year round. This circumstance contributed to the decision by Mallinckrodt Medical Inc., the only commercial supplier in the United States, to withdraw the isotope from the market. However, recently the feasibility of reactor production of $^{127}Xe$ from enriched $^{126}Xe$ has been studied here at Brookhaven as well as in Canada and the Soviet Union (*4*). This method has the potential to supply $^{127}Xe$ continuously and make the use of $^{127}Xe$ routine in the clinic.

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## Evaluation of Heparin and Anticoagulant Citrate Dextrose in the Preparation of Technetium-99m-Red Blood Cells with UltraTag® RBC Kit

**TO THE EDITOR:** Recently, the Food and Drug Administration approved a new kit for the in vitro preparation of $^{99m}Tc$-labeled red blood cells (RBCs). The UltraTag® RBC kit (Mallinckrodt Medical, Inc., St. Louis, MO) is a modification of the in vitro labeling RBC kit developed by the Brookhaven National Laboratory (*1,2*). We would like to bring attention to a potential problem we have found concerning the usage of the UltraTag® RBC kit for radiolabeling RBCs.

The package insert of the UltraTag® RBC kit recommends collecting the patient's blood sample (1.0 to 3.0 ml) using either heparin or ACD (anticoagulant citrate dextrose; acid citrate dextrose) as an anticoagulant (*3*). Unfortunately, the package insert fails to mention the amount of anticoagulant which should be used. For preventing coagulation of the laboratory blood sample, the package insert of Heparin Sodium Injection, USP (Elkins-Sinn, Inc., Cherry Hill, NJ) gives a recommended dosage of approximately 3.5–15 units heparin sodium per 1 ml of whole blood (*4*). ACD Solution, USP formula A (Baxter Healthcare Corporation, Deerfield, IL) is primarily designed to be utilized in apheresis procedures (*5*). There is no formal package insert available to instruct the user as to the volume of ACD which should be used to prevent coagulation of the whole blood sample (*personal communication*). However, Masouredis (*6*) suggests that a volume of 67.5 ml ACD can be added to 450 ml of whole blood. This equates to a ratio of 0.15 ml ACD to be employed as an anticoagulant solution for each milliliter of whole blood.

For our study, we collected 3-ml whole blood samples from a volunteer group using an anticoagulant of either 20-unit heparin dissolved in 1 ml 0.9% NaCl or 0.45 ml ACD diluted to 1 ml with 0.9% NaCl. Sodium pertechnetate eluted from a 3.0 Ci (111 GBq) technetium generator (Ultra-TechneKow® FM Generator, Mallinckrodt Medical, Inc., St. Louis, MO) with ingrowth time of either 24 or 72 hr was used as the $^{99m}Tc$ source. Typically, the $^{99m}Tc$ eluate with 24-hr ingrowth was obtained from a $^{99m}Tc$ generator which was eluted within the past 24 hr, whereas the 72-hr ingrowth eluate was obtained from a Monday generator (a generator manufactured on a Friday, but not eluted until the following Monday morning). Forty millicuries (1,480 MBq) of sodium pertechnetate $^{99m}Tc$ (in a volume of 1 ml) at different eluate ages of 0.25 hr, 2 hr, 6 hr, and 12 hr were added to the reaction vial for labeling RBCs. The labeling efficiencies (LE) of heparin versus ACD were then measured immediately and 30 min after preparation following the package insert's recommended method for assaying LE (*3*). According to the package insert of the UltraTag® RBC kit, LE is usually greater than 95% (*3*).

The results of these studies (Table 1) have indicated that the recommended dosages for both heparin and ACD give $^{99m}Tc$-RBCs LE greater than 90% when prepared with 24-hr ingrowth $^{99m}Tc$ eluate. However, unlike heparin, the recommended dosage for ACD was unsuitable for use as an anticoagulant with $^{99m}Tc$ eluate from a 72-hr ingrowth time generator in the preparation of $^{99m}Tc$-RBCs using the UltraTag® RBC kit (Fig. 1). Since the package insert of the UltraTag® RBC kit does not require that quality control be performed prior to reinjection of $^{99m}Tc$-RBCs to the patient, the patient could receive unnecessary radiation exposure due to the high percentage of unbound $^{99m}Tc$ with the use of eluate from a long-ingrowth-time generator.

Porter et al. have demonstrated that the usage of heparin in the preparation of $^{99m}Tc$-RBCs results in distinct renal and blad-

**TABLE 1**
Labeling Efficiencies of $^{99m}Tc$-RBCs Prepared With 24-Hr Ingrowth Tc-99m Eluate: ACD versus Heparin

| Eluate age (hr) | n | Heparin | | ACD | |
|---|---|---|---|---|---|
| | | 0 min | 30 min | 0 min | 30 min |
| 0.25 | 3 | 95.86 ± 1.28 | 98.93 ± 0.70 | 97.70 ± 0.27 | 98.93 ± 0.42 |
| 2 | 3 | 93.20 ± 2.13 | 98.93 ± 0.15 | 94.22 ± 1.04 | 98.52 ± 0.70 |
| 6 | 3 | 94.13 ± 1.49 | 98.91 ± 0.16 | 97.34 ± 0.29 | 99.33 ± 0.06 |
| 12 | 3 | 95.71 ± 0.82 | 98.93 ± 0.10 | 93.55 ± 2.62 | 98.61 ± 0.63 |

All differences between corresponding values are not statistically significant (two tailed t-test).

