Recommended Method for Indium-111 Platelet Survival Studies

International Committee for Standardization in Hematology
*Panel on Diagnostic Applications of Radionuclides**

Radioactive indium (^{111}In) has been introduced as an alternative to ^{51}Cr as a platelet label because of its shorter half-life, higher photon yield and greater affinity for platelets. Furthermore, platelet labelling with ^{111}In is possible at lower platelet counts than with ^{51}Cr. In healthy individuals, the kinetics of simultaneously injected ^{111}In-labeled and ^{51}Cr-labeled platelets are similar. The document describes the technical and analytic aspects of platelet survival determination with ^{111}In-labeled platelets and is intended to supplement the previously published recommendations for platelet survival studies.

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The International Committee for Standardization in Hematology has previously published recommendations for platelet survival studies by means of chromium-51- (^{51}Cr) labeled platelets (*1*). More recently radioactive indium (^{111}In) has been used as an alternative to ^{51}Cr as a platelet label because of its shorter half-life, higher photon yield and greater affinity for platelets. Platelet labeling with ^{111}In is possible at lower platelet counts than with ^{51}Cr. In healthy individuals the kinetics of simultaneously injected ^{111}I-labeled and ^{51}Cr-labeled platelets are similar.

This document describes the technical and analytic aspects of platelet survival determination with ^{111}In-labeled platelets.

The study of platelet survival may be of value in the investigation of the following.

1. The mechanism of thrombocytopenia and the quantitative evaluation of the factors contributing to it.
2. The effects of various diseases, environmental factors, prosthetic materials and therapies on platelet survival.

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Autologous platelets should be used. On rare occasions study of the survival of allogeneic platelets may help identify an intrinsic platelet defect rather than an extrinsic immunologic or consumptive mechanism as a cause of thrombocytopenia. In these circumstances platelets should be derived from a HBs antigen-negative, HIV-antibody negative, ABO-, Rh-, and HLA-compatible donor.

In addition, platelet survival studies may be of value in studying the effect of collection and storage on the survival of transfused platelets.

Indium-111 ligand is either oxine or tropolone. Tropolone has theoretical advantages over oxine as it is watersoluble and permits platelet labeling in plasma. However, tropolone has been introduced more recently and has therefore been used less extensively than oxine.

MATERIALS

All materials must be suitable for in vivo use.

Anticoagulants

ACD (A). dextrose, anhydrous: 25 g; trisodium citrate, $2H_2O$: 22 g; citric acid, H_2O: 8 g; distilled water to: 1 l.

Trisodium citrate (B). 38 g/liter distilled water.

ACD saline (C)

pH 6.5 made up as: ACD: 1 vol; NaCl 9%: 7 vol; adjust to pH 6.5 with NaOH, 1*M*. [^{111}In]oxine at 1 mCi ^{111}In/ml in ethanol-saline, in Tween or in Tris buffer. Tropolone stock solution: Tropolone in 20 mM Hepes saline buffer (pH 7.6) to obtain a concentration of 500 μg/ml. For Tropolone labeling, ^{111}In in 0.04*M* HCl will be used in a volume of not more than 50 μl.

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Tropolone stock solution. Tropolone in 20 m*M* Hepes saline buffer (pH 7.6) to obtain a concentration of 500 μg/ml.
For tropolone labeling. ^{111}In in 0.04*M* HCl will be used in a volume of not more than 50 μl.

METHODS

Sterile conditions should be used throughout the labeling procedure and manipulations should be carried out in a laminar air flow cabinet.

1. Using a 19-gauge needle, collect 43 ml of venous blood into 50 ml sterile plastic syringes containing 7 ml of ACD (A). Sufficient blood should be taken to provide at least 10^{10} platelets. This requires 30–40 ml when the platelet count is normal; platelet counts of 20–30 × 10^9/l may require 200 ml blood: lesser platelet counts may be too low for the study to be feasible.

