An Instant Kit Method for Labeling Antimyosin Fab′ with Technetium-99m: Evaluation in an Experimental Myocardial Infarct Model

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An instant kit method for labeling antibody Fab′ fragments was developed. The method utilizes a ligand exchange reaction between the intermediate complex ^{99m}Tc-D-glucarate and the free sulfhydryl groups on the antibody Fab′ fragment. Radiolabeling of the Fab′ using generator eluate achieves quantitative ^{99m}Tc incorporation in less than 30 min at room temperature. The radiolabel is stable in human plasma for at least 24 hr and stable to incubation with 10 m*M* diethylenetriaminepentaacetic acid (24 hr) and 1 m*M* diaminodithiol agent (up to 3 hr). Mouse biodistribution of ^{99m}Tc-antimyosin shows faster blood clearance and lower uptake in the lungs, liver, and spleen in comparison to ^{111}In-antimyosin. Technetium-99m-antimyosin and ^{111}In-antimyosin showed equivalent ability to detect myocardial infarct in a canine model.

J Nucl Med 1992; 33:144–149

Direct labeling of monoclonal antibodies with ^{99m}Tc has been reported previously (*1–9*) but has not been widely applied in routine practice due to a number of unresolved drawbacks (*10*). These issues have included the harsh conditions required for labeling, the low radiochemical purity of labeled preparations requiring subsequent purification, the instability of the radiolabel, decreased immunoreactivity, and the lack of a convenient method for use in the nuclear medicine clinic.

We report the development of an instant kit procedure for labeling monoclonal antibody Fab′ fragments with ^{99m}Tc which overcomes these drawbacks. Antimyosin (R11D10) Fab′ was labeled with ^{99m}Tc using this procedure. The resulting ^{99m}Tc-antimyosin Fab′ (^{99m}Tc-antimyosin) was compared in murine biodistribution studies and in a canine experimental myocardial infarct model to ^{111}In-antimyosin Fab-DTPA (^{111}In-antimyosin), which has been used widely in clinical studies (*23–25*).

Received Mar. 26, 1991; revision accepted Jul. 29, 1991.

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MATERIALS AND METHODS

Monoclonal Antibodies

Antimyosin (R11D10) is an IgG2a murine monoclonal antibody that was developed by standard hybridoma techniques as previously described (*5*).

Preparation of Antimyosin Fab′

Antimyosin was produced by continuous perfusion cell culture, and the resulting IgG was purified by Protein A affinity chromatography. The IgG was proteolytically cleaved to yield the $F(ab')_2$ fragment and subsequently purified by ion exchange chromatography. The $F(ab')_2$ fragment at a concentration of 5 mg/ml was reduced to the Fab′ fragment by treatment with 1 m*M* dithiothreitol (DTT) for 60 min at room temperature in 40 m*M* Tris buffer at pH 7.0. The Fab′ was purified by gel filtration chromatography on Sephadex G-25. The sulfhydryl content of the Fab′ was analyzed spectrophotometrically after reaction with Ellman's reagent (*11*). The Fab′ was found to contain 3 sulfhydryls per antibody fragment. The resulting product was formulated as 1.0 mg/ml of protein in 50 m*M* sodium phosphate, 1 m*M* EDTA pH 6.5, and sterile-filtered through a 0.22-μm filter.

Preparation of ^{99m}Tc-D-Glucarate

Various transfer chelates have been described in the literature for direct labeling of antibody fragments with ^{99m}Tc (*1–10*). The D-glucaric acid was selected for further evaluation of quantitative antibody labeling by varying the D-glucaric acid concentration and the antibody concentration. The concentration of D-glucaric acid was kept at the level of at least 12.5 mg/ml to minimize any colloid formation. The preparation of ^{99m}Tc-D-glucarate is summarized below. Monopotassium D-glucaric acid (25 mg) was added to 0.2 *M* sodium bicarbonate solution (1.0 ml) at pH 8.0. Stannous chloride in 0.1 *M* acetic acid (40 μl, 2.5 mg/ml) and sodium [^{99m}Tc]pertechnetate (500 μl, 15–20 mCi/ml) as generator eluate (^{99}Mo/^{99m}Tc generator, Mallinckrodt, Inc.) were added to the D-glucaric acid solution (500 μl, 25 mg/ml). The resulting solution was analyzed after 5 min at room temperature for radiochemical purity by chromatography on Whatman 3 MM paper (60% CH_3CN, 40% H_2O). The radiochromatograms were scanned with a Bioscan System 200 radiometric scanner. D-

glucarate was identified by an R_f of 0.3 to 0.4. In this system, TcO_2 had an $R_f = 0$ and TcO_4^- had an $R_f = 1.0$.

