An Assessment of Factors Which Influence the Effectiveness of the Modified In Vivo Technetium-99m-Erythrocyte Labeling Technique in Clinical Use

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This study assessed factors which may contribute to suboptimal image quality when the modified in vivo erythrocyte labeling technique is used with standard clinical ^{99m}Tc activities. For each assessment duplicate or triplicate blood specimens were withdrawn from ≥10 patients, into syringes containing 700–900 MBq ^{99m}Tc as pertechnetate. After incubation the percent of ^{99m}Tc which was not bound to erythrocytes at blood re-injection time (%Unbound ^{99m}Tc), was measured and compared when one of four factors was varied. The most significant results, in descending order of measured effect were:

		%Unbound ^{99m}Tc	
Generator ingrowth time:	≥48 hr	43% ± 15%	
	≤24 hr	13% ± 6%	p<<0.001
Volume of blood withdrawn:	1.5 ml	22% ± 13%	
	3.0 ml	10% ± 6%	p<0.001
Incubation time of blood with ^{99m}Tc:	10 min	19% ± 12%	
	20 min	11% ± 6%	p<0.001
Erythrocyte tinning time:	5 min	19% ± 7%	
	10min	13% ± 4%	p<0.05

Our data suggest that the requirements for optimal erythrocyte labeling with standard clinical ^{99m}Tc activities are: (A) Erythrocyte tinning time between 10 and 30 min; (B) blood volume ≥3 ml; (C) blood incubation time ≥20 min; and (D) Generator ingrowth time ≤ 24 hr.

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It is widely accepted that the modified in vivo method of labeling erythrocytes with ^{99m}Tc (*1*) more frequently provides clinically acceptable blood-pool images than the in vivo erythrocyte labeling technique (*2*). Despite this, some factors can impair this labeling technique including mixing ^{99m}Tc with large quantities of heparin prior to adding blood (*3*), large amounts of carrier ^{99}Tc present in the first elution of a new generator (*3*), and some medications, including dipyridamole (*4*). Even when these factors are avoided, suboptimal clinical results have still been observed with the modified in vivo technique, both by us and by others (*4*). Because of the expanding use of this technique (*5*), and because not all parameters that determine labeling efficiency are known (*4*), further attempts to increase the reliability of this labeling technique are desirable.

Most authors (*1,3,5,6*) have recommended that for the modified in vivo labeling technique, 0.5–1.0 mg of stannous ion should be administered intravenously, and after 20 min of in vivo erythrocyte "tinning", 3 ml of blood should be withdrawn and incubated with 700–900 MBq of [^{99m}Tc]pertechnetate for 10 min. The most important published experimental data in support of this protocol (*1*), used only 1–3 MBq of ^{99m}Tc for 3 ml of blood. Therefore these published results may not accurately predict labeling results when standard clinical ^{99m}Tc activities are used.

The aim of our study was to re-evaluate the combination of parameters which should most often produce optimal labeling efficiency when using standard clinical activities of ^{99m}Tc for the modified in vivo erythrocyte labeling technique.

The factors which have been assessed in this study are schematically illustrated in Figure 1 and were:

1. Tinning Time—the time interval between administration of stannous pyrophosphate and withdrawal of blood.
2. Blood Volume—the volume of blood withdrawn.
3. Incubation Time—the duration of time which was allowed for the blood to be incubated with ^{99m}Tc.
4. Generator Ingrowth—the time between the generator elution which produced the ^{99m}Tc sample being evaluated and the previous elution.

