

Laboratory for Advanced Electron and Light Optical Methods

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Preparation of Buffy Coats for Transmission Electron Microscopy

Method 1: (from Dykstra, M.J. 1993. *A manual of applied techniques for biological electron microscopy*. Plenum Press, NY)

1. Applications and Objectives: Analysis of blood samples for various types of pathology including the presence of organisms such as malarial parasites often are aided by segregating the different types of blood cells. If a subset of blood cells, such as leukocytes, is of interest, this technique concentrates each of the three major subsets of blood cells into separate bands. Thus, quicker analysis of these populations is possible than if all types were mixed together at random, as occurs if whole blood is fixed without prior centrifugation.

Method 1:

The technique produces a pellet of cells with platelets, nucleated white blood cells and red blood cells segregated into separate bands. This method is preferred with laboratory animals or sources of small blood volumes for sample preparation.

2. Materials Needed:

- EDTA blood collection tubes
- Wintrobe tubes
- McDowell and Trump's 4F:1G fixative
- molten 4% water agar
- glass microscope slide
- glass TEM sample vial
- 2-3 ml plastic syringe

3. Procedure:

- 3.1. Draw 2 ml of freshly collected whole blood into a plastic syringe.
- 3.2. Immediately inject the blood into a standard EDTA blood-collection (lavender-top) tube to prevent coagulation. Invert tube and transport to electron microscopy laboratory.
- 3.3. Fill Wintrobe tube with blood from EDTA tube. Centrifuge at low speed (250 rcf) for 5-10 min.
- 3.4. Gently remove plasma (transparent fluid) with Pasteur pipet and discard.
- 3.5. Gently pipet McDowells and Trump's 4F:1G fixative on top of cells in Wintrobe tube. Fill tube to top and refrigerate overnight (**Do Not Stir**).
- 3.6. Remove fixative with Pasteur pipet, score tube below buffy coat (pale band at top of pelleted cells) with a triangular file or diamond scribe. Wash outer surface of Wintrobe tube to remove glass dust, break tube and push buffy coat out of tube with applicator stick.
- 3.7. Place buffy coat pellet onto a glass microscope slide and carefully cut buffy coat plug lengthwise with a razor blade. Put a small pool of molten 4% water agar onto the glass slide and immediately add the longitudinally bisected buffy coat to the agar. Cover the buffy coat with more molten agar.
- 3.8. After the agar has solidified, trim around buffy coat and transfer the agar-encased buffy coat plug to a sample vial containing buffer and process as usual for any other tissue sample.
- 3.9. Flat-embed the sample so that a subsequent semi-thin section of the block face will contain a longitudinal section of the buffy coat from which the cell population of interest can be identified prior to block trimming for thin-sectioning.

4. Results Expected: Sectioning the buffy coat will reveal platelets at one end, nucleated leukocytes in the middle, and red blood cells at the other end.

5. Cautionary Statements: Be sure to collect the blood sample in an EDTA tube. If the cells coagulate, the layering of the cell populations by centrifugation will not occur. Be careful not to agitate the centrifuged blood sample during plasma removal and fixative addition to the Wintrobe tube. If agitation occurs, the layers of cells will become intermixed.

Method 2:

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Procedure to Harvest Buffy Coat Overlaid with Fixative and in Plastic Vacutainer Blood Tubes (the Method of Choice for Human Blood Samples)

- Uncap the vacutainer and gently pour the overlying fixative off into an approved container
- Using a spatula type instrument “rim” the buffy coat to separate it from the vacutainer tube wall
- As per a suggestion from Mike Toth , Huntingdon Life Sciences, NJ: Cut vacutainer tube enough below the buffy coat so as to not disturb it with a small tubing cutter (Lowes Hardware #29123, \$10.97):



- Insert a smaller glass tube or some blunt object with a flat leading surface into the vacutainer and push against the red cell layer, gently forcing the buffy coat and residual red cells out far enough to cut behind the buffy coat without damaging the sample
- Cut between the fixed buffy coat and red blood cells that were pushed out close enough to the buffy coat to leave a slight layer of RBCs. This will allow identification of the top and bottom of the fixed buffy coat sample (top light, bottom dark)
- Lay the fixed buffy coat sample flat in a tiny pool of fixative and make two cuts to produce 3 strips, each retaining a light top and darker bottom
- Place all 3 fixed buffy coat samples in 4 dram Wheaton tube and add adequate McDowell-Trump fixative to the samples (5-10 X the sample volume)
- Discard the remainder of the vacutainer and its contents as well as any cuttings of the vacutainer into an approved container for hazardous waste (if it contained human blood or potential human pathogens in other animal blood)