The Importance of Careful Filtration Technique in Obtaining High Recovery Yields of Indium-111 OncoScint CR/OV

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Objective: Indium-111 OncoScint CR/OV (satumomab pendetide) is the first FDA-approved radiolabeled monoclonal antibody for human diagnostic use. In our experience with preparing OncoScint CR/OV according to the manufacturer's package insert procedure, we have noted that the recovery of the labeled antibody after the required filtration through a 0.22-μm filter was variable; sometimes filtration yields as low as 60% of the initial activity injected into the reaction vial were observed. In this study we investigated whether or not the filtration yield of the radiolabeled immunoconjugate in the injection syringe could be improved.

Methods: We used either a scrupulous technique in withdrawing the material from the reaction vial into the dose syringe through the 0.22-μm filter or the dilution of the radiolabeled antibody solution with saline before careful withdrawal in our attempts to improve the yield.

Results: Our results indicate that it is possible to obtain a consistent recovery of greater than 80% of the labeled antibody without using any diluent if care is taken to completely remove the liquid from the reaction vial.

Conclusions: In our studies performed according to the manufacturer's directions, the yield was acceptable. An improvement in recovery less than 5% was achieved with the addition of saline which was statistically significant. Therefore, we do not recommend using saline to dilute ^{111}In OncoScint CR/OV before filtration.

Key Words: Indium-111 OncoScint CR/OV, saline, radiolabeled monoclonal antibody.

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Indium-111 OncoScint CR/OV (satumomab pendetide) is the first FDA-approved radiolabeled monoclonal antibody (Mab) for human diagnostic use. The antibody used in the OncoScint CR/OV kit is whole murine IgG_1 immunoglobulin known as B72.3. It targets a large tumor-associated glycoprotein known as TAG-72 which is expressed in a broad range of epithelial-derived cancers with little or no expression in most normal tissues (*1–3*). It is indicated for detection of recurrent colorectal and ovarian cancer (*4*).

The antibody is conjugated to DTPA through the saccharide portion of the antibody (*5*). Radiolabeling is performed by incubating the antibody with ^{111}In in the form of buffered indium chloride for 30 min at room temperature in a reaction vial. The labeled antibody is then withdrawn from the vial into the dose syringe through a 0.22-μm filter. The purpose of filtration of the radiolabeled antibody into the dose syringe is twofold: (1) to ensure the sterility of the final product and (2) to remove any protein aggregates which may have formed in the reaction vial. In our experience with preparing OncoScint CR/OV by this procedure, we have noted that the filtration yield of the labeled antibody was variable; sometimes as low as 60% of the initial activity injected into the reaction vial. A lower dose of radiolabeled antibody for patient injection was a concern because of the potential loss of diagnostic information (sensitivity) in the images resulting from the administration of as much as 40% less activity and correspondingly less antibody than recommended by the manufacturer.

This concern led us to investigate whether dilution of the radiolabeled antibody solution before filtration would improve the percentage recovered (yield) of the radiolabeled immunoconjugate in the injection syringe. We compared this to the filtration yields obtained by filtering the undiluted solution, with scrupulous attention paid to removing all the labeled antibody solution in both cases. This paper describes the results from this investigation and makes recommendations for assuring increased and consistent recovery of ^{111}In OncoScint CR/OV.

MATERIALS AND METHODS

OncoScint CR/OV kits and Indichlor ^{111}In-chloride were used as supplied by the manufacturers (Cytogen, Princeton, NJ, and Amersham, Arlington Heights, IL, respectively).

Antibody labeling was performed according to the manufacturer's package insert (*4*). Specifically, the vials of antibody, acetate buffer and ^{111}In-chloride solution were allowed to come to room temperature. All transfers were

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performed using the aseptic technique. Acetate buffer (0.5 ml) was added to the vial containing the ^{111}In-chloride. The volume of the buffered ^{111}In solution containing approximately 6 mCi was drawn into a syringe and the activity measured. This material was aseptically added to the vial containing the antibody solution. To avoid any potential losses of immunoconjugate by this route, the syringe was not rinsed with the protein solution. The empty syringe was counted again and the starting activity for the labeling was calculated to be the initial activity in the syringe minus the activity in the empty syringe. The vial was kept at room temperature for 30 min. Care was taken to avoid the formation of foam in the reaction vial.

