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STATE OF THE SCIENCE OF BLOOD CELL LABELING

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ABSTRACT

Blood cell labeling can be considered a science in as far as it is based on precise knowledge and can be readily reproduced. This benchmark criterion is applied to all current cell labeling modalities and their relative merits and deficiencies are discussed. Mechanisms are given where they are known as well as labeling yields, label stability, and cell functionality. The focus is on the methodology and its suitability to the clinical setting rather than on clinical applications per se. Clinical results are cited only as proof of efficacy of the various methods. The emphasis is on technetium as the cell label, although comparisons are made between technetium and indium, and all blood cells are covered.

KEY WORDS

Cell labeling, Blood cells, Technetium-99m, Indium-111, Red blood cells, Leukocytes, Platelets, Monoclonal antibodies.

INTRODUCTION

Blood cell labeling has progressed closer to being a science because much has been learned over the last several years about how and why certain radioisotopes attach to the various cell types. Consequently, a number of clearly defined, reproducible methods have evolved for labeling some or all blood cell types with either ^{111}In or ^{99m}Tc. This article is an attempt to summarize these methods with regard to their mechanics, mechanisms, effectiveness, and suitability to clinical use.

The general types of methods to be covered are: (i) direct in situ reduction of pertechnetate; (ii) non-specific uptake of labeled lipophilic complexes; (iii) phagocytosis of labeled colloids; and (iv) specific binding of labeled monoclonal antibodies. Emphasis will be placed on technetium labeling although indium will

also be considered and the merits and deficiencies of the two labels will be discussed. Clinical results will be cited only where necessary to demonstrate the efficacy of a method since clinical applications are beyond the scope of this article.

RED BLOOD CELLS

By far, the most common labeling modality for red blood cells is in situ reduction of the pertechnetate ion by stannous ions which were previously introduced into the cells ("pre-tinning") either in vivo or in vitro. The reduced technetium gets bound to hemoglobin and is therefore retained in the cell as shown from our data in Table 1. Successful labeling, that is, labeling yields of >90%, depends on the complete removal or inactivation of extracellular stannous ion. The amount of extracellular stannous ion (which varies according to various protocols and must be removed) is the difference between the first two columns in this table. In the in vivo method (Pavel, Zimmer and Patterson, 1977) this is done by normal metabolic processes, mainly excretion by the kidney. Certain drugs interfere with this process as do various illnesses, thus limiting the effectiveness of the in-vivo labeling method (Atkins and co-workers, 1985; Ballinger and co-workers, 1988). In addition, following a single Sn-PYP injection, substantial amounts of tin persist in the blood for a very long time thus complicating subsequent studies with many other ^{99m}Tc agents (Srivastava and co-workers, 1982; 1986; Table 2).

TABLE 1 Tin and Technetium Distribution in In-vitro Labeled ^{99m}Tc-RBC[a]

Tin In Kit μg	Bound Tin μg	%RBC-Bound Sn		%RBC-Bound ^{99m}Tc	
		Membrane	Hemoglobin[b]	Membrane	Hemoglobin[c]
2	0.4	4.0±2.0 (11)	17.5±9.6 (11)	1.7±0.3 (10)	94.8±2.0 (10)
5	2.1	8.3±1.3 (2)	34.2±14.1 (2)	1.6±0.4 (2)	84.5±4.6 (2)
15	3.6	5.7±2.9 (10)	18.3±5.1 (10)	1.3±0.4 (8)	93.3±3.2 (8)
50	5.3	0.5±0.1 (3)	10.0±5.1 (3)	21.8±8.7 (3)	76.0±8.9 (3)

[a] Whole blood method (using 1 ml) was used except for 2 μg tin kits (all prepared using ^{113}Sn or ^{117m}Sn). Data are average of n observations, n in parentheses, ± 1 std. deviation.

[b] 90.1 ± 4.5% of tin bound to heme; 12.9 ± 4.1% to globin.

[c] 18.3 ± 9.8% of ^{99m}Tc bound to heme; 80.5 ± 10.0% to globin.

The in-vitro methods remove extracellular stannous ion by either physical or chemical means. Two successful protocols developed at BNL , shown in Table 3, illustrate two different approaches for tin removal. The major objection to the former (physical removal) is the need to centrifuge and wash the tinned cells. The latter method, first described by Srivastava, Babich and Richards (1983), involves chemical inactivation of stannous tin through the addition of an oxidant, NaOCl, and a chelating agent, citrate in the form of ACD solution, to the blood after tinning and before pertechnetate addition. The chelating agent is needed to remove last traces of plasma-bound stannous tin and make it accessible for oxidation as can be seen from the data presented in Fig. 1. This method evolved

TABLE 2 ^{99m}Tc-RBC Labeling of Blood Samples with Time After One Sn-PYP Injection[a]

Time After Sn-PYP Injection	Percent Labeling Yield: Time (min) of Incubation, RBCs + $^{99m}TcO_4^-$ 15	60	300
30 min	98 ± 1	97 ± 1	98 ± 1
24 hr	99 ± 1	98 ± 1	95 ± 2
7 D	56 ± 4	85 ± 6	97 ± 2
21 D	30 ± 4	48 ± 7	89 ± 5
42 D	21 ± 4	27 ± 7	62 ± 11
63 D	21 ± 14	32 ± 28	57 ± 30

[a]Periodic blood samples were obtained, the RBC separated and then labeled with ^{99m}Tc. Normal human volunteers (n = 4) were used.

