

Syringe Apparatus for Radiolabeling Cells

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A simple and inexpensive procedure for radiolabeling cells using a sterile disposable plastic 30-ml syringe is presented. The procedure can be performed using available equipment and supplies to provide a sterile product of high binding efficiency with minimal radiation exposure.

Radiolabeled cells have been widely employed for research and routine diagnostic procedures. Tagged red blood cells (RBCs) have been used in red cell mass determinations (1,2), red cell survival studies (3), splenic sequestration studies (4), blood pool imaging (5), and after heat damaging for splenic imaging (6,7). Platelets and white blood cells have also been radiolabeled (8).

Many cell labeling procedures have been described, and each one has required the use of a sterile, pyrogen-free tagging vessel. The vessel, in many cases, is not fully described other than as a "sterile container," and the exact approach is left to the ingenuity of the individual conducting the tagging procedure. For labeling RBCs, many vessels have been employed: centrifuge tubes (9), Vacutainers (10), plastic bags (Unitag Bag, Abbott Laboratories) (11), glass syringes (12), and probably countless other unreported vessels. In each procedure reactants must be aseptically added (patient's whole blood, anticoagulant, saline, etc.); nonparticulate fluids must be aseptically removed; and finally a patient dose and counting standards must be accurately prepared. The Unitag Bag has been in use for many years and has been found convenient at this laboratory, but the nonavailability of this item from Abbott Laboratories prompted a search for an alternate vessel which is readily available and in which the tagging of RBCs can be accomplished with efficiency, relative ease, and minimal cost.

Materials and Methods

The simplified tagging system reported here consists of a sterile disposable 30-ml plastic syringe (IPCO Hospital Supply, NO. 600-39592), a sterile disposable 30-in. plastic tube (Abbott, No. 4481 Venotube), and a Luer tip cap (Becton, Dickson and Co.). Additional supplies include: disposable needles (20-26 gage) and syringes (3

ml); laboratory clamp and stand; sterile normal saline, sodium heparin 1:1000, and ACD solution (Abbott Special Formula); two plastic test tubes (Falcon, No. 2050 12 × 75 mm or similar), small rubber bands, centrifuge, and 100-ml centrifuge buckets.

Labeling is carried out using the following procedure.

Heparinization of 30-ml syringe. The 20-gage needle is placed on the 30-ml syringe and 0.5 ml of 1:1000 sodium heparin is drawn into the syringe. The plunger is withdrawn to the 25-ml volume mark and swirled so that the walls of the syringe become coated with heparin. The excess heparin is expelled from the syringe and discarded.

Collection of 20-ml whole-blood sample. Twenty milliliters of the patient's whole blood is aseptically withdrawn into the preheparinized syringe. Ten milliliters of this blood is transferred to a heparinized, nonsterile test tube and kept for a patient blood background count if a blood volume determination is requested. The syringe containing the heparinized whole blood is secured in an upright position in a clamp attached to a laboratory stand (Fig. 1). The plunger is withdrawn to the 25-ml mark and the needle is removed. Two milliliters of ACD solution is added through the syringe port. A sterile Luer tip cap is used to replace the needle and the syringe and clamp are manually oscillated to ensure complete mixing of the contents.

Addition of radiopharmaceutical. (This step can be performed behind lead-brick shielding to reduce radiation exposure.) The syringe is repositioned in the upright orientation and the Luer tip cap is removed. One hundred microcuries of ^{51}Cr -sodium chromate is added to the syringe through the syringe port. The sterile Luer tip cap is placed on the syringe and the syringe is again manually oscillated to ensure complete mixing. The mixture is allowed to incubate at room temperature for 20 min.

Centrifugation and removal of supernatant. Sterile normal saline is added to the mixture via the syringe port to bring the volume of the mixture to 25 ml. The syringe is manually oscillated to ensure complete mixing. The syringe and plunger are secured at the 25-ml mark by inserting two small plastic test tubes (as plunger supports) between the base of the syringe and the flange of the plunger (Fig. 2). Two small rubber bands are used to secure the test tubes in place. The syringe is then inserted (Luer tip cap in the upright position) into a 100-ml centrifuge bucket and placed upon one pan of a beam