Technetium-99m-Antimyosin Fab'

Technetium-99m-D-glucarate solution (500 μl) was added to antimyosin Fab' in 50 m*M* sodium phosphate, 1 m*M* EDTA at pH 6.5 (500 μl, 1 mg/ml) at room temperature. The resulting product was analyzed for radiochemical purity by paper chromatography and gel filtration HPLC (9.4 mm × 25 cm, DuPont GF 250 column; 200 m*M* sodium phosphate pH 6.8). Immunoreactivity was determined by myosin-Sepharose affinity chromatography. The effect of antibody concentration on radiolabeling performance was studied by varying the protein concentration of this preparation from 33 μg/ml to 1250 μg/ml.

Reaction of ^{99m}Tc-D-Glucarate with IgG, F(ab')$_2$, Fab-DTPA and Fab'-NEM (N-Ethylmaleimide)

In order to demonstrate that there was no nonspecific binding of ^{99m}Tc to sites other than sulfhydryl groups, antimyosin IgG (0.2 mg/0.1 ml), F(ab')$_2$ (0.2 mg/0.1 ml), Fab-DTPA and Fab'-NEM (0.1 mg/0.1 ml) were each reacted with ^{99m}Tc-D-glucarate (0.1 ml, 7.5 mCi/ml) for up to 3 hr at room temperature. Products were analyzed by paper chromatography. The Fab-DTPA was prepared by the method of Hnatowich (*11*) from Fab that was subjected to prior reaction with iodoacetamide. The sulfhydryl groups on the Fab' fragment (0.2 mg/0.1 ml) were blocked by adding an equal volume of 200 m*M* NEM. The absence of sulfhydryl groups in the Fab'-NEM preparation was confirmed by Ellman's technique (*12*).

Preparation of the Instant Kit for Radiolabeling Antimyosin Fab' with ^{99m}Tc

Radiolabeling was performed by adding 1.0 ml of sodium [^{99m}Tc]pertechnetate (15–20 mCi) as generator eluate to the single vial containing a lyophilized composition of 12.5 mg of potassium D-glucaric acid, 100 μg of stannous chloride, 16.8 mg of sodium bicarbonate, and 0.5 mg of antimyosin Fab'. The solution was allowed to react for 30 min at ambient temperature. All operations were performed under aseptic conditions. Incorporation of ^{99m}Tc into the antimyosin Fab' fragment was determined by thin-layer chromatography (ITLC-SG, Gelman Sciences, 100 m*M* sodium citrate, pH 5.0).

Immunoreactivity Assay

Immunoreactivity of the radiolabeled antimyosin was determined by diluting an aliquot of the test sample and applying this to a myosin-Sepharose affinity column. The column was washed with 10 ml of sodium phosphate, 145 m*M* sodium chloride pH 7.0 (PBS) containing 1% bovine serum albumin to elute unbound radiolabeled components. The column was then washed with 10 ml of 100 m*M* glycine pH 2.5 to elute the myosin bound radiolabeled component. Immunoreactivity is expressed as percent bound, calculated by dividing bound activity over total activity eluted and multiplying by 100.

Stability of ^{99m}Tc-Antimyosin

Stability of ^{99m}Tc-antimyosin was analyzed at 37°C in: (a) 90% fresh human plasma, (b) 50 m*M* sodium phosphate, 1 m*M* EDTA pH 6.5, (c) 200 m*M* sodium acetate, pH 4.5, (d) 10 m*M* DTPA, (e) 1 m*M* N_2S_2, 2,4-bis (mercaptoacetylamino) butanoic acid, and (f) 5 m*M* DTT at 1, 3, and 24 hr. Samples were analyzed by chromatography (gel filtration HPLC and paper chromatography).

