

## Blood Pool Imaging With Tc-RBC's

### 1. OVERVIEW

**Blood Pool Imaging** is a radionuclide diagnostic imaging study most commonly used for cardiac first pass and gated equilibrium imaging and for detection of sites of gastrointestinal bleeding and to localize hepatic hemangiomas.

Specific indications include

- a) Global ventricular systolic function
- b) Regional wall motion
- c) Ventricular volumes (qualitative or quantitative)
- d) Responses of above parameters to exercise or other interventions
- e) Systolic and diastolic function indices
- f) Stroke volume ratios

Gated equilibrium radionuclide ventriculography (RVG) is a procedure in which the patient's red blood cells (RBCs) are radiolabeled and electrocardiograph (ECG)-gated cardiac scintigraphy is obtained. Single or multiple measurements of left and/or right ventricular function are obtained. Alternative terminologies for this technique include gated cardiac blood-pool imaging, multi-gated acquisition (MUGA), and gated equilibrium radionuclide angiography (RNA).

Data are collected from several hundred cardiac cycles to generate an image set of the beating heart that is presented as a single, composite cardiac cycle. The method can be used to assess (a) regional and global wall motion; (b) cardiac chamber size and morphology; and (c) ventricular systolic and diastolic function, including left and right ventricular ejection fractions (LVEF and RVEF, respectively). An RVG may be acquired at rest, during exercise, or after either pharmacologic or mechanical interventions.

### 2. RADIOPHARMACEUTICAL UTILIZED

- a) The only radiopharmaceutical utilized for blood pool imaging today is autologous Tc-99m labeled red cells (Tc-RBCs). Autologous refers to cells from the patient himself rather than from a donor
- b) There are multiple procedures available for radiolabeling red cells; these procedures will be described in section 5 of this tutorial.

### 3. CHARACTERISTICS OF THE RADIONUCLIDE

Tc 99m decays by isomeric transition with a physical half-life of 6.02 hours. The principal photon that is useful for detection and imaging studies has a percent abundance of 89.07 % and the energy is 140.5 KeV.

- a) The specific gamma ray constant for Tc 99m is 0.78 R/millicurie-hr at 1 cm.
- b) The first half-value layer is 0.017 cm of lead (Pb) and the first tenth value layer is 0.08 cm of Pb.

### 4. DRUG AVAILABILITY

- a) A freeze-dried cold kit called Ultratag is commercially available. It is the preparative method of choice for labeling Red Cells to be used for performing a GI Bleeding study where quantitative labeling is required. It is a completely *in vitro* procedure.
- b) A freeze-dried cold kit containing stannous pyrophosphate (often referred to as “Cold PYP”). It is used to tin red cells prior to radiolabeling with Tc-99m and may be used for general purpose red cell labeling.

### 5. RED CELL RADIOLABELING METHODS

- a) *In vivo/in vivo* method
- b) *In vivo/in vitro* method
- c) Modified *In vivo/in vitro* method
- d) *In vitro/in vitro* method

**QUIZ:** To what does the term to the left of the slash mark and the term to the right of the slash mark refer in each of the procedures listed above?

**ANSWER:** 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)

**a) *In vivo/in vivo method***

- i. Inject ~1 mg  $\text{Sn}^{2+}$  as pyrophosphate (“cold PYP”) intravenously
- ii. 20 min waiting period to permit mixing of the Sn PYP in body and diffusion of  $\text{Sn}^{2+}$  into RBC.
- iii. IV injection of 25 mCi of Tc-99m pertechnetate
- iv. 10 min waiting period to permit diffusion of pertechnetate into RBC's where radiolabeling takes place.
- v. expected labeling efficiency: 75-85%
- vi. Advantages: quick, simple, inexpensive, reliable
- vii. Disadvantage: lowest labeling efficiency of all commonly used procedures, but perfectly acceptable for routine work, e.g., MUGAs.