TABLE 3 Comparison of BNL In-vitro Kit Methods for ^{99m}Tc-RBC Labeling

	Original Kit	New Kit
1.	Draw 4-6 ml blood in heparinized syringe and add to kit tube	Draw 1-3 ml blood into heparinized syringe and add to the kit vial
2.	Incubate 5 min	Incubate 5 min and mix
3.	Add 4-6 ml 1% EDTA solution in saline	Add 0.6 ml 0.1% NaOCl (contents of syringe I) to kit vial; mix
4.	Centrifuge upside down for 5 min	Add 1 ml ACD solution (contents of syringe II) to kit vial; mix
5.	Withdraw RBCs, transfer to pertechnetate vial	Add pertechnetate to kit vial; mix
6.	Incubate 5-10 min	Incubate 15 min
7.	Assay and inject	Assay and inject

from extensive mechanistic studies (Srivastava, Straub, and Richards, 1984) using ^{113}Sn labeled kits, which supported the following conclusions:

1. Only Sn(II) and not Sn(IV) is taken up by the RBC.
2. The Sn(II) uptake is irreversible and dependent on time, tin concentration, citrate concentration and temperature.
3. The limiting factor for Sn(II) uptake by the RBC (upon incubating 0.5-50 μg Sn(II) per ml blood) is not cell saturation.
4. A small amount of plasma-bound Sn(II) species resists oxidation but is slowly dissociable.
5. Chelants, such as EDTA or citrate, compete successfully for the plasma-bound Sn(II) and render it oxidizable.

6. Technetium-99m uptake by "tinned" RBC is adversely affected by low blood hematocrits (greater plasma content).
7. Both Sn(II) and ^{99m}Tc bind predominantly to hemoglobin within the cell.
8. The membrane does not appear to be the limiting factor in uptake rates.

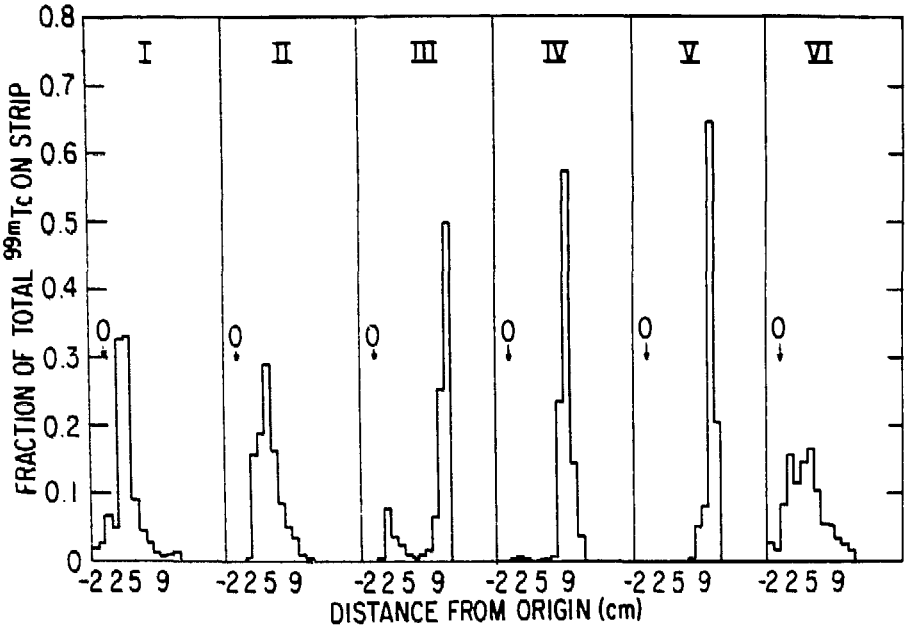

Fig. 1 . Cellulose acetate electrophoresis of plasma ^{99m}Tc (25 μg Sn(II) citrate kit, 1 ml whole blood method). I - kit + 1 ml $^{99m}TcO_4^-$ in saline, only; II - same as I, with 1 ml plasma added; III - standard labeling procedure without chelator; IV - standard labeling procedure with EDTA; V - standard labeling procedure with ACD; VI - standard labeling procedure with EDTA and without NaOCl.

This new in-vitro kit technique was further developed and refined to achieve simplicity and convenience comparable to the in-vivo method (Srivastava and co-workers, 1986; Srivastava, Straub, and Meinken, 1989). It allows quantitative (>95%) and selective labeling of RBC using small (~1 ml) whole blood samples in a closed one-vessel operation. The kit consists of a stable, lyophilized mixture of stannous citrate (50 μg tin), 3.7 mg trisodium citrate dihydrate, 5.5 mg anhydrous dextrose, and ≤2 mg sodium chloride. Another significant advantage is the ability to reduce pertechnetate from various commercial sources including those containing considerable amounts of ^{99}Tc carrier (Table 4). Use of the first milking, yesterday's milking, "instant" technetium, etc. usually causes low labeling yields when less tin is used. Studies in dogs (Srivastava and co-workers, 1986) demonstrated that the in-vivo stability of the label with time was superior than that using in-vitro procedures involving cell separation after the tinning step. To summarize, the advantages of this technique are as follows:

1. No need to separate plasma -- Cells remain in their native environment
2. No centrifugation required -- RBC handling greatly reduced
3. One vessel operation, no transfers -- More convenient; aseptic handling
4. Greater tolerance for carrier ^{99}Tc -- ^{99m}Tc from any source can be used
5. No technique-related damage -- Cells maintain their function
6. High, reproducible labeling yield -- 98±2% incorporation of ^{99m}Tc.

TABLE 4 Effect of Carrier ^{99}Tc on ^{99m}Tc-RBC Labeling Yields (%) Using the New BNL Kit Method[a]

^{99}Tc, μg[b]:	0	0.209	0.334	0.417	0.626	0.667	0.834
%Labeling Yield:	97.8 ±0.4	96.9 ±0.5	96.5 ±0.7	92.6 ±3.9	86.0 ±6.2	90.2 ±4.1	89.55 ±2.58

[a]Standard labeling conditions, 1 ml blood and tin citrate kits (50 μg Sn^{2+}), were used. Percent labeling yields are average of 6 determinations ± 1 standard deviation

[b]Approximately 1.27×10^{16} atoms or 2.085 μg of ^{99}Tc are produced upon the decay of 1 Ci ^{99}Mo.