At the end of the 30-min reaction time, either no diluent (regular method) or 3 ml of 0.9% saline (Abbott Laboratories, Abbott Park, IL) (saline dilution method) was added to the reaction vial. The labeled protein was withdrawn from the vial into a 10-ml syringe through a 0.22-μm filter and a 23-gauge needle. The following steps were taken to ensure maximum efficiency of this transfer for both the regular method and the saline dilution method: (1) care was taken to avoid causing the protein solution to foam; (2) the needle was placed with the beveled edge next to the inner wall of the vial neck just above the septum as illustrated in Figure 1; and (3) the syringe plunger was kept withdrawn until the filter/needle assembly had been removed from the syringe and a fresh needle had been placed on the syringe. The syringe was counted to obtain the yield of the procedure. The reaction vial and filter/needle assembly were also counted.

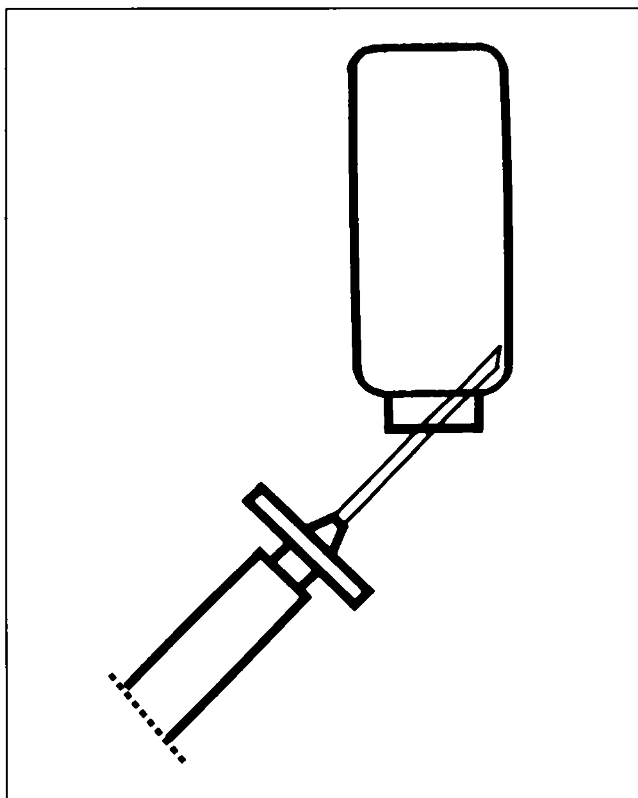

FIGURE 1. The proper placement of the needle in the OncoScint CR/OV reaction vial to ensure maximum recovery of the contents: place the needle with the beveled edge next to the inner wall of the vial neck just above the septum.

The radiochemical purity of the labeled antibody was determined chromatographically with silica gel-impregnated instant thin-layer chromatography strips (ITLC SG) (Gelman, Ann Arbor, MI) by the method of Zimmer et al (*6*). Briefly, one drop of the filtered ^{111}In-labeled OncoScint CR/OV was added to 100 μl of 0.05 *M* DTPA solution to challenge the binding of the radiolabel to the protein. After a 2-min incubation, a drop of the resulting solution was chromatographed on an ITLC SG strip using 0.9% saline as the solvent. In this system, the protein-bound label remains at the origin (together with any protein dimers or aggregates) while the ^{111}In-DTPA moves to the solvent front. The strip was cut in half and the upper and lower halves were counted in a well counter. The radiochemical purity of the material was expressed as the counts in the lower half divided by the counts on the entire strip.

Tests of the regular method and saline dilution method were repeated on five different days with different lots of indium chloride 1–4 days pre-calibration. Statistical comparisons of the recovered yields were performed using Student's t-test (*7*).

The holdup volume of the filter/needle assembly was measured by attaching a new filter and needle to a syringe containing 1.0 ml saline. The plunger of the syringe was depressed until the first drop of liquid appeared at the needle tip. The change in volume in the syringe was noted and taken to represent liquid contained in the filter and needle.

RESULTS

We found that it was possible to remove virtually all the labeled antibody solution (by visual evidence) from the reaction vial whether or not saline dilution was used, if the transfer was made as shown in Figure 1. This necessitated being able to observe the contents of the vial, taking care to use appropriate shielding.

