A SIMPLE KIT FOR THE PREPARATION OF ^{99m}Tc-LABELED RED BLOOD CELLS

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A simple kit for the preparation of ^{99m}Tc-labeled red blood cells in a closed sterile system is described. Whole blood (3–6 ml) is automatically drawn from the patient into a 10-ml Vacutainer tube, containing a freeze-dried stannous citrate formulation, with heparin as an anticoagulant. The kit provides consistent 97% yields in 20 min with small blood samples. The radionuclide is added at the end of the labeling sequence to minimize operator exposure. Freeze-dried kits have performed well after more than 12 months. Some technetium solutions produced low labeling yields. Experimental evidence suggests that the poor yields were due to carrier ^{99}Tc. This effect may explain low labeling yields obtained with some other radiopharmaceuticals.

Labeled red blood cells (RBCs) have conventionally been used for imaging the placenta (*1*) and for RBC mass determinations (*2,3*). Damaged labeled cells have been used for spleen imaging (*4–7*). More recent applications have included cardiac blood pool dynamics and imaging, where radiopharmaceuticals that are retained in the vascular system are best used (*8*). The advantages of ^{99m}Tc for imaging have been well documented (*9*). Technetium-99m-pertechnetate and ^{99m}Tc-labeled human serum albumin are not suitable for many bloodflow studies, however, because of the tendency of these radiopharmaceuticals to diffuse out of the vascular system. Although ^{99m}Tc-labeled RBCs do not have this disadvantage, their application has been limited by the need to withdraw and label a sample of the patient's blood just prior to clinical study—a laborious task with existing labeling methods. Earlier preparation methods, in which no reducing agents were used, had but minimal success (*1,4,10*). Reductive methods using stannous ion produced the first dependable labeling, but yields were limited to the 50–60% range (*3,5,6,8,11*). These low yields made it necessary to separate the unbound ^{99m}Tc before injection. In later efforts, improved yields were achieved by decreasing the stannous ion content, but these systems required considerable handling in the preparation of fresh solutions just before use, sometimes including washing steps (*12–14*).

As a result of the experiments reported here, a simplified and reproducible kit has been developed for the preparation of ^{99m}Tc-labeled RBCs. With few mechanical steps, it consistently produces high labeling yields in a closed sterile system.

During the development of this kit, an unexpected and possibly important problem was encountered with some technetium solutions (*15*). Technetium chemical effects are not usually considered in the preparation of routine radiopharmaceuticals. However, in this case the carrier ^{99}Tc in some generator eluates apparently exceeded the reductive capacity of the added stannous ion, causing depressed labeling yields. This same problem may exist with other radiopharmaceuticals that use stannous ion, particularly when the quantity used is very small or when poor formulations make the stannous ion unusable.

MATERIALS AND METHODS

All experiments were conducted with human blood from volunteers. The percent labeling (percent of ^{99m}Tc associated with the RBCs) was determined by cell sedimentation, separation, and ^{99m}Tc assay either in an ionization chamber or with use of an auto-gamma counter with a NaI(Tl) crystal. Stannous citrate was purchased from Pfaltz and Bauer (Flushing, N.Y.); the stannous citrate contained 90% stannous ion based on sample weight according to potentiometric titration with iodine. All other chemi-

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cals were reagent grade products from local suppliers.

Determination of optimal labeling parameters. Optimal labeling parameters were determined according to a modification of the aqueous-solution method of Schwartz and Krüger (*14*). Instead of an initial cell-separation step, whole blood was first treated with an isotonic aqueous solution of Sn^{+2}, followed by separation of pretinned RBCs and their incubation with $^{99m}TcO_4^-$. Our kit-testing procedure, given later, follows the same procedure, except that the Sn^{+2} solution is not freeze-dried.

The stability of aqueous stannous ion solutions was evaluated for the preparation of this kit by determining RBC-labeling yields with each solution after storage for various times and conditions. Incubation temperatures, incubation times, reagent addition order, and a step of washing the cells before labeling were varied one at a time and their effects assessed by determining RBC-labeling yields.

