# Iodination of Monoclonal Antibodies for Diagnosis and Radiotherapy Using a Convenient One Vial Method

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We have developed a convenient system that can be used to iodinate monoclonal antibodies for diagnosis or therapy. A vial, previously coated with 1,3,4,6-tetrachloro-3a, 6a-diphenyl glycouril (iodogen), is used as a reaction vessel. Iodination and separation of bound and free iodide, using AG1-X8 ion exchange resin, are both accomplished in this vial. We found 90 ± 4% of the iodide which was added was incorporated, respectively, into each of four different monoclonal antibodies evaluated. Approximately 90% of labeled antibody was recovered in each case. The monoclonal antibody OC125 was labeled to specific activities up to 25 mCi/mg. Immunoreactivities of 82 ± 2% using $^{125}$I and 66 ± 5% using $^{131}$I were achieved. As the radioiodination is done in one sealed vial and takes <15 min, this procedure is safe and can be performed in any nuclear medicine laboratory. The final product, which is sterile and apyrogenic, is suitable for diagnostic and radiotherapeutic applications.

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Several laboratories have investigated the use of radiolabeled polyclonal (*1,2*) and monoclonal antibodies (*3–6*) as reagents for diagnostic tumor imaging. Recently, bifunctional chelates also have been used to label antibodies with indium-111 ($^{111}$In) or gallium-67 ($^{67}$Ga) (*7,8*) for radioimmunoscintigraphic applications; however, iodine-123 ($^{123}$I) or iodine-131 ($^{131}$I) have remained radiolabels of choice in most instances (*1–6,9*). Also, the use of radioiodinated polyclonal (*10, 11*) and monoclonal (*12*) antibodies as therapeutic agents has been investigated. As monoclonal antibodies (*13*) with more restricted tumor specificities become available it is likely that specific clinical indications for their immunoscintigraphic or therapeutic applications will increase. To achieve widespread use of such reagents for these clinical applications, convenient, rapid and safe radioiodination procedures need to be developed.

This paper describes a radioiodination technique for antibodies which is rapid, simple, efficient and reproducible, and which can be accomplished in most radiopharmaceutical laboratories with minimal hazard to personnel.

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## MATERIALS AND METHODS

Iodine-131 was obtained at high specific activity as a reductant free solution in NaOH pH7-11, containing 5 μg I per 100 mCi $^{131}$I at 200 mCi/ml. Iodine-125 ($^{125}$I) was obtained in a comparable solution at 500 mCi/ml. iodogen (1,3,4,6-tetrachloro-3a, 6a-diphenyl glycouril) and AG1-X8 ion exchange resin (100–200 mesh) were both obtained from commercial sources.*

Four monoclonal antibodies were used in these studies: OC125, 1116NS 19-9, 115D8, and R11D10. Antibody OC125 ($IgG_1$) is directed against the antigenic determinant, CA125, found on the majority of epithelial ovarian tumors (*14*), and has been used to develop an immunoradiometric assay (*15*). Similarly, antibody 1116NS 19-9 ($IgG_1$), originally prepared against a colorectal carcinoma cell line (*16*), has been used to develop an immunoradiometric assay (*17,18*) and for tumor visualization through radioimmunoscintigraphic techniques (*5,6,9*). Antibody 115D8 was prepared against human milk fat globules and binds to the majority of breast carcinomas (*19*). Antibody R11D10 is directed against human cardiac myosin (*20*). All other chemicals used were of reagent grade quality and were prepared as sterile, apyrogenic solutions.