**b) *In vivo/in vitro method (in vitro method)***

- i. Inject ~1 mg  $\text{Sn}^{2+}$  as pyrophosphate (“cold PYP”) intravenously
- ii. 20 min waiting period to permit mixing of the Sn PYP in body and diffusion of  $\text{Sn}^{2+}$  into RBC.
- iii. Withdrawal of 5-10 ml of blood anticoagulated with heparin or ACD solution into a syringe containing 25 mCi Tc-99m pertechnetate
- iv. 10 min waiting period to permit diffusion of the pertechnetate into RBC's and to permit labeling to reach equilibrium.
- v. Reinjection of labeled cells into patient.
- vi. Expected labeling efficiency: ~92%
- vii. Advantages: quick, simple, inexpensive, reliable. Achieves higher labeling efficiency than *in vivo/in vivo* technique since incubation with RBC is extracorporeal. Not recommended for GI Bleeding Studies
- viii. Disadvantage: takes 5 min extra tech time

**c) Modified *In vivo/in vitro* method**

- i. Inject ~1 mg  $\text{Sn}^{2+}$  as pyrophosphate (“cold PYP”) intravenously
- ii. 20 min waiting period to permit mixing of the Sn PYP in body and diffusion of  $\text{Sn}^{2+}$  into RBC.
- iii. Withdrawal of 5-10 ml of blood anti-coagulated with heparin or ACD solution into a vacutainer
- iv. Centrifugation of the vacutainer in inverted position for 5 min at 3000 rpm.
- v. Removal of 1-2 ml of packed tinned cells through a 20 ga or larger needle.
- vi. Aseptic addition of these tinned, packed cells to a sterile vial containing 35-50 mCi of Tc-99m pertechnetate.
- vii. 10 min incubation to permit labeling reaction to go to completion. Expected labeling efficiency: 98-100%
- viii. Reinjection of 25 mCi of Tc RBC
- ix. Advantages: 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 best delayed images.
- x. Disadvantage: takes extra tech time; requires clinical centrifuge

**d) *In vitro/in vitro* method: Ultratag Kits**

- i. To vial containing  $\text{Sn}^{2+}$  compound, add 3-5 ml of anticoagulated blood
- ii. Incubate 15 min
- iii. add 25 mCi Tc-99m pertechnetate
- iv. add NaClO (sodium hypochlorite) to destroy extracellular  $\text{Sn}^{2+}$  ion
- v. Add Citrate Buffer
- vi. incubate 20 min. Expected labeling efficiency 98-100%
- vii. Advantages: quick, simple, inexpensive method; achieves higher labeling efficiency than *in vivo/in vivo* technique since incubation with RBC is extracorporeal. More suitable for GI Bleeding Studies than previously described technique.
- viii. Disadvantage: takes 5 min extra tech time

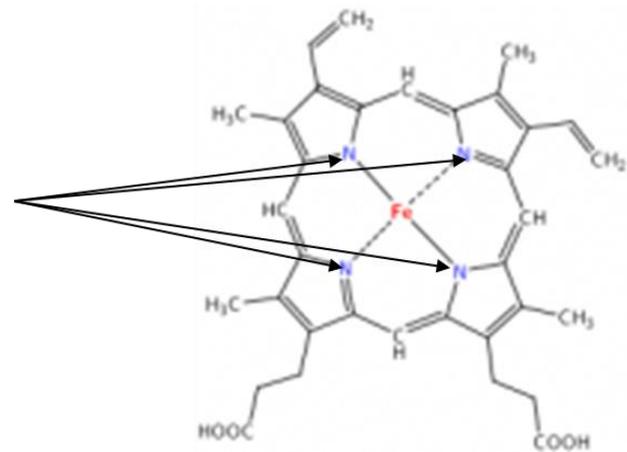
## QUIZ

When performing RBC Labeling with Tc-99m, what molecule is labeled? What portion of the molecule is labeled?