This method has been used by Jameson and Surveyor (1988) in a series of 548 patients in Australia. It has also been adapted by Kelbaek (1986) in Denmark. The first clinical trial in the United States has been completed using the commercial version, a kit developed by Mallinckrodt Medical, Inc. under the tradename of TechneScan®RBC. The results of that trial on five patients, reported by Atkins and co-workers (1989), were as follows: 1) The mean labeling yield was 97.4 ± 1.6%; 2) Blood radioactivity levels, after mixing time, remained essentially unchanged over three hours following injection; 3) Blood clearance half-time of the major, slow, component was 26.9 hours; 4) Plasma activity was 5.7 to 8.0% of the total blood activity; 5) Cumulative urinary excretion averaged 1.3, 5.8, and 24.3% over 0.5, 3.0, and 24 hr, respectively; and 6) No adverse reactions or toxicological effects were evident. Fig. 2 is a representative whole body blood pool scan from this study. Fig. 3 illustrates a typical determination of left ventricular ejection fraction from the acquired images. Further clinical trials are currently underway.

LEUKOCYTES

Although methods for labeling leukocytes with ^{99m}Tc (Uchida and Kariyone, 1973) and ^{67}Ga (Burleson, Johnson, and Head, 1973) appeared earlier, the standard labeling modality for clinical use is the ^{111}In oxine method first proposed by McAfee and Thakur (1976). This method owes its popularity primarily to its reproducibility. The mechanism, non-specific transport of a neutral lipophilic complex into the cell, applies to all cell types, therefore necessitating cell separation before labeling. Also, plasma proteins interfere, consequently cells must be suspended in an artificial medium, usually saline, for labeling. With commercially available ^{111}In-oxine, labeling yields of 80-90% are routinely reported when 10^8 cells are used (Desai and Thakur, 1986). For most clinical purposes sedimentation of RBCs with hydroxyethyl starch followed by centrifugation of the supernatant to separate the mixed WBCs from plasma is adequate. Other ^{111}In complexes have been proposed which are more effectively taken up by cells in the presence of plasma, namely ^{111}In-tropolone and ^{111}In-mercaptopyridine-N-oxide (Desai and Thakur, 1986 and references therein).

Technetium-99m is clearly superior to ^{111}In for imaging quality, radiation dose, half-life, and in-vivo kinetics and is less costly and more readily obtainable. However, lipophilic complexes of ^{99m}Tc are relatively less stable. Until Peters and co-workers (1986) and Schümichen and Schölmerich (1986) first used ^{99m}Tc-HMPAO (^{99m}Tc (±)-hexamethyl-propyleneamineoxime) to label leukocytes, no suitable complex