Table 1 shows the results of radiolabeling procedures, five using the regular method (no diluent), and five using 3 ml of saline to dilute the labeled antibody before withdrawal through a 0.22-μm filter (saline dilution method). The average filtration yield for the regular method was 82.2% ± 2.3%. This increased with the use of 3 ml of saline as a diluent to 86.4% ± 1.9%. The difference in filtration yields for the two methods is significant at the 2.5% confidence level by the Student's t-test (*7*).

Table 1 also shows the percent activity measured in the vial and filter/needle assembly after the dose was withdrawn. These figures are lower than the true values owing to geometry differences with the material counted in the syringe. Nevertheless, it is clear that the use of saline diluent caused less activity to be retained in the filter/needle assembly, and to a lesser extent, in the vial. The smaller percent of activity retained by the filter/needle assembly when 3 ml of saline was used as a diluent is statistically significant at the 1%

TABLE 1
Comparison of OncoScint CR/OV Recovery Yield by Regular and Saline Dilution Methods

	Regular Method			Saline Dilution Method		
Run	Percent yield	Percent in vial	Percent in filter	Percent yield	Percent in vial	Percent in filter
1	86.1	3.2	5.8	84.8	4.4	4.6
2	82.0	4.9	6.7	89.4	2.9	3.5
3	82.3	5.9	5.6	85.3	3.5	4.2
4	81.9	5.0	6.1	88.0	2.3	4.5
5	78.9	7.6	6.0	84.7	5.1	3.4
Average	82.2	5.3	5.3	86.4	3.6	4.0

confidence level, but the difference in the activity retained in the vial is not statistically significant.

These results are summarized in Figure 2, which shows the average proportions of activity in the dose syringe, reaction vial and filter/needle assembly after filtration by the regular method and the saline dilution method. A portion of the initial activity is not accounted for because of geometrical differences between the syringe, and the reaction vial and the filter/needle assembly. This activity must, in fact, be in the reaction vial and the filter/needle assembly since the initial activity was also measured in a syringe.

In all of the preparations, the radiochemical purity was 97.7% or greater by ITLC analysis.

DISCUSSION

A number of factors combined to make withdrawing the labeled antibody into a syringe through the sterilizing filter a challenge. The solution had a tendency to foam if manipulations were not performed smoothly. The resulting foam took several minutes to break up and made complete recovery of the liquid in the vial difficult.

The 0.22-μm filter complicated matters. Significantly more effort was required to withdraw the plunger of the syringe with the filter attached. On two occasions the filter appeared to lock (i.e., it did not permit continued withdrawal of solution remaining in the reaction vial) before all the liquid had been removed from the vial. It was possible to correct this by depressing the plunger of the syringe, reinjecting some of the liquid into the vial and then smoothly withdrawing the plunger of the syringe to remove the rest of the vial contents.

The 0.22-μm filter and needle combination had a significant volume of 0.5 ml and this, we believe, represents a significant site of lost activity. The manufacturer's package insert for the 0.22-μm filter indicates that its holdup volume is ≤0.1 ml after an air purge (*8*). Such a purge, of course, is not practical in this application. However, it was possible to

