

Cell Labeling

There are important indications for performing imaging procedures with Tc-99m labeled red cells, including MUGA studies, GI Bleeding Studies, and evaluation of patients for hepatic hemangioma. Special handling, careful selection of needle gauge, and anticoagulation techniques are required to achieve optimal labeling.

There are three generalized methods of labeling Red Blood Cells with Tc-99m. All are simple, rapid, inexpensive and reliable. It is important to compare the advantages and disadvantages of each, and to identify the labeling efficiencies obtained with each method.



GENERALIZED METHODS OF RBC LABELING:

- * *In vivo / In vivo* Method
- * *In vivo / In vitro* Method
- * *In vitro / In vitro* Method

Stannous ion is used as the reducing agent to convert pertechnetate to Tc^{4+} which is then able to radiolabel the red cells. This process is referred to as “tinning” the red cells. The term to the left of the slash refers to where the tinning of the red cells took place (inside or outside the body). The term to the right of the slash mark refers to where the radiolabeling took place (inside or outside the body).

When performing RBC Labeling with Tc-99m, the molecule labeled is hemoglobin; the portion labeled is the β -globin chain. The heme portion cannot be labeled since it already has an iron atom (Fe) in the center of a square planar array of nitrogen atoms. Since the Fe atom cannot be displaced by another metal under physiological conditions, the globin portion of the molecule binds the Tc-99m. Similar chemistry applies to Cr-51 labeling of RBCs: again, the radiometal binds to the β -globin chains.

To determine RBC labeling efficiency, a sample of blood containing radiolabeled RBCs is spun in a sealed microhematocrit tube. The tube is then scratched with a file and broken at the dividing line between the plasma and the packed cells and the two halves are counted. The labeling efficiency is equal to

$$\frac{\text{activity in red cells} \times 100\%}{\text{activity in red cells} + \text{plasma}}$$

There are also important indications for performing in vivo non-imaging procedures with Cr-51 labeled red cells, including Red Cell Mass, Red Cell Survival Studies, and Splenic Sequestration Studies. Special handling and anticoagulation techniques are also required to achieve optimal labeling when labeling with Cr-51.

It is very easy to label red cells with Cr-51. One simply adds Cr-51 Na chromate to an anticoagulated sample of blood and incubates for 15 minutes. The reaction may then be terminated by adding ascorbic acid (Vitamin C) or by centrifuging the sample and removing the plasma. Isolation of RBCs is not required.

As in the case of Tc-99m red cells, Cr-51 binds to β -globin chains of hemoglobin.

Other labeling protocols involve preparation of In-111 White Blood Cells, Tc-99m White Blood Cells, and In-111 platelets. It is relatively easy to label White Blood Cells with either Tc-99m or In-111. Both preparations are quite labor-intensive and involve isolation of the WBCs and incubation with a lipophilic intermediate to effect radiolabeling.

In Vivo / In Vivo Method

1. 1 mg of Sn²⁺ in the form of pyrophosphate (“cold PYP”) is given IV
2. 20 min wait to permit mixing of the Sn PYP in body and diffusion of Sn²⁺ into RBC.
3. IV injection of 25 mCi 99mTc pertechnetate
4. 10 min waiting period to permit diffusion of pertechnetate into RBC's where radiolabeling takes place.
5. Expected labeling efficiency: 80-85%

In Vivo / In Vivo: Advantages / Disadvantages

Advantages: quick, simple, inexpensive

Disadvantage: lowest labeling efficiency of all commonly used procedures, but perfectly acceptable for routine work, e.g., MUGAs

In vivo / in vitro Method (in vitro Method)

1. 1 mg of Sn²⁺ in the form of pyrophosphate (“cold PYP”) is given IV

2. 20 min wait to permit mixing of the Sn PYP in body and diffusion of Sn²⁺ into RBC.
3. Withdrawal of 5-10 ml of blood anti-coagulated with heparin or ACD solution into a syringe containing 25 mCi Tc-99m pertechnetate
4. 10 min waiting period to permit diffusion of the pertechnetate into RBCs and to permit labeling to reach equilibrium.
5. Reinjection of labeled cells into patient.
6. Expected labeling efficiency: ~92% In vivo / in vitro:

In Vivo / In Vitro: Advantages / Disadvantages

Advantages: quick, simple, inexpensive method; achieves higher labeling efficiency than in vivo/in vivo technique since incubation with RBC is extracorporeal. Not optimal for GI Bleeding Studies.

Disadvantages: takes extra tech time; potential for breaking sterility

Modified In vivo / in vitro method

1. 1 mg of Sn²⁺ in the form of pyrophosphate (“cold PYP”) is given IV.
2. 20 min wait to permit mixing of the Sn PYP in body and diffusion of Sn²⁺ into RBC.
3. Withdrawal of 5-10 ml of anti-coagulated blood (heparin, ACD) into vacutainer.
4. Centrifuge the vacutainer in inverted position for 5 min at 3000 rpm.
5. Removal of 2-3 ml of packed cells through a 20 gauge or larger needle.
6. Aseptic addition of these tinned, packed cells to a sterile vial containing 35 mCi of Tc-99m pertechnetate.
7. 10 min incubation to permit labeling reaction to go to completion. Expected labeling efficiency: 98-100%
8. Reinjection of Tc RBC

Modified In vivo / in vitro Method Advantages / Disadvantages

Advantages: quick, simple, inexpensive method; achieves highest labeling efficiency of all procedures since reaction of Tc with plasma proteins has been eliminated. Ideally suited for GI Bleeding Studies; produces excellent delayed images.

Disadvantage: Takes extra tech time; requires clinical centrifuge; potential for breaking sterility.