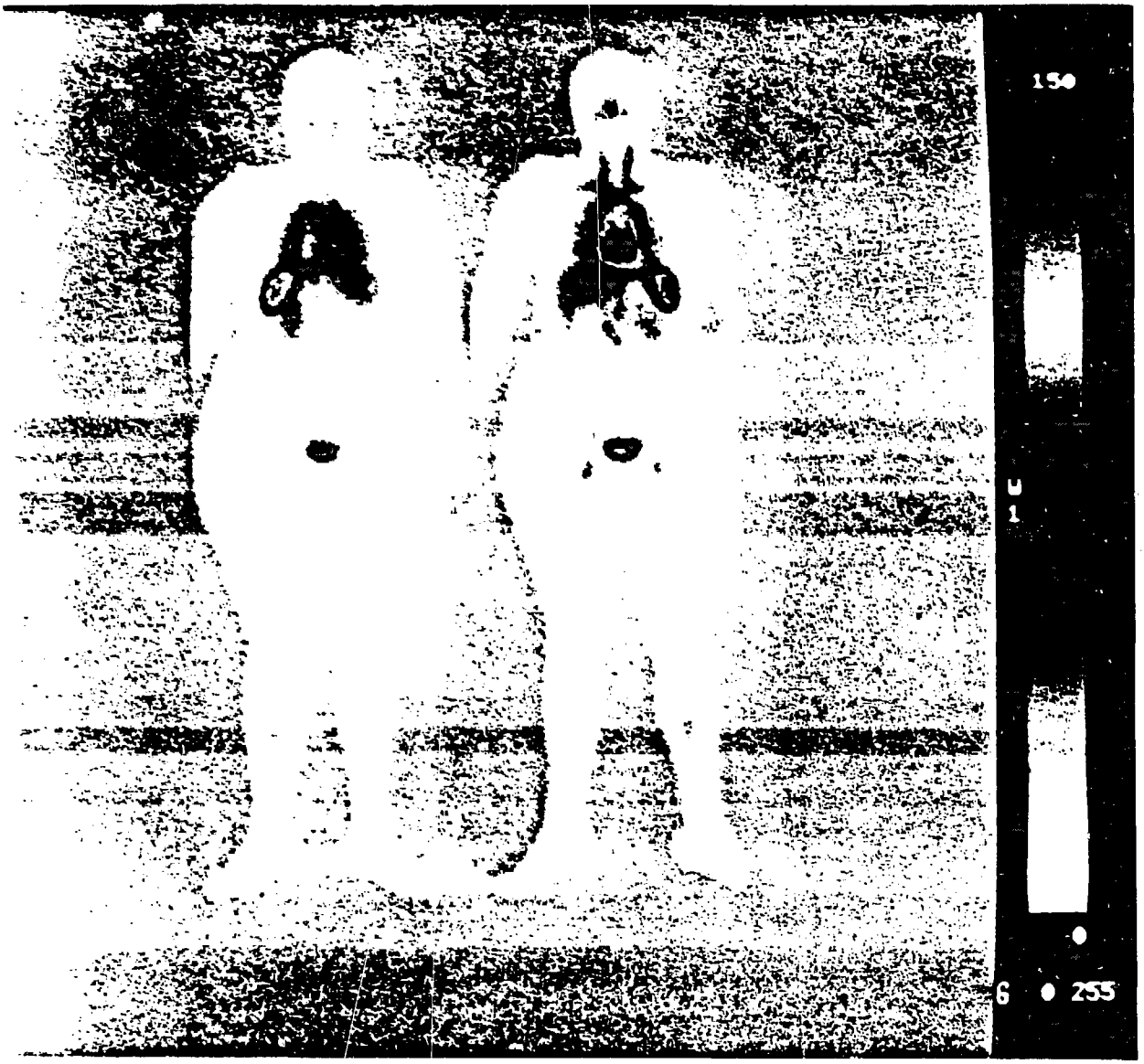

Fig. 2. These whole body images were obtained in a patient one hr following the administration of red blood cells labeled with ^{99m}Tc using the new BNL kit method (Mallinckrodt TechneScan®RBC). This method offers a combination of superior labeling yields, better in-vivo performance and quantification of data, and enhanced convenience over current in-vitro methods. Both the anterior (right) and the posterior (left) views clearly outline the heart blood pool, spleen, and the major blood vessels.

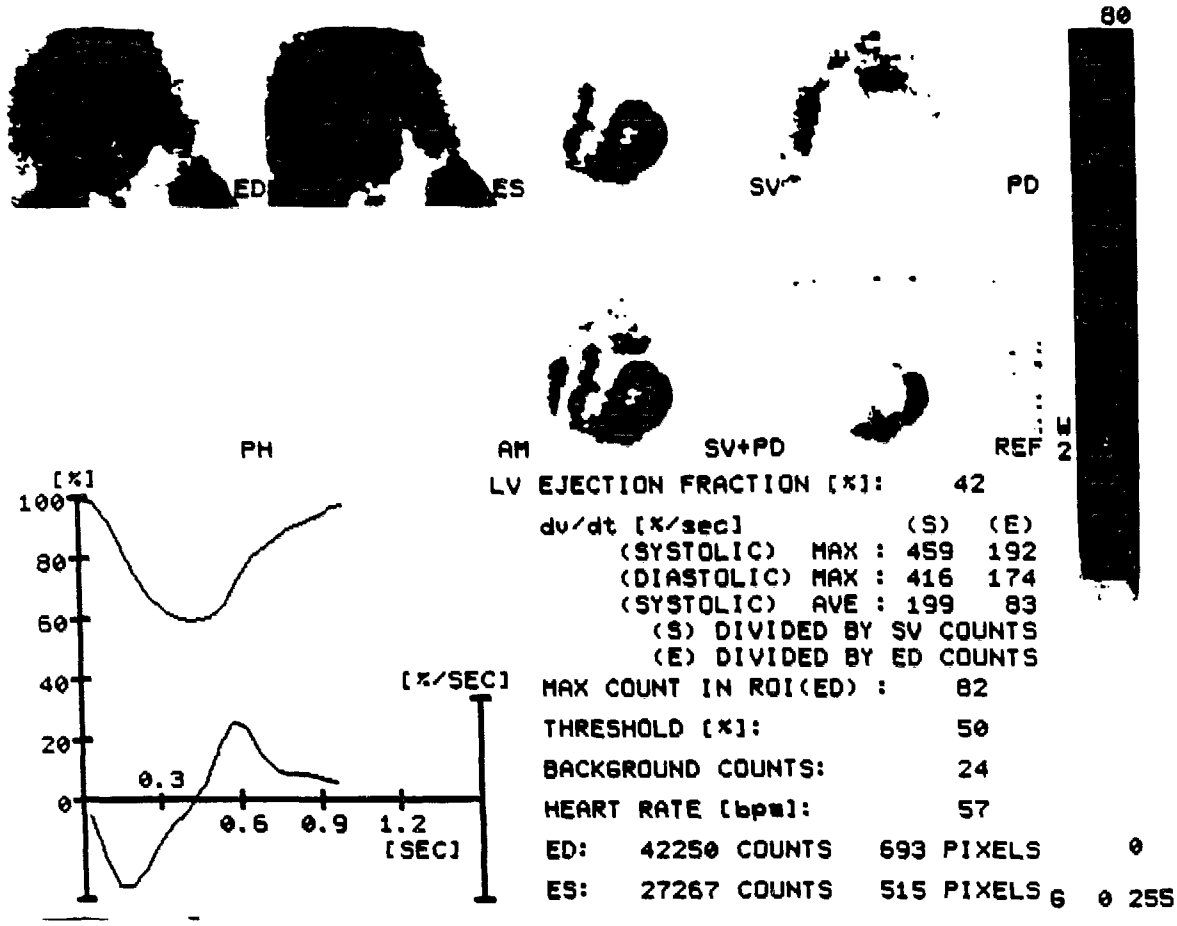

Fig. 3. Quantification of left ventricular ejection fraction from gated cardiac blood images of patient from Fig. 2.

with ^{99m}Tc was known. This approach is mechanistically similar to the ^{111}In-oxine labeling method. The transport of the complex into the cells supposedly follows a lipophilic mechanism; however, retention within the cells is lower than that of ^{111}In-oxine owing perhaps to differences in binding and ligand exchange reactions inside the cells. In general (Becker and co-workers, 1988), mixed leukocytes from 50-100 ml blood suspended in 20% plasma/ACD are mixed with ^{99m}Tc-HMPAO (freshly prepared) from a commercial kit, incubated 10 min at room temperature, washed with plasma, resuspended in plasma, and injected. Labeling yield is about 50-60% with approximately 80% of the cell-bound activity found on granulocytes. The label stability is about 90% for 1 hr (in-vitro) in plasma (Becker and co-workers, 1988). In-vitro cellular function is preserved and in-vivo distribution and function were found to compare favorably with ^{111}In-tropolonate labeled leukocytes in dogs by Mock and co-workers (1988) and in man by Peters and co-workers (1988).

