

Improved Modification for In Vitro Labeling of Red Blood Cells with Technetium-99m

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We have tested a modification of the Brookhaven method for in vitro labeling of red blood cells (RBCs) with technetium-99m by adding an initial centrifugation step and performing the labeling on packed RBCs. This results in reproducible, high labeling efficiencies ($99.3\% \pm 0.4\%$, $n = 50$) after 15 min of incubation. The use of packed RBCs also results in a higher concentration of labeled RBCs (smaller bolus for injection) and less radiation exposure to the technologist. This technique has proved useful for radionuclide angiography, venography, gastrointestinal bleeding studies, and red cell mass determinations. It is particularly advantageous for RBC labeling in patients receiving chemotherapy.

Technetium-99m (^{99m}Tc) labeled red blood cells (RBCs) are widely used in nuclear medicine. The in vivo labeling technique (1) is adequate for most patients undergoing radionuclide ventriculography, but higher labeling efficiencies are required for venography, gastrointestinal bleeding studies, and red cell mass determinations (2–5). The highest labeling efficiencies are obtained with in vitro techniques (4,6,7).

The current Brookhaven kit procedure for in vitro labeling of RBCs with ^{99m}Tc is the result of a progression of improved techniques over ~ 15 yr (7). Early techniques required washing of the RBCs to remove unbound pertechnetate. Srivastava modified the technique with the use of EDTA which scavenges extracellular stannous ions and prevents extracellular reduction of pertechnetate (7). The need for centrifugation was eliminated with the current Brookhaven procedure by the use of sodium hypochlorite to oxidize extracellular stannous ion. The higher level of stannous chloride in this kit (50 μg) also overcomes the effect of ^{99}Tc carrier build-up in the first eluate in a new generator (7).

Following on the work of Srivastava et al. (7), we investigated a number of parameters in the Brookhaven technique, including choice of anticoagulant, use of whole blood or packed RBCs, and optimal incubation time with sodium [^{99m}Tc]pertechnetate. Based on these results, we devised an improved procedure for evaluation in patients with a variety of conditions and medications. These results will be compared with those obtained using in vivo and modified in vivo labeling techniques.

METHODS

Preparation of Stannous Citrate Kits

A solution was prepared which contained 50 μg stannous chloride (anhydrous), 3.67 mg sodium citrate, 5.5 mg dextrose, 1.4 mg sodium chloride, and 0.5 mg gentisic acid per ml of distilled water. The solution was purged with nitrogen and dispensed in 1-ml aliquots through a sterile, 0.22- μm membrane filter into sterile, septum-sealed, nitrogen-flushed vials which were then stored at -70°C until use.

Investigation of In Vitro Method

Venous blood from healthy volunteers was collected in evacuated tubes with heparin, ACD, or EDTA as anticoagulant. For experiments which used packed RBCs, the whole blood was centrifuged for 5 min at ~ 1,000 G with the tube upside down to aid in removal of packed cells. The desired volume of whole blood or packed cells was added to a freshly thawed stannous citrate kit and incubated at room temperature for 5 min, after which 0.6 ml freshly diluted 0.1% sodium hypochlorite solution and 1 ml 4.4% EDTA solution were added with mixing. Sodium [^{99m}Tc]pertechnetate, 16–21 mCi (600–800 MBq), was then added and the kit was incubated for 5–60 min at room temperature. In one set of studies, [^{99m}Tc]pertechnetate obtained from a generator with 3 days of ingrowth was used.

Determination of Labeling Efficiency

Samples of 0.2 ml labeled blood were removed from the kit, diluted with 2 ml saline and centrifuged for 5 min at ~ 1,000 G. The supernatant was transferred to a fresh tube, and 2 ml water was added to the red cell pellet. The labeling efficiency was calculated as the activity in the red cell pellet divided by the total activity in the pellet and supernatant.

Clinical Comparison of Labeling Methods

The optimal method for in vitro labeling developed using blood from healthy volunteers was evaluated clinically in patients referred for radionuclide ventriculography, venography, gastrointestinal bleeding studies and red cell mass determinations. These patients were receiving a variety of medications, and included patients treated with doxorubicin in whom cardiotoxicity was monitored by radionuclide ventriculography. Labeling efficiency results were compared for the optimized in vitro ($n = 26$), in vivo ($n = 13$), and modified in vivo ($n = 11$) labeling in similar groups of patients.

In Vivo Method. The in vivo method of Pavel et al. (1) was

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used with 13 patients. Stannous pyrophosphate equivalent to ~ 700 µg stannous ion was administered intravenously, followed 30 min later by 20 mCi (740 MBq) sodium [^{99m}Tc]pertechnetate. A blood sample was obtained 30 min later for measurement of labeling efficiency as described above.

Modified In Vivo Method. The modified in vivo method of Porter et al. (2) was used in 11 patients. Thirty minutes after i.v. injection of stannous pyrophosphate, an 8-ml sample of blood was drawn through a butterfly into a syringe which contained 1.5 ml ACD solution and 20mCi (740 MBq) sodium [^{99m}Tc]pertechnetate. The syringe contents were mixed gently for 10 min, then reinjected through the butterfly which had been kept open with saline. Labeling efficiency was measured either in a sample of the injectate or in a blood sample obtained 30 min after reinjection.

