

EVALUATION OF TECHNETIUM-LABELED RED CELLS USING Sn-kit

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ABSTRACT

The labeling yield of ^{99m}Tc -erythrocytes by our method is higher than 97% for blood drawn from 35 patients with polycythemia vera (Hct. 50%-74%). Quality control for tagging efficiency was done both in vitro and in vivo by using ionization chamber and scintillation counter respectively. All experiments were conducted with sodium pertechnetate which was not the first elution from a new generator. As a results of experiments reported here, a simplified and reproducible method has been developed for the preparation of ^{99m}Tc -labeled red blood cells, it consistently produces high yield in a closed sterile system with few mechanical-steps.

INTRODUCTION

Technetium-99m-pertechnetate has been proposed as a radionuclidic label for red blood cells¹⁻⁹, however all methods were conducted with a laborious task. This paper described the preparation of an inhouse simple Sn-kit for ^{99m}Tc -labeled red blood cells in a closed sterile system with few mechanical steps. The Sn-kits have performed well after more than 12 months with 12 ml of whole blood from patients who have hematocrit higher than 50%. The labeling efficiency is higher than 97% with only 5 minute incubation time.

MATERIALS

All experiments were conducted with human blood from 35 patients with possible polycythemia referred to division of Nuclear Medicine for red cell mass measurements. The percent of ^{99m}Tc associated with red blood cells was determined by cell sedimentation. Ionization chamber was used for ^{99m}Tc assay in vitro and gamma well counter was used for ^{99m}Tc assay in vivo. Stannous

freeze dried kits were prepared in our laboratory. All other chemicals were reagent grade. Surflo Int Type Wing Infusion Set with releasable injection site was used to reduce repetition of venipunctures. Methylenediphosphonic acid was purchased from Sigma Chemical Co, St.Louis, Mo. and Stannous chloride dihydrate was purchased from E-Merck, Darmstadt, Germany.

METHODS

PREPARATION OF Sn-FREEZE DRIED KITS.

Cleaned glassware and equipment were wrapped in wrapping paper, and sterilized in autoclave.

A. Dissolve $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ 1 g in 5 ml of concentrated HCl. Filter the solution through 0.22 μm millipore filter. Stir the filtered solution at least 30 minutes.

B. In a 250 ml beaker, dissolve 500 mg Methylene diphosphonic acid in 150 ml sterile distilled water.

C. To solution B add 1.3 ml of solution A containing 260 mg of stannous chloride and adjust the pH to 5.0-6.0 using 40% NaOH 2.2 ml.

D. Sterilize the solution by 0.22 µm membrane filtration into presterilized evacuated vials. Aseptically transfer 1 ml each of this solution to sterile 7 ml serum vials. Freeze dry for 24 h, seal under vacuum after freeze drying, each vial contains 1.7 mg stannous chloride and 3.25 mg methylenediphosphonic acid.

IN VITRO QUALITY CONTROL FOR ^{99m}Tc-RBC USING INHOUSE Sn-KIT.

35 adult patients suspicious of polycythemia vera with hematocrit range from 50% to 74% were studied. Erythrocytes were labeled with ^{99m}Tc using the following procedure.

1. Add 1.5 ml of normal saline to the Sn-kit, then mix the vial and withdraw the solution from the vial by using 2.5 ml syringe. Inject stannous solution to the blood circulation of the patient, after waiting for 10 minutes and withdraw 12 ml of blood from the same patient, and transfer to 50 ml sterile Falcon centrifuge tube #2098 containing 2.5 ml of ACD.

2* Mix the withdrawn blood with 200 mCi of Na^{99m}TcO₄. This amount can be prepared by using Na^{99m}TcO₄ 10 mCi/ml 2 drops from tuberculin syringe with needle mix and wait for 5 minutes.

3. Add 25 ml of normal saline to the sample, mix and centrifuge at 2500 rpm for 5 minutes.

4. Read the volume of the supernatant for example Y ml (Fig 1). Measure the activity of volume X (Fig 1) by using the ionization chamber.

5. Pipette 10 ml of the supernatant and assay the activity, calculate in vitro percent bound to red cells with the following formula.

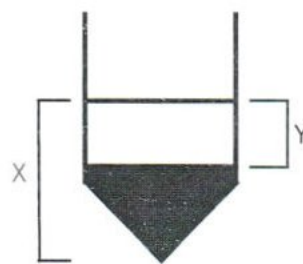


Fig 1. Sedimentation of ^{99m}Tc - RBC in Falcon tube.

$$= \frac{\text{Activity of volume X} - \frac{\text{Volume Y}}{10} (\text{activity of 10 ml supernatant})}{\text{Activity of volume X}}$$

The in vitro percent bound of ^{99m}Tc -RBC in 35 patients, were greater than 97%. This indicates that neither washing nor centrifugation is needed in the labeling process in the future for high hematocrit patients.

IN VIVO QUALITY CONTROL FOR ^{99m}Tc -RBC USING INHOUSE Sn-KIT.

To examine in vivo stability of ^{99m}Tc -labeled red cells, the blood activity was followed in four patients at intervals of 10, 20, 30, 60 and 120 minutes after injection of 6 ml (about 100 μCi) of ^{99m}Tc -labeled red blood cells from 2*. 5 ml of postinjection samples of blood were drawn in heparinized syringes from a vein on the opposite limb at intervals of 10, 20, 30, 60 and 120 minutes.

The samples were pipetted triplicately and the activity was counted by using scintillation well counter. Red cell volumes at 10, 20, 30, 60 and 120 minutes were calculated.

RESULTS

The labeling efficiency in vitro was found greater than 97%, and in vivo stability of ^{99m}Tc -labeled red blood cells was confirmed by comparing the sequential red cell volume measurement at various times after injection. The results expressed as the percentage change between the values obtained with 10 minute sample. The figures are shown in table 1. The red cell volumes are almost identical for the first hour with slight increase in the second hour sample.

Table 1. Sequential Red Cell mass measurements at various time after injection

Subjects	Time (minutes)	% 10-minute value (ml)				Mean
		1	2	3	4	
	10	100	100	100	100	
	20	100.2	100.1	100.3	100.2	100.2
	30	100.4	100.3	100.4	100.5	100.4
	60	100.3	100.6	100.5	100.6	100.5
	120	102.4	103.3	104.5	101.8	103.0

DISCUSSION

From the study, a new method for labeling red blood cells with pertechnetate ion without using the centrifugation was developed. From this technique more red cells could absorb almost all ^{99m}Tc in order to gain high yield. The results showed that the technique is useful for adult patients with high hematocrit.

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