Other lipophilic ^{99m}Tc complexes evaluated for leukocyte labeling include dihydroxybenzoic acid in buffer by Sundrehagen and co-workers (1986), diethyldithiocarbamate (DDC) by Sampson and Solanki (1988), and 1,2-bis-(dimethylphosphino) ethane (DPO) in plasma by Endert, Franke and Kleinert (1989). The latter two complexes are claimed to give over 70% labeling yields; however, clinical effectiveness remains yet to be demonstrated.

Indium-111 forms more stable complexes with intracellular proteins than does ^{99m}Tc. On the other hand, ^{99m}Tc does not readily complex with extracellular transferrin as does ^{111}In, hence free ^{99m}Tc is more rapidly cleared from the body. This allows much earlier visualization of inflammatory processes and therefore an earlier diagnosis is possible compared to ^{111}In-oxine imaging.

Most other methods for labeling WBC with technetium are non-specific in-vitro methods which entail prior cell separation. They employ in-situ reduction of pertechnetate via pre-tinning as in the classic RBC methods. The importance of the pre-tinning agent, especially when dealing with WBC, is shown in Fig. 4 (Srivastava, Straub and Meinken, 1989, in press). Two representative pretinning methods, first reported in 1986 (Gil and co-workers, 1986; Kelbaek, 1986a) differ in the tinning agent used and give different results (Table 5). With the use of stannous glucoheptonate and one saline wash, as few as 10^7 cells can be labeled with ~80% labeling yields in saline (Gil and co-workers, 1986). The higher the labeling yield the more stable the label, with more than 85% of the label remaining attached after 24 hr incubation in plasma. Plasma interferes dramatically with labeling. Its effect is partially on the tinning process as demonstrated by Fig. 5 and partially on the uptake of pertechnetate by the tinned cells as shown in Fig. 6. ((Srivastava and co-workers, 1986); although red cell kinetics are shown, similar effects were seen when WBCs were studied). This method requires very careful technique and detail to avoid unnecessary damage to the cells; however, it is applicable to all cell types as shown in Table 6.

TABLE 5 Two Representative Pre-Tinning Kit Methods for ^{99m}Tc Granulocyte Labeling

	Method 1[a]	Method 2[b]
Blood sample	50 ml	3-10 ml
Separation method	Sedimentation; percoll double gradient or RBC lysis	Single or double Ficoll-Hypaque gradient
Tinning agent	Sn-PYP	Sn-GH
Amount of tin	85 μg (as Sn-PYP)	5-10 μg (as Sn^{2+})
Labeling medium	Saline	Saline or PBS
Incubation times	5 min/10 min	15 min/15 min
Incubation temperature	20°C	37°C
Labeling yield, %	17.8 - 32.3	75 ± 10
Stability (in-vitro) in plasma	37% (1 hr)	85% (24 hr)

[a] Kelbaek, 1986a
[b] Srivastava, et al., 1986

TABLE 6 Technetium-99m Labeling Yields (Leukocytes and Platelets) with the Pre-tinning Approach Using Stannous Glucoheptonate[a]

Cells Used[b]	Human	Dog	Rabbit	Rat
Mixed WBC	75 ± 5 (21)	86 ± 2 (2)	--	76 ± 11 (3)
Lymphocytes	77 ± 5 (13)	87 ± 6 (2)	81 ± 5 (4)	--
Granulocytes	65 ± 10 (4)	77 ± 3 (2)	71 ± 5 (5)	87 (1)
Platelets	77 ± 5 (9)	96 (1)	78 ± 6 (2)	--

[a]Data represented as percent ^{99m}Tc bound to cells after labeling and washes (n in parentheses) (Srivastava and co-workers, 1986). Higher labeling yields (~90%) have recently been reported using this method. (Gil and co-workers, 1988, personal communication)
[b]From an average of 4 ml blood