Since the optimal quantity of stannous ion has been reported to be 0.5 μg per 4 ml of whole blood (*12*), this was evaluated in our laboratory for a 3-ml whole-blood sample with 5-min incubations at room temperature. All stannous solutions were prepared in a diluent containing 1.5 gm of sodium chloride and 1.0 gm of sodium citrate (dihydrate) per 200 ml of aqueous solution. The stock stannous citrate solution contained 6.5 mg of stannous citrate in 50 ml of the diluent. These dilutions of the stock solution provided concentrations of 0.5–5.0 μg of stannous ion. Red blood cells were labeled over this range of concentrations, and labeling yields were determined (Fig. 1). The optimal stannous ion capacity for this system was 0.5–1.0 μg per 3 ml of whole blood.

Determination of technetium capacity. To evaluate the technetium capacity of the kit, the stannous ion was maintained constant in a series of samples containing increasing amounts of $^{99}TcO_4^-$.

A stock solution of $^{99}TcO_4^-$ purchased from Oak Ridge National Laboratory was diluted in 0.9% saline to prepare two working solutions: Solution A (9.84×10^{-7} *M*) and Solution B (9.84×10^{-8} *M*). The solution concentrations were determined by spectrophotometric assay (*16*) and confirmed several months after preparation by liquid scintillation counting of ^{99}Tc using an Amersham/Searle ^{99}Tc standard. Aliquots of Solutions A and B were dispensed into separate tubes to provide a series of technetium concentrations; 0.9% saline was added where necessary to maintain the geometry of all technetium samples. All samples were spiked with a few thousand counts of $^{99m}TcO_4^-$ for assay purposes, and studies of RBC-labeling yield were conducted according to the kit procedure using aqueous Sn^{+2} solutions (Fig. 2). Further studies were then conducted to find suitable conditions for preparing a freeze-dried product.

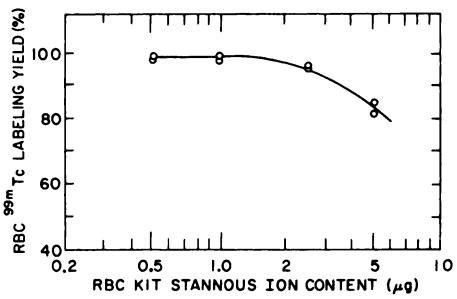

FIG. 1. Effect of stannous ion content on RBC labeling yield. All stannous ion solutions were freshly prepared. One ml of RBCs from 3 ml whole blood was contacted with tracer ^{99m}Tc according to procedure for testing freeze-dried kits.

Preparation of freeze-dried reagent kits. All glassware and equipment were washed with soap, rinsed with distilled water, then rinsed in 10% HCl, and finally rinsed in distilled water. Cleaned glassware was drained, wrapped in aluminum foil, and sterilized in a hot-air oven at 190°C for 3 hr. The final Tc formulation was dispensed into 10-ml Vacutainer tubes (Becton-Dickinson & Co., Rutherford, N.J.; tube 3200 NT [100 × 15 mm]) that had been washed and sterilized as above. The rubber stoppers were washed by the same procedure but were not sterilized until later. (Autoclaved rubber stoppers tended to retain moisture, even after vacuum-drying. The moisture escaped slowly from the stoppers into the dried formulation during storage, significantly shortening the shelf life of the kits.) A final radiation sterilization was included in the kit preparation.

Concentrated and diluted stannous citrate solutions were prepared in an aqueous citrate–dextrose diluent* containing 1.0 gm of sodium citrate (dihydrate) and 1.5 gm of anhydrous dextrose per 200 ml of solution.

Stannous citrate concentrated solution. Citrate–dextrose diluent was added to 6.5 mg of stannous citrate (initially dissolved on the glassine weighing paper in three drops of a solution of 1 ml citrate–dextrose diluent and 4 ml saline) to obtain 50 ml.

