Rapid and Simple Methods for Labeling White Blood Cells and Platelets with Indium-111-Oxine

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Simple procedures in a kit form for labeling white blood cells (WBCs) and platelets with commercially available indium-III (^{111}In)-oxine have been developed for the convenience of small community hospitals. The time required for the labeling procedure is less than 2 hr. The resulting scintigrams from the clinical studies in both WBCs and platelets showed that the ^{111}In-labeled cells have a high degree of viability.

Indium-111-oxine for labeling leukocytes was first introduced in 1976 by McAfee and Thakur (*1*). Since then many modifications of the procedures for labeling leukocytes (*2,3*) and platelets (*2,4,5*) have been introduced. Some new ^{111}In chelating agents—tropolone (*6*), acetyl acetone (*7*), and merc (*8,9*)—have also been successfully used for leukocyte and platelet labeling. Because of its availability and approved status as a radiopharmaceutical, ^{111}In-oxine has been the choice of many institutions.

Since the introduction of ^{111}In-oxine for labeling leukocytes and platelets, several investigators have successfully applied these techniques in various clinical settings. The clinical diagnosis of inflammatory conditions such as abscesses (*10,11*), occult infection (*12*), bowel infarction (*13*), and skeletal photopenic lesions (*14*) have been reported in literature using ^{111}In-labeled leukocytes. Similarly, ^{111}In-labeled platelets have been used successfully for imaging the sites of vascular thrombi (*15*), possible occlusion of grafts due to platelet deposition (*16*), pulmonary emboli (*17*), and evaluation of transplanted kidney rejection (*18,19*). Because it is useful in many clinical applications, ^{111}In-oxine has become a valuable radiopharmaceutical in diagnostic nuclear medicine.

We have developed a simple and fairly rapid procedure for both leukocyte and platelet labeling using commercially available ^{111}In-oxine.

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MATERIALS AND METHODS

The necessary laboratory equipment required for cell labeling includes a refrigerated variable speed centrifuge, a pH meter, a radioisotope dose calibrator, a laminar flow hood, sterile polypropylene centrifuge tubes, and sterile transfer pipettes.

The chemical reagents and radiotracer required for cell labeling include ^{111}In-oxine*, sterile isotonic saline, anticoagulant-citrate-dextrose solution (ACD) modified, sterile water, and sodium hydroxide.

Sodium hydroxide solution was prepared by weighing out 4 g of sodium hydroxide and dissolving it in 100 ml of sterile water, then filtering through a 0.22 μm millipore membrane filter (sterile) into 10-ml sterile vials. This gave a 1 N sodium hydroxide sterile solution. Ten milliliters of 1 N sodium hydroxide was diluted to 100 ml with sterile water to prepare 0.1 N sodium hydroxide, which was stored in 10-ml sterile vials. These two concentrations are helpful in adjusting the buffers.

The ACD-saline kit was prepared by mixing ACD solution with sterile isotonic saline in 1:7 proportions, i.e., 36 ml of ACD solution was mixed with 250 ml of isotonic saline and the pH was adjusted to 6.3 using both 1 N and 0.1 N sodium hydroxide. The solution was filtered through a 0.22 μm millipore membrane filter and 10-ml aliquots were transferred to sterile vials. The vials were stored in the refrigerator.

Sodium chloride 5% solution was made by dissolving 5 g of sodium chloride in 100 ml of sterile water and filtering the solution through a 0.22 μm millipore membrane filter. The solution was transferred to 10-ml sterile vials and stored in the refrigerator.

Indium-111-oxine* is shipped in 1 ml HEPES buffer, and generally the pH is in the range of 7. For labeling white blood cells, 3 ml of sterile isotonic saline (0.9% sodium chloride, pH 6.0) was added to the vial and the pH adjusted itself close to 7. Generally no pH adjustments were needed. Platelet labeling requires ^{111}In-oxine tracer in a citrate medium. Three milliliters of ACD-saline, pH 6.3, were added to the vial containing ^{111}In-oxine. The pH adjusted itself to 6.5 and usually

no further pH adjustments were necessary. If needed, the pH adjustment was made with 0.1 N sodium hydroxide.

Indium-111 Leukocyte Labeling Procedure

All procedures described were performed in a laminar flow hood using sterile reagents and labware.

