

Labeling of Red Blood Cells with ^{99m}Tc : A Very Simple Kit

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Summary. A very simple kit to obtain red blood cells labeled with ^{99m}Tc at 37 and 50 °C is presented. These radiolabeled blood cells can be used in several nuclear medicine procedures. The described kit is easily manufactured and contains only sodium and stannous chloride. It takes only 3 h to be lyophilized and is not expensive. With this kit, ^{99m}Tc -red blood cells with high stability and high efficiency are obtained for at least 12 months.

INTRODUCTION

^{99m}Tc (as sodium pertechnetate or many other ^{99m}Tc -products, accounts for almost 80% of the radiopharmaceuticals used in nuclear medicine procedures.¹) The reasons for such a preeminent position are: (1) its electron emission is negligible and thus the radiation dose for patients is minimal; (2) its gamma-ray energy of 140 keV, with an abundance of 90%, is suitable for scintigraphy with a gamma camera; (3) its half-life of 6 h is sufficient to allow different clinical evaluations and causes minimal environmental impact and disposal wastes; (4) its chemical form permits the labeling of several structures with biomedical interest, used as radiopharmaceuticals or in basic research.¹⁻⁴)

Kits to be labeled with ^{99m}Tc have been developed. These have permitted the obtaining of various radiopharmaceuticals: glucoheptonic acid for cerebral and renal studies, dimercaptosuccinic and dietilentriaminopentaacetic acid for renal evaluations, methylenodiphosphonic acid and sodium pyrophosphate for bone studies, stannous colloidal and phytic acid for

hepatic splenic studies, derivatives of iminodiacetic acid for biliary evaluations and others.^{1,2,5-7}) Besides these molecules, red blood cells (RBC), leukocytes and platelets have been labeled with this radionuclide using specific kits and procedures.^{1,2,8-12})

The labeling procedures of different molecules and structures of biomedical interest with ^{99m}Tc , as sodium pertechnetate, frequently depend on a reducing agent. Stannous chloride is employed for this purpose.^{2,13})

^{99m}Tc labeled red blood cells are employed in various procedures, including determining the red cell mass, localizing the sites of gastrointestinal hemorrhage, for cardiovascular studies and for studying splenic sequestration.^{1,2,14-16})

Different kits have been utilized to obtain radiolabeled red blood cells with ^{99m}Tc .^{8,9}) Many of these kits are expensive and are not often available when necessary. In this work, we present a very simple and inexpensive kit to label RBC with ^{99m}Tc . Its application in obtaining, with high efficiency, ^{99m}Tc -red blood cells at 37 and 50°C, is also described.

MATERIALS AND METHODS

This kit is always produced using fresh distilled water. The following steps were taken in the preparation of this kit: (1) 300 μg of stannous chloride, as $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (E. Merck, Darmstadt, Germany), were added to NaCl (Reagen, Brazil) 0.9% solution and set up to 50 ml, under a nitrogen atmosphere; (2) sterilization of this mixture was carried out by filtration through a membrane (0.22 μm); (3) volumes of 1 ml of

this solution were distributed in 50 sterile and closed vials, kept under nitrogen atmosphere and frozen at -20°C ; (4) lyophilization was carried out in a lyophilization system (model freeze Dryer 3, Labconco, MO, USA) with a 37-port manifold for 3 h; and (5) the lyophilizate kits were sealed in a nitrogen atmosphere and stored.

To determine the optimal time for lyophilizing, the vials in the lyophilization system were removed during different periods of time and their masses were determined in an analytical balance. The percentage of residual mass (%RM) was determined as previously described.⁵⁻⁷ Briefly, the %RM was calculated by the ratio of the mass at different periods of time and the original mass of the kits.