Stannous citrate diluted solution. Sodium heparin (10,000 units in 1 ml, Upjohn) was dissolved in about 20 ml of citrate–dextrose diluent in a second 50-ml volumetric flask and 2 ml of stannous citrate concentrated solution

* For the freeze-dried kit preparation, dextrose was substituted for sodium chloride in the diluent.

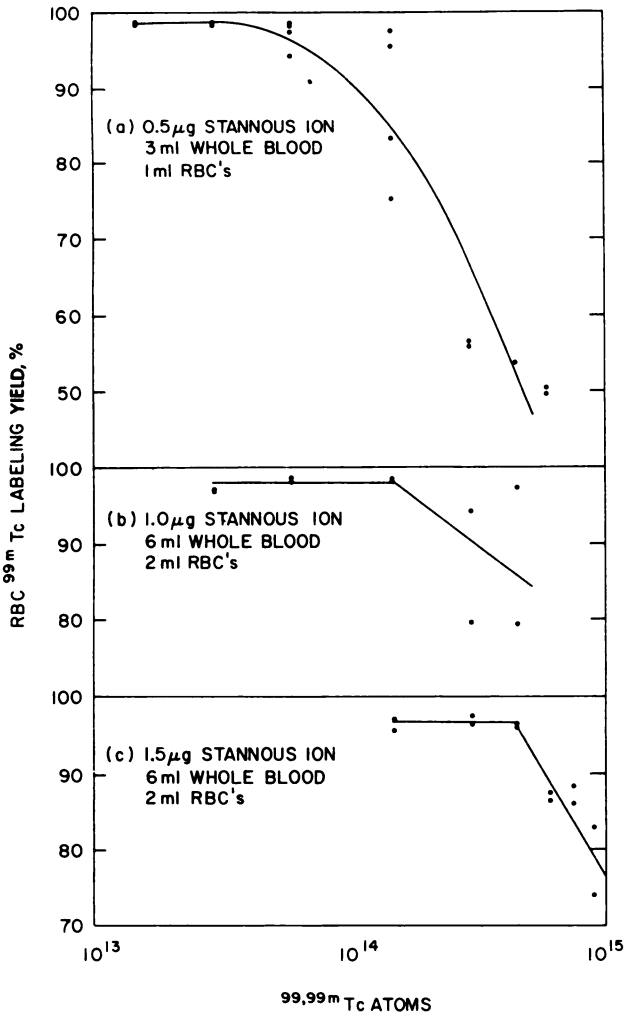

FIG. 2. Technetium capacity of RBC labeling kits with three different quantities of stannous ion. Test solutions were freshly prepared and tested according to procedure for testing freeze-dried kits.

added. The dilution was made up to volume with citrate–dextrose diluent.

Half-milliliter aliquots of the stannous citrate diluted solution were filtered from an all-glass system (modified 25-ml buret) through an 0.22-μm Millipore filter into the sterile Vacutainer tubes. A slight positive nitrogen pressure was used to force the solution through the filter. Loaded tubes were transferred to an aluminum block (with holes to accept 100 × 15-mm tubes) and the tube contents were frozen. Slit-stoppers were inserted into the tubes for freeze-drying.

The frozen samples were lyophilized while still in the aluminum block for a minimum of 36 hr at a shelf temperature of 40°C (105°F) and a pressure of 10–30 μm Hg. The freeze-drier chamber was then backfilled to 400 mm Hg with nitrogen, and the tubes were remotely stoppered in situ. Tubes stoppered under these conditions automatically draw 6 ml of whole blood from the patient. The correct partial vacuum was determined by stoppering several tubes under different partial vacuums and testing their blood-draw volumes on animals. Tubes stoppered at 240 mm Hg drew 3 ml of whole blood. The finished product was given a final sterilization with ^{60}Co radiation (2.5 Mrad) (*17*). A representative fraction of the final kits was tested for both sterility and the presence of pyrogens. The labeling efficiency was determined and all clinical preparations were conducted according to the procedure below.