1. Set the centrifuge temperature to 20–25 °C.
2. Take 7 ml of ACD[+] in 60-ml syringe with an 18 gauge needle and draw blood to 40–60 ml. Tilt the syringe a few times to mix.
3. Take two 50-ml polypropylene centrifuge tubes, add 10 ml hydroxyethyl starch (6% in saline) to each tube (total 20 ml). Transfer the whole blood in equal volumes into both tubes and mix with the transfer pipette.
4. Sediment for 30–45 min at a 45° angle.
5. Transfer the supernate leukocyte rich plasma (LRP) into 15-ml or 50-ml polypropylene tubes and centrifuge for 8 min at 150 g.
6. Transfer the supernate, platelet rich plasma (PRP); save for future use. Leave the white blood cell button in the bottom.
7. To the WBC button, add 9 ml of sterile water for injection and suspend the cells with the transfer pipette for 15–20 sec. Immediately add 2 ml of 5% sodium chloride and mix gently. Add another 10–15 ml of isotonic saline and centrifuge for 8 min at 150 g.
8. Remove the supernate carefully and discard it. To the WBC button, first add 1 ml of ^{111}In-oxine, suspend the cells; then add the remaining 3 ml ^{111}In-oxine and mix well. The total ^{111}In-oxine radioactivity is maintained between 600–800 μCi. Incubate for 15 min at room temperature. Agitate two or three times during the incubation period. Measure the radioactivity and note on the log sheet.
9. Centrifuge the PRP from step 6 at 1200–1500 g for 10 min. Separate the platelet poor plasma (PPP) and save for future use. This is done during incubation to save time.
10. After a 15-min incubation period (step 8), centrifuge for 8 min at 150 g, remove the supernate, measure the radioactivity, and note on the log sheet.
11. Suspend the WBC in 4 ml of PPP and centrifuge for 8 min at 150 g.
12. Remove the supernate, measure the radioactivity, and record on the log sheet.
13. Suspend the WBC in 4–5 ml of PPP and measure the radioactivity.
14. Draw the dose into a syringe with an 18 or 19 gauge needle. Adjust the dose to 500 μCi or less and inject the patient as soon as possible. Enter the dose on the log sheet.

Indium-111 Platelet Labeling Procedure

1. Set the centrifuge temperature at 20–25 °C.
2. Take 7 ml of ACD into a 60-ml syringe with an 18 gauge needle and draw blood to 40–50 ml. Tilt the syringe a few times and mix gently.
3. Transfer the blood to two 50-ml or four 15-ml polypropylene centrifuge tubes and centrifuge for 10 min at 180 g.
4. Remove PRP and transfer to either 15- or 50-ml tubes. Discard red blood cells.
5. Centrifuge PRP for 3 min at 1000–1200 g and transfer PPP into 15- or 50-ml tubes; save for later use.
6. Remove all the plasma from the platelet button and incubate with 600–800 μCi of ^{111}In-oxine in ACD-saline, pH 6.5, for 15 min at room temperature. To obtain best labeling efficiency, add 1 ml of ^{111}In-oxine to the platelet button, suspend the platelets; then add the rest of the same and mix the radioactivity-cell mixture gently for a few seconds. Measure the radioactivity in a dose calibrator and note on the log sheet.
7. Centrifuge for 8 min at 1000–1200 g. Remove the supernate, measure the activity, and record on the log sheet.
8. Suspend the platelets in 4 ml of PPP and centrifuge for 8 min at 1000–1200 g. Remove the supernate, measure the radioactivity, and note on the log sheet.
9. Suspend platelets in 4–5 ml PPP and draw a dose of 500 μCi or less, and record on the log sheet.

RESULTS AND DISCUSSION

Schematic representation of separation and labeling procedures for both ^{111}In-labeled WBCs and platelets are shown in figures 1 and 2 as described. The procedures described in this study are carried out with relative ease and the time required for the labeling procedure in both cases was approximately 2 hr or less, depending on the familiarity with the procedures and laboratory skills. The labeling efficiencies of WBCs and platelets were on the order of 75%–80% and 70%–75%, respectively. The amounts of radioactivity with cells initially, with supernates, and finally the amount with the dose are recorded in a log sheet shown in Table 1.

