

Standard Operating Procedures (SOP) for the Preparation of Cytospin Slides of Peripheral Blood Mononuclear Cells (PBMCs) Isolated from Venous Blood

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1. Objective

The objective of this procedure is to standardize the method for preparing air-dried cytospin slides of PBMCs isolated from venous blood specimens obtained by standard phlebotomy procedures for use in LHTP assays.

2. Scope and Applicability

This SOP applies to all personnel in the Predictive Toxicology, Formulation Development, and Pharmacodynamics Assay Development & Implementation sections of the LHTP using cytospin slides of PBMCs for assay development or testing.

3. Definitions

- a. **Environment, Health and Safety (EHS):** The department at the National Cancer Institute at Frederick responsible for maintaining and leading the development of safety programs and regulations intended to achieve full compliance with all applicable federal, state, and local occupational safety and environmental laws and regulations.
- b. **PBMCs:** A collection of a subset of blood cell types including lymphocytes, monocytes, stem and progenitor cells, and platelets that is defined by the density gradient centrifugation procedure used to isolate the cells. PBMCs are defined as cells that do not sediment into buffers of density >1.077 g per cc, collecting just above the interface between physiologic fluids and buffers of that density. They can then be isolated from more dense cells in peripheral blood, such as erythrocytes and granulocytes. PBMCs are also known as “buffy coat” cells, because of the fuzzy-white visual appearance of the cells above the density interface after centrifugation.
- c. **Standard Operating Procedures (SOPs):** Detailed written descriptions of how a laboratory executes a particular procedure or method. They are intended to standardize the performance of the procedure or method.

4. Summary of Method

Venous blood is obtained by any standard phlebotomy technique from a peripheral access point, or from a central line by trained personnel if allowed by protocol, into a specialized Vacutainer tube known as a CPT (“Vacutainer Cell Preparation Tube”). The CPT is processed according to approved SOPs to isolate PBMCs, some of which will be directed to this SOP for preparation of cytospin slides for use in histochemical or immunostaining procedures.

5. Health & Safety

Safety measures that are set forth by the EHS, the Safety Plan and Procedures section of the LHTP’s Quality Assurance Plan, and any situation-specific SOPs will be followed to ensure the safety of all LHTP members. Prior to starting work under this SOP, laboratory staff should review the EHS Pathogen Program, Section C-3 at <http://home.ncifcrf.gov/ehs/uploadedFiles/c->

[3_Pathogen_Program\(1\).pdf](#). Lab staff should initial the Batch Record and enter the date of the EHS version reviewed to indicate review and understanding of parts IV.C about employee responsibilities and to confirm compliance with Pre-Operational Laboratory procedures in part V.B. Prior to starting work under this SOP, employees should also review, and indicate review and understanding by initialing the Batch Record, the EHS Chemical Exposure Hazard Assessment and Control, Section C-10 at http://home.ncifcrf.gov/ehs/uploadedFiles/C-10_Chemical_Exposure_Hazard_Assessment.pdf, and also the LHTP's Chemical Inventory to find out the Hazard Class and Level of chemicals to be used.

6. Equipment and Supplies

- Shandon Cytospin 4
- Shandon EZ Single Cytofunnel with white filter card (Ref #A78710003)
- Plasma-Lyte A pH 7.4, USP (Baxter Healthcare Corporation; NDC 0338-6317-03 or NDC 0338-6317-04)
- 15 cc BD Falcon* centrifuge tubes, polypropylene, conical-bottom, sterile (BD Biosciences Ref #352097, #352196, or #352096)
- P100 Pipetman with sterile, single-use yellow tips
- P1000 Pipetman with sterile, single-use blue tips
- Sterile, plastic single-use, graduated 3 cc Transfer Pipet (BD Ref #357575)
- Sterile individually wrapped 2.0 cc polypropylene pipet
- Batch Record template for this SOP
- Sharpie permanent marker, fine point
- Cavicide surface disinfectant
- Class II Biological Safety Cabinet