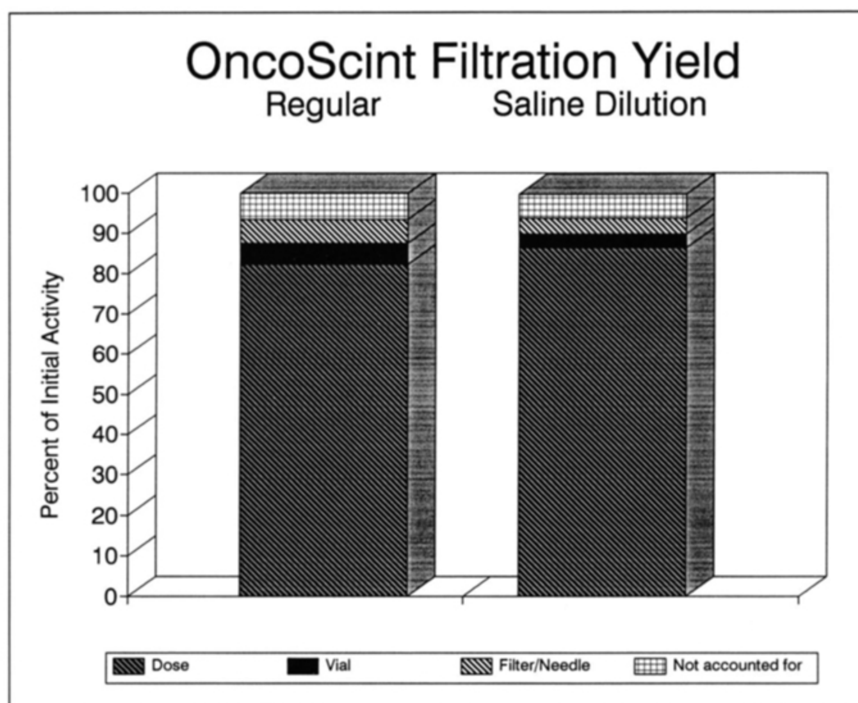

FIGURE 2. Bar graph illustrating the relative activity balances for the regular method and the saline method. Note that the activity not accounted for is due to geometrical differences between the syringe and the reaction vial and filter/needle assembly. This activity is in the reaction vial and filter/needle assembly, but its distribution cannot be determined.

TABLE 2
Effect of Dilution on Activity Concentration

Method	mCi	ml	mCi/ml
Regular	6.0	2.5	2.4
Saline	6.0	5.5	1.1

transfer a portion of the liquid in the filter to the dose syringe by maintaining tension on the withdrawn plunger while the filter was removed from the syringe.

Table 2 shows that the concentration of the labeled protein solution was 2.4 mCi/ml before dilution with saline and 1.1 mCi/ml after. At the undiluted concentration of 2.4 mCi/ml, 1.0 mCi corresponds to 0.42 ml, which is essentially identical to the volume determined for the filter/needle combination. This suggests that losses experienced in the filtration process are largely due to holdup in the filter and needle when due care is taken to withdraw all of the contents of the reaction vial. It also suggests that in cases where a greater proportion of the starting activity is not recovered, it is likely that some of the protein solution remains in the reaction vial. For example, a 60% recovery could be caused by a total volume loss of 1.0 ml which, with the holdup volume of the filter/needle at 0.5 ml, would mean an additional 0.5 ml left in the vial.

The use of saline to dilute the solution prior to filtration was effective in permitting increased filtration yields of the labeled protein. In other words, if the volume loss in the filter is essentially constant, then the lower concentration of the antibody solution after dilution with saline will result in less labeled antibody being retained on the filter.

SUMMARY

In all five preparations of ^{111}In-labeled OncoScint CR/OV performed for this study according to the manufacturer's directions and using a careful filtration technique, the yield was acceptable (78.9%–86.1%). While the addition of saline to the vial of labeled OncoScint CR/OV does permit a somewhat improved recovery of the radiopharmaceutical (84.8%–89.4%), it is important to note that there are no published data regarding the stability and immunoreactivity of ^{111}In OncoScint CR/OV diluted in saline for any length of time. Further, the improvement in recovery, while statistically significant, was less than 5%. For this reason *we do not recommend* using saline to dilute ^{111}In OncoScint CR/OV before filtration.

We have shown that it is possible to obtain consistent recoveries of labeled OncoScint CR/OV in the range of 80% or higher, if the following recommendations are followed:

1. Use a vial shield for the radiolabeling procedure with a clear lead window which will permit observation of the contents so that the complete removal of the contents can be verified.
2. Take care to avoid the formation of foam in the protein solution, which will reduce the recovery of activity.
3. Place the needle with the beveled edge next to the inner wall of the vial neck just above the septum.
4. Withdraw the plunger of the syringe in a smooth, steady fashion to a volume of about 1 ml greater than the contents of the vial.
5. Maintain outward tension on the plunger until the needle is out of the vial and the filter is removed from the dose syringe.

CONCLUSION

Indium-111 OncoScint is the first approved radiolabeled monoclonal antibody for use in nuclear medicine. The use of a sterilizing filter in its radiolabeling has resulted in inconsistent yields and significant losses. We have shown that it is possible to perform the filtration step and obtain consistent yields by ensuring maximum fluid recovery. We have also demonstrated that the use of a saline dilution step prior to filtration permits improved recovery of the labeled antibody. This technique will be helpful once data are available on the stability and immunoreactivity of ^{111}In OncoScint CR/OV diluted in saline.

The filtration techniques we have described are simple and easily implemented. As each OncoScint CR/OV kit preparation is a unit dose, high recovery of the radiolabeled monoclonal antibody will permit better quality images due to the higher count rated obtained with injected doses close to the recommended 5 mCi.

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