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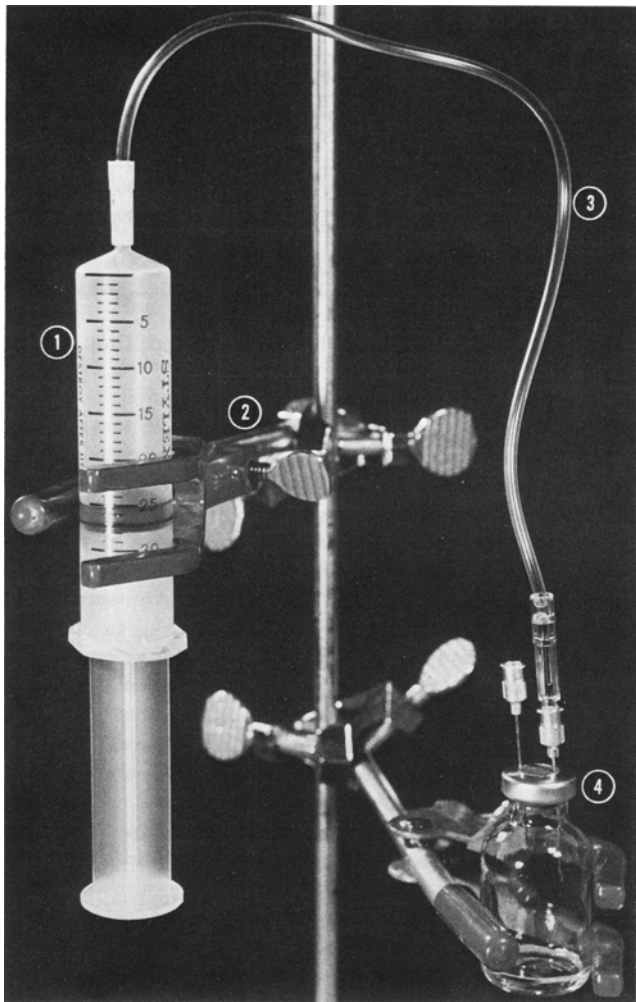


FIG. 1. Setup of radiolabeling apparatus. (1) 30-ml syringe, (2) laboratory clamp, (3) Venotube, and (4) vial.

balance. A syringe containing 25 ml of water and similarly secured to two plastic test tubes is inserted into another 100-ml centrifuge bucket. This bucket is placed on the other pan of the balance. A few drops of water are added to the lighter bucket until both weigh the same. The buckets are centrifuged at 2,000 rpm for 20 min. The syringe is repositioned in the laboratory clamp, the Luer tip cap is removed, and a Venotube is attached to the port of the syringe (Fig. 1). The other end of the Venotube is inserted into a shielded vial or test tube. The plunger supports are carefully removed and the supernatant fluid is expelled into the vial via the Venotube by lifting the plunger into the syringe. This centrifugation step is performed twice.

Preparation of patient's dose. The labeled cells are transferred to a sterile, pyrogen-free dose vial. A label describing the contents is prepared and affixed to the vial. If the labeled cells are to be used for red cell mass determination or red cell survival, the packed cells are brought to a 12-ml volume by adding sterile normal saline. If the labeled cells are to be used for splenic imaging, the packed cells are brought to 7-ml volume for heat damaging.

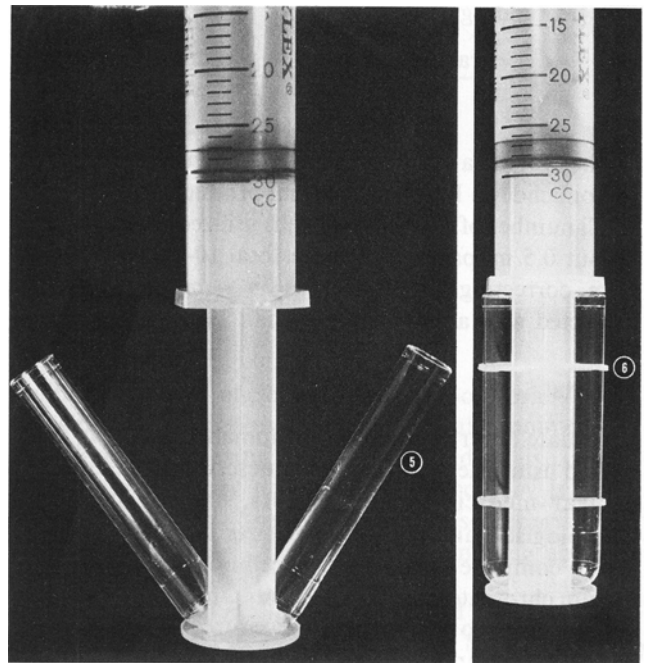


FIG. 2. Placement of plunger supports. (5) Small plastic tubes and (6) small rubber bands to secure tubes in place.