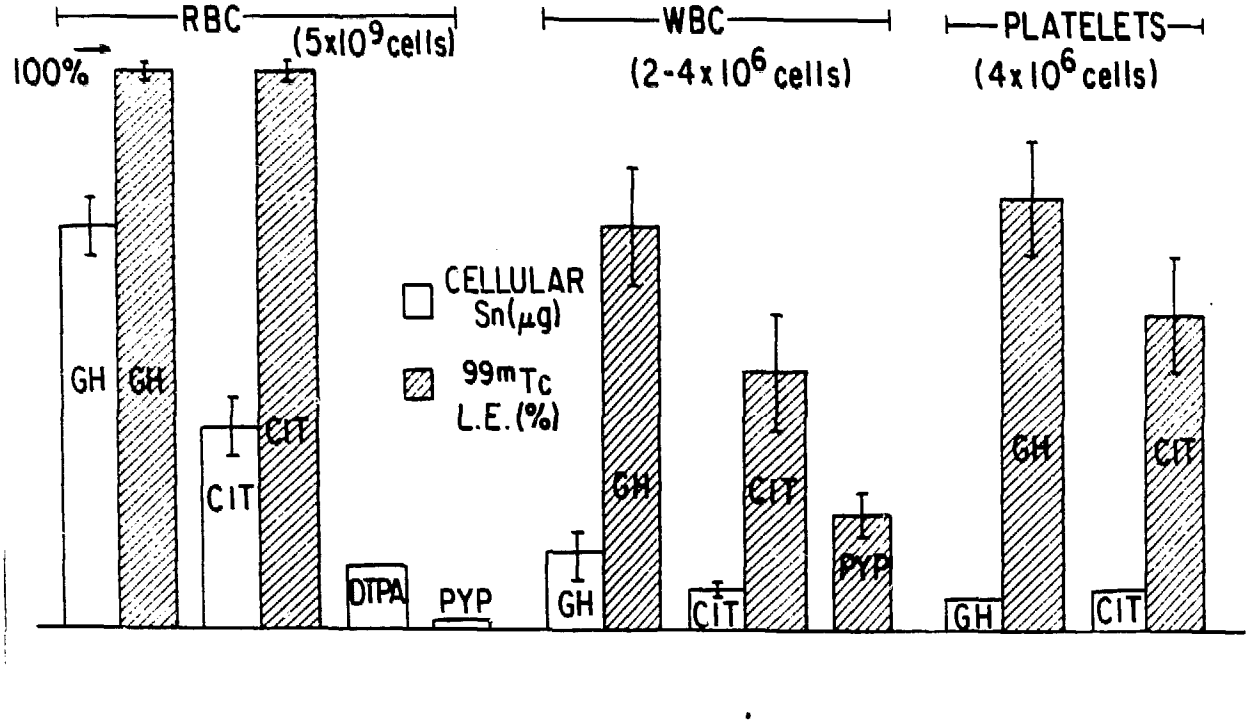

Fig. 4. A graphical representation of ^{99m}Tc labeling yields (per cent) for red blood cells (RBC), mixed leukocytes (WBC), and platelets as a function of tin uptake using various tinning agents (GH: glucoheptonate; PYP: pyrophosphate; CIT: citrate). Kits were prepared using ^{113}Sn or ^{117m}Sn as radiotracers. Careful methodology was followed to ensure >85% of total tin as being Sn^{2+}. RBCs were labeled in 1 ml blood by the whole blood labeling method. Leukocytes and platelets were labeled at 37°C by incubating tinned cells, once washed, with $^{99m}TcO_4^-$ for 15 min. All individual cell preparations were treated with identical quantities of tin, except in the case of platelets, where GH kit used 10 μg tin and the CIT kit used 50 μg tin.

Of all the in-vitro methods for labeling leukocytes in whole blood with ^{99m}Tc, only phagocytosis has given successful results. Hanna and Lomas (1986) reported a labeling yield of 80% (60% on neutrophils) using a freshly prepared prelabeled SnF_2 colloid. Preparation of the colloid took over 1 hr and incubation with the blood took an additional hour. They stressed the importance of particle size and other characteristics of the colloid on labeling. Mock and English (1987) repeated this work and found that neutrophils exhibited normal in-vitro and in-vivo function but claims were made that the mechanism is adherence rather than phagocytosis. In any event, unless preformed colloid can be supplied in kit form the method is not practical for clinical use. A kit preparation has recently been reported by Hirsch and co-workers (1989) which according to the authors, gives a very uniform SnF_2 colloid of optimal size for labeling. Furthermore, at this meeting Penglis, Baker and Bellow (1989) reported on the clinical application in a series of 200 patients of the Hanna and Lomas method simplified into a kit form. Since this meeting, Baker and Hanna (1989) have reported on clinical use in more than 2000 patients and the recent availability of a commercial kit in Australia.

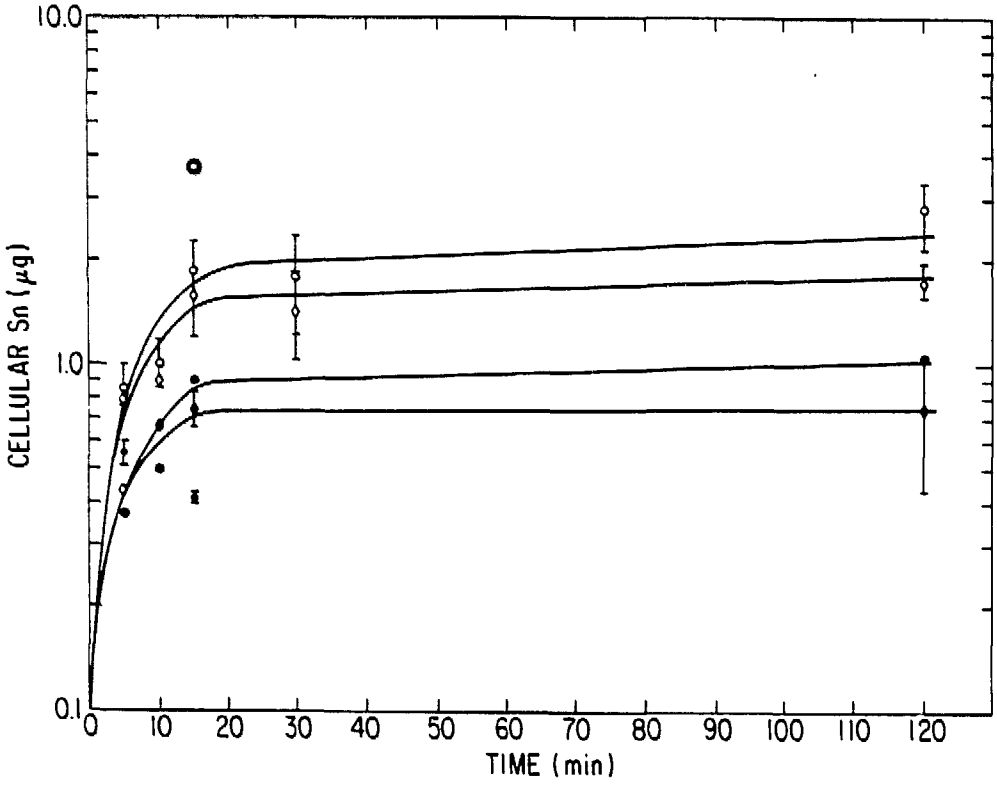

Fig. 5. Kinetics of WBC tin uptake (0.6 ml saline or plasma) using Sn^{2+} glucoheptonate kits (9.6 μg Sn) at 37°C.
● WBC in saline; ○ lymphocytes and monocytes in saline;
◊ PMN in saline; • platelets in saline;
• lymphocytes and monocytes in plasma; ♦ PMN in plasma;
• platelets in plasma.

