Rapid Assay for Total Unbound Tc-99m in Preparations of Tc-99m Macroaggregated Albumin: Concise Communication

J. R. McLean

Radiation Protection Bureau, Ottawa, Canada

A rapid economic filter-paper assay for determining total unbound Tc-99m in preparations of Tc-99m macroaggregated albumin (Tc-99m MAA) is described. The procedure uses Whatman 542 filter-paper discs and aqueous 0.009% NaCl as wash solution. The principal radiochemical impurities found in Tc-99m MAA kits are not significantly adsorbed to the filter matrix. The procedure can be completed in 1–2 min and gives results comparable to those obtained by the centrifuge assay (USP XIX).

J Nucl Med 19: 1045–1048, 1978

The widespread use of Tc-99m-labeled kits in nuclear medicine has made it necessary to develop rapid, economic, and simple methods for quantitating the total concentration of unbound Tc-99m in radiopharmaceuticals. Several radiochemical impurities can exist in Tc-99m MAA preparations, and these include a colloidal reduced and hydrolyzed fraction (Tc-Sn colloid), free pertechnetate ($^{99m}TcO_4^-$), and in some formulations a soluble Tc-99m albumin (Tc-99m HSA) (*1,2*). The currently popular methods of monitoring radiochemical purity use paper- or instant thin-layer chromatography (PC or ITLC) with methanol as solvent, and give labeling efficiencies that are based on the presence of only $^{99m}TcO_4^-$. The other radiochemical impurities that can be present remain undetected (*1–3*). The USP (XIX) centrifugation assay (*4*), which requires 10–15 min to complete, is a more satisfactory quality-control procedure because it determines total unbound Tc-99m in the preparation. The use of particle filtration would be a rapid alternative to the centrifuge assay if the nonspecific adsorption of radiochemical impurities to the filter matrix could be minimized. The purpose of this report is to demonstrate the statistical equivalency of such a filtration procedure to the USP centrifugation assay. No attempt will be made to correlate the in vitro test results with clinical efficacy because of the many other variables that would also have to be considered.

MATERIALS AND METHODS

Macroaggregated albumin (MAA) kits were obtained from several manufacturers as a part of a routine quality-assessment program. The kits were reconstituted as directed by the manufacturers using eluates from a technetium-99m generator. A Whatman 542 filter-paper disc (2.4 cm dia.), moistened with dilute saline, is placed on the fretted glass support of a filter-funnel stem* that is connected to a vacuum system. A 0.1-ml aliquot of Tc-99m MAA is drawn into a 1-cc syringe (20 gauge × 1½-in. needle) and is assayed for radioactivity in a dose calibrator. The sample suspension is discharged onto the filter-paper disc. The syringe is rinsed onto the filter paper with 1 ml of a wash solution consisting of 0.009% NaCl. The residual radioactivity in the syringe and the radioactivity on the filter-paper disc are then determined. The filter disc is assayed by placing it in a standard plastic liquid-scintillation vial. The disc can also be assayed in a 16- × 125-mm plastic test tube, in which case it can be folded or left to lie along the contour of the vial without any significant geometry effect. The geometry difference

Received Oct. 31, 1977; revision accepted Feb. 21, 1978.

For reprints contact: J. R. McLean, Radiation Protection Bureau, Ottawa, Canada.

TABLE 1. NONSPECIFIC ADSORPTION OF RADIOCHEMICAL IMPURITIES TO FILTER DISCS (THREE REPLICATES)

Filter Type	% of applied sample remaining on filter (mean + range) (using 1 ml of 0.009% saline as wash)		
	$^{99m}TcO_4^-$	$^{99m}TcO_2$	^{99m}TcHSA
Whatman 542 (paper)	<0.1	12.7 (10.5–14.9)	1.3 (1.2–1.4)
Whatman GF/C glass fiber	<0.1	63.8 (56.6–71.0)	8.1 (7.1–9.1)

between the syringe and filter disc has no appreciable effect on radioactivity measurements. The labeling efficiency for the preparation is calculated as:

$$\% \text{ labeling efficiency} = \frac{\text{filter cpm} \times 100}{\text{(gross syringe) cpm} - \text{(residual syringe) cpm}}.$$

This assay was repeated using 2.4-cm glass fiber discs†. The filters retain particles down to 1 μm. The USP (XIX) centrifuge assay (*4*) and paper-methanol chromatography (*5*) were also performed on the reconstituted Tc-99m MAA preparations in parallel with the filter-disc assays. In addition, Whatman 542 filter discs and glass fiber GF/C filters were tested for their ability to adsorb nonspecifically $^{99m}TcO_4^-$, Tc-99m HSA‡, and Tc-99m Sn colloid (*6*). Retention of these substances by the filters was determined using the method described for Tc-99m MAA. In addition, binding of colloid was determined by diluting a 0.1-ml aliquot with 5 ml of 0.001 *N* HCl and then allowing this solution to filter slowly through the matrix by gravity. This was repeated with Tc-Sn colloid in 100 m*M* phosphate buffer at pH 7.2. Finally, aliquots of 0.025 ml of Tc-99m Sn colloid were allowed to dry on the discs and then eluted with 1 ml of 0.009% NaCl. This was repeated using 0.001 *N* HCl and 100 m*M* phosphate buffer at pH 7.2.