2. Platelet labeling with In-oxine is as follows. Dispense all but 9 ml of the citrated blood into one or more 50 ml conical sterile plastic tubes, label containers, A. Dispense 9 ml citrated blood into a single sterile plastic container with 1 ml trisodium citrate (B), label container, B.

Platelet suspension. Centrifuge each container A at 200 *g* for 12 min. Aspirate supernatant avoiding red cell contamination and retain in containers marked, PRP. Bring an equal volume of solution C to 37°C and add it to PRP. Centrifuge tube(s) at 640 *g* for 10 min. Aspirate and store supernatant. Label container, D. Wash the remaining platelet pellet(s) without resuspension with ~5 ml ACD saline (solution C). Decant as much of the buffer as possible. Resuspend the platelets in 4 ml ACD saline (solution C) and keep at 37°C.

Platelet-poor plasma. Centrifuge container B at 800 *g* for 10 min. Aspirate and retain supernatant in container marked PPP.

Platelet labeling. (a) Draw up the required volume of [^{111}In] oxine into a 1-ml plastic syringe. The oxine concentration should be 5 μg/10^9 platelets/ml. For platelet survival studies the patient dose should not exceed 4 MBq; (b) Add this [^{111}In] oxine gently to the resuspended platelets; (c) Incubate the platelet ^{111}In mixture in a water bath at 37°C for 1 min; (d) After 1 min pour 5 ml of solution D (1:1 plasma: buffer supernatant) and mix by inversion. Leave platelet suspension for 5 min; (e) Centrifuge at 640 *g* for 10 min.; (f) Decant supernatant; (g) Resuspend platelet button using 5 ml PPP (see Platelet-poor plasma); (h) Centrifuge the labeled platelet suspension at 100 *g* for 3 min to remove any contaminating red cells; (i) Aspirate the labeled platelet suspension into a 10-ml plastic syringe. The platelets are now ready for injection.

3. Platelet labeling with indium tropolone as follows. (a) Dispense the ACD blood into one or more sterile plastic tubes; (b) Centrifuge each tube at 200 *g* for 12 min; (c) Aspirate supernatant avoiding red cell contamination and dispense into tube(s) marked PRP; (d) Add ACDA 1 volume to 10 volumes PRP to reduce the PH to 6.4; (e) Centrifuge tube(s) at 640 *g* for 10 min; (f) Aspirate and retain supernatant in container marked PPP; (g) Resuspend and collect pelletted platelets in not more than 0.5 ml residual plasma in a single plastic tube; (h) Add 50 μl tropolone solution; (i) Add the required activity of ^{111}In in 0.04*M* HCl in a volume not >50 μl; (j) Let stand at room temperature for 5 min; (k) Add 10 ml PPP (see step (f)); (l) Centrifuge at 640 *g* for 10 min; (m) Discard supernatant; (n) Resuspend the platelet button using 5 ml PPP (see step (f)); (o) Centrifuge the labeled platelet suspension at 100 *g* for 3 min to remove red cells; (p) Aspirate the labeled platelet suspension in a syringe. The platelets are now ready for injection.

4. Administration and standard. (a) To prepare a standard add an aliquot of the platelet suspension (e.g., 0.5 ml) to a volumetric flask (e.g., 100 ml). Deliver known volumes of the diluted standard thus obtained into duplicate counting tubes for radioactivity measurement; (b) Weigh the syringe with the labeled platelet suspension. Inject the platelets through a butterfly infusion set. Remove the syringe and rinse the butterfly with saline to ensure infusion of all the injected material. Weigh the platelet syringe. (c) Keep an aliquot of the platelet suspension for evaluation of red cell, platelet and lymphocyte contamination.

5. Sampling. The number and timing of the samples will be decided by the purposes of the study and the anticipated mean survival. As a minimum samples should be taken at 20 min, 2 hr, 3 hr, and 4 hr after injection, and thereafter daily for up to 10 days. Where survival is expected to be short, additional samples should be taken on the first day. The anticoagulant of choice is either the dipotassium or tripotassium salt of EDTA at the concentration of 1.5 ± 0.25 mg per ml of blood.