Description of kit components:

One sterile disposable Vacutainer needle multiple sample, 20-gage 1½-in.
One Vacutainer adapter
One 20-gage $^{9}/_{32}$-in. hypodermic needle (special)
One Vacutainer reagent tube, 100 × 15 mm, 10 ml capacity, evacuated to draw 6 ml whole blood and containing:
- 100 units of sodium heparin
- 2.6 μg stannous citrate (1.0 μg stannous ion)
- 2.5 mg sodium citrate (dihydrate)
- 3.7 mg dextrose (anhydrous)

Procedure for using the RBC kit to prepare ^{99m}Tc-labeled RBCs. (Use aseptic techniques throughout.)

1. Add 1–5 ml of saline ^{99m}Tc-pertechnetate to a sterile and pyrogen-free 15-ml pharmaceutical vial and assay. *Important:* Determine the maximum ^{99m}Tc activity for satisfactory performance of the kit. The technetium atoms added to the kit must not exceed the number of atoms of technetium (^{99m}Tc plus ^{99}Tc) generated by the decay of 10 mCi of ^{99}Mo. Store in a lead shield.
2. The Vacutainer tube is evacuated to draw about 6 ml of whole blood. Use 20-gage 1½-in. sterile multiple-sample Vacutainer needle and Vacutainer adapter.
3. Mix immediately to dissolve the freeze-dried solids in the blood and gently mix the tube contents for 5 min at room temperature.
4. Add 4 ml of sterile saline to the blood. *Caution:* To avoid pressure buildup in the Vacutainer tube, draw 4 ml of sterile saline into a 10-ml syringe, push the hypodermic needle through the Vacutainer stopper, and, with the tube upright so that the blood will not be drawn back into the syringe, pull the syringe plunger back to the 8-ml mark. Allow the created vacuum to draw the saline into the tube.
5. Mix briefly and centrifuge the tube stopper end down for 5 min at 1300 G.

6. Maintain the tube in inverted position to avoid disturbing the packed RBCs. Withdraw 2 ml of RBCs using a 20-gage $\frac{5}{32}$-in. needle. This needle length will just penetrate the stopper.
7. Transfer the RBCs to the premeasured technetium solution prepared in Step 1.
8. Incubate the technetium–RBC mixture for 5 min at room temperature, with gentle mixing.
9. Assay and dilute appropriately for injection. Cell separation and yield determination at this point consistently give 97% yields.

Three-milliliter blood samples work equally well if the total of ^{99m}Tc and ^{99}Tc atoms is decreased proportionately. In this case only 1 ml of RBCs is transferred in Step 6. A spleen-seeking agent can be prepared conveniently by replacing Step 8 with a 15-min incubation period at 49°C, with gentle mixing (*18*).

Figure 3 shows a blood sample being drawn directly into the reagent kit tube.

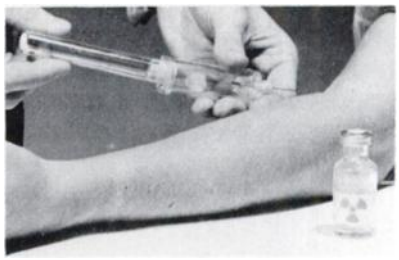

FIG. 3. Brookhaven RBC reagent kit. Patient blood samples are automatically drawn into evacuated tube containing reagent.

RESULTS AND DISCUSSION

Determination of optimal labeling parameters. A modification of the Schwartz and Krüger method (*14*) was selected as a basis for the development of optimal kit labeling conditions: addition of stannous ion to the blood sample preceded the radionuclide addition, and reported high yields would negate the need for extra washing steps. Their method had the disadvantages of requiring initial separation of RBCs from plasma and 37°C incubation, of not using a closed system to minimize bacterial contamination, and of requiring freshly prepared stannous chloride solutions.

