careful not to overfill. Allow cell suspension to settle in hemacytometer for at least 10 sec before counting.

26 *Observing and Counting Cells:* Place hemacytometer on the stage of microscope and adjust focus using 10X magnification, then change to 20X and refocus if necessary.

27 Count live cells in the four large corner squares. ***Include cells that touch either the top line or left vertical perimeter line of any corner square.*** Do not count any cells that touch either the bottom line or right vertical perimeter line of any corner square. **Blue-stained cells are dead and clear are alive.** COUNT ONLY VIABLE CELLS. It may help to use a hand-held counter if available. See figures below.



28 *Formula to Determine Cell Counts:* **Viable cells/mL = (Total # viable cells/squares counted) x 10^4 x dilution factor** (dilution factor is **1.2** since 5:1 of cell suspension:stain)

29 *To calculate total viable cells:* **Total viable cells = viable cells/mL x volume of original cell suspension in mL** (volume of original cell suspension is 9-10 mL: See Steps 11-12 under Washing Protocol)

30 To clean, rinse hemacytometer and cover slide with H_2O and 70% ETOH and wipe dry.

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31

Note

Cells should be thawed quickly but diluted slowly to remove DMSO. Cells with DMSO intercalated into their membranes are very fragile and must be pelleted and handled gently to prevent decrease in cell viability and recovery.

32 Set aside media or DPBS at Room temperature before thawing procedure to wash out DMSO.

33 Add desired amount of room temperature media or DPBS in 50 mL falcon tube.

34 When cryovial containing cells are thawed at room temperature for 1-2 min, transfer cells to 50mL falcon tube containing room temperature media or DPBS.

35 Centrifuge cells at 1500 rcf, Room temperature, 00:10:00. Decant supernatant and gently finger flick tube to break up pellet.

36 Resuspend in desired volume of media.

37 Determine cell number and viability.

38 Adjust cell volume for functional assay.

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https://dx.doi.org/10.17504/protocols.io.n92ld9w87g5b/v1