TITLE: SURFACE STAINING OF HUMAN CELLS

PRINCIPLES OF THE PROCEDURE:

White blood cells, originally classified on the basis of morphology, can now be classified according to the pattern of certain cell surface markers. These markers can be demonstrated by the use of monoclonal antibodies which are "tagged" by fluorochromes. These labeled cells are acquired using a flow cytometer. When analyzing the data from the flow cytometer, a cell surface marker pattern emerges which can be useful in classification, prognosis, and treatment.

SPECIMEN REQUIREMENTS:

1. BONE MARROW or WHOLE BLOOD collected in Sodium Heparin, Sodium Citrate, or EDTA tube. Keep at room temperature. Specimens have to be processed within 48 hours of the time of collection, EDTA specimens within 24 hours.
2. CSF, PLEURAL FLUID AND BRONCIAL WASHES, collected in sterile containers. Keep at room temperature. Specimens have to be processed within 6 hours of time collected.
3. **TISSUE/LYMPHNODE SPECIMENS** should be sterilely minced using a scalpel. Cut into sections from 0.01 to 0.25 cubic cm in size. They should be placed into a screw top, leak proof container with RPMI 1640 or alternatively HANKS Balanced Salt Solution.

NOTE: both should be supplemented with Gentamicin sulfate, 50ug/ml, to retard bacterial growth.
Keep specimens on ice or at 4°C. . Specimens have to be processed within 12 hours of collection. Samples with viability of less than 85% will not be reported.

ALL REQUISITIONS MUST INDICATE:

a) PATIENT NAME AND OR ID NUMBER.
b) DAY AND TIME OF COLLECTION.

CRITERIA FOR SPECIMEN REJECTION:

1. Any unlabeled specimen will be rejected. All specimens must be labeled with the patient name or number and the date drawn.
2. The requisition slip must have the time drawn; if it does not, the specimen will not be processed. Rather, we will call the appropriate individual for a time of draw. Once this is accomplished, the specimen will be processed.
3. Tissue samples which are more than 12 hours old (lymph nodes more that 12 hrs) will be rejected.
4. Viability of <85%

5. For the complete list of rejection criteria see Criteria for Sample Rejection page.

REAGENTS/SUPPLIES:

1. Ammonium Chloride Lysing Reagent. Make fresh daily. See procedure in this manual.

2. Phosphate Buffered Saline Solution PBS-Heparin and PBS. These are located on the inside door of the clinical refrigerator. (See procedure in this manual.)

3. Methanol Free Formaldehyde. 2% EM grade 10% buffered Formaldehyde (Polysciences 08379) dilute 2 parts Formaldehyde in 8 parts PBS.

4. Immunophenotyping panels. The panels in use are kept in small amber glass bottles located on the second shelf of the clinical refrigerator and labeled according to the Panel Look Up Table.

5. Mouse IgG. (Cat # CUST MPKG Caltag/Invitrogen) This is located in the white canister on the second shelf of the clinical refrigerator.

6. LIVE/DEAD® Fixable Green Dead Cell Stain Kit for Flow Cytometry (Invitrogen Molecular Probes cat # L-23101). See procedure in this manual.

EQUIPMENT & INSTRUMENTATION:

12 by 75 test tubes.

Pipettors and tips. These are located in the clinical lab on the bench, or on the shelves

Vortex

Absorbent towels for blotting

Vortex

Centrifuge

BD Falcon Cell Strainer 70um (Cat # 352350 BD)

COULTER AcT 10

PROCEDURE:

1. Perform a WBC on each sample (see Coulter Act 10 procedure in this manual).

2. Pipette 1.5 - 2.0 ml (see sample dilution chart) whole blood or bone marrow filtered* from each patient's sample into a 15 ml centrifuge tube and q. s. to 15 ml with Phosphate Buffered Saline containing heparin (1 ml/liter).

*Filter all bone marrow samples using a 75 micron mesh. (Clean the mesh with 10% bleach followed by distilled water after use.

3. Centrifuge @ 1500 x g (3200 rpm) for 3 min., aspirate supernatant carefully. Mix cell suspension well. Repeat this wash with PBS (without Heparin) and repeat centrifugation.

4. Add 67 µl (3mg/ml) normal mouse IgG for each ml of the above cell suspensions and incubate 10 min. on ice.

5. Label 12 x 75 mm plastic tubes with patient name, date and panel number (see SOP on how to make labels); put in racks and place on ice.

6. Dispense Panels, standards, and special mAbs into all appropriately labeled tubes. Place on ice.

7. To the LIVE/DEAD tube add 5ul of the 1:50 dilution working stock solution to the appropriate tubes. This solution will be placed in the Clinical Lab Refrigerator with the 4-Color Antibodies.

8. Pipette 50 µl of the appropriate cell suspension into the appropriate tubes (labeled in 3 above) containing the dispensed antibodies and controls. Rack or vortex each tube after adding cells to mAb's. Incubate on ice another 30 min.

9. Rack or vortex all tubes, two at a time, place into centrifuge adaptors, add 3.5 ml of lysing reagent, and place parafilm securely over the top of each adaptor. Invert twice. Let stand for 5 min. Centrifuge @ 1500 x g for 3 minutes (3200 rpm). Lyse a maximum of 36 tubes at a time and continue until all tubes are lysed. Remove parafilm, decant and blot each tube <u>three times</u> on a towel, rack or vortex tube(s) and return to adaptors.

10. Add 3.5 ml PBS and centrifuge as above. Decant and blot as above. Rack or vortex each tube.

11. Add between 0.3 to 0.5 ml (one drop of auto pipettor) of 2% formaldehyde to all samples, cover with parafilm, and label rack with date.

12. Store capped to prevent evaporation in refrigerator no longer than 5 days until ready to put on the Flow Cytometer.

PROCEDURAL NOTES:

INTERFERENCES: Protect stained cells from light to prevent quenching.

QUALITY CONTROL GUIDELINES:

Blood from a Healthy Donor should be stained daily (excluding weekends) to ensure all panels are working properly. As a new lot of Reagent is prepared it should be tested with that day's healthy donor or similar sample in parallel with current lot to confirm that both give comparable results.

EXPECTED VALUES:

Samples with viability less that 85% should be reported out as unacceptable.

REPORTING RESULTS & CALCULATIONS:

The results are calculated based on the % gated, % of total and the number of cells/µl.

See reporting of results.

REFERENCES:

1. Stewart, C. C. and Stewart, S. J. Cell Preparation for the Identification of Leukocytes. In: Methods of Cell Biology, Vol. 64, p. 218-70. New York: Academic Press, 2001.
2. Stewart, C. C. and Stewart, S. J. Multiparameter Data Acquisition and Analysis of Leukocytes by Flow Cytometry, Vol. 64, p. 289-312. New York: Academic Press, 2001.

TITLE: **SURFACE STAINING OF HUMAN CELLS**

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REVISED BY		sjs	sjs	aec/mbd	aec		

		6-4-97	3-18-98	7-12-4			
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APPROVED BY		Ccs	Ccs	pkw			

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