Indium-111-Antimyosin

Antimyosin Fab-DTPA (Centocor, Inc.) was labeled with ^{111}In-chloride (4 μCi/μg protein) in 200 m*M* sodium citrate, pH 5.0. The resulting product was analyzed by thin-layer chromatography (ITLC-SG strips, Gelman Sciences; 100 m*M* sodium citrate, pH 5.0), gel filtration HPLC and a myosin-Sepharose affinity column.

Biodistribution Study

A comparative biodistribution study was performed by coinjecting ^{99m}Tc-antimyosin Fab' (2.5 μg/150 μCi) and ^{111}In-antimyosin (2.5 μg/10 μCi) into mice. CF-1 female mice (8–10 wk, Charles River, Canada) were injected intravenously via a lateral tail vein with a total volume of 0.2 ml. Groups of mice (n = 3) were killed by cervical dislocation at 0.5, 2, 6 and 24 hr after injection. The heart, lung, liver, spleen, kidneys, stomach, small intestine, large intestine and muscle were excised, rinsed of residual blood, and weighed. Blood was also collected and weighed at each of the times. Samples were counted for radioactivity in a multichannel gamma counter (LKB, Model 1282) with window settings set to detect the 140 KeV photopeak of ^{99m}Tc and 247 keV upper energy photopeak of ^{111}In. The technique used to determine the crossover activity of one tracer to the window of the other tracer has previously been reported (*13*). Briefly, sources of either ^{111}In or ^{99m}Tc were placed in petri dishes (1.5 × 6 cm) and imaged in the 20% window. With the ^{111}In source, there was 16% crossover into the ^{99m}Tc window. In contrast, the ^{99m}Tc source had less than 0.5% crossover into the ^{111}In window. Organ activity was expressed as %injected dose per gram. Data from the gamma counting of both ^{111}In and ^{99m}Tc activities were corrected for physical decay and compared with a standard sample used to calibrate the efficiency of the gamma counter.

Experimental Myocardial Infarct Model

Purpose-bred dogs (15–20 kg) were anesthetized with intravenous pentobarbital (30 mg/kg) and intubated. Respiration with room air was maintained by a Harvard respirator. Femoral venous and arterial lines were placed for intravenous injection and monitoring of arterial pressure, respectively. Experimental acute myocardial infarction was produced by the method described by Beller et al. (*14*). A left thoracotomy was performed at the fifth intercostal space and the heart suspended in a pericardial cradle. A segment of the left anterior descending (LAD) coronary artery was isolated by blunt dissection and snared with O silk. The LAD was occluded for a period of 3 hr and then reperfused for 15 min. On completion of reperfusion, ^{99m}Tc-antimyosin (15 mCi/0.5 mg) and ^{111}In-antimyosin (1 mCi/0.5 mg) were administered intravenously. Serial imaging was initiated immediately upon tracer administration and continued for up to 5 hr. Images were acquired with a large field of view gamma camera (Union Carbide) equipped with a medium-energy collimator with the pulse-height analyzer set to detect the 140 keV photopeak for ^{99m}Tc with a window setting of ±20%. Only the high energy photopeak of ^{111}In (247 keV) with a window setting of ±20% was used for ^{111}In. Blood samples were taken for evaluation of blood clearance. Upon termination of the in vivo portion of the protocol, animals were sacrificed by triphenyltetrazolium chloride (TTC) infusion. The heart was then excised, rinsed of blood, and transverse sliced into rings (each approximately 1 cm in thickness) parallel to the atrioventricular groove. Each slice, after imaging, was divided into epicardial and endocardial sections, and approximately 1-g pieces were cut, weighed and counted in a multichannel well counter. In addition, com-

parable samples from the liver, spleen, kidneys, lung and urine were obtained and analyzed for radioactivity at 5 hr. Infarct avidity was determined by expressing the uptake of ^{99m}Tc-antimyosin and ^{111}In-antimyosin in infarcted myocardium relative to the uptake in normal myocardium. The counts per minute per gram of myocardium (cpm/g) for both ^{99m}Tc and ^{111}In were determined. Uptake in infarcted tissue was determined by averaging the cpm/g for all pieces that were completely infarcted (showing no visual evidence of staining by TTC). Uptake in normal tissue was determined by averaging the cpm/g for all endocardial pieces in the most basal ring of the heart (chosen to be representative of normal tissue), because it was distal to the site of the LAD occlusion and would be least affected by the occlusion.