7. Procedures

- a. Record the Batch Record information and the Specimen Identifier of the PBMC isolate that was received from the processing laboratory into the cytospin slide Batch Record
- b. Record the concentration of PBMCs per cc plasma measured by the processing lab into the cytospin slide Batch Record
- c. Adjust the PBMC concentration into the optimal range of 200,000 cells per cc and record the action taken into the Batch Record:
 - i. For PBMC concentrations greater than 250,000 cells per cc, dilute the sample to 200,000 cells per cc using Plasma Lyte A USP, using a second BD Falcon 15 cc blue top tube, if necessary
 - ii. For PBMC concentrations between 200,000 – 250,000 cells per cc, do NOT alter the sample; it is already optimal for the procedure
 - iii. For PBMC concentrations less than 200,000 cells per cc, concentrate the sample using the following steps:
 - Load the 15 cc tube into a swinging bucket rotor and confirm that it will clear the rotor arms during centrifugation and that the centrifuge is appropriately balanced

- Centrifuge the sample using settings of 430 x g RCF, 18-25°C, 10 minutes, without braking
 - Using a new transfer pipet, aspirate all of the supernatant without disturbing the cell pellet and discard the supernatant into biohazardous liquid waste. (Use a different transfer pipet for each patient)
 - Using at least 0.5 cc, resuspend the cell pellet in the correct volume of Plasma-Lyte A USP to achieve a PBMC concentration of 200,000 cells per cc, by gently flicking the bottom of the tube with the index finger and then gently triturating 5 times using a 2 cc pipet. If the cell yield is too low to complete this, resuspend the available cells in 0.5 cc, record this deviation in the Batch Record, and continue
- d. Place the correct number of EZ Cytofunnels into the sealed head of the Cytospin 4 not to exceed the number approved in the clinical protocol, and make sure they are distributed evenly so the Cytospin 4 is not out of balance
 - e. Load the EZ funnels **after** they have been inserted into the sealed head of the Cytospin 4.
 - f. While agitating the PBMC suspension in the BD Falcon tube between the filling of each EZ funnel, use a transfer pipet to load exactly 0.5 cc of PBMC suspension into the sample chamber of each EZ funnel with white filters. Make sure that the sample is deposited directly into the bottom of the sample chamber and do not allow the sample to drip down the sides of the chamber
 - g. Set the Cytospin 4 for 1,000 rpm for 10 minutes, with medium acceleration, and hit "Start"
 - h. When the Cytospin 4 lid automatically unlocks at the end of the 10 minute run, remove the sealed head from the instrument and transfer it to a biological safety cabinet ("BSC")
 - i. Once placed inside the BSC, wipe the outside of the sealed head and lid with Cavicide disinfectant
 - j. Open the sealed head lid, remove the EZ Cytofunnel assemblies one at a time, disassemble, and place the microscope slide face up onto a paper towel in the BSC to air dry for 30 minutes (acceptable range 25-35 minutes)
 - k. Wipe the inside of the lid and head with Cavicide disinfectant and place back inside the Cytospin 4 unit
 - l. Apply a LHTP identifier label to each dried slide, log them in LHTP inventory
 - m. Place the slides into a plastic microscope slide box, and apply a LHTP identifier label to the box, and log the box into LHTP inventory
 - n. Place the slide box containing the slides into a Ziplock freezer bag containing an "Humidity Sponge" (indicator-desiccant pouch)
 - o. Place the box of slides into -80°C storage, and record the storage location in the Batch Record
 - p. Document ANY and ALL deviations from this SOP in the Batch Record

- q. Fax a copy of the original Batch Record to the QC Coordinators of the LHTP and the NCTVL, and file the original Batch Record into the Batch Record Binder in the laboratory of origin
 - r. Complete the procedure within 1 hour of receiving the PBMC preparation
- 8. Data/Records Management**
- a. Store the completed original Batch Record in the Batch Record Binder in the laboratory of origin, in the section labeled "PBMC Preparations"
 - b. Send a copy or fax copy of the completed Batch Record to the QC Coordinator of the LHTP and the NCTVL for filing in the QA records
 - c. A copy/fax copy of the Batch Record may be pasted into a serially-numbered laboratory notebook in the pages corresponding to the current day's procedure

HISTORY AND CHANGE CONTROL NOTES:

- (i) This SOP is similar to LHTP SOP 003.4.12, but is NOT identical and therefore should not be exchanged with the LHTP SOP.