Meanwhile, Marcus and co-workers (1988) have developed a method of ^{99m}Tc labeling of leukocytes starting with a commercial albumin colloid kit (DuPont, Microlite) and leukocytes separated from 40 ml heparinized blood. Twenty to 30% of the labeled colloid remained unbound to cells. Incubation time was cut to 15 min by using mixed WBC in saline. Acute infections were visualized by 30 min while chronic or low-level infections/inflammations took several hours before visualization. The dosimetry compares favorably with ^{111}In-WBC and pediatric as well as fetal doses are also quite low (Marcus and co-workers, 1988a). Pending further clinical testing by other investigators, this method could have substantial clinical usefulness.

In Table 7 representative data from three different approaches for labeling WBC with ^{99m}Tc are compared. Each method requires cell separation. All have been used successfully, clinically, somewhere in the world, but clearly, each leaves a lot to be desired.

TABLE 7 A Comparison of Three Approaches (Kit Methods) for ^{99m}Tc-Leukocyte Labeling[a]

Method	^{99m}Tc-HSA Colloid[b]	Pre-tinning[c]	^{99m}Tc-HMPAO[d]
Labeling medium	Saline	Saline	20% Plasma/ACD
Label preparation	Reconstitute kit with H_2O and add $^{99m}TcO_4$	Add cell suspension directly to kit	Reconstitute kit with $^{99m}TcO_4^-$, add to cells
Incubation(s)	15 min, 37°	2 x 15 min, 37°C	10 min, R.T.
Washes	1	2-3	1
Centrifugation	1 x 35 g/10 min	4-5 x 400 g/10 min	2 x 150 g/5 min
Total time (hr)	1.25	2	1.5
Labeling yield, %	60 - 90	75[e]	55
In-vitro stability in plasma	> 90% in 3 hr	85% in 24 hr	~ 90% in 1 hr

[a]Representative examples only of the phagocytic, pre-tinning and lipophilic chelate approaches are included. A variety of reagents and conditions have been utilized by different investigators. "Standard" methodology is lacking but is expected to evolve in the future

[b]Marcus et al, Nucl. Med. Biol. 15:673 (1988)

[c]Gil et al, J. Nucl. Med. 27:946 (1986)

[d]Danpure et al, Nucl. Med. Commun. 9:465 (1988)

[e]Higher labeling yields (~90%) have recently been reported using this method (Gil and co-workers, 1988, personal communication)

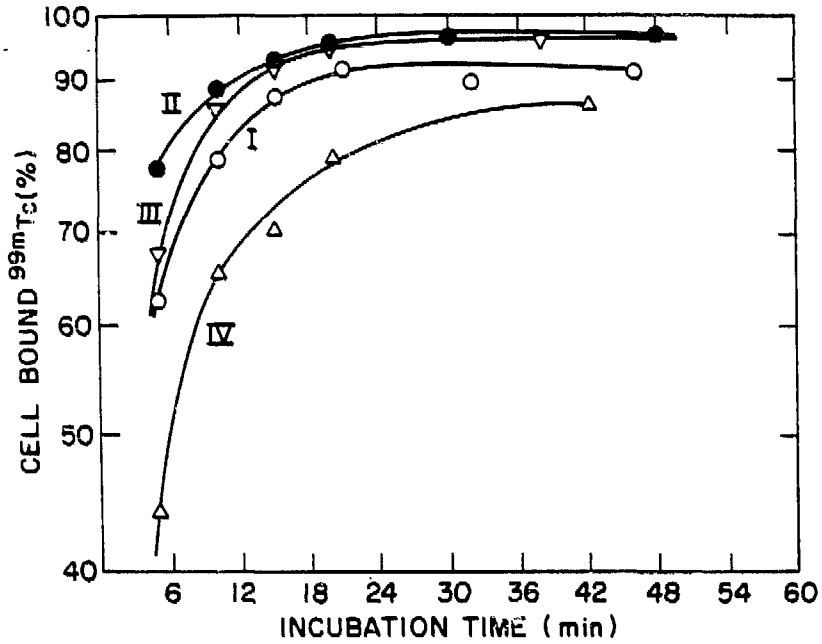

Fig. 6. Kinetics of ^{99m}Tc uptake into RBC (1 ml blood) using Sn^{2+} citrate kits (50 μg tin, whole blood procedure).
I ○ - NaOCl only;
II ● - NaOCl + $CaNa_2EDTA$;
III ▽ - 1 ml saline added;
IV △ - 1 ml plasma added.

The goal of selective in-vivo labeling of granulocytes with ^{99m}Tc via labeled antigranulocyte monoclonal antibodies appears to have gotten closer with the identification of several candidate antibodies (Locker and co-workers, 1986; Thakur, Richard and Luettgen, 1988) and the development of improved methods for labeling antibodies with technetium-99m (Fritzberg and co-workers, 1988; Schwarz and Steinsträsser, 1987). Joseph and co-workers (1988) used a monoclonal murine antibody (BW250/183) which reacts with non-specific crossreacting antigen (NCA) and carcinoembryonic antigen (CEA) and was labeled with ^{99m}Tc by the method of Schwarz and Steinsträsser (1987). In a study in 37 patients suspected of abscesses or other inflammatory processes, scans were positive in 10 patients, all confirmed as true positive by surgery, endoscopy and radiology. Less than 30% of the injected radioactivity was found associated with the granulocytes and the kinetic patterns were found to be generally comparable with those of ^{111}In-oxine or ^{99m}Tc-HMPAO labeled granulocytes although differences were observed at early periods due to the necessity of slow antibody injections (Becker and co-workers, 1989).