## ANSWER

The molecule labeled is hemoglobin; the portion labeled is globin chains; the specific chain is the  $\beta$ -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. This also applies to Cr-51 labeling of RBCs.

4 N-atoms in  
square planar  
array



### Stannous Pyrophosphate for tinning red cells

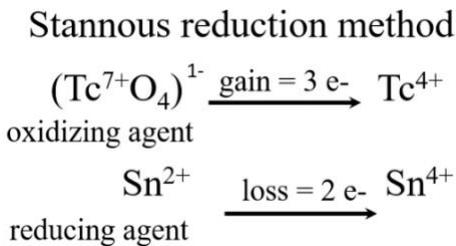
- Aseptically add 5 mL of oxidant free, sterile and non-pyrogenic 0.9% NaCl solution to the vial.
- Swirl the contents of the vial for 15-30 sec to insure complete dissolution of the freeze-dried pellet.
- Aseptically withdraw 2.5 ml of the Stannous Pyrophosphate into a sterile syringe
- Inject intravenously into patient. **A direct venipuncture is required. Do not use a butterfly or an indwelling catheter as this can cause radiolabeling problems.**

## 6. Tc-99m RBCs: DETERMINATION OF RADIOLABELING EFFICIENCY

- 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 triangular shaped file at the dividing line between the plasma and the packed cells
- The tube is then carefully broken and the two halves are placed in separate test tubes and counted in an appropriate well counter.
- The labeling efficiency =  $\frac{\text{activity in red cells} \times 100\%}{\text{activity in red cells} + \text{plasma}}$

## 7. RADIOCHEMICAL REACTION

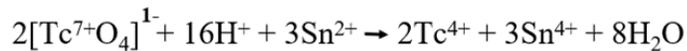
- It is necessary to convert the electronegative pertechnetate to an electropositive form as pertechnetate with its -1 charge is unable to bind to the metal binding sites on the red cells. That conversion of pertechnetate takes place via a reduction/oxidation reaction as shown below.