In our hands, the Schwartz–Krüger method gave only 90% RBC tagging yields; this would require the introduction of a final washing step in any developed kit. Equivalent labeling yields could be achieved by incubation at room temperature and addition of the technetium before the stannous ion, but addition of technetium first was not suited to kit development. Little or no labeling occurred if the stannous ion and pertechnetate were mixed before contact with the separated RBCs. No labeling occurred in the absence of Sn^{+2}.

In an effort to simplify the procedure, subsequent experiments evaluated the Schwartz–Krüger method without the initial cell separation and room-temperature incubations. Labeling yields of 70% were obtained without any cell separation when 0.5 μg of Sn^{+2} was used. These yields were improved to ~90% by extending both incubation periods to 30 min each at room temperature. The lower yield without cell separation supported earlier reports that plasma decreased the labeling yield (*11*).

Further work showed that whole blood (without the initial cell separation) could be incubated with stannous ion to produce 97% yields with 5-min incubations at room temperature if the plasma is separated from the pretinned RBCs before adding the technetium. [A similar approach was used by Nouel and Brunelle (*13*)]. To avoid a wash to remove plasma residues, 4 ml of 0.9% saline was added to the sample before cell sedimentation; this diluted any plasma trapped in the cell sediment. Since separation was still required, the kit was centrifuged upside down, which caused the cells to settle against the stopper. A sterile needle just long enough to penetrate the stopper could then be used to remove an aliquot of sedimented cells without breaking the sterility of the tube. The aliquot was transferred to a second vial containing ^{99m}Tc-pertechnetate for the last incubation.

More stable stannous chloride solutions are produced by initial solution in minimal amounts of water before diluting to volume (*19–20*). Although the stability of aqueous stannous chloride solutions was further improved by adding 0.5% sodium citrate (*21*), such aqueous solutions were not stable for extended times (Table 1). However, freeze-dried aliquots of solutions prepared with 0.5% sodium citrate still performed well after 12 months.

Room temperature incubations for 5 min were optimal. Neither extended incubation times nor 37° incubations improved yields. Technetium could be added in 1–5 ml of 0.9% saline.

Maximum yields (approaching 100%) were obtained when 0.5–1.0 μg of stannous ion was used per 3 ml of whole blood (Fig. 1). Although further increases in stannous ion resulted in correspondingly lower yields, large quantities of Sn^{+2} still produce high yields if washes are employed to remove trapped plasma and excess Sn^{+2}. The implication is that RBCs have a limited capacity to take up stannous ion. Excess reductant remains in the trapped plasma during cell separation and, if not removed, reduces the pertechnetate before it can penetrate the cell membrane. Reduced technetium apparently cannot

TABLE 1. STABILITY OF STANNOUS CITRATE SOLUTIONS IN AQUEOUS VEHICLES BASED ON RBC-LABELING YIELD STUDIES

Vehicle	Preparation and storage* atmosphere	Preparation and storage* time	RBC-labeling yield (%)
A. 5% dextrose	Air	Initial	98.8
	Air	3 hr	42.4
B. 0.75% sodium chloride 0.5% sodium citrate	Air†	Initial	97.2
	Air	3 hr	92.3
	Air	3 days	35.6
	N_2	3 days	58.0

* All solutions were incubated at room temperature.
† A fraction of the initial sodium chloride/sodium citrate test solution prepared in air at room temperature was flushed with N_2 before storage.

cross the cell membrane: mixing stannous ion and pertechnetate and then adding RBCs produces very low labeling yields.