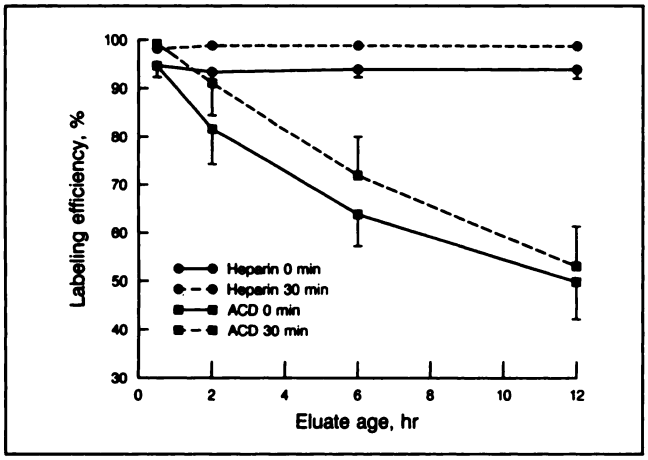


**FIGURE 1.** Comparison of labeling efficiency of $^{99m}$Tc-RBCs prepared with initial eluate (72-hr ingrowth) of Monday generator: ACD versus heparin (n = 3).

der activity compared with those using ACD (*7*). Thus, due to the superiority of $^{99m}$Tc-RBCs/ACD images versus those of $^{99m}$Tc RBCs/heparin, we are currently involved in further investigation to determine the optimal concentration of ACD to achieve maximum LE, as well as to find the mechanism by which ACD couples with old eluate to drastically reduce the LE.

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**REPLY:** We thank Drs. Wilson and Hung for their interest in the use and evaluation of the UltraTag® RBC kit for labeling red blood cells (RBCs) with $^{99m}$Tc. This kit (available from Mallinckrodt Medical, Inc., St. Louis, MO, since June 1991) utilizes the whole blood in vitro method developed at Brookhaven National Laboratory (BNL), first reported at the SNM Annual Meeting in June 1983 (*1*) and described in detail in later publications (*2–4*).

Wilson and Hung in their letter refer to a "potential problem" they have found concerning the use of this kit for radiolabeling RBCs. This relates to reduced labeling yields when $^{99m}$Tc from a long ingrowth generator is used in conjunction with patient blood that has been collected in ACD. Presence of carrier $^{99m}$Tc is by no means a problem unique to the UltraTag® RBC kit; in fact, all $^{99m}$Tc labeling kits have a range of tolerance for $^{99m}$Tc and workers in the field are well aware of this problem. The issue of technetium carrier has been studied in great detail (*5–7*), and one has to be very mindful of this problem when "instant" technetium or $^{99m}$Tc from a >24 hr ingrowth between milkings is used. The capacity of UltraTag® RBC kit for carrier $^{99}$Tc is more than adequate to handle the eluates from all commercially available generators when Heparin is used as the anticoagulant, as shown in Table 1 (*8*). It will tolerate $^{99m}$Tc produced from at least 200 mCi of $^{99}$Mo decay and still produce ~95% labeling yields. For example, one can use ~200 mCi of $^{99m}$Tc from a fresh eluate of a 3 Ci generator that has grown-in for 72 hr before milking. If the eluate is allowed to sit 12 hr, 50 mCi $^{99m}$Tc could be used. When ACD-collected blood is used, however, the levels of $^{99}$Tc that can be tolerated are substantially reduced as corroborated by Wilson and Hung. Ample quantities of $^{99m}$Tc from a <24 hr ingrowth generator can nonetheless be used without any problems.

The reduced tolerance for $^{99}$Tc results from a diminished uptake of stannous tin into the RBC during the tinning process in the presence of ACD. Extensive mechanistic work done at BNL (*3*, and Straub RF, Srivastava SC, unpublished data) has demonstrated the negative effects on tin uptake as a function of ACD concentration (Table 2). Even though the exact reasons are not clear, it would appear that the nature of the chemical species of tin in the presence of excess citrate, and/or the adverse effects of ACD on the RBC (*9*), are responsible for the reduction in tin uptake. It should be noted that the kit itself contains 0.0125 mmol citrate, and the addition of 0.15 ml ACD per ml blood (widely recognized as the recommended upper limit) will increase the total citrate to 0.063 mmol if 3 ml blood are used (Fenwal ACD, formula A). This much citrate will reduce tin uptake to the extent that the tolerance for $^{99m}$Tc present in longer ingrowth milkings will be reduced and labeling yields will be lower as Wilson and Hung have also noted. Given the mechanisms involved in our kit method, there is really no "optimum" ACD concentration that could overcome this situation (Table 2). It is

**TABLE 1**
**Effect of Carrier $^{99}$Tc on $^{99m}$Tc-RBCs Labeling Yields (%) Using the BNL Kit Method* (*8*)**

| $^{99}$Tc (μg)† | 0 | 0.209 | 0.334 | 0.417 | 0.667 | 0.834 |
|---|---|---|---|---|---|---|
| %Labeling yield | 97.8 ± 0.4 | 96.9 ± 0.5 | 96.5 ± 0.7 | 92.6 ± 3.9 | 90.2 ± 4.1 | 89.6 ± 2.6 |

* Standard labeling conditions, 1 ml blood and tin citrate kits (50 μg $Sn^{2+}$), were used. Percent labeling yields are average of six determinations ± 1 s.d.

† Approximately $1.27 \times 10^{16}$ atoms or 2.085 μg of $^{99}$Tc are produced upon the decay of 1 Ci $^{99}$Mo.