

TECHNIQUE OF LABELING LEUKOCYTES WITH In-111-OXINE
UPDATED: MARCH 2006 (Do not use, but keep in book)

CPT CODE: N/A

Technical Notes: This entire procedure is carried out in the Laminar flow hood. Ensure hood is turned on at least 30 minutes before procedure is started. All reagents, tubes, pipettes, syringes, etc. are sterile. Use only plastic (polypropylene) sterile pipettes. **LABEL ONLY ONE PATIENT AT A TIME.**

Hydroxyethyl starch should be removed from refrigerator and human serum albumin (HSA) and ACD removed from the freezer when Laminar flow hood is turned on.

Before centrifuging, be sure that tubes and metal holders are balanced.

Re-suspend pellet with gentle pipette mixing or tube inversion.

Tubes:

Tube "A" (black top)
Falcon 2045 16 x 150 mm 19 ml capacity
polystyrene, sterile, and individually wrapped

Tube "B" (white top)
Falcon 2001 17 x 100 mm 16 ml capacity
polystyrene, sterile, and individually wrapped

Tube "C" (blue top)
Falcon 2071 30 x 115 mm conical 50 ml capacity
polypropylene, sterile

Reagents:

Preparation of HSA/Saline

Pipette 0.2 ml of 25% HSA into tube "C". Bring volume up to 40 ml with saline. Label the tube "HSA/Saline."

Preparation of In-111-Oxine

Indium-111-Oxine is calibrated to be 1.0 mCi in 1.0 ml at 12:00 Noon Greenwich Mean Time on Wednesday (6:00 AM) and is used as supplied.

Preparation of Trypan Blue

A 0.5% solution should be used in this procedure, therefore when necessary, dilute the 1% stock solution to 0.5% (1:1) with normal saline and store in a small capped test tube.

Since trypan blue tends to crystallize rapidly it is important to filter the solution frequently through millipore filter paper. Store the filtrate in a capped polypropylene test tube.

Preparation of Human Serum Albumin (HSA) Aliquots

Obtain stock solution of HSA from radiopharmacy.

In Laminar flow hood aseptically inject 1-3 ml HSA into sterile vials (5 ml size). Label each vial with:

Name of Solution Lot # Date Aliquoted

Store in freezer. Discard individual vials immediately after use. Unused vials should be discarded after expiration date of stock solution has been reached.

Preparation of Hetastarch Aliquots

Aliquots of 6% hydroxyethyl starch should be prepared monthly. Obtain stock solution from radiopharmacy. Notify supervisor or radiopharmacist when there is only one stock bottle remaining.

In Laminar flow hood aseptically inject 15 ml hetastarch into 15-30 ml sterile vials. Label each vial with:

Name of Solution Lot # Date Aliquoted

Store in refrigerator. Discard individual vials immediately after use. Unused vials should be discarded one month after date aliquoted.

Preparation of Anticoagulant Aliquots

Aliquots of Citrate, Phosphate, Dextrose (CPD) solution should be prepared monthly. Obtain stock solution from radiopharmacy. Notify supervisor or radiopharmacist when there is only one bag of stock solution remaining.

In a Laminar flow hood aseptically inject about 7 ml CPD into 10 cc sterile vials. Label tube with:

Name of Solution Lot # Date Aliquoted

Store in refrigerator. Discard individual tubes immediately after use. Unused tubes should be discarded one month after date aliquoted.

Harvesting Procedure:

Use Laminar flow hood.

