Ultratag® RBC Kit for Combined Cardiac First-Pass and Multigated Acquisition Studies

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The authors developed a procedure to use the in vitro Ultratag® (Mallinckrodt, St. Louis, MO) red blood cell (RBC) labeling kit for both first-pass (FP) and multigated acquisition (MUGA) studies with a high specific activity in a reduced volume (50 mCi/0.5 ml) and a high labeling efficiency that can be used with a single-crystal camera to yield a quality study.

Methods: A packed red blood cell (PRBC) bolus was created by two methods: (a) reducing the volume of the components of the Ultratag® kit and (b) centrifuging the final dose volume. The labeling efficiency of each bolus was evaluated, each PRBC bolus was visually inspected for clots and percent hemolysis was assessed using a hemocytometer at 30 min, 1 hr and 2 hr postcentrifugation.

Results: Use of the first method, the 50% kit, provided the best results. However, the resulting volume from this kit only approached 1 ml, which is not clinically adequate for a first-pass study. In the second method, the total volume was centrifuged to form a PRBC bolus, which appeared to be stable in the syringe for at least 2 hr. A combined FP/MUGA study from a centrifuged 50% reduced kit was performed in one normal subject as a preliminary assessment of the clinical utility of this procedure. The image quality of the scan is diagnostically adequate.

Conclusion: By using the in vitro Ultratag® kit, a compact PRBC bolus was created that was stable in the syringe and could be reinjected safely into the patient for combined cardiac FP/MUGA studies.

Key Words: first-pass studies; multigated acquisition; technetium-99m-red blood cell labeling

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In routine clinical practice, a multigated acquisition (MUGA) study may be performed after a first-pass (FP) study. Therefore, it is important to consider the requirements of both tests when selecting the best RBC labeling method. The FP study places more restrictions on the radiopharmaceutical than does the MUGA study. It requires a compact bolus of 0.5 ml or less (*1*), which is essential to assure minimal mixing of the radioactive tracer with the blood and to achieve a full width at half maximum (FWHM) $\leq$ 8.0 sec. Thus, changes in counting rate will be directly proportional to changes in ventricular volume.

The clinical benefits of combining the two diagnostic measures exceed those from either test alone. Both provide a qualitative and quantitative measure of cardiac function. The FP study enables evaluation of right and left ventricular ejection fraction, while the MUGA provides a determination of left ventricular ejection fraction. The FP study is also indicated for detection and quantitation of a left-to-right cardiac shunt and for assessment of valvular heart disease to estimate the severity of mitral and tricuspid valve insufficiency and the necessity of valve replacement. Finally, the MUGA provides an evaluation of wall dyskinesia.

FP studies have traditionally required multicrystal cameras because of their high sensitivity. However, the high cost and lower resolution of these cameras have rendered them impractical for daily use in nuclear medicine clinics. Nichols et al. (*2*) demonstrated that a single-crystal camera equipped with an ultra-high sensitivity collimator can reliably measure ejection fractions obtained from FP studies. If Ultratag® can be used for both FP and MUGA studies, using a single crystal camera, it would be cost-effective in terms of pharmacy and technologist preparation time and convenient to incorporate into a clinic's daily schedule.

There are several methods available to perform gated equilibrium studies. The first is the in vivo method in which the patient is injected with stannous pyrophosphate (PYP), given approximately 15 min "tinning" time, and is then injected with a small bolus of concentrated technetium. One problem with this method is that some of the tin reduces the ^{99m}Tc extracellularly, resulting in a lower labeling efficiency (LE) and lower target-to-background ratio (*3*).

A modified in vivo technique was created in response to this problem. After injection of stannous PYP, a sample of blood is drawn from the patient and labeled in a reaction vial with ^{99m}Tc for subsequent reinjection into the patient. However, the dose volume using this method approaches 3 ml, exceeding

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the volume requirement for the FP study. Furthermore, LE decreases as the volume of blood tagged decreases (*4*).

