Practical Platelet Labeling with Indium-111-Oxine

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Interest in platelet scanning with ^{111}Indium-oxine labeled platelets is increasing. Although many methods are available, critical details of platelet labeling and quality control are often omitted. We describe a detailed technique that should be helpful to those unfamiliar with the special requirements of platelet labeling. The method requires ~ 90 min, uses common reagents, and yields a viable population of platelets with labeling efficiencies in excess of 95%.

Since the introduction of ^{111}In-oxine labeled blood products by McAfee and Thakur in 1976 (*1*), the use of labeled leukocytes has increased tremendously. The use of labeled platelets has not been widespread, perhaps because of the difficulties inherent in the handling of platelets. Furthermore, there are multiple methods described in the literature with proponents claiming improvements in labeling efficiency, labeling time, or cellular viability. Undoubtedly, many of the published methods work well in the hands of the originators, but they may be difficult to implement by others, particularly those unaccustomed to the special requirements of platelet handling. Critical details of platelet labeling and quality control are often left out of published reports, leading to frustration on the part of the beginning platelet labeler. We present a simple, effective platelet labeling procedure adapted from the work of Heyns, et al. (*2*). The procedure requires only common reagents and includes a complete description of the technique. This method yields a viable population of platelets with labeling efficiencies of > 95%. The platelet handling techniques presented should prove useful even if a different labeling method is to be employed.

GENERAL CONSIDERATIONS

Platelet separation and labeling is easily confounded by the tendency for the platelets to remain irreversibly aggregated in a pellet after centrifugation. This problem can be minimized by the following procedures:

1. Withdraw the blood directly from a vein using a 21-gauge or larger needle. (Do not use an i.v. line, heparin lock, portacath, etc. for either drawing blood or injecting labeled platelets.)
2. Discard the first 5–7 cc of blood withdrawn, or use it for a peripheral platelet count. Contact with the damaged endothelium and metal needle can activate the platelets and cause clumping.
3. The blood and subsequent platelet concentrate should not come in contact with either glass or metal. Sterile polypropylene tubes should be used as centrifugation vessels. Plastic pipettes or intravenous catheters (minus the needles) should be used when pipetting the cells.
4. Prolonged exposure to elevated temperatures (i.e., 37° C) is to be avoided. Operations are best performed at room temperature except for incubation with the ^{111}In-oxine. Labeling efficiency is improved and the procedure time reduced if a short incubation is carried out at 37° C (*3*).
5. The technique, which follows, provides the optimal pH range of 5.5–6.5. Other techniques may not, and this factor should not be taken for granted. Spontaneous aggregation will occur at higher pH levels. Frothing of the cell suspension during attempts to resuspend the platelet pellet will raise the pH and thus further inhibit the resuspension of the cells. Check the pH of the solutions during practice labeling sessions to ensure that the pH is correct; subsequent measurements can then be dispersed.
6. The rest periods described in this technique are essential to the procedure and must be included if complete platelet resuspension is to be achieved after centrifugation.

MATERIALS AND METHODS

All of the procedures should be performed in a laminar flow hood with sterile technique. The pH of the blood, plasma, and wash solutions should be maintained between 5.5 and 6.5. The following equipment is used in the procedures:

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Tourniquet	Ringstand and clamps
Alcohol wipes	5 cc 0.125 N NaOH
Sterile gloves	20 μL 0.0575 M $CaCl_2$
(2) 20-gauge or larger sterile needles	600 μCi ^{111}In oxine
	Balance
3-way stopcock/IV extension	Centrifuge
50-cc syringe	(5) sterile polypropylene centrifuge tubes
10 cc sterile normal saline	
Test tube rack	(1) 30-cc syringe
(2) 10-cc syringes	(4) sterile plastic pipettes
20 cc ACD-A solution*	

Platelet labeling is carried out as follows:

1. Flood laminar flow hood with alcohol, and allow it to evaporate. Set up water bath at 37° with lead shield.
2. Put 10 cc of sterile normal saline in a sterile syringe and place it in the hood. Draw 8 cc of acid-citrate dextrose solution A (ACD-A) into the 50-cc syringe.
3. Assemble stopcock, 50-cc syringe, one 30-cc syringe, tubing, and needle. Withdraw atraumatically and aseptically 25 cc (only 7 cc if no aggregation studies are to be performed) of blood into the 30-cc syringe and send for the appropriate laboratory tests. Withdraw 42 cc of blood into the 50-cc syringe, mixing the blood with the anticoagulant.
4. Transfer the blood to a sterile centrifuge tube directly from the syringe. Prepare a balance tube and centrifuge at 225 G for 15 min.
5. Pipette platelet rich plasma (PRP) into a new centrifuge tube. If there is no clear separation between the PRP and red cells, risk using a few red blood cells (RBCs) and perform steps 6 and 7. Add 5 cc ACD-A solution/100 cc of PRP.
   Steps 6 and 7 are optional. Perform only if significant RBC contamination of the PRP is present.
6. Prepare balance tube and spin PRP at 225 G for 10 min.
7. Using a sterile plastic pipette, carefully withdraw the PRP into a new centrifuge tube. Take care not to transfer any RBCs.
8. Prepare the balance tube and centrifuge the PRP at 650 G for 10 min.
9. Separate the platelet poor plasma (PPP) with a new sterile plastic pipette and store in a sterile centrifuge tube at room temperature.
10. Add 2 cc of saline solution to the pellet. Place the tube aside and let stand at room temperature for 10 min. Thereafter the button may be agitated *gently,* without frothing, to completely resuspend the cells.
11. When the pellet is completely resuspended, place the tube in the water bath, add 600 μCi of ^{111}In-oxine (800–850 μCi if platelet aggregation studies are to be performed on the labeled cells), and incubate at 37° C for an additional 5 min.
12. Prepare a balance tube and centrifuge the resuspended platelets at 180 G for 15 min. (Note: Although this gentle spin may not completely remove all platelets from suspension, it does allow rapid resuspension of the platelet button. If complete platelet separation is desired, this spin should be accomplished at 225 G.)
13. Decant radioactive supernatant (the empty balance tube may be used for this). Gently layer 2 cc of saline solution over the pellet and decant to the same tube used previously. Add 6 cc of PPP to the pellet as soon as possible, and let stand for at least 10 min at room temperature. Thereafter, the pellet may be agitated *gently,* without frothing, to resuspend the cells.
14. Compute labeling efficiency based on: 100 × (activity of resuspended platelets) / (platelet activity + ACD-A supernatant activity). Dispose of the radioactive supernatant properly.
15. When the platelets are completely resuspended, 2 cc of radioactive PRP and 1 cc of nonradioactive PPP may be removed for platelet function studies.
16. Draw the labeled PRP into a syringe through an 18-gauge angiocath. Remove plastic catheter and replace with a 21-gauge or larger needle for reinjection. Measure the syringe in the dose calibrator and aseptically inject the PRP directly into a vein. *(Do not use i.v. tubing.)*
17. If aggregation studies are being performed, adjust the pH of the remaining 2 cc of PRP and 1 cc of PPP to 7.45–7.50 using 0.125 N NaOH. Transport to the special coagulation laboratory as soon as possible thereafter. Studies to be performed are a manual platelet count and assessment of aggregation in response to adenosine diphosphate (ADP) and collagen stimuli.

