# Comparison of Pretinning Methods to Label Leukocytes with Technetium-99m

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***Leukocytes labeled with technetium-99m using pretinning with stannous complexes of pyrophosphate, glucoheptonate, and gentisic acid were compared with respect to labeling efficiency, time required for labeling, in vitro cell viability, initial lung transit, and image quality following reinjection into normal volunteers. Gentisic acid pretinning was the best method, offering the shortest preparation time, moderate labeling efficiency, rapid lung clearance, and greatest in vivo stability.***

Indium-111-labeled leukocyte imaging is an important method of diagnosing infection and localizing abscesses (*1*). However, $^{111}$In suffers the disadvantages of being expensive and inconvenient to supply; there is also concern about its dosimetry (*2–5*). Because of these factors, there is interest in alternative methods using technetium-99m ($^{99m}$Tc) for labeling leukocytes.

There have been two main approaches to leukocyte labeling with $^{99m}$Tc. One approach makes use of $^{99m}$Tc colloids which are engulfed by phagocytic leukocytes (*6*). In the second approach, leukocytes are pretinned with a stannous agent, the excess agent is washed off, and the pretinned leukocytes are incubated with [$^{99m}$Tc]pertechnetate (*2–3*). Recently, a third approach has used [$^{99m}$Tc]hexamethyl propyleneamine oxime (HM-PAO), which is trapped inside leukocytes (*4*).

We studied three of the pretinning methods, using stannous complexes of pyrophosphate (*2–3*), glucoheptonate (*7*), and gentisic acid (*5*). The methods were compared with respect to labeling efficiency, time required for labeling, and in vitro viability. Leukocytes labeled by each method were assessed in normal volunteers and the lung wash-out time was measured as an index of cell function (*8–9*).

## MATERIALS AND METHODS

### Preparation of Kits

All kits were manufactured in-house and were stored at −70 °C. The pyrophosphate (PYP) kit contained 10 mg sodium pyrophosphate, 0.5 mg gentisic acid, and 1 mg stannous chloride dihydrate at pH 6.8 in 1 ml sterile water. The glucoheptonate (GH) kit contained 50 mg sodium glucoheptonate, 0.5 mg gentisic acid, and 0.5 mg stannous chloride dihydrate at pH 6 in 1 ml sterile water. The gentisic acid (GA) kit contained 0.5 mg gentisic acid, 0.05 mg sodium tartrate, and 0.02 mg stannous chloride at pH 7 in 0.5 ml sterile water.

### Separation of Leukocytes

Forty milliliters of venous blood anticoagulated with heparin and mixed with 10 ml 6% hetastarch was allowed to stand 30–60 min at room temperature to allow the erythrocytes to settle. The supernatant leukocyte-rich plasma was centrifuged to produce a leukocyte pellet from which the residual erythrocytes were removed by hypotonic lysis.

### Labeling with PYP or GH

The leukocyte pellet was resuspended in a PYP or GH kit with 2.5 ml saline and incubated for 15 min at 37 °C. The cells were washed once with saline, then incubated with 1 ml [$^{99m}$Tc]pertechnetate (25 mCi) for 10 min at 25 °C. The labeled cells were washed once with saline, then resuspended in 4 ml of saline. Before reinjection, aggregates were removed by passing the suspension through either a sterile 100-$\mu$m stainless-steel filter or a blood filter.

### Labeling with GA

Technetium-99m pertechnetate (25 mCi) was added to a GA kit and allowed to stand for 1 min. The leukocyte pellet was resuspended in the [$^{99m}$Tc]GA solution and incubated 15 min at 25 °C. The cells were washed once with saline, then resuspended in a 4 ml saline and filtered.

### Effect of pH

To determine the effect of pH on labeling efficiency, the pH of the PYP kit was adjusted with hydrochloric acid prior to the pretinning step.

### In Vitro Tests

The stability of the label in vitro at 25 °C was studied by

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pelleting samples after various incubation times and measuring the cell-bound and supernatant activity. Cell viability was assessed by the trypan blue exclusion test.