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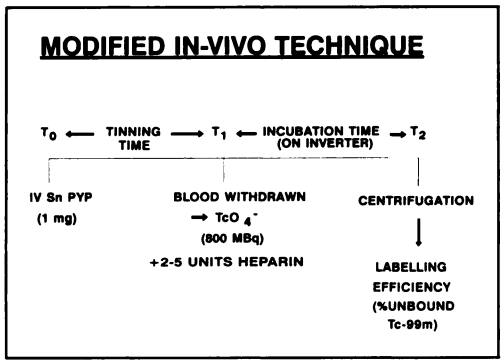

FIGURE 1. This laboratory's implementation of the modified in vivo erythrocyte labeling technique: T_0 = time intravenous stannous pyrophosphate (i.v. SnPyP) was administered. T_1 = time blood was withdrawn for labeling with ^{99m}Tc. T_2 = time incubation finished and measurement of labeling efficiency commenced. %Unbound ^{99m}Tc = percentage of ^{99m}Tc not bound to erythrocytes prior to re-injection.

MATERIALS AND METHODS

Patient Population

Sixty adult patients in whom ^{99m}Tc-erythrocyte labeling was being performed for clinically indicated bloodpool scintigraphy were included in this study. Patients undergoing scintigraphy for gastrointestinal bleeding, or who were known to have recently received any blood transfusion, were not included.

Study Protocol

For each study patient at least two blood samples were analyzed, for a total of 151 labeled blood samples. In order to compare the effect on labeling efficiency of varying individual parameters, radiolabeling of blood specimens from any one patient was performed identically except for variation of one of the studied variables at a time. Many specimens were used in more than one comparison, which means that there was no constant "reference" value for the nonvaried parameters, although these were usually close to the optimal value, and were always constrained within the range of variation being studied for each parameter.

Erythrocyte Labeling Technique

At the commencement of labeling, 1 mg of stannous ion as pyrophosphate was injected intravenously through a 26 gauge needle (Time T_0, Fig. 1). At the time required by the protocol (Time T_1, Fig. 1) blood was withdrawn from a previously inserted 22 gauge intravenous cannula, and through the side arm of a three way tap into a 5-ml shielded syringe containing 800 ± 50 MBq of [^{99m}Tc]pertechnetate in isotonic saline. In each case, the ^{99m}Tc was prepared from undiluted eluate of a generator within 4 hr of elution (mean volume = 0.6 ml, range 0.2 to 1.5 ml). Heparinized saline, 0.2 to 0.5 ml (containing 2–5 units of Heparin), was then mixed with the blood and ^{99m}Tc via the three-way tap. The shielded syringe was immediately placed on a rotating invertor for the duration of the incubation time.

Immediately after the conclusion of incubation time required by the protocol (Time T_2, Fig. 1) analysis of the labeled blood specimen was performed in order to identify the percentage of [^{99m}Tc]pertechnetate which was free in plasma and not bound to the erythrocytes (%Unbound ^{99m}Tc).

Measurement of Labeling Efficiency

For measurement of %Unbound ^{99m}Tc, the blood was removed from the rotating inverter and placed in a laminar flow cabinet, transferred to a 12 ml shielded centrifuge tube, made up to 12 ml with isotonic saline and mixed, and then centrifuged at 1000 g–2000 g for 2–4 min. The supernatant was pipetted off into a second tube, and %Unbound ^{99m}Tc was calculated as follows:

$$\%\text{Unbound } ^{99m}\text{Tc} = \frac{\text{Activity in supernatant} \times 100}{\text{Activity in erythrocytes} + \text{activity in supernatant}}.$$

Assessment of Individual Parameters

Tinning Time. We compared the effect of tinning times of 5, 10, 15, 20 and 30 min, keeping other factors constant. In one group of 13 patients, 5 min and 15 min tinning times were compared, and in a second group of 13 patients (in whom triplicate blood specimens were used) 10, 20 and 30 min tinning times were compared.

Blood Volume. In 13 patients, the conventional 3.0 ml of blood was compared to 1.5 ml of blood, which could more readily permit the combination of first-pass cardiac studies with equilibrium cardiac blood-pool studies (*6*).

Incubation Time. Incubation times of 10, 20 and 30 min were compared in two patient groups, each of 13 patients. In one group, 10 min was compared with 20 min, while in the other group 20 min was compared with 30 min.