To evaluate this stannous-sodium kit stability, the percentage of radioactivity uptake by red blood cells (%RU) for 14 months at 37 and 50°C ¹⁷ was determined, as described before.^{10,14,17} Briefly, after the labeling process and centrifuging, aliquots of pellets (RBC) and the supernatant were counted in a scintillation counter using a NaI (TI) crystal. To calculate the RU% into RBC, the activity in the pellet was divided by the sum of the activities in the pellet and supernatant.

RESULTS

In Table 1, the steps employed to manufacture a very simple kit to label red blood cells with $^{99\text{m}}\text{Tc}$ are shown. It can be observed that only two salts, stannous and sodium chloride, both easily available, are necessary to prepare this kit.

Table 2 shows the values of the %RM, plus/minus standard deviations, at different periods of time in the lyophilization process. It can be seen that the %RM reaches constant values after 1 h. As a safe

Table 1. Manufacturing steps of a stannous-sodium kit to label red blood cells with $^{99\text{m}}\text{Tc}$.

- 1- Add 300 μg of stannous chloride to NaCl 0.9% solution (*).
- 2- Set volume at 50 ml (*).
- 3- Filtrate this mixture using 0.22 μm membrane.
- 4- Distribute 1 ml of this mixture in 50 vials (*).
- 5- Freeze vials at -20°C .
- 6- Lyophilize the vials for 3 h.
- 7- Close the vials (*).
- 8- Store the kits.

(*). These steps were carried out in a nitrogen atmosphere.

Table 2. %RM of stannous-sodium kit at different lyophilization periods of time.

Time (h)	% of Residual mass (*)
0.0	100.00
0.5	97.84 \pm 0.08
1.0	96.41 \pm 0.19
1.5	95.79 \pm 0.21
2.0	95.79 \pm 0.22
2.5	95.79 \pm 0.20
3.0	95.79 \pm 0.15

(*). The values (mean \pm SD) were obtained from an average of three isolated experiments.

Table 3. % of radioactivity uptake for red blood cells labeled with $^{99\text{m}}\text{Tc}$, at 37 and 50°C , using a stored stannous-sodium.

Time (month)	% of radioactivity (*)	
	37 $^{\circ}\text{C}$	50 $^{\circ}\text{C}$
0	98.71 \pm 3.5	97.30 \pm 1.8
3	97.39 \pm 4.1	96.81 \pm 4.0
6	98.11 \pm 2.9	98.15 \pm 3.6
8	96.20 \pm 1.9	95.91 \pm 4.1
12	97.81 \pm 3.7	98.01 \pm 1.1
14	85.01 \pm 5.8	87.98 \pm 6.4

(*). These values (means \pm SD) were obtained from an average of three isolated tested kits, during each month of the study.

condition, we decided to choose the period of 3 h to lyophilize this kit.

In Table 3 is shown the percentage of radioactivity uptake by red blood cells, labeled with $^{99\text{m}}\text{Tc}$, at 37 and 50°C in different months after this kit's production. These values were always independent of the temperature and higher than 95%, for at least 12 months.

DISCUSSION

The analysis of the obtained results shows that a good product has been achieved. The necessity of only two inexpensive and easily available salts has enabled the manufacture of a very simple kit. There are other simple kits described in the literature.^{8,9} The one described in this paper is a good alternative should it is necessary to label red blood cells with $^{99\text{m}}\text{Tc}$.

The time of lyophilization is important; sometimes it can be long.¹⁸ As in other kits^{5-7,19} a short lyophil-

ization period of time of only 3 h is required in the stannous-sodium kit presented in this paper. This fact contributes to reducing its cost.

The percentage of ^{99m}Tc uptake by RBC, at 37 and 50°C shows that a product with high and stable labeling has been obtained, even after several months of production. This kit can be stored up to 12 months. Some authors have already reported kits that perform well even after 12 months, although this time (12 months) is very sufficient for storing a kit.

The great advantage of this kit is its simplicity. It is manufactured with only two inexpensive and easily obtained salts. A short lyophilization time is required. This kit is thus very useful in its application to obtain ^{99m}Tc-labeled RBC.

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