PLATELETS

The non-specific methods for labeling leukocytes with ^{99m}Tc have all been used for labeling platelets with some success. They include the pre-tinning kit method (Srivastava and co-workers, 1989), based on an adaptation of the method for leukocyte labeling (Srivastava and co-workers, 1986) which utilizes stannous glucoheptonate as the tinning agent. High labeling yields (~90%) are obtained with minimal effect on platelet aggregability as measured in-vitro, and good in-vitro stability in plasma (~90% at 24 hr). The labeling medium is saline, with pH critical to platelet survival. In-vivo testing is presently under way. A pre-tinning procedure using the supposedly neutral complex, stannous mercaptopyridine N-oxide, was recently reported by Dewanjee and co-workers (1989). In this study, labeling yields and in-vivo kinetics of the labeled platelets in dogs were shown to be similar to ^{99m}Tc-HMPAO labeled platelets.

Recent effort is also being focused on platelet labeling with ^{99m}Tc-HMPAO (Becker, Börner, and Borst, 1988a; Danpure and Osman, 1988). As in pre-tinning methods, platelets must be separated from all other blood cells. Labeling yields (~55%) are similar to those obtained with leukocytes, and in-vitro stability of 85% after 1 hr incubation with plasma has been reported (Danpure and Osman, 1988). Initial clinical results by Becker and co-workers (1989a) appear promising.

The only specific method for labeling platelets in whole blood in-vitro or in-vivo is through the use of antiplatelet monoclonal antibodies. This approach was first investigated by groups at BNL (Oster and co-workers, 1985; Som and co-workers, 1986; Ezekowitz, Coller, and Srivastava, 1986). In one study the antibody 7E3 was used. This antibody (IgG_1 subclass, directed against the fibrinogen receptor on the platelet surface, a glycoprotein IIB/IIIA complex) has been extensively studied and characterized by Coller and co-workers (1983,1985). The antibody inhibits both ADP-induced platelet aggregation and ADP-induced binding of fibrinogen to platelets, and cross reacts with dog platelets. There are 50,000 antibody binding sites per human platelet, and 75,000 binding sites per dog platelet (Coller and co-workers, 1983).

In-vitro studies with human and dog platelets showed that: there is only one type of binding site (linear Scatchard plot); greater than 90% tracer antibody dose binds to human platelets; ~70% of tracer dose binds to dog platelets; there is negligible binding to other blood components; virtually all platelet-bound 7E3 becomes incorporated into thrombin produced clots; 10 μg antibody/ml blood causes total inhibition of platelet aggregation; and 0.5-1 μg antibody/ml blood does not produce any significant change in platelet function (Coller and co-workers, 1983,1985). The antibody was both iodinated and labeled with ^{111}In (Srivastava and Meinken, 1988). Both iodine and indium labeled 7E3 displayed >90% binding specificity in the fibrinogen-coated bead assay (Oster and co-workers, 1985). In dogs, 7E3-labeled platelets generate early and clear images of experimental arterial and venous thrombi without the need for blood pool subtraction (Oster and co-workers, 1985; Ezekowitz, Coller and Srivastava, 1986). Labeling of 7E3 as well as its $F(ab')_2$ fragments with ^{99m}Tc is presently being attempted. A similar antibody and its fragments have also been labeled with ^{111}In and studied by other investigators in dogs (Thakur and co-workers, 1987) as well as humans (Lavender and co-workers, 1988).

Som, Oster, and co-workers (1986) at BNL utilized ^{99m}Tc-labeled antiplatelet antibody fragments to visualize experimental thrombi in dogs. Briefly, monoclonal antibody 50H.19 which reacts with human platelets and cross reacts with dog platelets was fragmented, pretinned, and lyophilized as a kit. Technetium-99m pertechnetate was added to the kit, and the mixture incubated for 1 hr. Before injection, the mixture was filtered to remove unbound pertechnetate. The labeled preparation consisted mainly of F(ab') (85%), and $F(ab')_2$ (15%) fragments. When injected into dogs having freshly induced thrombi (1-3 hr old), positive images

were obtained at 1-2 hr. A non-specific antibody used as a control, did not visualize the thrombi. This method has also been used to detect acute mesenteric ischaemia in dogs (Oster and co-workers, 1989, 1989a).

CONCLUSION

Simple kit methods for efficient labeling of red blood cells with ^{99m}Tc are now, or soon will be, available to the nuclear medicine community. Not-so-simple kit methods for labeling leukocytes and platelets with ^{99m}Tc are in the developmental stages, requiring further refinement and optimization before any of them replace ^{111}In-oxine as the labeling method of choice. Which one of the methods under development will ultimately give the best clinical results with reasonable simplicity and cost cannot be predicted. At the moment, the phagocytic methods using ^{99m}Tc colloids appear to have a slight edge for labeling granulocytes but the lipophilic ^{99m}Tc chelates may eventually prove to be as good or better for both leukocytes and platelets. Monoclonal antibodies may hold the greatest promise for specific and in-vivo labeling of various blood cells with ^{99m}Tc. However, many problems remain to be resolved before in-vivo labeling with antibodies becomes an accepted clinical method. Among these are specificity, background, cross-reactivity with other tissue antigens, clearance of unbound antibody, and the elevated HAMA levels in some patients resulting from the use of murine antibodies.

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DISCLAIMER