Finally, Ultratag® is the commercially available product used for in vitro labeling, in which the entire labeling process occurs within a closed reaction vial. The volume contributed by the constituents ranges from 2.9 to 4.9 ml: 1–3 ml of whole blood, 0.6 ml sodium hypochlorite, 1.0 ml sodium citrate/citric acid buffer and 0.25 ml of concentrated ^{99m}Tc (100 mCi/ml). The components contributing the most to the final volume are the blood and the contents of syringes 1 and 2, not the technetium. One possible way to circumvent this high dose volume would be to utilize highly concentrated ^{99m}Tc and put 100 mCi (the maximum activity specified in the package insert) in 0.5 ml. This would effectively cut the dose volume to 0.7–1.2 ml. The availability of highly concentrated technetium (200 mCi/ml) would be the limiting factor, which would determine when the test could be done, an obvious inconvenience to the nuclear medicine clinic.

There are advantages and disadvantages to each labeling method. PYP and technetium are both relatively inexpensive compared to the Ultratag® kit. With the in vivo method, a compact bolus of concentrated ^{99m}Tc can be injected for a FP study but at the cost of a decreased target-to-background ratio (*3*). Certain drugs and pathophysiological conditions cause consistent decreases in LE when the in vivo technique is used. Many of these same drug interactions have been problematic with the modified in vivo method (*3–17*).

In contrast, the in vitro method results in a LE greater than or equal to 95% and can be prepared in less than 30 min. The major drawback preventing the use of the Ultratag® kit for FP studies is the size of the bolus. To overcome this obstacle, we attempted to minimize the volume of blood and kit components necessary for RBC labeling. This would result in a single radiopharmaceutical that would be adequate for both studies, with a high specific activity of 50 mCi, in a reduced volume of 0.5 ml, while not compromising LE.

MATERIALS AND METHODS

In all studies, whole blood was collected from the patient in a syringe containing anticoagulant citrate dextrose (ACD) in the ratio of 0.15 ml ACD/ml whole blood.

In the first experiment, the stannous chloride reaction vial was reconstituted with 0.5 ml NS and 0.25 ml was discarded to reduce the amount of stannous ions by 50%. One-half milliliter of whole blood was added, which is half of the minimum volume of blood specified in the Ultratag® package insert. After a 5-min blood incubation time, 50% of the contents of each syringe were added to the reaction vial, followed by 50 mCi ^{99m}Tc in a volume of 0.5 ml. After the usual 20-min incubation time, LE was determined using silica gel and acetone and calculated from the following equation:

$$\text{LE} = \frac{\text{Activity}_{\text{origin}}}{\text{Activity}_{\text{total}}}.$$

Reductions in volume were also attempted by decreasing the kit to 33% and 20%. Procedures were followed similar to those outlined above, keeping the proportions of the constituents constant.

The 50% kit reduced the dose volume to about 1 ml, which is an improvement but is still not clinically adequate for a FP study. As a second experiment, the total volume from the 50% kit was centrifuged in a capped 3-ml syringe, plunger end down, at 600 rpm for 5 min to separate the plasma from the tagged RBCs. The LE of the packed red blood cells was determined immediately after centrifuging and again at 15, 30, 60 min and 120 min after centrifugation. In addition, the PRBCs were visually inspected for clots, and the percentage of hemolysis was assessed using a hemocytometer at 30 min, 1 hr and 2 hr postcentrifugation.

A combined FP and MUGA study from a centrifuged 50% reduced kit was performed in one normal subject as a preliminary assessment of the clinical utility of this procedure. FP/MUGA imaging was performed with a gamma camera connected to a computer for image reconstruction with a 64 × 64 matrix using a low-energy, high-resolution collimator. A dose of 25 mCi ^{99m}Tc-labeled RBCs were administered to the subject and imaging began immediately postinjection. Images were examined by the nuclear medicine staff and evaluated for diagnostic adequacy.