**Radioiodination**

Antibody was iodinated using a modification of the iodogen method (*21*). Labeling was done in 1 ml of 0.1*M* borate buffer at pH 8.3 and at room temperature for 10 min in a 20-ml vial previously coated with iodogen. The vial was coated as follows: 2 ml of chloroform containing 50 μg/ml iodogen were added to the vial; the chloroform was allowed to evaporate overnight in a fume hood at room temperature. Fifty micrograms to one milligram of antibody was reacted with 1 to 10 mCi of $^{125}$I or $^{131}$I. The reaction was quenched with 2 ml ion exchange resin (AG1-X8) suspension, v/v 90% resin in phosphate buffered saline at pH 7.4 containing 1% bovine serum albumin (BSA). After 1 min the suspension was withdrawn from the vial and sterile filtered into another uncoated and sterile vial ready for use (Fig. 1). The recovery of radiolabeled antibody was calculated as follows. At the completion of the reaction and prior to adding the ion-exchange resin suspension, the total radioactivity in the vial was determined. Then a 5-μl sample was removed from the reaction vial which was used to determine the fraction of radioactivity which was trichloroacetic acid precipitable. From this data a theoretical yield of radioiodinated antibody could be computed. Following filtration into another vial the radioactivity was again measured. The fraction of recovered radioactivity compared with the theoretically calculated radiolabeled antibody was defined as the antibody recovered.

$$\text{Antibody recovered} = \frac{\text{total radioactivity recovered}}{\text{total radioactivity added} \times \text{fraction radioactivity TCA precipitable}} \times 100.$$

**Resin Capacity/Concentration/Time**

To determine the capacity of the anion exchange resin for iodide, 1 ml of a 60% (v/v) resin suspension was prepared in a 1, 0.1, 0.01, or 0.001*M* NaI solution. Approximately 0.01 μCi of Na$^{125}$I was added to this suspension. After 5 min the samples were centrifuged at 1,000 *g* for 10 sec and the amount of free iodide was assayed by counting 100 μl of the supernatant, and computing the percentage of total counts remaining in solution. The minimal concentration of resin (v/v) required to bind free iodide was determined in a similar fashion. Different concentrations of resin (1–90%, v/v) in 0.1*M* borate, pH 8.3, were exposed to 0.01 μCi Na $^{125}$I which was added and incubated for 10, 30, 60, 120 and 300 sec. Free iodide was assayed as above; the removal of free iodide was virtually complete by 10 sec.

**Assay of Percent Incorporated Iodine and Free Iodine in Final Product**

Prior to the addition of ion exchange resin, a sample was withdrawn from the iodination vial and assayed for free and bound iodide using gel filtration high performance liquid chromatography (HPLC). A TSK 3000 column (30 cm) with 0.2*M* phosphate buffer as eluent at 1 ml/min was used. HPLC equipment was fitted with a uv monitor (214 nm) and a radiation detector. Final product, which had been mixed with ion exchange resin and filtered, was assayed in identical fashion. Percent iodide incorporation was accomplished by calculating the surface area under the peak of the radiation profile of antibody and free iodide.

**Immunoreactivity**

Assay of the immunoreactivity was performed as described (*22*) for both OC125 and 115D8 antibodies, respectively. To one concentration of radiolabeled antibody serial dilutions of OVCA 433 (*23*) cells were added and incubated for 4 hr at 4°C. The cells were washed and cell bound radioactivity was determined. To calculate the immunoreactivity, the ratio of total applied to specifically bound radioactivity (A/S) was plotted against the inverse of cell concentration (1/C).

Estimates of the immunoreactivity of 1116NS 19-9 or R11D10 were made by determining the fractional binding of a sample of the radiolabeled antibodies to an affinity chromatography column. Either cardiac myosin or 19-9 monoclonal antibody was coupled to CNBr-activated Sepharose 4B. Columns of 0.5 × 2 cm