6. Counting of radioactivity. (a) Radioactivity may be measured in whole blood if there are no contaminating ^{111}In labelled red cells or lymphocytes and if the mean survival is longer than 24 hr. In this procedure 3–5 ml samples of EDTA anticoagulated blood are placed in counting tubes and the blood cells are lysed by saponin or SDS (sodium dodecyl sulfate). The volume of the standard solution to be counted should be identical to the volume of the blood samples; (b) The counting rates of the standards, patient samples and background should be measured in a well-type scintillator counter to give a standard deviation of 2% or less, i.e., a total of 3,000 counts.

Expression of the Results

1. If all circulating activity is linked to platelets, the platelet-bound radioactivity of whole blood sampled at time t_i is

$$N_i = C/V$$

where V is the volume of the blood sample (ml) obtained at time t_i and C is the total count rate of the whole blood sample.

2. To express circulating platelet radioactivity at time t_i as a fraction of the total platelet-bound radioactivity injected into the subject (No), the following formula may be used:

$$\frac{N_i}{No} = \frac{C_i/V_i}{R/B}$$

where R is the total platelet-bound activity injected into the subject (cps/ml standard × dilution factor of standard × volume in ml injected) and B is the total measured blood volume of the subject (ml).

Analysis of Data

The validity of the methods of analysis is based on the assumption that the blood volume of the recipient is constant and the pattern of disappearance of each successive cohort of platelets from the circulation remains constant during the course of the study.

1. The ICSH document of 1977 gives several methods to obtain the mean survival. Although all the proposed methods are valid, a procedure based on the use of iterative procedures for nonlinear estimation may be most appropriate. This method ("multiple hit method") has the advantage of greater coherence and flexibility. It also yields confidence limits in the individual case.

Here follows a brief description of the method. Those values of c, a, and n that minimize the residual sum of squares of the m data points from the appropriate gamma function are found by iteration on a computer. The quantity minimized is:

$$\sum_{i=i}^{m} (N_i - H_i)^2,$$

where

$$H_i = \frac{c}{n} \sum_{i=0}^{n-1} \left(\frac{(n-i)}{i!} e^{-at}(at)^i \right)$$

and Ni is the observed radioactivity at time t_i. The constants have the following significance: a is the reciprocal of the mean waiting time between hits; n is the number of hits before the platelet is destroyed; and c is the y-intercept. The mean platelet survival is n/a and the variance n/a^2. The computer program for obtaining the estimation is available from the ICSH secretariat (Dr. R. L. Verwilghen, University Hospital, Herestraat 49, B-3000 Leuven, Belgium).

2. If appropriate computer facilities are not available, the weighted mean survival may be calculated. This method can also be used for comparison with the multiple hit method.

The weighted average estimate of mean survival, MW, is given by:

$$MW = \frac{AS_B + BS_A}{SB + SA}$$

where SA and SB are the residual sum of squares associated with linear and exponential regression curves. A is the estimate of the linear function and B the estimate of the exponential function. The exponential regression constants are best determined by non linear least squares calculation.

3. The recovery should be defined as the value at the y-intercept radioactivity (zero time) obtained by back-extrapolation.

TABLE 1
Dose Received to Various Target Organs (MGy/MBq)

Spleen	7.4 ± 2.5
Liver	0.97 ± 0.2
Total body	0.25 ± 0.04
Red marrow	0.28 ± 0.04
Ovaria	0.22 ± 0.04
Testes	0.14 ± 0.04

RESULTS AND DISCUSSION

The normal values for the platelet survival, using the multiple hit model range from 7.3 to 9.5 days. The normal values for the initial labeled platelets recovery range from 55 to 72% of the injected dose.

Data relating to the radiation dose to an adult subject in the procedure described above are shown in Table 1. It should be emphasized that the calculations on which these data are based involve many assumptions. The data in pathological conditions can be very different.

The data in Table I have been calculated assuming that once all activity has been cleared from the blood circulation ^{111}In was not redistributed, and that no ^{111}In was leaving the body (*2*).

REFERENCES

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