RESULTS

Table 1 illustrates the results of the first experiment, the effect of reducing the volume of the Ultratag® kit constituents on the LE and final dose volume. Reducing the kit components by 50% resulted in a final dose volume of approximately 1 ml, which produced a LE of 99.3% ± 1.2%. Further constituent reduction via 33% and 20% reductions produced an unacceptable LE, possibly due to the presence of insufficient stannous ions for reduction of the technetium to its active form. Reducing the ^{99m}Tc activity to 30 mCi did not improve the LE sufficiently for clinical use.

Table 2 illustrates the results of the second experiment: centrifuging the 50% kits to create a PRBC bolus with an average volume of 0.3 ml (range 0.2–0.5 ml). The LE of the PRBCs remained high throughout the measurement period (>99%), and the percent hemolysis was <3% at 2 hr postcentrifugation. Since the PRBC represents a highly concentrated bolus, there may be considerable loss of activity in the hub space of the syringe. Therefore, after completion of the trial, the PRBC bolus was injected into a waste vial and the empty syringe was assayed. It was determined that approximately 10% of the dose was lost in the syringe hub.

Figure 1 provides a comparison of the reduction and centrifugation methods in terms of LE and final dose volume. By using the reduction method, only the 50% kit provides an acceptable LE, but the dose volume does not meet the requirements for the FP study. The centrifugation method is clearly superior in terms of final dose volume, and the LE is equivalent to that obtained using a full kit or 50% kit.

The images in Figure 2 represent the FP and MUGA performed in a normal volunteer. The FP ventriculogram illustrates the complete passage of the bolus through the right

TABLE 1
Quality Control and Dose Volume of Various Ultratag® Reductions

Ingredients	Volume				
	100% kit (50 mCi)	50% kit (50 mCi)	33% kit (30 mCi)	20% kit (50 mCi)	20% kit (30 mCi)
Whole blood	1–3 ml	0.5	0.33	0.2	0.2
Reaction vial*	—	0.25	0.1	0.1	0.1
Syringe I	0.6	0.3	0.2	0.12	0.12
Syringe II	1	0.5	0.33	0.2	0.2
Technetium-99m†	0.5	0.5	0.3	0.5	0.3
Total volume	3.1–5.1	2.05	1.36	1.12	0.92
Dose volume	1.55–2.55	1.03	1.13	0.56	0.77
LE (%)	100 ± 0.0 n = 3	99.3 ± 1.2 n = 3	62 ± 5.0 n = 3	9.7 ± 4.7 n = 3	25.3 ± 4.1 n = 3

*Reaction vial reconstituted with 0.5 ml normal saline for 50% and 20% kits, 0.6 ml ns for 33% kit.
†Technetium-99m minimal concentration used = 100 mCi/ml.

ventricle within 10 frames (or 5 sec). Although a FWHM was not determined electronically, it would certainly be less than 5 sec. The MUGA in Figure 2B provides a diagnostically adequate ejection fraction and the cine image (not shown) generated from the data can adequately evaluate left ventricular wall motion.

DISCUSSION

Effect of Reducing the Kit Components

Reducing the kit components by 50% produced a quality control result of 99.3% ± 1.2% and resulted in a final dose volume of approximately 1 ml which, although an improvement compared to the dose volume obtained from using a full kit, is not clinically adequate for a FP study. The 20% and 33% kit reductions produced unacceptable quality control results, possibly due to the presence of insufficient stannous ions for reduction of the technetium to its active form. Attempts to avoid this problem by reducing the amount of technetium to 30 mCi (dose plus 5%) failed to improve the QC sufficiently for clinical use.

Effect of Centrifugation

The centrifugation method provided a more reliable means of creating a compact bolus for use in combined cardiac FP/MUGA studies. Although the PRBC boluses in this study were obtained by centrifuging the 50% kits, a preliminary evaluation of one full kit provided results that, as expected, were comparable to that of a reduced kit. Moreover, creating a PRBC bolus using a full kit will remove unnecessary handling steps, thus, minimizing the potential for contamination. Handling can be reduced further by centrifuging only the dose plus 10% (hub loss) rather than the contents of the entire vial.

