# A Modified Method for the In Vivo Labeling of Red Blood Cells with Tc-99m: Concise Communication

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**The rate of incorporation of Tc-99m into red blood cells pretinned in vivo was measured by collecting blood samples in stannous DTPA solution, which served as a competing ligand for Tc-99m. This collection technique permitted a measurement of high-affinity red-cell labeling efficiency at the instant of sampling. At 0.5 min after injection only 62% of technetium is tightly bound to the red cell; this rises to 94.5% at 10 min. Based on the graded labeling of the red cells, the in vivo labeling procedure was modified by isolating pertechnetate and red blood cells tinned in vivo in a syringe during the first 10 min of labeling. The pertechnetate is thus prevented from distributing to extravascular compartments, and 90% of the injected Tc-99m is firmly bound to red blood cells at the time of injection. In a series of 23 patients, seven were tested with the in vivo method and seven with the modified in vivo method, and nine patients were tested with each method on separate occasions. A decrease in gastric activity and improved image quality were found with the modified method compared with the standard method of in vivo red-cell labeling.**

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In 1974, McRae et al. (*1*) observed that the tissue distribution of pertechnetate (Tc-99m) in rats was altered for up to 13 wk following administration of stannous ion. The tin prolonged the disappearance of technetium from the blood. A similar effect was observed in humans by Walker (*2*) and by Chandler et al. (*3*), who noted increased intravascular radioactivity on brain scans performed with pertechnetate following Tc-99m pyrophosphate bone scan. These observations led Pavel et al. (*5*) and Stokely et al. (*4*) to suggest that red cells could be intentionally labeled in vivo for blood-pool images based on the intravenous injection of stannous pyrophosphate 20 min to 24 hr before the administration of Tc-99m. Pavel, administering the stannous ion 20 min before the pertechnetate, reported in vivo labeling efficiency of 96%, which persisted for up to 60 min following pertechnetate injection. In our laboratory, however, it was observed clinically that patients studied for gastrointestinal hemorrhage with Tc-99m red blood cells labeled by the in vivo technique showed variable gastric and urinary activity (*6*). To understand better the source of this extravascular radioactivity with Tc-99m RBCs labeled in vivo, we studied the rate of incorporation of Tc-99m into RBCs in vivo and in vitro using a competing ligand for Tc-99m in the collecting solution. The in vivo RBC-labeling technique was subsequently modified to improve the quality of gated cardiac images. The change also precluded the need for continuous gastric suction during studies of gastrointestinal hemorrhage.

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## MATERIALS AND METHODS

**Measurement of in vivo labeling rate.** Ten patients referred for a gated blood-pool examination received ~500 μg of stannous ion as stannous pyrophosphate.*

Twenty minutes later, 20 mCi of pertechnetate (Tc-99m) was administered intravenously. At 0.5, 1, 2, 4, 6, and 10 min following pertechnetate injection, 0.5-ml blood samples were withdrawn into two kinds of heparinized syringes, one kind for each of two groups. Each syringe contained either (a) 1 ml of 0.15 *M* sodium chloride solution or (b) stannous DTPA solution prepared by adding 10 ml of 0.15 *M* sodium chloride solution to a radiopharmaceutical kit containing 5 mg of DTPA and 0.25 mg stannous chloride.† This solution was thus 1 m*M* in DTPA and 0.13 m*M* in stannous ion and provided a ligand capable of competing for Tc-99m not bound to RBCs.

After the final sample was taken, all samples were transferred to test tubes and centrifuged at 150 *g* for 15 min. Aliquots of the supernatant plasma and red-cell fractions were counted in a gamma scintillation counter with window spanning 120–160 keV. Labeling efficiency was calculated according to the following formula:

$$\text{L.E.} = \frac{\text{Cpm}_{\text{RBC}} - \text{bkg}}{(\text{Cpm}_{\text{RBC}} - \text{bkg}) + (\text{Cpm}_{\text{plasma}} - \text{bkg})} \times 100.$$

**Modification of in vivo labeling technique.** Patients received approximately 500 $\mu$g of stannous ion as stannous pyrophosphate intravenously. Twenty minutes later, a butterfly infusion set fitted with a four-way stopcock was placed in an antecubital vein. The line was heparinized with a solution containing 10 units/ml of heparin. Three milliters of blood were then withdrawn into a shielded syringe containing 20 mCi of pertechnetate (Tc-99m). Anticoagulation was provided by the residual heparin in the line, which is reheparinized after withdrawing the blood. After 10 min of incubation with gentle agitation at room temperature, the labeled RBCs were reinjected using a standard Oldendorf technique.