Statistical Analysis

All data was expressed as mean ± s.d. Statistical analysis was performed using Student's t-test for unpaired data. All tests were two-sided, and $\alpha = 0.05$ was used to determine statistical significance.

RESULTS

Technetium-99m Labeling of Antimyosin Fab'

The reaction of sodium [^{99m}Tc]pertechnetate with D-glucaric acid at a concentration of 25 mg/ml in the presence of stannous chloride proceeds at room temperature to form a complex with ^{99m}Tc having an R_f on paper chromatography of 0.3 to 0.4. Under these conditions, this complex was obtained in yields of greater than 95% after one min as analyzed by paper chromatography.

Reaction of the ^{99m}Tc D-glucarate complex at room temperature with antimyosin Fab' yields ^{99m}Tc-antimyosin. The extent of this reaction at 1 hr is dependent on the antibody concentration as shown in Table 1. For a concentration of potassium D-glucaric acid of 12.5 mg/ml and an antimyosin Fab' concentration of 0.5 mg/ml, radiolabeling of the Fab' at room temperature reaches greater than 95% in 30 min. Reaction of the ^{99m}Tc-D-glucarate complex with antimyosin IgG, F(ab')$_2$, Fab-DTPA, or Fab'-NEM does not result in any detectable radiolabeling of the antibody, as shown in Table 2.

TABLE 1
Effect of Protein Concentration on ^{99m}Tc Labeling with ^{99m}Tc-D-glucarate

Antimyosin Fab' (μg/ml)	% ^{99m}Tc-Ab*	% ^{99m}Tc-DG†
1250	100	0
340	100	0
165	72	28
133	66	34
100	67	33
33	53	47
0	0	100

* Percent of ^{99m}Tc-labeled antibody fragment.
† Percent of ^{99m}Tc-labeled D-glucarate.

TABLE 2
Selective Labeling of Sulfhydryl Group Containing Protein with ^{99m}Tc-D-glucarate

Antimyosin antibody	% ^{99m}Tc-Ab* (1 hr)	% ^{99m}Tc-Ab (3 hr)	-SH / Antibody
IgG	0	0	0
F(ab')$_2$	0	0	0
Fab-DTPA	0	0	0
Fab'	100	100	3.27 ± 0.05
Fab'-NEM	0	NA	0

* Percent of ^{99m}Tc-labeled antibody.
† Mean ± s.d.
NA = not available.
SH = sulfhydryl.

Stability of ^{99m}Tc-Antimyosin

The ^{99m}Tc-antimyosin was challenged under various conditions designed to test the stability of the radiolabeled antibody. As shown in Table 3, ^{99m}Tc-antimyosin is stable in 50 m*M* sodium phosphate buffer pH 6.5, 200 m*M* acetate buffer pH 4.5, 10 m*M* DTPA and 90% human plasma. The ^{99m}Tc-antimyosin showed no loss of immunoreactivity after 24 hr incubation with 90% human plasma at 37°C. Approximately 90% of radioactivity remains associated with the Fab' antibody fragment after 1 and 3 hr challenge with 1 m*M* N_2S_2, whereas challenge with the sulfhydryl-containing reagent DTT results in loss of the ^{99m}Tc radiolabel (Table 3).

In Vivo Biodistribution in Mice

The results of the mouse biodistribution study are presented in Figure 1. At 30 min, the percent of injected dose in blood is the same for both radiolabeled species. However by 2 hr and at later time points, ^{99m}Tc-antimyosin clears more rapidly from the blood and has lower uptake in the

TABLE 3
Stability of ^{99m}Tc-Antimyosin as a Function of Time Under Various Conditions

Challenge conditions	% ^{99m}Tc-antimyosin (1 hr)	(3 hr)	(24 hr)
Human plasma*	100	100	100
50 m*M* sodium phosphate pH 6.5	100	100	100
200 m*M* sodium acetate pH 4.5	100	100	100
10 m*M* DTPA	100	100	100
1 m*M* N_2S_2	90	87	NA
5 m*M* DTT	13	4	3

NA = not available.
* Immunoreactivity of ^{99m}Tc-antimyosin after prolonged incubation with human plasma showed no loss of activity. Immunoreactivity was determined to be 95.85 ± 1.88 (n = 4) at 1 and 24 hr using a myosin-Sepharose affinity column.