A technetium carrier problem. Technetium-99m samples of equivalent activity were found to contain very different chemical amounts of technetium. In clinical quantities of ^{99m}Tc activity, the carrier technetium can be appreciable. Almost all of the commercial instant ^{99m}Tc samples evaluated exceeded the stannous capacity of the RBC kit. Even generator samples exhibited the same problem unless generator ingrowth times were carefully controlled. Although the technetium capacity of the kit can be extended by keeping the blood-to-stannous ion ratio constant and increasing the kit size (Fig. 2a and 2b), the larger volume detracts from the simplicity and usefulness of the kit especially where bolus injections are required. With a constant Sn^{+2} content of 1.0 μg the kit can accept 1.48×10^{14} atoms of ^{99}Tc (Fig. 2b).

Since all Tc atoms present in a generator eluate originated from a like number of ^{99}Mo atoms, one can easily calculate the generator ingrowth time required for 1.48×10^{14} atoms of ^{99}Mo to decay. The time required for 1.48×10^{14} atoms of ^{99}Mo (11.65 mCi) to decay will depend on the size of the generator used and also on the fraction of the total elution to be used with the RBC kit. If only one-fourth of the eluate will be used in the kit, then four times as much decayed ^{99}Mo (4×11.65 mCi) may be represented in the total elution.

From a strict theoretical standpoint, there would appear to be an excess of stannous ion in the kit. But when one assumes that technetium is reduced to the +4 oxidation state, as might be suggested from the previously determined citrate case (*22*), and then considers that only about 0.3 μg of stannous ion is actually available [only one-third of the added 1.0 μg of Sn^{+2} is retained with the sedimented RBCs by ^{113}Sn tracer studies (*23*)], there is only a sevenfold excess. Other factors such as the approximately 10% impurity in commercial stannous salts, the tendency of stannous salts to hydrolyze during reagent preparation, and the oxidative nature of blood itself would further decrease the apparent excess. Stoichiometric agreement is therefore quite good.

The significance of this discovery may be even more important when one realizes that this same problem may occur with other commercial stannous radiopharmaceutical kits. Not all the added tin may be available as usable stannous ion in the final preparation; the stated amount of stannous ion in some commercial kits is not all available in a useful form. A more detailed study of commercial technetium sources and kits is in progress (*24*).

Although the observed technetium carrier effect might be explained by oxidants in technetium solutions, this seems unlikely since up to 50 μl of 3% H_2O_2 added to technetium solutions containing 1.48×10^{14} atoms of ^{99}Tc caused no decrease in labeling yield. Addition of the H_2O_2 to the kit reagents prior to drawing the blood sample, however, inhibits the labeling. This suggests that peroxide does not cross the cell membrane and cannot oxidize the stannous tin once it is inside the cell. Furthermore, we have been able to predict the performance of various technetium sources with the Brookhaven RBC kit from the observed Sn^{+2}/TcO_4^- ratio (Fig. 2).

The possibility that stable molybdenum might affect the stannous reductant was also considered. The addition of up to 15 μg of molybdenum as MoO_4^{-2}, both to the technetium solution and the tin reagent, before drawing the blood sample had no noticeable effect on the yield. Apparently no reduction of molybdate by stannous ion occurs at this pH.

Simple preventive measures can minimize technetium-saturation problems. Generator eluates from systems permitted to grow-in for long times should not be used because the number of technetium atoms continues to increase on a generator column with time. However, the ^{99m}Tc activity on the column decreases after an initial ingrowth. Since many generators milk with only about 80–90% efficiency, they should be washed with a second saline elution immediately after each milking to remove any residual technetium, which would exist mainly as ^{99}Tc by the next milking. Eluates should be used immediately after elution from the generator column. Once ^{99m}Tc has been removed from the generator, the number of technetium atoms remains constant but ^{99m}Tc decays to ^{99}Tc, which decreases the specific