**Evaluation of modified method.** To measure the rate of Tc-99m incorporation into RBCs with the modified labeling technique, the DTPA competitive collection technique was used as above. Heparinized blood samples (3 ml) were obtained from ten patients 20 min after injection of stannous pyrophosphate. Twenty-five to 100 $\mu$Ci of pertechnetate (Tc-99m) were then added to the blood sample, and aliquots were collected into stannous DTPA solution at 1-min intervals up to 10 min following pertechnetate addition. After the last aliquot was collected, the labeling efficiencies were determined in all samples. To determine the effect of stannous-ion dose on the rate and extent of RBC labeling, five patients received a dose of 2.1 mg stannous ion as stannous pyrophosphate. The time-dependent labeling efficiencies were then determined as above.

To define the biological behavior of RBCs labeled in vivo, compared with the modified method, two groups of seven patients, each undergoing gated cardiac imaging, were selected at random. In one group the standard in vivo technique was used, in the other group the modified method. At completion of the gated blood-pool scan, about 45 min after injection, 1-min images of the epigastrium were obtained. With other information withheld, two observers rated the gastric activity on a scale of 0 to +++, with 0 indicating no gastric activity and +++ indicating significant gastric activity, comparable to that of the liver or spleen.

The ratio of counts/pixel in the left ventricle to background radioactivity was computed in nine patients who had undergone gated blood-pool scans with Tc-99m RBCs labeled by each method on separate occasions.

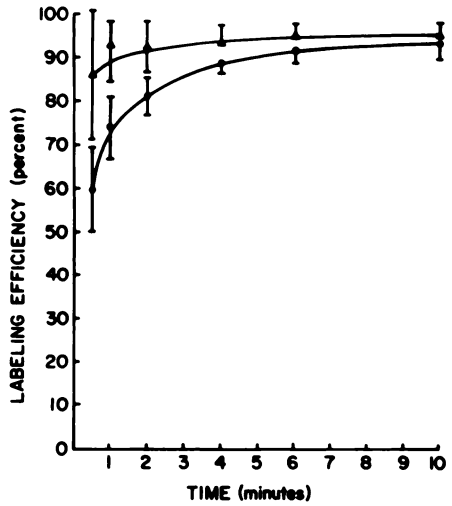


**FIG. 1.** In vivo RBC labeling efficiency as a function of time after pertechnetate injection when blood samples are collected in saline (-▲-) or in stannous DTPA solution (-●-). (Mean ± s.d., n = 10).

## RESULTS

**Determination of labeling rates.** Figure 1 summarizes the labeling efficiency for in vivo labeled red cells as a function of time after pertechnetate injection, as measured in saline and in DTPA in ten patients. When samples were collected in saline, a labeling efficiency > 90% was reached 1 min following pertechnetate injection and remained relatively constant up to 10 min. The stannous DTPA sample collection technique demonstrated that labeling efficiency increased slowly from a value of 62% at 0.5 min to an equilibrium value of 94.5% at 10 min. A Student's t-test performed at each time period showed a significant difference in labeling efficiency between the two methods at the 0.5-, 1.0-, and 2.0-min time periods ($p < 0.05$).

Figure 2 shows the labeling efficiency as a function of time when the syringe was handled with the modified labeling technique. Labeling proceeds at an initial rate of 12.1% per minute over the first 5 min, then more slowly, reaching 90% at 10 min.

Figure 3 shows labeling efficiency as a function of time using the modified technique in patients who received 2.1 mg of stannous ion. Labeling proceeds at an initial

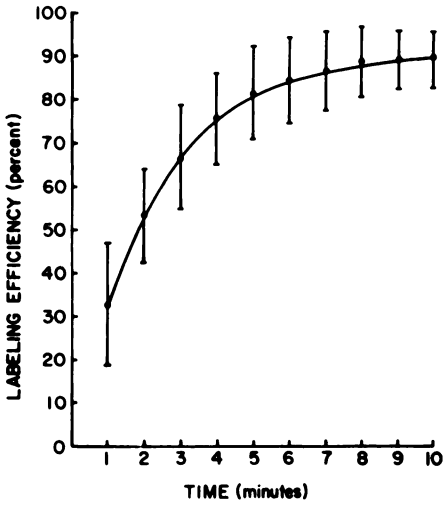


**FIG. 2.** Efficiency of RBC labeling with Tc-99m by the modified method as a function of time after pertechnetate addition. Blood samples collected in stannous DTPA solution. Patients received 0.5 mg sannous ion. (Mean ± s.d., n = 10).