In Vitro Method. The optimized method was evaluated in 26 patients for labeling efficiency comparison and in an additional group of 24 patients. A 3–5-ml sample of whole blood was obtained in an evacuated, heparinized tube which was then centrifuged upside down for 5 min at ~ 1,000 G. A 1.0–1.5-ml sample of packed red cells was transferred to a stannous citrate kit and incubated for 5 min followed by addition of 0.6 ml 0.1% sodium hypochlorite and 1 ml 4.4% EDTA solutions. Sodium [^{99m}Tc]pertechnetate, 21 mCi (800 MBq), was added to the kit and allowed to incubate 15 min before reinjection. Labeling efficiency was measured as for the modified in vivo method.

RESULTS

In Vitro Volunteer Studies

In preliminary studies, we confirmed that 50 µg stannous chloride was the optimal amount per kit and that an incubation time of 5 min after addition of the blood to the kit was sufficient (8). These parameters were kept constant for the remaining studies.

Heparin was the standard anticoagulant. Equally high labeling efficiencies were obtained with ACD (mean ± s.d.: 99.2% ± 0.3%, n = 5). However, when EDTA was used, the labeling efficiency was much lower (33.5% ± 0.7%, n = 3).

TABLE 1. Time Course of In Vitro RBC Labeling Using 1 ml Packed RBCs and 3 ml Whole Blood

Incubation time (minutes)	% Labeling efficiency*	
	Packed RBCs	Whole blood
5	97.2 ± 1.3	—
10	99.4 ± 0.2	—
15	99.6 ± 0.2	95.6 ± 0.2
30	—	99.4 ± 0.3
60	99.4 ± 0.3	99.2 ± 0.1

*Each value is mean ± s.d. for three determinations.

Table 1 shows that high labeling efficiency was achieved much faster with 1 ml packed RBCs than with 3 ml whole

blood. Within 10–15 min > 98% labeling efficiency was routinely obtained. In 50 clinical studies, the mean labeling efficiency was 99.3% ± 0.4%. When pertechnetate obtained from a generator with three days of ingrowth was used, labeling efficiency remained high (99.3% ± 0.3%, n = 3).

TABLE 2. Comparison of Three RBC Labeling Methods

	In vivo	Modified in vivo	In vitro
Number of patients	13	11	26
% labeling efficiency ± s.d.	94.7 ± 1.0*	96.1 ± 1.5*	99.3 ± 0.4
Correlation coefficient between labeling efficiency and:			
Hematocrit	0.298	0.883**	0.085
Hemoglobin concentration	0.826**	0.860**	0.051

*p < 0.001, t-test versus in vitro results.

**p < 0.001, p-value of correlation coefficient.

Clinical Studies

The data in Table 2 show that the labeling efficiency of the in vitro method was significantly higher than that of the in vivo (1) and modified in vivo (2) techniques used in this laboratory (t-test, p < 0.001). For the in vivo and modified in vivo techniques, there was a significant correlation between labeling efficiency and the patient's hematocrit or hemoglobin concentration (p < 0.001). Labeling efficiency was independent of these parameters for the in vitro method.

In a total of 50 studies in patients with a variety of conditions and medications, the mean labeling efficiency of the optimal in vitro method was 99.3% ± 0.4%. The stability of the label after reinjection was shown in blood samples taken within the first hour (98.8% ± 0.6% labeling efficiency, n = 5) and up to 24 hr (> 95% bound to RBCs).

In a subgroup of patients in whom radionuclide ventriculography was used to monitor cardiotoxicity of doxorubicin, the labeling efficiency was 99.3% ± 0.3%, n = 12.

DISCUSSION

This modification to the current Brookhaven kit procedure (7) involves the addition of an initial centrifugation step. This resulted in high labeling efficiencies after short incubation periods and reduced the effect of patient condition and medication on labeling efficiency. The shorter incubation time more than offset the centrifugation time.

The use of heparin and ACD as anticoagulants resulted in similar labeling efficiencies. This allows flexibility in procurement of the blood sample for labeling. However, EDTA as anticoagulant resulted in poor labeling, presumably by chelating the stannous ion before it could enter the RBC.

We have noted elsewhere with in vivo and modified in vivo RBC labeling that labeling efficiency showed a significant correlation with the patient's hematocrit and hemoglobin concentration (9). With the improved in vitro technique, labeling was independent of hematocrit and hemoglobin. The initial centrifugation step maximizes the hematocrit and hemoglobin concentration in each sample and reduces the variability between patients.

Radionuclide ventriculography is an important technique with which to monitor the cardiotoxicity of chemotherapeutic agents such as doxorubicin. However, RBC labeling in these patients is felt to be poorer than that in general cardiac patients (9). The improved in vitro technique produced excellent results in these patients.

We generally measure the labeling efficiency before the labeled cells are reinjected. On the rare occasions when the labeling efficiency has been low, the blood can be centrifuged and the packed RBCs reinjected.

In conclusion, the improved procedure results in a reproducible high labeling efficiency > 98% despite a variety of patient conditions and medications, a higher concentration of labeled RBCs (smaller bolus for injection), and less radiation exposure to the technologist because the activity is added to a shielded vial in the final step. The total time required is 25–30 min. The technique has proved useful for radionuclide ventriculography, venography, gastrointestinal bleeding studies, and red cell mass determinations. It is particularly advantageous for use in patients treated with doxorubicin.

NOTE

This work was presented in part at the 34th Annual Meeting of the Society of Nuclear Medicine (8).

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