Assessment of the biological quality and stability of the PRBC bolus is of paramount importance to both scan quality and patient safety. If a large percentage of cells in the bolus are hemolyzed, FP extraction of these cells by the spleen may remove enough of the activity to diminish the quality of the MUGA.

The labeling efficiency of the PRBCs remained high throughout the measurement period. However, care must be taken in the interpretation of the LE in this instance. LE testing using silica gel and acetone only detects free pertechnetate in the sample and is not a good indicator of the viability or hemolysis of cells. Technetium-99m bound to nonpolar components of a ruptured RBC, such as a piece of the RBC membrane or the hemoglobin may remain bound to the silica

TABLE 2
Labeling Efficiency of Packed RBC Bolus over Time*

Time following centrifugation (min)	% LE n = 28	% Hemolysis
0	99.9 ± 0.5	1.8 ± 0.4
30	99.6 ± 0.26	1.8 ± 0.41
60	99.7 ± 0.85	2.0 ± 0.4
120	99.5 ± 0.62	2.7 ± 0.54

*Mean dose volume = 0.3 ml (range = 0.2–0.5 ml).

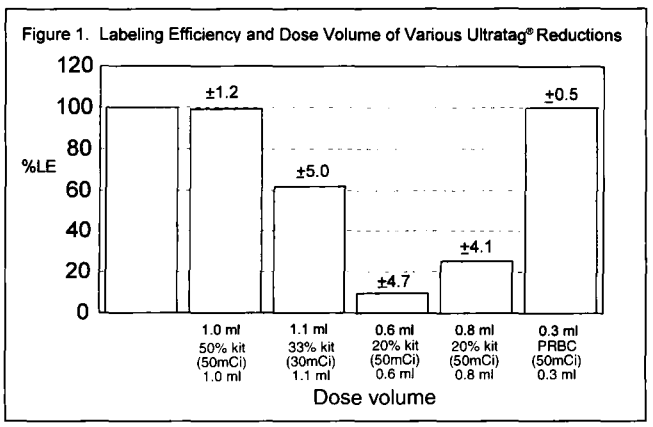

FIGURE 1. Comparison of the reduction and centrifugation methods in terms of LE and final dose volume.