### In Vivo Tests

Autologous leukocytes labeled with $^{99m}Tc$ (3–10 mCi) by the three methods were reinjected into normal volunteers. Scintillation camera images were obtained including a dynamic study of lung transit of radiolabeled cells using 1-min frames for 30 min and static images of the chest and abdomen taken at 0.5, 4, and 24 hr after injection. From the dynamic study a half-time for lung wash-out of radioactivity was calculated.

## RESULTS

The labeling efficiencies of the three methods are presented in Table 1. The PYP and GH cell labeling required 2.5 hr to complete while the GA method required 2 hr.

The effect of pH on leukocyte labeling efficiency with PYP is shown in Table 2. At lower pH, the labeling efficiency was higher; however, cell viability was adversely affected, as shown by loss of ability to exclude trypan blue dye.

**TABLE 1. Labeling Efficiencies with Three Pretinning Agents**

| | % Labeling Efficiency | |
|---|---|---|
| Agent | Mean ± s.d. | n |
| PYP | 22.8 ± 11.2 | 14 |
| GH | 48.3 ± 12.6 | 4 |
| GA | 39.9 ± 9.7 | 13 |

**TABLE 2. Effect of pH on Labeling Efficiency with PYP**

| | % Labeling Efficiency | |
|---|---|---|
| pH | Mean ± s.d. | n |
| 6.8 | 22.8 ± 11.2 | 14 |
| 6.5 | 30.4 | 1 |
| 6.0 | 59.5 ± 5.2 | 4 |
| 5.1 | 70.3 ± 20.6 | 3 |
| 2.5 | 88.2 ± 0.7 | 2 |

**TABLE 3. Effect of Labeling Method on Half-Time for Lung Transit**

| | Half-Time in Minutes | |
|---|---|---|
| Method | Mean ± s.d. | n |
| PYP | 52.5 , 94.5 | 2 |
| GH | 59.0 | 1 |
| GA | 23.9 ± 2.7 | 4 |
| $^{111}In$ oxine | 16.4 ± 8.8 | 9 |

The half-times for clearance of labeled cells from the lungs are shown in Table 3. Activity cleared from the lungs into the liver, spleen, and bone marrow. At later times, activity was also seen in the kidneys, bladder, thyroid, and intestines.

## DISCUSSION

Labeling efficiency with PYP was 22.8% ± 11.2%, which is similar to the 20.5–33.5% reported by Kelbaek (*3*). Higher labeling efficiencies, 81% ± 6%, were reported by Farid (*2*).

The pH of the pretinning solution with PYP had a profound effect on labeling efficiency. As noted previously (*10*), labeling efficiency was higher at lower pH. However, increased labeling efficiency was achieved at the cost of cell viability. Hence, lowering the pH is not a practical way to improve labeling efficiency.

Labeling efficiency was higher with GH than with PYP, as reported previously (*7*), although this may be partially due to the lower pH of the GH kit. GH appeared to produce less impairment of cell function as shown by the more rapid lung transit. However, lung transit of GH-labeled cells was still considerably slower than that observed with $^{111}In$ oxine. The present studies used the total stannous ion content of a GH kit; use of lower amounts as suggested by Straub (*7*) may result in less impairment of cell function.

Although labeling efficiency was somewhat lower with GA than with GH, the cells labeled with GA showed even more rapid lung transit, similar to that observed with $^{111}In$ oxine. The labeling efficiencies obtained in the present study, 39.9% ± 9.7%, are higher than the 18–31% originally reported by Sundrehagen (*5*). The half-times for lung transit in the present study are similar to those reported by Sundrehagen.

The GA method produced the best image quality with least evidence of in vivo breakdown and release of pertechnetate. However, activity was apparent in the kidneys, and the 24-hr images demonstrated considerable intestinal activity. Similar problems have been reported with the use of [$^{99m}Tc$]HM-PAO for leukocyte labeling (*11*).

In conclusion, GA appears to be the best of the three pretinning methods for leukocyte labeling, offering the shortest preparation time, moderate labeling efficiency, rapid lung clearance, and greatest in vivo stability. The GA kit is inexpensive to prepare (*5*). We are now assessing the clinical utility of leukocytes labeled with $^{99m}Tc$ by the GA method.

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