Immediately prior to the addition of ADP and collagen, the calcium concentration should be adjusted to 1.25 M by the addition of 10 μL of a solution containing 319 mg CaCl in 50 cc of water.

QUALITY CONTROL

A large variety of quality control evaluations are possible. We recommend that as many as possible be employed when first developing a technique for labeling. Once each laboratory has confirmed its ability to label viable platelets a less time-consuming approach may be utilized.

Quality control can be determined in three ways: assessment of platelet harvest and cellular purity, assessment of labeling efficiency, and assessment of platelet viability and function.

Percent harvest reflects the ability of the procedure to provide complete extraction of the platelets from the whole blood. Additionally, knowledge of the total number of platelets in the PRP may provide insight into an unexpectedly low labeling efficiency. Approximately 10^9 platelets are required to achieve optimum labeling efficiencies.

Platelet harvest is expressed as the number of platelets in the PRP relative to the total number of platelets available in the sample of whole blood. A manual platelet count should be obtained on a sample of whole blood and on the PRP. The percent harvest is calculated as follows:

$$\%\text{Harvest} = 100(\text{Plt ct (PRP)} \times \text{cc (PRP)} / \text{Plt ct (WB)} \times \text{cc (WB)})$$

Cellular purity is evaluated by performing a differential cell

count on the purified cell suspension. Contamination with red and white cells should be minimal.

Labeling efficiency is the percent of cell-associated ^{111}In activity relative to the total ^{111}In activity available. This step is built into the technique described above and will not be discussed further.

In vitro aggregation studies assess the ability of platelets to aggregate in response to various stimuli such as ADP, epinephrine, and collagen. Samples of whole blood as well as labeled platelet suspension should be provided to the hematology laboratory. The aggregation response of the labeled cells is reported as a percentage of control response. This prevents confusion as to whether the labeling process affected the function or whether the patient may have had underlying platelet function abnormalities. An aggregation response of at least 60% of the control should be obtained. In vitro procedures are time consuming, and after experiments have proved the validity of the technique, injection of the labeled cells should not be delayed to perform them. The extra delay may have deleterious effects on platelet function.

It is important to adjust the pH and Ca^{++} concentration of the aliquot of labeled cells that are to be used for in vitro aggregation studies just prior to testing (*4*). Failure to do so will lead to erroneous conclusions about the effects of labeling on platelet function. The pH and Ca^{++} concentration of the cells that are injected will be corrected in vivo.

Electron microscopy of the labeled cells can be performed to assess cellular shape changes associated with platelet activation. Activated platelets will show pseudopod formations whereas "resting" platelets are discoid in shape.

Perhaps more important than the in vitro testing is an assessment of in vivo survival after injection. Platelet survival determinations are based on standard methods and require multiple blood samples over several days. The finding of normal platelet survival confirms minimal platelet damage by the labeling procedure. Survival determinations are described elsewhere and will not be discussed in detail here (*5,6*).

Finally, if patients undergoing platelet scanning have had a recent arteriotomy or other vascular trauma, an image of the affected vessel should be obtained. Platelet deposition at a site of known vascular damage confirms in vivo function if the patient is not actively bleeding at the time of the study.

CONCLUSION

The method described has been in use for over a year at this institution, and has been used successfully at other institutions as well (Kasi L and Hightower D, personal communications). Labeling takes ~ 90 min, and has been taught to our radiology residents as well as our pharmacy technician. The procedures described are essential to platelet separation and labeling regardless of the actual method used, and should prove useful to those interested in implementing a platelet scanning protocol.

FOOTNOTE

*Acid-citrate-dextrose solution A (ACD-A), E.R. Squibbs, New Brunswick, NJ.

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Answer Sheet to Self-Assessment Quiz

1. a	6. b	11. b	15. c	19. d
2. b	7. b	12. b	16. d	20. b
3. b	8. a	13. d	17. d	21. c
4. a	9. b	14. c	18. b	22. d
5. a	10. b			