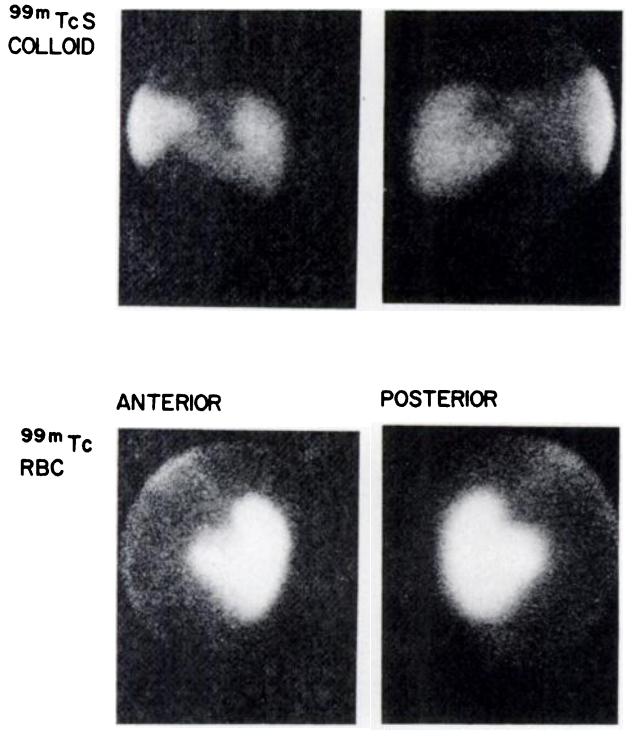

FIG. 4. Spleen image comparison between ^{99m}Tc-sulfur colloid and kit-labeled red cells. Note absence of activity in liver in RBC study of patient with previous history of hepatitis.

activity of the eluate. Technetium-99m eluates of low specific activity may exceed the reductive capacity of the stannous ion.

The simultaneous development of a kit procedure that uses stannous glucoheptonate has been reported by Gutkowski and Dworkin (*18*). Tests at Brookhaven showed that this tin compound is limited by total technetium much as the citrate is. Moreover, the reported procedure is much more involved mechanically than that described in this report.

Biologic behavior. Preliminary in vivo evaluation of erythrocytes labeled with the Brookhaven kit shows considerable promise for a number of applications, both from the standpoint of label stability and the ability to label small volumes of cells for bolus administration (*25*). In four patients, comparison with ^{51}Cr in a double-tagging experiment showed excellent correlation. When red-cell mass is determined with both ^{99m}Tc and ^{51}Cr, the ratio of the results was 1.01 ± 0.03 at 15 min, 0.98 ± 0.03 at 30 min, 1.04 ± 0.02 at 60 min, and 1.09 ± 0.03 at 2 hr.

Kit-labeled cells appear promising for measurement of the ejection fraction in cardiac studies, for cerebral perfusion imaging (dynamic and static), and for peripheral vascular imaging. Damaged cells prepared by heating at 49° for 15 min were used for spleen imaging (*18*).

The ability to label small volumes of erythrocytes is advantageous for two reasons. A comparison of dynamic and static imaging for peripheral vascular studies was possible after a small bolus injection. In spleen imaging approximately 90% of the dose localized in the spleen in 1 hr. This rapid and high uptake was thought to be due to the small volume of injected cells. Figure 4 shows a comparison between ^{99m}Tc-sulfur colloid and labeled red cells. The red-cell study gives a much clearer picture of the spleen, with negligible interference from liver activity.

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REFERENCES

1. HAUBOLD U, PABST H-W, HÖR G: Scintigraphy of the placenta with ^{99m}Tc-labelled erythrocytes. In *Symposium on Medical Radioisotope Scintigraphy*, vol 2, Vienna, IAEA, 1969, pp 665–674

2. KORUBIN V, MAISEY MN, MCINTYRE PA: Evaluation of technetium-labeled red cells for determination of red cell volume in man. *J Nucl Med* 13: 760–762, 1972

3. ECKELMAN WC, REBA RC, ALBERT SN: A rapid simple improved method for the preparation of Tc^{99m} red blood cells for the determination of red cell volume. *Am J Roentgenol Radium Ther Nucl Med* 118: 861–864, 1973