Generator Ingrowth. Generator ingrowth times of ≥48 hr were compared with those of ≤24 hr. Comparisons were first performed in 10 patients using a "wet column" generator (Australian Nuclear Science and Technology Organisation, Sydney, Australia), also used for all other experiments, in which generator ingrowth was ≤24 hr. In order to assess the potential effect of radiolysis from the wet column generator, this experiment was repeated in 10 additional patients using a dry column generator, (Amersham, England). For each type of generator, a comparison was performed between results obtained using the first elution after at least 48 hr free of generator elution and results obtained using a second blood specimen drawn at the same time and incubated with the same total ^{99m}Tc activity obtained from a repeat elution performed approximately 1–3 hr after the first elution.

Statistical Analysis

Values of %Unbound ^{99m}Tc have been expressed as mean ± s.d. Comparison of paired measurements was performed with a paired Student's t-test where two groups were to be compared. In the experiment where triplicate blood specimens were to be compared, analysis of variance was performed.

RESULTS

The effect of varying tinning time is illustrated in Figure 2. There was a significantly higher ($p < 0.01$) %Unbound ^{99m}Tc with a tinning time of 5 min (19% ± 7%) compared with that found with a time of 15 min (11% ± 5%). No statistically significant difference in %Unbound ^{99m}Tc was found between 10, 20 and 30 min of tinning time. However there was a trend for lowest %Unbound ^{99m}Tc results to be found at 15 min with a trend to higher and more variable %Unbound ^{99m}Tc measurements by 30 min.

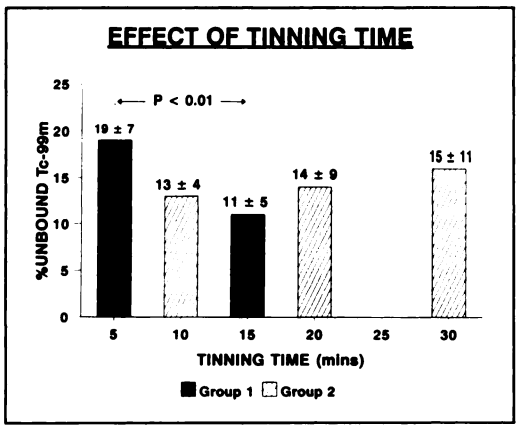

FIGURE 2. The effect on %Unbound ^{99m}Tc (mean ± s.d.) of varying tinning time (see text) between 5, 10, 15, 20 and 30 min. Group 1 (solid bars) contained 13 paired samples. Group 2 (hatched bars) contained 13 triplicate samples.

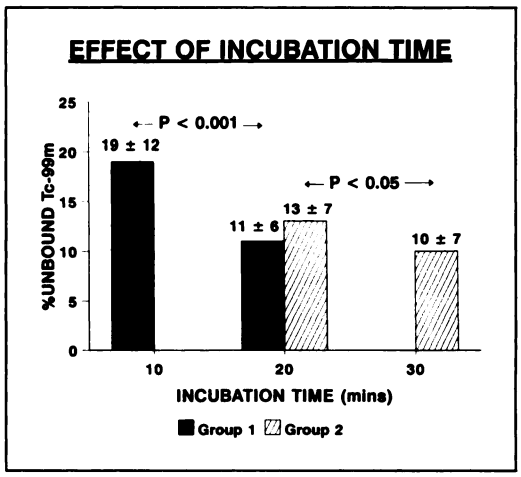

FIGURE 4. The effect on %Unbound ^{99m}Tc (mean ± s.d.) of varying incubation time of blood with ^{99m}Tc between 10, 20 and 30 min is shown. Both Group 1 and Group 2 contained 10 paired samples.

The effect of varying blood volume is shown in Figure 3. The %Unbound ^{99m}Tc was much lower (10% ± 6%) when 3.0 ml of blood was used, than when 1.5 ml was used (22% ± 13%) ($p < 0.001$).