The simple procedure described above can easily be altered for other cell-tagging procedures. For example, ^{99m}Tc -stannous glucoheptonate tagged RBCs (7) can be prepared by altering (A) the addition of the radiopharmaceutical and (B) the preparation of the patient's dose as follows.

(A) Two hundred milligrams of stannous glucoheptonate in 1 ml of sterile normal saline are added to the tagging syringe instead of the 100- μCi ^{51}Cr -sodium chromate. The mixture is gently mixed and allowed to incubate at room temperature for 5 min. One milliliter of a 5% solution of EDTA is added to the syringe and mixed again. Continue on to centrifugation.

(B) The mixture is brought to the indicated volume with the addition of ^{99m}Tc -sodium pertechnetate and sterile normal saline. Allow the mixture to incubate an additional 10 min at room temperature so that the ^{99m}Tc radiolabeling can take place. Continue with the preparation as described above.

Quality Control

Labeled RBC percent binding was determined using the following procedure. One milliliter of the diluted cells from the dose vial was brought to a 10-ml volume with normal saline. A 1-ml aliquot (A) of this mixture was placed in a test tube for counting. The remaining 9 ml is centrifuged for 10 min at 2,000 rpm. A 1-ml aliquot (B) of the supernatant fluid was placed in a test tube for counting. Both aliquots were counted in an NaI(Tl) well scintillation counter and the results were expressed in terms of radioactivity/ml.

Percent binding

$$\approx \left(1.0 - \frac{\text{radioactivity/ml supernatant (B)}}{\text{radioactivity/ml mixture (A)}} \right) \times 100.$$

This formula approximates the percent binding of radionuclide to RBCs in the final product. In view of the small number of radiolabeled cells utilized for the analysis (about 0.5 ml packed cells in a total 10-ml volume), the term correcting for variations in hematocrit may be neglected with an error of less than 1%.

Results

To date, over 60 batches of human RBC have been labeled using the syringe method described here. Student nuclear medicine technologists, nuclear medicine technologists, and radiopharmacists have averaged 75 min to complete the tagging procedure using either ^{51}Cr -sodium chromate or $^{99\text{m}}\text{Tc}$ -stannous glucoheptonate. The time required to perform the procedure is comparable to that required for the Unitag Bag method previously used. The percent binding of the final products was comparable in both the syringe and Unitag Bag methods (Table 1).

Our experience has shown that the syringe method is an easy procedure to master and has reduced technical errors compared to the Unitag Bag method. Less manipulation of equipment is required, disposal of materials presents no special problems, and setup procedure is less complicated than in the Unitag Bag method.

Sterility checks on all $^{99\text{m}}\text{Tc}$ -stannous glucoheptonate tagged RBC batches and occasional checks on the ^{51}Cr -sodium chromate tagged RBC batches have yielded "no growth" reports in every case.

Discussion

The syringe method does not require the purchase of a special tagging vessel and fewer syringes are needed compared to the Unitag Bag system since expelling of the supernatant fluids is accomplished via a Venotube that can be reused throughout the procedure if sterility is maintained. Each of the items used in the syringe method is easily obtainable through hospital supply houses and is relatively inexpensive.

Radiation safety is a concern when dealing with radiolabeling procedures. Although syringe shields are available at additional expense, we have been able to limit radiation exposure by decreasing the handling time of the radioactive vessel itself. The entire procedure may

TABLE 1. Percent Binding Range of Radiolabeled RBCs

Method	Radiopharmaceutical	
	^{51}Cr -sodium chromate	$^{99\text{m}}\text{Tc}$ -stannous glucoheptonate
Unitag bag	98.9% - 99.7% (10)	95.0% - 99.0% (10)
Syringe	98.8% - 99.6% (10)	96.0% - 99.0% (10)

() = number of determinations.

be performed behind lead bricks if desired, thus further reducing exposure.

Acknowledgment

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