Some minor improvements are necessary in some cases to achieve better labeling yields. The presence of traces of plasma

TABLE 1. Indium-111-Labeled White Blood Cells for Clinical Studies

Patient name	Date	
Patient no.	Tech.	
	Rad. Pharm.	
Initial radioactivity		μCi
Radioactivity in supernate 1		
Radioactivity in supernate 2		
Final radioactivity with WBCs		
Labeling efficiency (%)		
Dose in syringe	Volume	
Radioactivity in syringe after injection		
Injected dose		
Comments		

WBC, white blood cell.

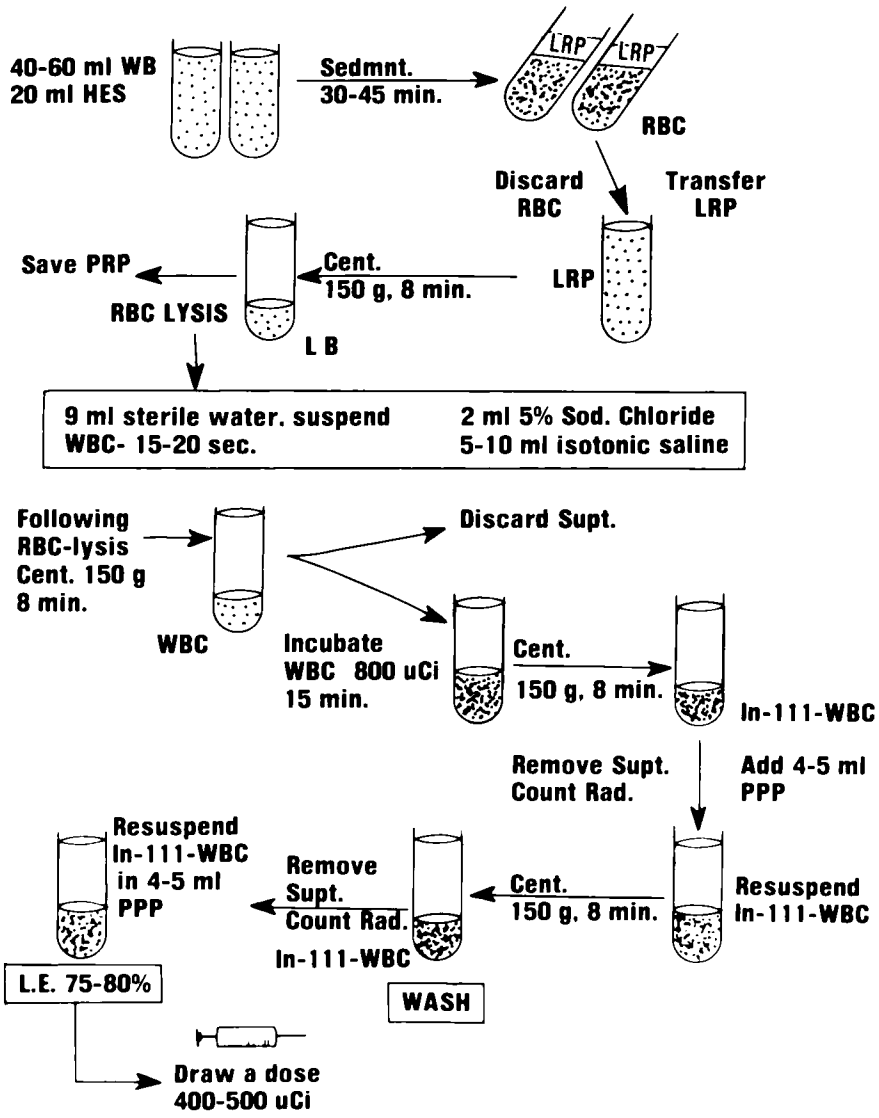

FIG. 1. Schematic representation of separation and labeling of white blood cells with ^{111}In-oxine. HES, hydroxy ethyl starch. LRP, leukocyte rich plasma. LB, leukocyte button. PRP, platelet rich plasma. PPP, platelet poor plasma. WB, whole blood. RBC, red blood cell. WBC, white blood cell.

will reduce the yield approximately 10%–20%, especially when less than a normal platelet count is present. If the patient platelet count is much lower than normal, one washing step with 4 ml ACD–saline solution, pH 6.3–6.5, before incubating with ^{111}In-oxine will result in a higher labeling yield. One low-speed centrifugation for 2 to 3 min at 50–100 g is necessary if clumps and large numbers of RBCs are present in the sample, before drawing the dose.