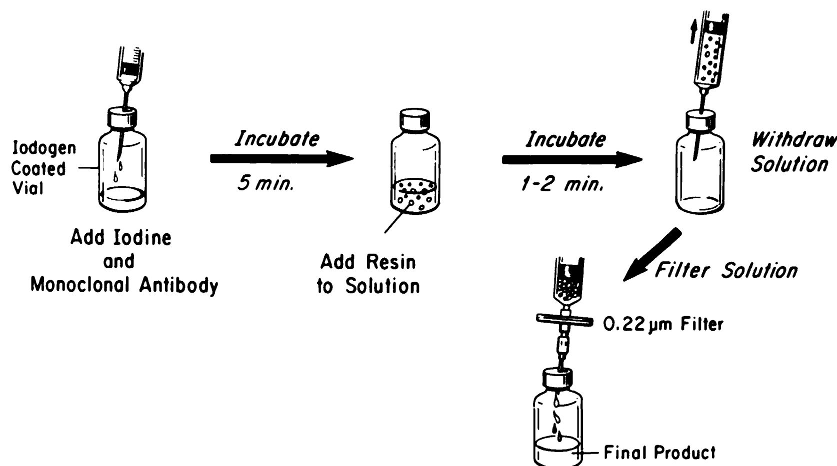


**FIGURE 1**
Schematic diagram of iodination method. To a vial previously coated with iodogen, monoclonal antibody and radioactive iodide are added. After 5 min of incubation AG1-X8 resin is injected into vial and after another 1–2 min solution is drawn from vial. After passing through 0.22-μm filter, product is ready for injection

were used in each case. Columns with 19-9 antibody attached were exposed to a multivalent CA 19-9 antigen preparation prior to use. A small sample of radiolabeled monoclonal antibody either 19-9 or R11D10 (~0.01 μCi) was applied to the appropriate column in each case and the eluant was monitored for radioactivity. In the case of 19-9, bound radiolabeled antibody was eluted from the 19-9/CA 19-9 column using 3*M* $NH_4SCN$. Radiolabeled R11D10 was eluted from the myosin column using 0.1*M* glycine HCl buffer at pH 3.5. Immunoreactivity was defined as the percentage of retained antibody applied to the column. Nonspecific binding to either column was always <2%.

### Quality Control

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on antibody samples prior to and after radioiodination to verify purity of the antibody preparations and identify protein containing radiolabel.

Ten percent polyacrylamide gels were utilized. Antibody preparations were reduced in dithioerythritol (DTT), boiled in 1% SDS solution in 0.1*M* Tris-HCl pH 6.8 and applied to stacking gels (*24*).

Product sterility prior to and after radiolabeling was tested by incubating 1 ml of purified product with thioglycolate medium for 2 wk at 37°C. Potential pyrogenicity was tested using the Limulus Amebocyte Lysate (LAL) method. Material proved to be sterile and contained less than one endotoxin U/ml in all cases, which was used as an operational definition of apyrogenicity.

## RESULTS

### Radiolabeling of Monoclonal Antibodies

Figure 1 illustrates schematically the system developed for radioiodination of monoclonal antibodies. The system utilizes a sealed iodogen coated reaction vial, a buffered monoclonal antibody solution, ion exchange resin suspension and 0.22 μm filter to obtain, in <10 min, a final sterile, apyrogenic product ready for injection.

Typical results of iodination using this system are illustrated by the reaction of the antibody OC125. Incorporation of iodide into OC125 usually appeared to reach its maximum by 5 min of incubation; however, incubation was often done for 10 min to assure complete reaction. Free iodide was removed using a strong anion exchange resin with a high affinity for iodide. One milliliter of 60% resin could bind up to 0.01*M* of NaI. There was no increase in the percentage iodide removed after 0.5 min of incubation (Materials and Methods). Usually, incubations were allowed to proceed for 1–2 min. Typical HPLC profiles of the reaction mixtures prior to and after removal of free iodide by the resin are shown in Figs. 2A and 2B, respectively. Additionally, the reaction mixtures were electrophoresed on polyacrylamide SDS gels which were then scanned for radioactivity. Only immunoglobulin heavy and light chains contained radioactivity. No other radioactive peaks were noted, as illustrated in Fig. 3 with the OC125 antibody. The anion exchange resin buffer