- Overall reaction



THEREFORE,

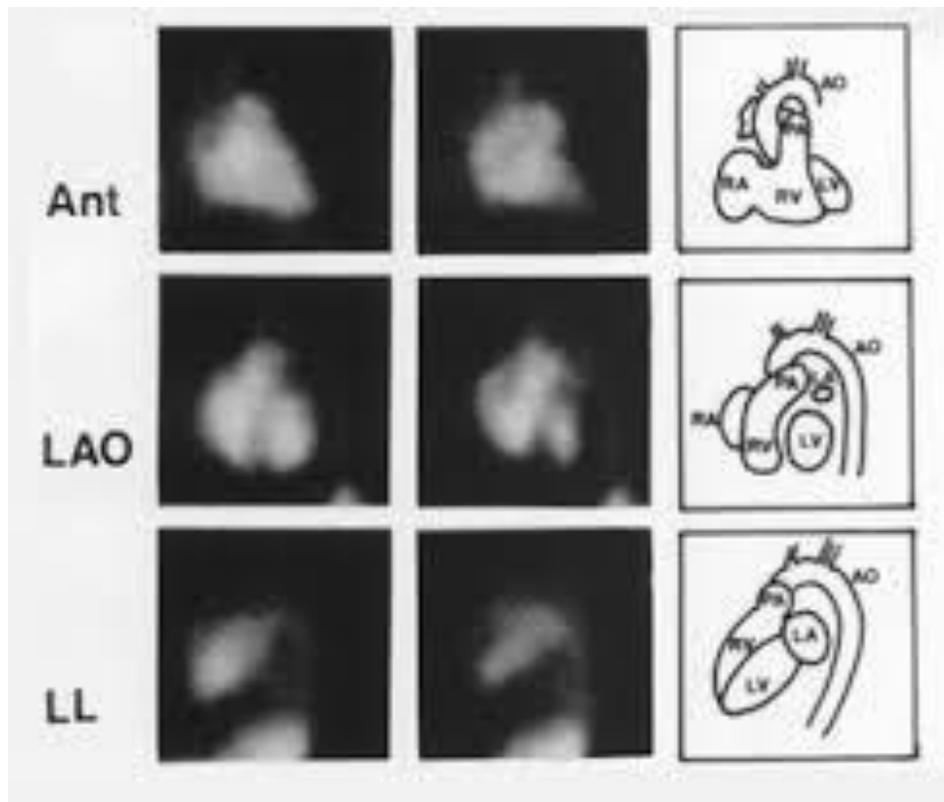
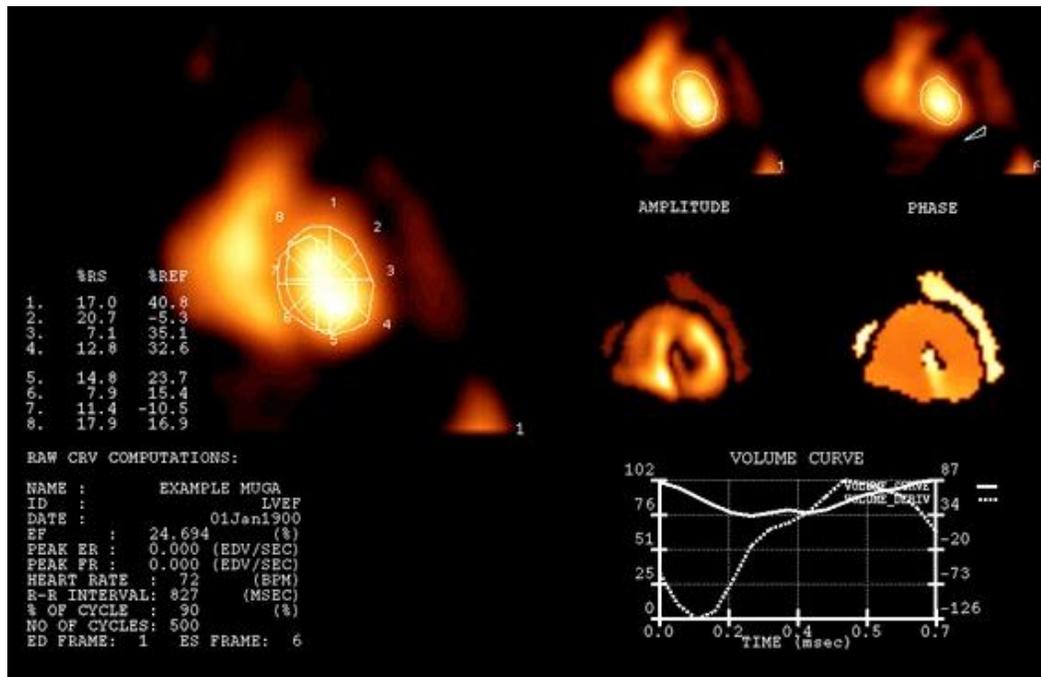


- $\text{Tc}^{4+}$  then binds to  $\beta$ -globin chains on the hemoglobin molecule.