The red blood cell (RBC) lysis technique (*20*) used in WBC separation to remove RBCs has yielded a much higher fraction of labeled WBCs than any other method used previously for clinical studies. The viability of the ^{111}In-oxine-labeled WBCs was tested in several clinical samples using Trypan blue test and found to be of the order of 80%–90%.

The quality control procedures for ^{111}In-labeled platelets and leukocytes have been previously described in detail (*6,21*). We suggest that tests for viability of ^{111}In-labeled leukocytes, platelet aggregation, and sterility of the labeled cells should be performed periodically, if not for each clinical study.

We suggest that the separate washing step with platelet poor plasma, in both the WBCs and platelet procedures, could be entirely avoided to save more time by adding 10 ml of PPP at the end of incubation time to the ^{111}In-oxine–cell mixture, and centrifuging to remove unbound ^{111}In-activity from the cells. This method yielded as little as 1% to 2% unbound ^{111}In-activity with the cells, and this negligible amount will not be a significant factor for clinical studies. This modification will save 15 to 20 min with no variation of results. We also suggest to new investigators that they should first establish the procedures well, and then venture on to shortcut methods for faster completion. Appropriate cell viabililty studies and quality control results must ensure cell performance in vivo.

The clinical utilization of ^{111}In label for both WBCs and platelets prepared by the methods described in this study revealed very good results in the diagnosis of acute infection and renal transplant rejection. Figure 3 shows a posterior lower abdominal and pelvis image of ^{111}In-WBC study performed in a patient with fever and a large fluctuant swelling in the lumbo-sacral region. Ultrasound examination of this area revealed a fluid collection suggesting an abscess. No abnormal

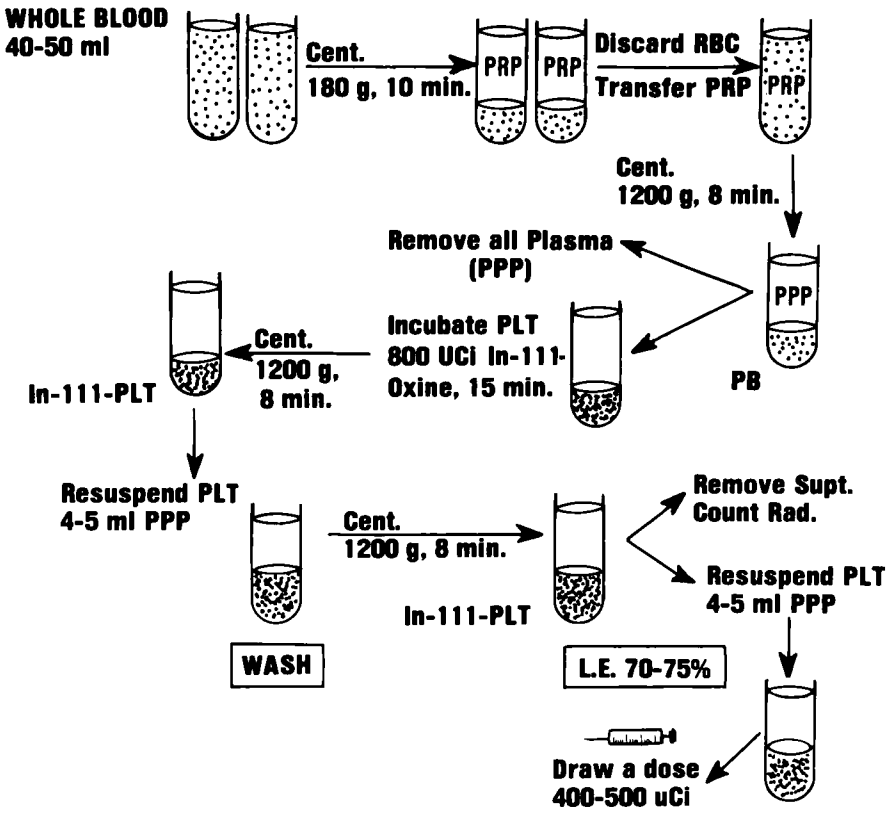

FIG. 2. Schematic representation of separation and labeling of platelets with ^{111}In-oxine. PRP, platelet rich plasma. PPP, platelet poor plasma. PB, platelet button. PLT, platelets. RBC, red blood cell.

uptake of ^{111}In-WBCs is found in the lumbo-sacral region; however, there is grossly abnormal accumulation bilaterally in the superior gluteal area, suggesting injection abscess. Subsequent surgical drainage of the lumbo-sacral fluid collection revealed evidence of hematoma. The gluteal abscesses were successfully treated with antibiotics.