***In vitro/in vitro* method Ultratag* Kits**

1. To vial containing Sn^{2+} compound, add 3-5 ml of anticoagulated blood
2. Wait 5 min.
3. Add Syringe A- shake lightly (Na hypochlorite to destroy extracellular Sn^{2+} ion)
4. Add Syringe B- shake lightly (Citrate buffer)
5. Add 25 mCi Tc-99m pertechnetate
6. Incubate 20 min.
7. Assay and reinject back into patient
8. Expected Labeling > 98%
9. --Remember to use 20g needles or larger
10. --Remember to draw blood and infuse it back SLOWLY

In vitro/in vitro (Ultratag*): Advantages/Disadvantages

Advantages: Quick, simple, inexpensive method; achieves higher labeling efficiency than *in vivo/in vivo* technique since incubation with RBC is extracorporeal. Ideally suited for GI Bleeding Studies; produces excellent delayed images.

Disadvantage: Takes extra tech time; slight potential for breaking sterility

Cr-51 Labeled RBCs

Uses

1. RBC Mass
2. RBC Survival and Sequestration

Properties of Cr-51

1. Half-life = 28 days
2. Gamma energy = 320 keV
3. Principle of Labeling Cr-51 RBCs
4. Cr-51 in the form of Na_2CrO_4 (6⁺ oxidation state) is incubated with RBCs. The Cr-51 binds to RBCs
5. Reaction terminated by adding a reducing agent such as ascorbic acid

Procedure for Labeling Cr-51 RBCs

1. Obtain 25-30 mL of anticoagulated blood
2. Combine blood with 75 - 150 μCi Cr-51
3. Incubate 20-30 minutes at 37°C or at room temp
4. Add 100 mg ascorbic acid and incubate 5 more minutes
5. Remove and use the labeled cells

Tc-99m Labeled WBCs

1. Used for localizing infection and abscesses
2. Principle of labeling
3. Tc-99m Ceretec is lipophilic and crosses lipid bilayer of cell membranes. Inside the cell the Ceretec complex is broken down and the resulting charged Tc-99m species is trapped in the cell.
4. All cell types are labeled by Ceretec so prior leukocyte separation is necessary

Tc-99m WBC Labeling Procedure

1. Check patient's white count (must be > 2K)
2. Obtain ~50 mL anticoagulated whole blood from pt (larger volume if WBC count is low)
3. Optional: add Hetastarch as a sedimentation aid
4. Centrifuge at 15 x g for 10 min
5. Aseptically remove the platelet-rich & leukocyte-rich supernatant
6. Centrifuge at 200 x g for 10 min.
7. Decant platelet-rich plasma and then wash cells with 0.9% NaCl
8. Add ~40 mCi freshly prepared Tc-99m Ceretec dropwise to leukocyte pellet and incubate for 20 min. Do NOT use Methylene Blue.
9. Centrifuge, remove unbound Tc-99m, and wash pellet.
10. Resuspend labeled WBC in either platelet poor plasma or 0.9% NaCl
11. Labeling 50 - 80 %.
12. Draw up dose and inject patient. If patient has been on dialysis, wait until procedure is complete

Quality Control of Tc-99m WBC

1. Place a drop of labeled cells on a hemocytometer
2. Add a drop of Trypan Blue dye
3. Add a cover slip and examine under microscope
4. Viable cells exclude dye; dead cells are stained blue
5. If >10% are dead or large clumps are present, don't use

In-111 Labeled WBCs

Used for localizing infection and abscesses

Principles of labeling

1. In-111 Oxine is lipophilic and crosses lipid bilayer of cell membranes
2. Inside the cell the Oxine complex is broken down and the resulting charged In-111 species is trapped in the cell
3. All cell types are labeled so prior leukocyte separation is necessary

In-111 WBC Labeling Procedure

1. Check patient's WBC count; adjust if necessary
2. Separate WBC fraction as previously described
3. Add 500-1,000 μ Ci In-111 Oxine.
4. Radiolabel as previously described. Labeling typically 50-95%.
5. QC is the same as for Tc-99m WBC

WBC Separation by Osmotic Lysis

1. RBCs are more susceptible to lysis from osmotic pressure than WBCs
2. Add sterile H₂O to WBC pellet contaminated w/ RBCs
3. This lowers the concentration of ions, etc.
4. The result is increased pressure in cells
5. RBCs lyse (burst open and die)
6. Quickly add 5% NaCl to restore tonicity
7. Centrifuge
8. WBC sediment, Platelets and RBC debris remain in supernatant
9. Remove supernatant
10. Resuspend WBC
11. Label WBC with Tc-99m or In-111

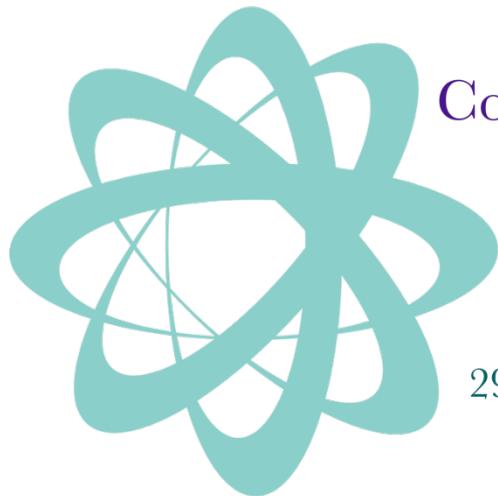
In-111 Labeled Platelets

Used for

1. Localization of thrombi
2. Platelet survival studies

Procedure

1. Separate platelets from whole blood by centrifugation
2. Label by incubation with lipophilic complexes of Tc-99m or In-111



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