## 8. CLINICAL PHARMACOLOGY BASED ON ULTRATAG KIT

- a) *In vitro* Tc 99m red blood cell labeling is accomplished by adding 1.0 to 3.0 milliliters of autologous whole blood, anticoagulated with heparin or Anticoagulant Citrate Dextrose Solution (ACD), to the reaction vial.
- b) A portion of the stannous ion in the reaction vial diffuses across the red blood cell membrane and accumulates intracellularly.
- c) Sodium hypochlorite is then added to the reaction vial to oxidize the extracellular stannous ion. Since hypochlorite does not cross the red blood cell membrane, the oxidation of stannous ion is selective for the extracellular tin.
- d) ACD solution (a citric acid, sodium citrate and dextrose solution) is then added to the reaction vial to sequester any residual extracellular stannous ion, rendering it more readily available for oxidation by sodium hypochlorite.
- e) Radioactive labeling of the red blood cells is completed by addition of sodium pertechnetate Tc 99m to the oxidized reaction vial. The pertechnetate Tc 99m diffuses across the red blood cell membrane and is reduced by the intracellular stannous ion. The reduced technetium Tc 99m cannot diffuse out of the red blood cell. The red blood cell labeling is essentially complete within 20 minutes of sodium pertechnetate Tc 99m addition to the reaction vial.
- f) Red blood cell labeling efficiency of  $\geq 95\%$  is typically obtained using this *in vitro* labeling procedure. *In vitro* Tc 99m red blood cell labeling efficiency can decrease when excessive amounts of Tc 99 are allowed to accumulate in the sodium pertechnetate Tc 99m generator eluate. Therefore, long Tc 99 in-growth times are to be avoided; the use of fresh ( $\leq 24$  hour in-growth time) sodium pertechnetate Tc 99m generator eluate is recommended.
- g) After the labeling procedure is completed, the Tc 99m-labeled RBCs are then reinjected intravenously into the patient for gamma scintigraphic imaging.
- h) Following intravenous injection, the technetium Tc 99m-labeled red blood cells distribute within the blood pool with an estimated volume of distribution of approximately 5.6% of body mass.
- i) The Tc 99m is well retained in the blood pool with an estimated biological half-life of approximately 29 hours. Of the total Tc 99m retained in the whole blood pool 24 hours after administration, 95% remains bound to the red blood cells. Approximately 25% of the injected dose is excreted in the urine in the first 24 hours.

## 9. NORMAL DISTRIBUTION OF Tc-RBCs: EXAMPLES OF MUGA SCANS



## **10. TYPICAL ADMINISTERED DOSE FOR ADULTS**

- a) The usual administered activity for adult patients is 15-25 mCi, injected intravenously.

## **11. PATIENT PREPARATION FOR BLOOD POOL IMAGING**

(adapted from Society of Nuclear Medicine Procedure Guideline for Gated Equilibrium Radionuclide Ventriculography version 3.0),” reprinted from <http://snmmi.files.cms-plus.com/docs/Gated%20Equilibrium%20Radionuclide%20Ventriculography%203.0.pdf>, © SNMMI Inc.)

- a) The rationale for performing the procedure and the details of the procedure itself should be explained to the patient in advance.
- b) Resting Study: No special preparation is required for a resting RVG. A fasting state is generally preferred. It is not necessary to withhold any medications. The electrodes used for cardiac gating must be placed securely on the skin to ensure an optimal ECG signal.
- c) Stress Study: The patient should be fasting for at least 3–4 hours before the study and should be both hemodynamically and clinically stable. Exercise stress, in the form of supine or upright ergometry, is generally preferred. Patients who are unable to exercise for noncardiac reasons may undergo pharmacologic stress with a positive inotropic agent. It is recommended that medications that may alter the heart rate response be withheld unless medically contraindicated or the efficacy of the medication is being tested by the exercise test.

## **12. DRUG ADMINISTRATION PROCEDURE**

- a) The injection is performed intravenously over a period of a few seconds.
- b) A small volume of blood is drawn back into the syringe and then re-injected to insure complete delivery of the bone agent.
- c) Hemostasis is accomplished using a gauze pad and pressure.
- d) The gauze pad at the injection site is covered with a Band-Aid or tape.

## IMAGING PROTOCOLS

(adapted from Society of Nuclear Medicine Procedure Guideline for Gated Equilibrium Radionuclide Ventriculography version 3.0),” reprinted from <http://snmmi.files.cms-plus.com/docs/Gated%20Equilibrium%20Radionuclide%20Ventriculography%203.0.pdf>, © SNMMI Inc.)