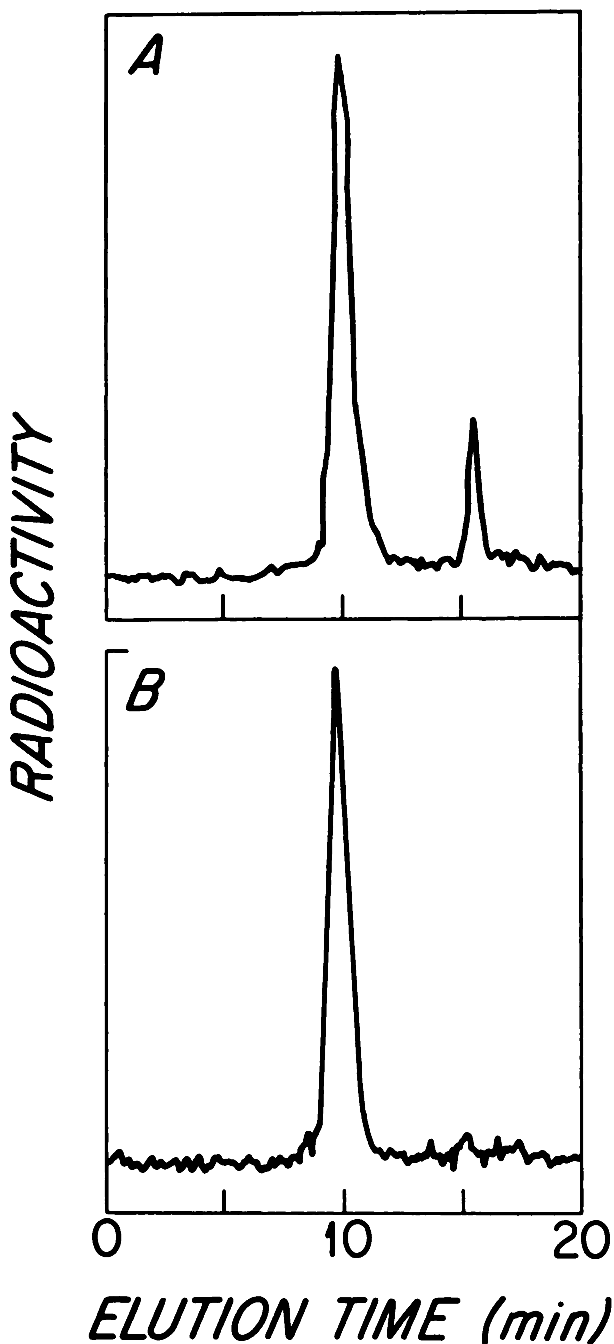


**FIGURE 2**
Removal of free iodide by AG1-X8 ion-exchange resin. Typical HPLC gel filtration profiles of radiolabeled OC125 IgG immediately following iodination (A) and after addition of AG1-X8 resin (B) are illustrated. Removal of iodide ions from antibody preparation is confirmed by disappearance of second peak (free radioactive iodide) which elutes at interstitial volume (Vi) of column