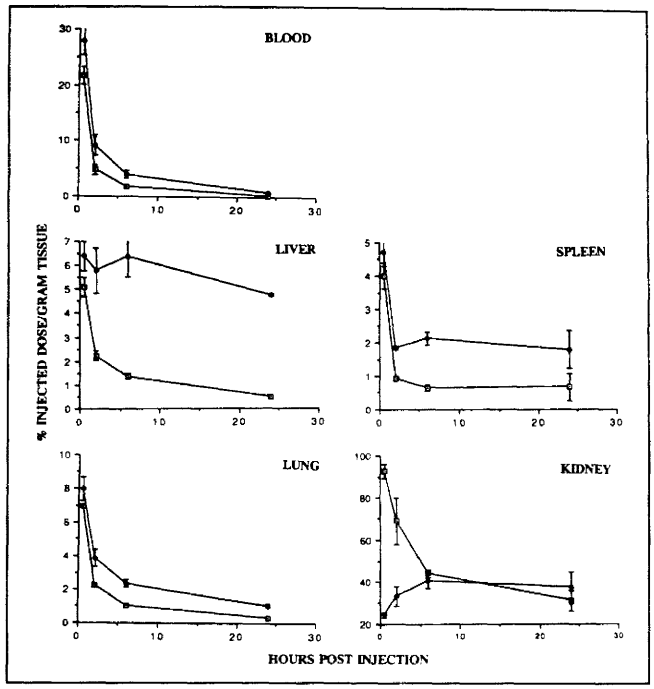

FIGURE 1. Biodistribution of ^{99m}Tc-antimyosin and ^{111}In-antimyosin in normal mice. Values are an average of three animals. Indium-111-antimyosin (●); ^{99m}Tc-antimyosin (□).

lungs, liver, and spleen in comparison to ^{111}In-antimyosin. Kidney uptake at 30 min and 2 hr is greater for ^{99m}Tc-antimyosin but is equivalent for the two radiolabeled species from 6 hr onwards. Massuger et al. (*15*) have compared the tissue distribution of ^{99m}Tc, ^{111}In and ^{123}I-labeled antibody fragments in tumor bearing mice. Both ^{99m}Tc and ^{111}In-labeled fragments were retained in kidneys due to interactions with metal-binding proteins. Similar results were observed with other antibody fragments labeled with either ^{99m}Tc or ^{111}In (*16,17*). Liver uptake is greater for ^{111}In-antimyosin and remains high, whereas the liver activity for ^{99m}Tc-antimyosin decreases with time.

In Vivo and Ex Vivo Results in Experimental Infarct Model

Data obtained from the canine infarct model experiments are presented in Table 4. The 5-hr biodistribution data show equivalent percent injected dose per gram in kidney-medulla, spleen and muscle for ^{99m}Tc-antimyosin in comparison to ^{111}In-antimyosin. There are trends indicating that the uptake (%ID/g) for ^{99m}Tc-antimyosin are lower than that for ^{111}In-antimyosin for both liver and lung and higher for kidney-cortex. However, due to the individual variation and limited samples sizes (n = 8), the results showed no statistical differences for these organs. The average results indicate no significant differences between infarct-to-normal (cardiac tissue) ratio (17 ± 9 versus 19 ± 9) and infarct-to-blood (5 ± 3 versus 5 ± 1) ratio for both radiolabeled species. Figure 2 shows the scintigraphic images of a canine experimental myocardial infarct at 3 hr after intravenous administration of ^{99m}Tc-antimyosin. The infarct area is clearly shown in the right and left lateral views. Figure 3 shows the infarct areas delineated by both ^{99m}Tc-antimyosin and ^{111}In-antimyosin. The identical cardiac slices are shown with ^{99m}Tc-antimyosin on the left and ^{111}In-antimyosin on the right. There is intense antimyosin uptake in the area of the myocardial infarction with a virtually identical distribution of the radiolabel in both sets of images.