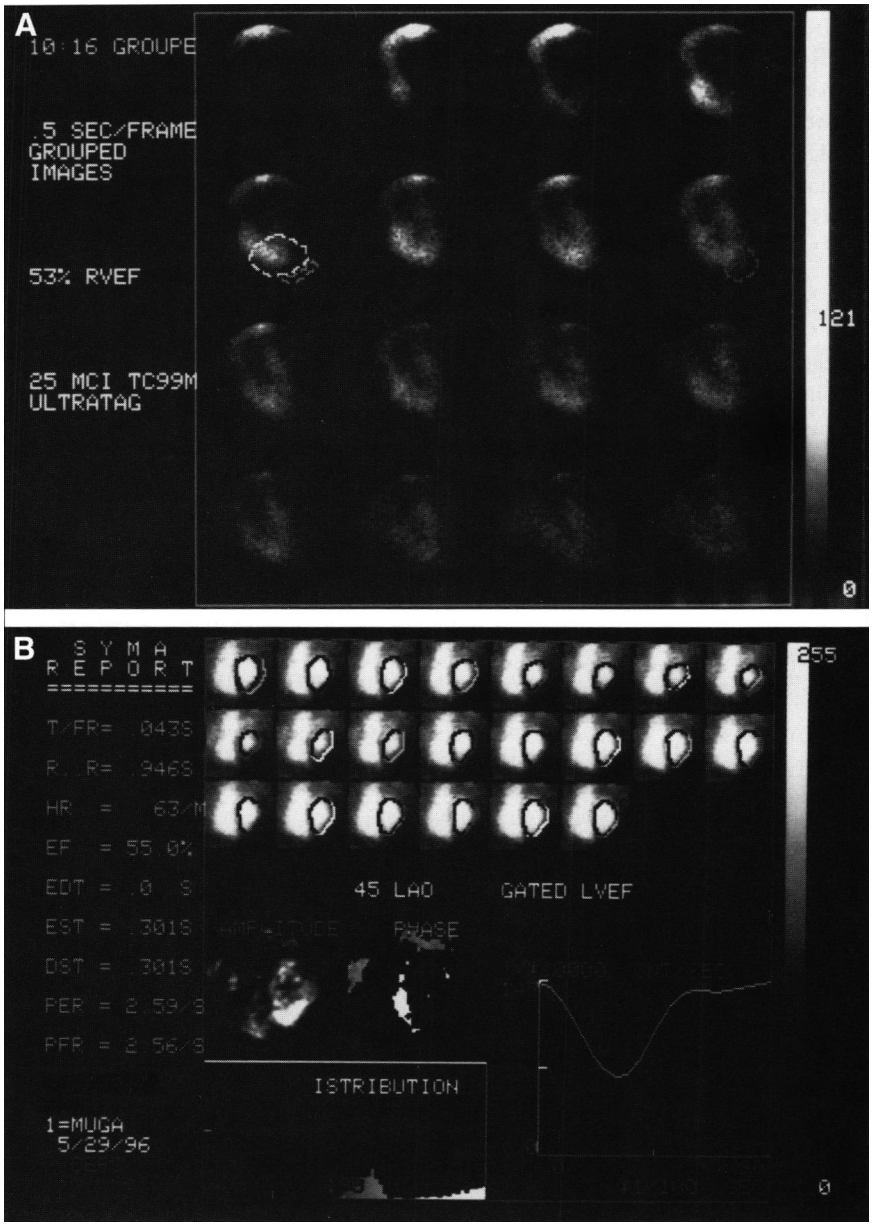

FIGURE 2. (A) First-pass ventriculogram and performed in a normal volunteer illustrates complete passage of the bolus through the right ventricle within 10 frames (or 5 sec). (B) MUGA study performed in the same normal volunteer.

gel rather than migrate with the polar acetone solvent. A more reliable indicator of cell hemolysis was obtained with use of a hemocytometer. A low percentage of the cells that were studied hemolyzed even at 2 hr. Visual inspection of the cells failed to reveal the presence of a clot in any sample.

The centrifugation method also alleviates several other potential problems. For example, the need for concentrated technetium is eliminated. Just as the sodium hypochlorite and sodium citrate/citric acid buffer are removed by centrifugation, the normal saline diluent from the ^{99m}Tc eluate is removed subsequent to incorporation of the pertechnetate ions into the RBC. Consequently, moderately dilute ^{99m}Tc may be used without compromising the final cell volume, adding some flexibility to the labeling process.

Furthermore, tagging the blood of a patient who has a hematocrit less than 30% often results in a poor labeling efficiency (*15*), a difficulty which may be mitigated through the use of a PRBC bolus. Since the final volume of the bolus is dependent on the number of RBCs, we could effectively, if not artificially, raise the hematocrit for purposes of the scan by increasing the blood component before labeling by 50%–75%.

Image Quality of the PRBC Bolus

A combined FP and MUGA study from a centrifuged 50% reduced kit was performed in one normal subject as a preliminary assessment of the clinical utility of this procedure. The FP ventriculogram illustrates the complete passage of the bolus through the right ventricle within 10 frames (or 5 sec). Although a FWHM was not determined electronically, it would certainly be less than 5 sec. Subsequently, a MUGA was performed and the image quality of both scans is diagnostically adequate. A follow-up study focusing on scan quality using centrifuged full kits is currently in progress.

CONCLUSION

Reducing the components of the Ultratag® RBC kit and centrifuging the final volume provided a reliably compact bolus of activity for use in cardiac First Pass studies. Because RBCs were labeled, rather that just using ^{99m}Tc, a MUGA can be performed after the FP study. The average labeling efficiency was greater than 94% and the PRBC bolus appears to be stable in a syringe for at least 2 hr postcentrifugation.

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