Rest study

### a. Instrumentation

Acquisition is performed by a gamma camera interfaced to a dedicated computer. Images may be acquired with either a low-energy all-purpose (LEAP) or high-resolution parallel-hole collimator. An appropriate ECG gating device should interface with the acquisition computer. The simultaneity of the gating device’s R-wave trigger and the patient’s QRS complex should be verified before initiation of the study. An appropriate RR interval beat acceptance window should be selected to account for heart rate variability and ectopy. Systolic function determinations are less susceptible to heart rate variability than diastolic function measurements. “List” mode acquisition is useful for making a composite cardiac cycle from a heterogenous population of beats and for retrograde gating for diastolic parameters.

### b. Acquisition parameters

A minimum of 16 frames per R-R interval are required for an accurate assessment of ventricular wall motion and assessment of ejection fraction. A higher framing rate (32–64 frames per R-R) is preferred for detailed measurement of diastolic filling parameters and is required for absolute volume measurements. Acceptable indices of diastolic function are achievable at 16 frames per cardiac cycle, if Fourier curve fitting is employed.

Images should be acquired so that the heart occupies ~50% of the usable field of view. Typical acquisitions are for a total of 3–7 million counts. Supine imaging is performed in a minimum of 3 views to visualize all wall segments of the left ventricle. The left anterior oblique (LAO) acquisition is obtained at 45° or at an angle that allows the best separation of the right and left ventricles (best septal or best separation view). An anterior acquisition is obtained in a straight (0°) anterior projection or at an angle ~45° less than the “best septal” view. The lateral acquisition is obtained as a left cross-table lateral or at an angle that is approximately 45° greater than the best septal view. The lateral view may also be acquired in the right-side down left lateral decubitus position. This altered positioning may improve visualization of the true posterobasal segment.

A 70° LAO acquisition may be used instead of a left cross-table lateral view. Left posterior oblique (LPO) or right anterior oblique (RAO) acquisitions may be of additional benefit. These angles often need to be altered in patients with congenital heart

or lung anomalies or right-sided overload. A slant-hole collimator may be used for angulation in the caudal–cephalic plane to help separate the ventricles from the atria.

Stress study

**Instrumentation and acquisition parameters are the same as for Rest study.**

However, a high sensitivity or LEAP collimator is preferred for the stress equilibrium study.

Sixteen frames per R-R interval are sufficient for assessment of ventricular wall motion and LVEF. SPECT imaging with 8 or 16 frames is an acceptable substitute. Images may be acquired on a bicycle ergometer in either a supine, semi-upright, or upright position using the best septal view as previously described or other views as appropriate to visualize a specific region of interest (ROI). The most accurate determination of the LVEF is usually obtained in the best septal view. Images may be acquired at multiple levels of exercise. A 2–3-minute acquisition may be attained at each new level of exercise once a stable heart rate is attained (usually beginning after 1 minute of exercise at the new level). The last stage of exercise may be extended to increase image statistics, but workload should not be decreased. A post exercise RVG is desirable to assess post exercise recovery; LVEF increases promptly in the great majority of patients.

Pharmacologic stress with inotropic agents, mental stress, and atrial or ventricular pacing are other, less common alternatives to exercise testing.

**13. ADVERSE REACTIONS FOLLOWING IV INJECTION OF Tc-LABELED RED CELLS**

None known.

**14. INTERNAL RADIATION DOSIMETRY OF Tc-RBCs (ULTRATAG)**

(Dose estimates calculated at Oak Ridge Associated Universities, Oak Ridge, TN.)

<b>Organ</b>	<b>mGy/740 MBq</b>	<b>rads/20 mCi</b>
Total Body	3.0	0.30
Spleen	22	2.2
Bladder Wall	4.8	0.48
Testes	2.2	0.22
Ovaries	3.2	0.32
Blood	8.0	0.80
Red Marrow	3.0	0.30
Heart Wall	20	2.0
Liver	5.8	0.58
Bone Surfaces	4.8	0.48