TABLE 4
Biodistribution of ^{99m}Tc-Antimyosin and ^{111}In-Antimyosin in Dogs at 5 Hours After Intravenous Administration

Organ	%ID/gram*	
	^{99m}Tc-antimyosin	^{111}In-antimyosin
Liver	0.014 ± 0.005	0.027 ± 0.014
Lung	0.010 ± 0.003	0.017 ± 0.008
Kidney—cortex	0.245 ± 0.124	0.164 ± 0.154
Kidney—medulla	0.016 ± 0.006	0.018 ± 0.012
Spleen	0.004 ± 0.001	0.004 ± 0.002
Muscle	0.002 ± 0.001	0.004 ± 0.002

* Mean ± s.d.

DISCUSSION

Attempts to develop a method for radiolabeling antibodies with ^{99m}Tc have utilized either direct (*1–9*) or indirect (*18–21*) techniques. Direct methods allow labeling the native antibody or antibody fragments without covalent modification employing a chelating moiety. Direct methods, although theoretically requiring less chemical manipulation prior to use, have suffered from one or more of the following practical drawbacks related to the final

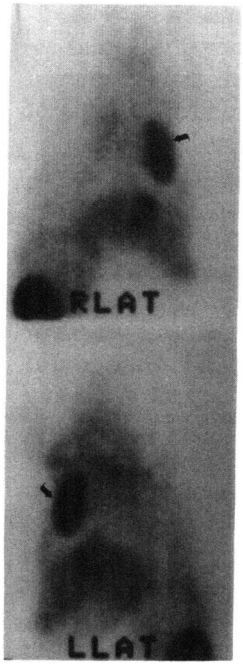

FIGURE 2. Scintigraphic images of a canine experimental myocardial infarct (arrow) at 3 hr after intravenous administration of ^{99m}Tc-antimyosin. (A) Right lateral gamma images (upper), (B) left lateral gamma images (lower).

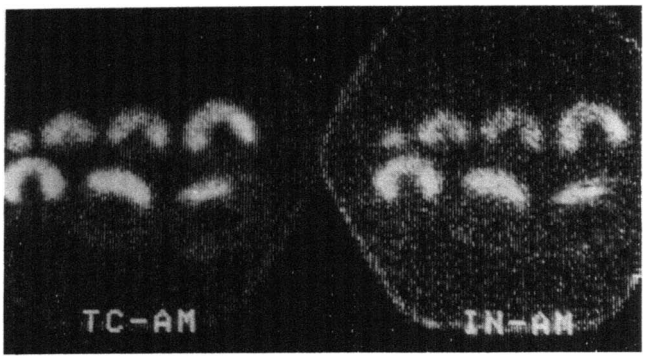

FIGURE 3. Comparison of infarct areas delineated by ^{99m}Tc-antimyosin (left) and ^{111}In-antimyosin (right) after intravenous administration in a canine experimental myocardial infarct 5 hr postinjection.

preparation: (a) low purity requiring subsequent purification, (b) instability of the radiolabel, (c) loss of immunoreactivity, (d) harsh conditions required for labeling, and (e) lack of convenience. Various attempts have been made for the direct labeling of proteins with ^{99m}Tc such as reducing agent, transfer chelate, pH, and temperature (*1–10*). The major problem encountered in the labeling of antibodies with ^{99m}Tc is the binding to nonspecific sites. Paik et al. (*7*) have identified two sites on antibodies for ^{99m}Tc labeling. They showed that the high affinity sites are related to sulfhydryl groups, and the low affinity sites are nonspecific binding sites. Several groups have shown that reduced antibody or fragment can be labeled quantitatively with ^{99m}Tc after the reduction process using a transfer chelate approach. Baum et al. (*26*) reduced the antibody with 2-mercaptoethanol and then labeled it with ^{99m}Tc-phosphonate. Pak et al. (*27*) have demonstrated that antibody fragments and reduced IgG can be labeled rapidly with ^{99m}Tc-D-glucarate upon reduction with dithiothreitol. Thakur et al. (*28*) have evaluated stannous chloride, 2-mercaptoethanol, dithiothreitol, dithioerythritol, and ascorbic acid as antibody reducing agents and determined that these methods yielded similar results in terms of labeling efficiency and immunoreactivity.