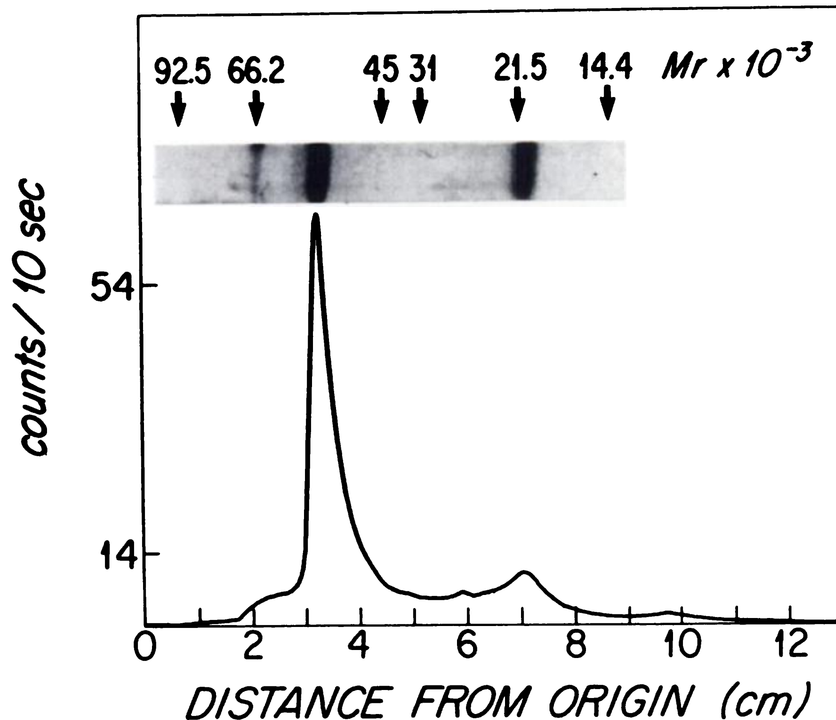


**FIGURE 3**
SDS polyacrylamide gel analysis and radiation profile of radiolabeled OC125 IgG under reduced conditions. To 10% polyacrylamide slab gel, 100,000 cpm of radiolabeled antibody in 1% BSA was applied. Unlabeled purified OC125 antibody was run side-by-side in same gel with small amount of BSA added to serve as internal standard. After development and drying, gel was scanned for radioactivity at 1 cm/min with 10 sec integration time. Significant radioactivity was detected only in heavy and light chains of reduced antibody preparation

utilized in these experiments contained 1.0% BSA, yet no significant labeling of this BSA could be observed (Fig. 3), despite the fact that the total protein applied to this gel contained a minimum of 99.5% BSA and only 0.5% or less antibody.

Free iodide was not detected in the final product in the majority of cases. Seven of 13 preparations were completely free of iodide. In the other six instances fractions of free iodide remaining ranged from (0.7 to 3.4%; mean 1.72 ± 0.95%).

The removal of the resin from the reaction mixture was achieved by filtration through a 0.22-$\mu$m Millex filter which at the same time sterilized the product.

The immunoreactivity of the iodinated OC125 was determined using the cell binding assay (*22*) described in the Methods section. Plots of A/S versus 1/C for [$^{125}$I]OC125 and [$^{131}$I]OC125 are shown in Fig. 4. Results of all iodinations are summarized in Table 1. The immunoreactivity of iodinated OC125 was 82 ± 8% (range 73–96%) for $^{125}$I and 65 ± 5% (range 59–71%) for $^{131}$I. The immunoreactivity slightly decreased with higher specific activities, being ~83% for 1–5 mCi/mg $^{125}$I and ~73% for 25–30 mCi/mg $^{125}$I. For $^{131}$I, immunoreactivity dropped from ~71% for 1–5 mCi/mg to ~63% for 25–30 mCi/mg.

For eight replicate experiments using $^{125}$I and four replicate experiments using $^{131}$I to label OC125 it was found that incorporation was 90 ± 4% in each case (Table 1) using the final reaction system. The final filtered product was always found to be sterile and apyrogenic. Following sterilization, recovery of iodide in the final product was 80–90% and final recovery of antibody was never <80%.

Besides OC125 other monoclonal antibodies were tested in this system. Both $IgG_1$ and $IgG_2$ antibodies could be iodinated successfully. In every case incorporation of iodide exceeded 90% and immunoreactivities

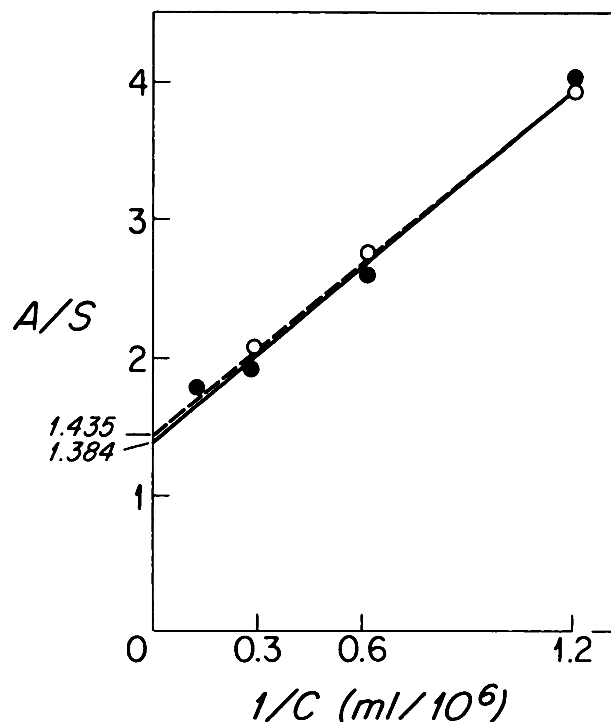


**FIGURE 4**
Plot of A/S versus 1/C for radiolabeled OC125 antibody. Immunoreactivity of $^{125}$I-labeled OC125 (●) and $^{131}$I-labeled OC125 (O) were determined as described in "Methods" section