rate of 9.6% per minute over the first 5 min, then more slowly, reaching 82% at 20 min. A Student's t-test indicated that the group receiving the high stannous ion had a lower equilibrium labeling efficiency than the group receiving the low dose ($p < 0.01$).

**Evaluation of modified labeling method.** Observer grading of the presence of gastric activity on gated blood-pool scans revealed that the standard in vivo method resulted in six of seven patients with ++ activity in the epigastrium and one patient who showed + activity in the epigastrium. With the modified in vivo labeling technique, six of the seven patients had no detectable gastric activity; there was a trace of activity in the remaining patient.

The ratio of left ventricle to background activity, computed in ten patients labeled by both techniques, revealed a mean ratio of 2.4 ± 0.55 (mean ± s.d.), in the in vivo group while the modified labeling group gave a mean ratio of 3.58 ± 1.41 ($p < 0.05$).

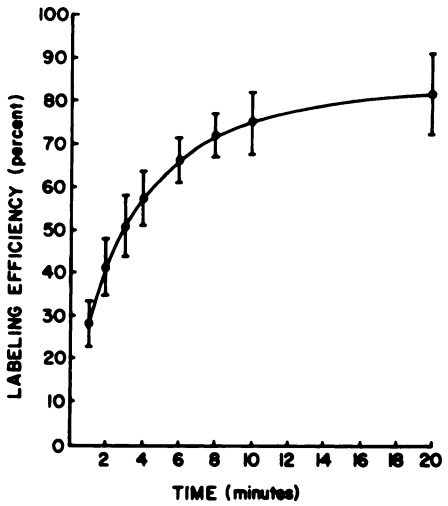


**FIG. 3.** Efficiency of RBC labeling with Tc-99m by the modified method as a function of time after pertechnetate addition. Blood samples collected in stannous DTPA solution. Patients received 2.1 mg stannous ion. (Mean ± s.d., n = 5).

## DISCUSSION

Currently, red blood cells can be labeled with Tc-99m by in vivo (4) and in vitro techniques (*7*). Clinical comparisons (*8*) have shown that the in vitro method results in a superior product. The need to remove a blood sample from the patient and the lack of a commercially available kit have prevented the method from gaining widespread acceptance. In vivo methods use readily available components and do not require blood samples to be removed from the patient. However, in our laboratory, the quality of images obtained with the standard in vivo method were often of poor quality, even though Pavel et al. (*5*) reported that a labeling efficiency of 96% was achieved 5 min after pertechnetate injection and that it remained greater than 95% for up to 60 min.

The labeling efficiency reported by Pavel represents the distribution of Tc-99m between red blood cells and plasma at the time of sampling but does not account for the fraction of the injected dose of pertechnetate that may have distributed to the extracellular fluid compartment. The DTPA and saline results (Fig. 1) suggest that labeling continues in blood samples after withdrawal until measurements are made unless the process is stopped by addition of competing ligand. Since free pertechnetate is injected in the standard in vivo technique, there is competition for the pertechnetate between red blood cells and the extracellular fluid space, thyroid, gastric mucosa, and salivary glands. The rate at which Tc-99m becomes firmly bound to the RBC will therefore determine the fraction of the administered dose that remains in the intravascular pool.

The modified labeling method presented here provides adequate time for labeling to take place by isolating the Tc-99m and pretinned red blood cells from competing body compartments during the first 10 min of the reaction. At the time of injection, approximately 90% of the injected dose of Tc-99m is firmly bound to the red cells. This results in increased intravascular concentration of Tc-99m and improved scintigram quality. In addition, the labeling procedure is carried out in a completely closed system, which minimizes the possibility of infection.

This modified labeling technique has been used in over 500 patient studies, yielding increased contrast between target and background in blood-pool images and eliminating the need for continuous gastric suction in studies of gastrointestinal hemorrhage.

## FOOTNOTES

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† Medi-Physics, Inc., Emeryville, CA.

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