The ideal ^{99m}Tc labeling method should be simple, efficient and easily prepared in a kit-form. The method we described for preparing ^{99m}Tc-antimyosin overcomes the previous drawbacks and provides a practical solution to preparing the ^{99m}Tc-labeled product. The method involves the addition of sodium [^{99m}Tc]pertechnetate as generator eluate to the lyophilized stannous chloride, D-glucaric acid and antimyosin Fab′ antibody. The extent of labeling within a given time is dependent on the concentration of the antimyosin Fab′. Greater than 95% radiolabeling is routinely achieved within 30 min at room temperature, and immunoreactivity is greater than 95% at an antibody concentration of 0.5 mg/ml. Since the radiolabeling is routinely greater than 95%, no further purification is required. The radiolabel is stable at 37°C in human plasma, at pH 4.5 and with 10 m*M* DTPA. The diamide dithiolate (N_2S_2) forms very stable tetradendate complexes with ^{99m}Tc, and Fritzberg et al. (*20*) have demonstrated that the ^{99m}Tc-N_2S_2-bound antibodies and fragments displayed high stability and retained immunoreactivity upon challenge with human serum, 10 m*M* DTPA, 10 m*M* 2,3-bis(thioacetamido) propanoate, and 6 *M* urea. Upon challenge with 1 m*M* diaminodithiol compound (N_2S_2), there was 87% of radioactivity associated with the antibody fragment after 3 hr incubation at 37°C. Thus, ^{99m}Tc and antimyosin Fab′ are tightly bound. Technetium-99m can be removed from the antibody in the presence of 5 m*M* DTT, however, this is far from the challenge conditions that might be encountered in vivo.

The instant kit described and evaluated in the current study produces reproducible conditions for ^{99m}Tc labeling of antibody fragments. In the presence of D-glucarate, stannous ion reduction of [^{99m}Tc]pertechnetate produces a weak ^{99m}Tc-D-glucarate complex. This complex stabilizes the ^{99m}Tc under the reaction conditions used and prevents reaction of the ^{99m}Tc with non-sulfhydryl components of the protein molecule. The ^{99m}Tc-D-glucarate radiolabels the antibody Fab′ fragment by a transfer reaction involving the free sulfhydryl groups on the Fab′. Evidence supporting the hypothesis that the free sulfhydryls of the Fab′ fragment participate in the binding of the ^{99m}Tc is provided by failure of ^{99m}Tc-D-glucarate to radiolabel antimyosin IgG, F(ab′)$_2$, Fab-DTPA, or Fab′-NEM under the conditions described, as well as the work of Steigman et al. (*22*). The current method allows stable ^{99m}Tc labeling of antibody Fab′ not only derived from IgG2a isotype, but also Fab′ fragments derived from IgG1 and IgG3 isotypes (*29*). Recently, the method described in this paper has been applied successfully to the labeling of three monoclonal antibodies with an IgG1 isotype, and specific uptake has been demonstrated in experimental models of venous thrombosis (*30*), atherosclerosis (*31*) and tumor xenograft (*32*).

Mouse biodistribution of ^{99m}Tc-antimyosin compares favorably to ^{111}In-antimyosin in showing faster blood clearance and lower uptake in lungs, liver, and spleen (except kidneys). The results of the canine infarct model demonstrate that ^{99m}Tc-antimyosin is functionally equivalent to ^{111}In-antimyosin in this model.

In conclusion, using this instant kit method, ^{99m}Tc radiolabeling can be completed in under 1 hr at room temperature by merely adding [^{99m}Tc]pertechnetate. No purification step is required, since radiolabeling yields are quantitative. Using this method, we have demonstrated that antimyosin Fab′ can be stably labeled with ^{99m}Tc and that the resulting ^{99m}Tc-antimyosin can be used to visualize myocardial infarcts in vivo.

ACKNOWLEDGMENTS

The authors would like to thank Roger Stewart, James Powers for technical support, Dr. I. Elaine Allen for assistance with the statistical analysis, and Joani Wendel for secretarial assistance.

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