1. Withdraw 40 ml whole blood from the patient into a 50-60 ml syringe containing 6 ml CPD anticoagulant (1.5 ml to 10 ml whole blood). An 18 gauge needle should be used. Total volume blood and anticoagulant should be 46 ml.
2. Aliquot the blood equally into each of 4 "A" tubes (approximately 12 ml in each tube). It is imperative that the "contaminated" syringe does not touch the inner walls of the sterile tubes during the transfer.
3. Add 3 ml of 6% hydroxyethyl starch, at room temperature, to each of the 4 tubes. Total volume should now be approximately 15 ml per tube.
4. Mix well by inverting the tubes several times. Remove caps, wipe away excess blood from inside of tube and cap using a sterile cotton tipped swab. Replace cap. Allow the sample to sediment for 60 minutes at a 45 degree angle in predrilled wooden block.
5. After the sedimentation period, use a sterile plastic pipette to transfer the leukocyte-rich plasma into 4 "B" tubes. Care must be taken not to include RBC's. Each "B" tube should have a volume of approximately 7 ml.
6. Centrifuge the "B" tubes at 150 x G for 8 minutes. Pour or pipette off the supernatant plasma and discard. The plasma should be cloudy due to the presence of platelets. A pellet or button of white cells should remain at the bottom of the tube. This pellet may be pink if there is RBC contamination.
7. Holding the test tube by the top, tap the bottom of the tube to begin loosening the pellet. Re-suspend the leukocyte pellets by gentle agitation with 2 ml of HSA/Saline (at room temperature). Avoid vigorous agitation.
8. Combine the re-suspended pellets into one of the original "B" tubes. Bring the volume up to 10 ml by adding HSA/Saline. Gently invert the tube several times to ensure uniform mixing.

9. Centrifuge at 150 x G for 8 minutes. Pour or pipette off and discard the supernatant. Re-suspend the leukocyte pellet in 2 ml of HSA/Saline. Gently mix the suspension and bring up to 10 ml with HSA/Saline.
10. Centrifuge at 150 x G for 8 minutes (925 rpm on IEC, ~ 40 on IEC setting). Pour or pipette off and discard supernatant.
11. Re-suspend the leukocyte pellet in 5 ml 0.9% saline WITHOUT HSA.

Labeling:

Start with 600 µCi Indium-111-Oxine.

1. Run Indium-111-Oxine down the side of tube with leukocyte suspension. Rinse syringe with saline several times. Incubate at room temperature for 30 minutes on test tube rotator to ensure optimum labeling.
2. Centrifuge the labeled cells at 150 x G for 8 minutes (setting at ~ 40 for 1000 rpm). Pour off the supernatant and save in a "B" tube (to maintain identical geometry when checking for % bound).
3. Re-suspend the pellet in 5 ml 0.9% saline. Be sure the suspension of WBC's is uniform with no gross clumps visible. If there are gross clumps, check with Nuclear Medicine staff physician before proceeding.

**QC, Re-injection,
& Viability:**

1. Assay the 5 ml supernatant in the "B" tube in dose calibrator.

Assay the 5 ml re-suspended WBC's in "B" tube in dose calibrator. Calculate the % bound.

$$\% \text{ bound} = \frac{\mu\text{Ci in WBC's}}{\mu\text{Ci in WBC's} + \mu\text{Ci in supernatant}} \times 100\%$$

Record % bound on request form. **If 90% tag is not achieved, check with Nuclear Medicine physician before injecting cells.**

If there is a question of cell viability, do step 2. If not, proceed to step 3.

2. Using sterile technique, remove 0.2 ml of the cell suspension using a tuberculin syringe. In a 12 x 75 mm test tube prepare a 5:1 suspension of cells to trypan blue. Mix well and incubate for 30 seconds. Transfer 20 microliters or less of the stained cell suspension to a hemocytometer and check for viability of cells.

Record results on request form.

3. In the Laminar flow hood, using a 20 gauge spinal needle, draw the entire contents of the tube containing the re-suspended WBC's into a 20 ml syringe and assay the radioactivity in the dose calibrator. Maximum dose for reinjection is 500 µCi.

Reading from dose calibrator must be corrected as discussed in radiopharmacy procedures.

4. The labeled cells are now ready to be re-injected into the patient. **Re-injection should be accomplished within one hour of completion of the labeling procedure.**

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