Figure 4 shows an ^{111}In-labeled platelet study on a patient with a cadaver renal transplant oliguira and increasing serum creatinine levels. This anterior abnormal pelvic image shows intense concentration of labeled platelets in the transplanted

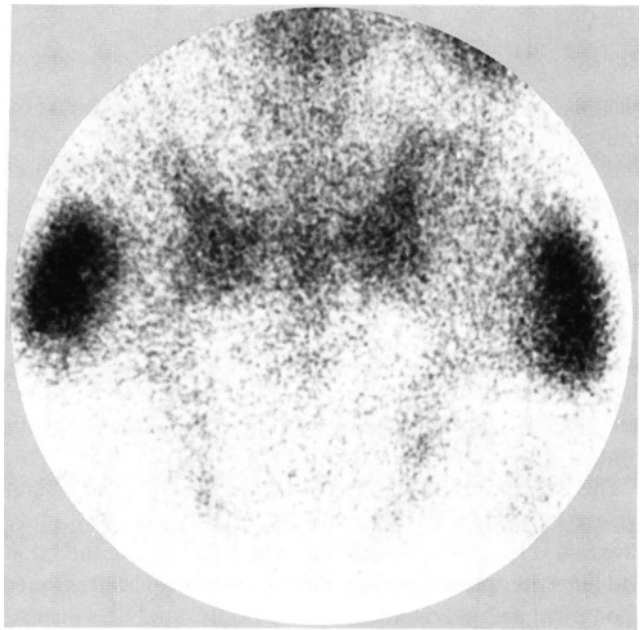

FIG. 3. Posterior lower abdominal, pelvic image of an ^{111}In-labeled WBC study showing gross abnormal accumulation of tracer in superior gluteal areas.

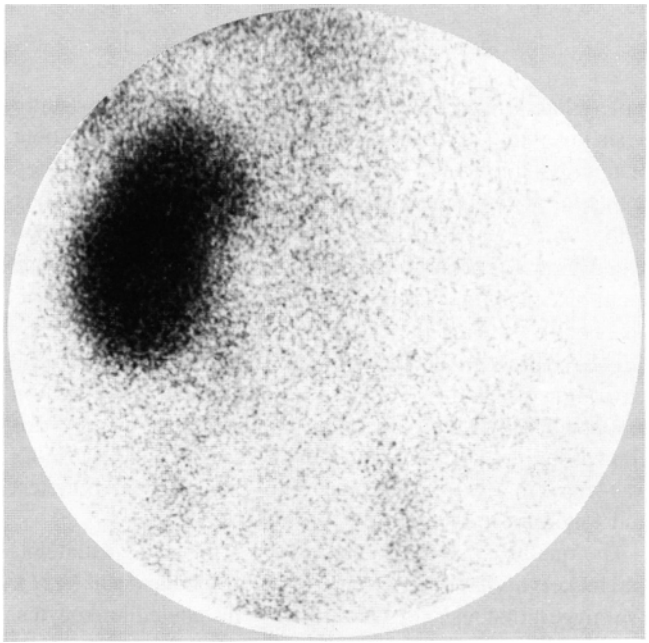

FIG. 4. Intense accumulation of ^{111}In-labeled platelets in a rejecting transplanted kidney. Right is on reader's left.

kidney as compared to adjacent vascular structures. Computer quantitation (*18*) revealed a kidney-to-contralateral iliac artery activity ratio of 11.7 (normal 1.0). A subsequent renogram showed poor perfusion and secretion consistent with rejection rather than acute tubular necrosis. The patient underwent transplant nephrectomy, and a biopsy specimen showed acute hemorrhage rejection.

In conclusion, the procedures described in this study are rapid and simple, and should take less than 2 hr. In addition to the simplicity, commercially available ^{111}In-oxine in kit form allows a convenient method of labeling for the small hospital. The viability of the ^{111}In-labeled WBCs and platelets are a clear indication from biological distribution radioactivity found in the images.

NOTES

*Amersham Corp., Arlington Heights, IL.
†Mallinckrodt, Inc., St. Louis, MO.

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