The effect of varying blood incubation time is illustrated in Figure 4. There was a highly significant reduction in %Unbound ^{99m}Tc (from 19% ± 12% to 11% ± 6%) when incubation time was increased from 10 min to 20 min ($p < 0.001$). When the incubation time was increased from 20 min to 30 min, there was a statistically significant further decrease ($p < 0.05$) in %Unbound ^{99m}Tc, but this was of small magnitude.

The effect of generator ingrowth is illustrated in Figure 5. With the wet column generator, %Unbound ^{99m}Tc averaged 43% ± 15% for generator ingrowth of ≥48 hr, compared with only 13% ± 6% when generator ingrowth was ≤24 hr. The dry column generator yielded almost identical results.

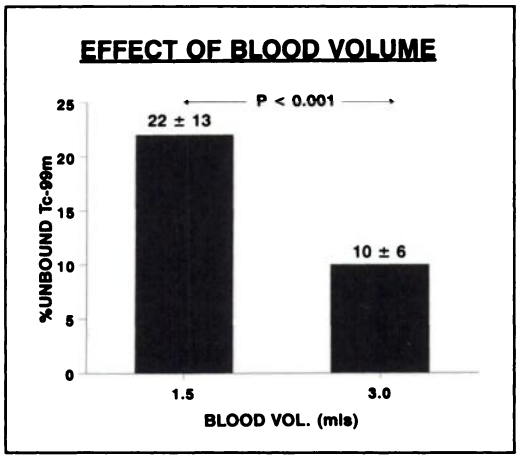

FIGURE 3. %Unbound ^{99m}Tc (mean ± s.d.) is compared using 1.5 ml of blood and 3.0 ml of blood (n = 13 paired samples).

DISCUSSION

This study suggests that when standard clinical activities of ^{99m}Tc are used optimum results with the modified in vivo erythrocyte labeling technique may be obtained using a significantly different protocol than has been recommended on the basis of experiments using smaller amounts of ^{99m}Tc (*1*).

The generally recommended tinning time is 20 min (*1*, *3*,*5*,*6*). Hamilton et al. suggested from animal experiments that 5 min to 30 min was acceptable, although they did not study any time intervals between 5 and 30 min (*7*). Our results suggest that 5 min is too short for optimal labeling of erythrocytes with ^{99m}Tc, presumably because

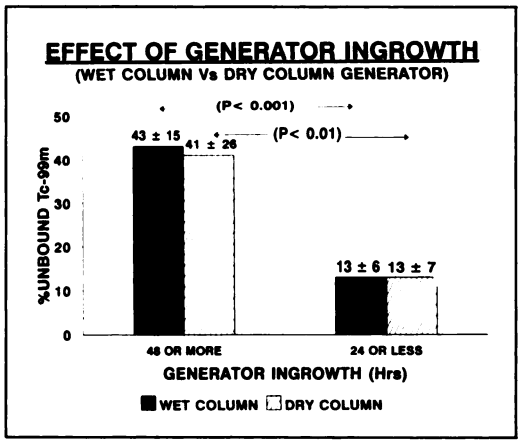

FIGURE 5. The effect on %Unbound ^{99m}Tc (mean ± s.d.) of ^{99m}Tc generator ingrowth time ≥48 hr compared with ingrowth times ≤24 hr. Results obtained with a wet column ^{99m}Tc generator (solid bars) are compared with those obtained using a dry column generator (hatched bars). (n = 10 paired samples for each class of generator).

plasma stannous ion levels have not yet fallen sufficiently, leading to increased reduction of pertechnetate by stannous ion in plasma (*6*). Although optimum labeling efficiency was found with a tinning time of 15 min, comparable results were found with tinning times of 10, 20 and 30 min.