4. FISCHER J, WOLF R, LEON A: Technetium-99m as a label for erythrocytes. *J Nucl Med* 8: 229–232, 1967

5. ECKELMAN W, RICHARDS P, ATKINS HL, et al: Visualization of the human spleen with ^{99m}Tc-labeled red blood cells. *J Nucl Med* 12: 310–311, 1971

6. ATKINS HL, ECKELMAN WC, HAUSER W, et al: Splenic sequestration of ^{99m}Tc-labeled red blood cells. *J Nucl Med* 13: 811–814, 1972

7. SMITH PHS: $^{99}Tc^{m}$ labelled erythrocytes for spleen scanning. *Int J Appl Radiat Isot* 25: 137–139, 1974

8. ATKINS HL, ECKELMAN WC, KLOPPER JF, et al: Vascular imaging with ^{99m}Tc-red blood cells. *Radiology* 106: 357–360, 1973

9. SMITH EM: Properties, uses, radiochemical purity and calibration of Tc^{99m}. *J Nucl Med* 5: 871–882, 1964

10. WEINSTEIN MB, SMOAK WM: Technical difficulties in ^{99m}Tc-labeling of erythrocytes. *J Nucl Med* 11: 41–42, 1970

11. ECKELMAN W, RICHARDS P, HAUSER W, et al: Technetium-labeled red blood cells. *J Nucl Med* 12: 22–24, 1971

12. BERGER R, JOHANNSEN B: Markierung von Erythrozyten mit ^{99m}Technetium. *Wiss Zeit Math-Naturwiss Leipsig Univ* 18: 635–638, 1969

13. NOUEL JP, BRUNELLE P: Le marquage des hématies par le technetium 99m. *Presse Med* 78: 73–74, 1970

14. SCHWARTZ K-D, KRÜGER M: Improvement in labeling erythrocytes with ^{99m}Tc-pertechnetate. *J Nucl Med* 12: 323–324, 1971

15. SMITH TD, RICHARDS P: A simple kit for the rapid preparation of ^{99m}Tc red blood cells. *J Nucl Med* 15: 534, 1974

16. BOYD GE: Technetium and promethium. *J Chem Educ* 36: 3–14, 1959

17. RUBBO SD, GARDNER JF: Sterilization by radiation. In *A Review of Sterilization and Disinfection*, Rubbo SD, Gardner JF, eds, Chicago, Yearbook, 1965, pp 79–80

18. GUTKOWSKI RF, DWORKIN HJ: Kit-produced ^{99m}Tc-labeled red cells for spleen imaging. *J Nucl Med* 15: 1187–1191, 1975

19. DONALDSON JD, MOSER W, SIMPSON WB: Primary standards in tin analysis. *Analyst* 86: 619–621, 1961

20. STECHER PG, ed: *The Merck Index*, 7th ed, Rahway, N.J., Merck and Co., 1960, p 975

21. DONALDSON JD: The chemistry of bivalent tin. In *Progress in Inorganic Chemistry*, vol 8, Cotton FA, ed, New York, Interscience, 1967, pp 319–320

22. STEIGMAN J, MEINKEN G, RICHARDS P: The reduction of pertechnetate-99 by stannous chloride. I. The stoichiometry of the reaction in HCl, in a citrate buffer, and in a DTPA buffer. *Int J Appl Radiat Isot* 26: 601–609, 1975

23. BROWN LC, MEINKEN G, SMITH T, et al: The behavior of ^{113}Sn(II) in ^{99m}Tc-labeled radiopharmaceuticals. *Int J Appl Radiat Isot*: to be published

24. SMITH TD, STEIMERS JR, RICHARDS P: Chemical effect of ^{99}Tc on ^{99m}Tc labeled radiopharmaceuticals. *J Nucl Med* 16: 570–571, 1975

25. ANSARI AN, ATKINS HL, SMITH T, et al: Clinical application of the BNL ^{99m}Tc RBC labeling kit. *J Nucl Med* 16: 512, 1975