**TABLE 1**
Incorporation of Radioactive Iodide and Immunoreactivity of Various Monoclonal Antibodies

| Antibody | Radionuclide | Replicates | Fraction of iodine incorporated (%) | Immunoreactivity final product (%) |
|---|---|---|---|---|
| OC125 ($IgG_1$) | $^{125}I$ | 8 | 90 ± 4 | 82 ± 8 |
| OC125 ($IgG_1$) | $^{131}I$ | 4 | 90 ± 4 | 66 ± 5 |
| 1116NS 19-9 ($IgG_1$) | $^{125}I$ | 4 | 90 ± 4 | 72 ± 4 |
| R11D10 ($IgG_{2a}$) | $^{125}I$ | 2 | 89 ± 1 | 86 ± 2 |
| 115D8 ($IgG_{2b}$) | $^{125}I$ | 2 | 88 ± 4 | 77 ± 7 |

all exceeded 70% (Table 1). Final reagents were always sterile and apyrogenic.

## DISCUSSION

In this paper we describe an easy and reproducible method for iodination of monoclonal antibodies. Antibody OC125, which reacts with >80% of ovarian cancers, was used as a model to evaluate the methodology. Additionally, three other antibodies, 1116NS 19-9, 115D8, and R11D10 also were tested in the system. Incorporation of iodide was ~90% and in each case the radiolabeled antibodies had immunoreactivities of 65–85%. We used an ion exchange resin to remove free from bound iodide. This was accomplished by injecting 2 ml of 90% resin into the reaction vial. From 98–100% of nonbound iodide was removed within seconds. Absorbance of antibody to the resin was 5–10% and did not change significantly after addition of carrier protein (e.g., BSA). However, carrier protein could be added to the resin solution to minimize radiation damage to the protein. This did not result in labeling of the carrier protein since the iodide was very rapidly bound to the resin. The reaction volume of 1 ml makes the method potentially useful for iodination of up to hundreds of milligrams of antibody with large amounts of radioactive iodine.

We utilized the iodogen labeling method to develop this vial system. This labeling method does minimal damage to the protein during the iodination (*21*). The reaction is slow relative to chloramine T iodination; however, it is consequently more controllable and still complete in ~5 min. These advantages and the performance noted in this study recommend the iodogen method in this system. With minor modifications to the one vial system, solid phase lactoperoxidase iodinations might also be performed.

The labeling system described is remarkably simple when compared to other iodination techniques (*25*, *26*). It eliminates the need for pumps, columns, etc., as well as transfers between various vessels with potential reductions in yield. All transfers to and from the single iodination vial are made with syringes so exposure of volatile iodide is minimized. If evacuated vials are used, no positive pressure is developed in the vial so leaking of radioactive material is minimized. Additionally, all buffers used in this system can be prepared in advance and can be stored for months. We used iodogen vials after up to 12 mo of storage (−4°C) with no loss of activity. Given the excellent reproducibility of the system it seems likely that the need for measurement of immunoreactivity of antibody obtained from each labeling procedure may also be reduced or eliminated.

One vial iodinations as described here can be performed conveniently in any radionuclide laboratory. Consequently, this system will allow broad use of radioiodinated monoclonal antibodies with less restriction to specialized centers. This eventually could extend the use of these reagents for immunoscintigraphic diagnostic procedures as well as antibody-guided radiation therapy.

## FOOTNOTE

* Pierce Chemical Co., Rockford, IL, and Bio-Rad Laboratories, Richmond, CA, respectively.


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