Our finding that %Unbound ^{99m}Tc is halved when a blood volume is 3.0 ml compared to 1.5 ml, favors use of the 3.0 ml volume originally and most widely recommended (*1,3,5,6*). One likely explanation of our results could be the lower ratio of erythrocytes to [^{99m}Tc]pertechnetate solution when blood volume is smaller, effectively producing a low hematocrit, which has been shown to impair erythrocyte labeling with ^{99m}Tc (*8*). Another factor, shown by Callahan et al. (*4,8*) could be the greater cooling of erythrocytes that occurs when a smaller blood volume at body temperature is mixed with a significant volume of ^{99m}Tc in saline (average 0.6 ml) and heparinized saline (average 0.3 ml), both at room temperature, as occurs when standard clinical activities of ^{99m}Tc are used.

Callahan et al. have previously demonstrated the importance of incubation time in reducing %Unbound ^{99m}Tc, using an experimental model in which approximately 1–3 MBq ^{99m}Tc in a volume of less than 0.1 ml was used to label 3 l of blood (*1*). In their experiments, when in vivo tinning was performed with 0.5 mg of stannous ion, %Unbound ^{99m}Tc decreased rapidly as incubation time was lengthened to 10 min, but did not change significantly thereafter. Our results differ from those of Callahan et al. in finding a clinically significant degree of continuing reduction in %Unbound ^{99m}Tc between 10 and 20 min, and to a much lesser extent between 20 and 30 min. Possible causes of these disparate findings include the differing amounts of stannous ion, and the differing volumes and activities of [^{99m}Tc]pertechnetate solution.

Our study used 1 mg of stannous ion, while Callahan et al. used 0.5 mg of stannous ion is those experiments in which binding was maximal within 10 min. However, they also found that labeling took longer when 2 mg of stannous ion was used. It is possible therefore that the use of 1 mg of stannous ion, as widely practiced clinically (*3,5,6*), and as used in our study, may have an intermediate effect on rates of labeling.

The difference in volumes of [^{99m}Tc]pertechnetate solution is in all likelihood an important factor. Our clinically based experiments used much greater ^{99m}Tc activities (mean = 800 MBq) and proportionately larger saline volumes (mean = 0.6 ml) than those of Callahan et al. (1–3 MBq ^{99m}Tc in <0.1 ml saline). In view of the marked dependence of ^{99m}Tc uptake by erythrocytes on hematocrit (*8*) and temperature (*4,8*), this volume factor may contribute to the longer time taken for optimal labeling in our experiments.

With respect to generator ingrowth, the marked increase in %Unbound ^{99m}Tc in our experiments when heavily ingrown generators were used, is compatible with the clinical observation that suboptimal erythrocyte labeling is most common on those Mondays when a generator has not been eluted over the weekend for urgent clinical studies. The results shown in Figure 5A are compatible with the results obtained by Porter et al. (*3*), and others (*9*), who postulated that buildup of ^{99}Tc from decay of ^{99m}Tc, may exceed the reducing capacity of stannous ions in the sample of erythrocytes (*5,6*). In view of our virtually identical results for wet column and dry column generators, radiolysis appears not to be a significant factor.

CONCLUSION

We conclude that when 1 mg of stannous ion and approximately 800 MBq of [^{99m}Tc]pertechnetate are used for the modified in vivo erythrocyte labeling technique, optimum erythrocyte labeling (%Unbound ^{99m}Tc prior to re-injection of blood of approximately 10%) occurs if the following conditions apply:

1. The delay between administering stannous ion and withdrawing blood for labeling is between 10 min and 30 min.
2. The volume of blood withdrawn is at least 3 ml.
3. The delay between withdrawing blood and re-injecting into the patient is at least 20 min.
4. Technetium-99m-pertechnetate is eluted from a generator without excessive ingrowth, in that its previous elution had occurred no more than 24 hr earlier. If generator ingrowth is >24 hr then a second elution should be obtained one or more hours after the first and used for all erythrocyte labeling procedures.

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