Two MAA kits—one with and one without normal serum albumin—were selected to undergo storage at 60 ± 5°C in the presence of air, in an attempt to effect changes in their radiochemical purity. The assay methods could then be compared for their ability to detect any changes that might occur in the labeling efficiency of the preparations. The compromised MAA kits were then reconstituted and assayed like the intact Tc-99m MAA preparations, except that the albumin-containing kit was assayed for $^{99m}TcO_4^-$ and T-99m Sn colloid by TCA precipitation (*2*), whereas chromatography with HSA-loaded paper and N_2-purged saline (*7*) was used to determine whether protein fragments were present in the non-albumin kit.

Correlation coefficients were calculated for various pairs of assay procedures (*8*). Assay results were then subjected to a two-way analysis of variance (*9*).

RESULTS AND DISCUSSION

The nonspecific binding of the various radiochemical impurities to the filters is shown in Table 1. The nonspecific binding of Tc-99m Sn colloid was highly variable on all filter materials tested but absolute binding was much lower for Whatman 542 paper. The adsorption of Tc-99m Sn colloid did not depend on the pH of the eluting medium, since elution efficiencies were about the same for all samples. The level of adsorption of Tc-Sn colloid by the Whatman 542 filter is probably not significant because it would represent only about 13% of the small percentage of the preparation's total activity that is due to the Tc-Sn colloidal impurity.

The results for assays performed on the commercial preparations of Tc-99m MAA are shown in Table 2. Assay procedures 1–3 give labeling efficiencies based on the total radioactivity that is dissociated from the MAA particles, whereas procedure

TABLE 2. RADIOCHEMICAL PURITY OF COMMERCIAL Tc-99m MAA KITS BY FILTRATION, CENTRIFUGATION, AND PAPER CHROMATOGRAPHY (FOUR REPLICATES)

Company	Lot number	% of radioactivity bound to MAA particles (mean + range)			
		Whatman 542	USP cent. assay	Whatman GF/C	USP-paper chromatography
CE Frosst	03294	99.0 (98.8–99.2)	98.4 (98.0–98.8)	98.7 (97.9–99.5)	97.8 (96.6–99.0)
Diagnostic isotopes	LG2486	96.7 (95.0–98.4)	96.9 (95.7–98.1)	95.5 (93.8–97.2)	98.6 (97.7–99.5)
Mallinckrodt	7BA	97.8 (97.4–98.2)	97.5 (95.9–99.1)	N/A	99.4 (99.3–99.5)
3M	F-067110	98.2 (97.1–99.3)	98.6 (98.2–99.0)	N/A	>99.9
NEN	7021	92.4 (91.2–93.6)	92.2 (92.0–92.4)	93.7 (92.3–95.1)	97.5 (96.7–98.3)
Squibb	6M970	93.9 (93.2–94.6)	92.9 (92.7–93.1)	N/A	97.5 (97.2–97.8)
Medi-Physics	99.4-216	96.2 (95.6–96.8)	96.5 (96.1–96.9)	N/A	99.8 (99.7–99.9)

TABLE 3. LABELING EFFICIENCY (%, MEAN + RANGE) OF PRODUCTS TREATED AT 60 ± 5°C IN PRESENCE OF AIR FOR SEVERAL DAYS (REPLICATES, n AS INDICATED)

Product	Assay procedures				
	Filter disc 542 assay	USP cent. assay	TCA cent.* assay	Paper with methanol chromatography	Paper with saline chromatography
1. With normal HSA	81.2 (80.2–82.2) n = 4	83.0 (92.7–83.3) n = 4	96.4 (96.1–96.7) n = 4	96.0 (95.6–96.4) n = 4	N/A
2. Without normal HSA	99.0 (98.1–99.9) n = 4	99.2 (98.9–99.5) n = 4	N/A	82.8 (81.1–84.5) n = 3	99.3 (99.2–99.4) n = 3

* Radioactivity remaining in supernatant can be assumed to be an index of $^{99m}TcO_4^-$ and Tc-99m-Sn colloid in the preparation.

4 is based on the dissociation of only $^{99m}TcO_4^-$ from the particles. All preparations show <3% $^{99m}TcO_4^-$ by paper-methanol chromatography. The two preparations that contain normal HSA (Squibb and NEN) show lower labeling efficiencies than the other products when subjected to the filter-disc and USP centrifuge assay procedures. Nevertheless, they still meet the USP limit of not more than 10% supernatant activity.

The results of assays performed on the heat-treated preparations are shown in Table 3. Centrifugation of Tc-99m MAA under USP conditions leaves $^{99m}TcO_4^-$ and Tc-99m Sn colloid largely in the supernatant. If the preparation contains normal serum albumin as part of its formulation, Tc-99m HSA will also be present in the supernatant (*1*). Centrifugation of Tc-99m MAA containing normal HSA in the presence of 10% TCA will pellet both labeled particles and Tc-99m HSA (*2,3*). Some Tc-99m Sn colloid is probably occluded in the precipitate, and some of the label is probably dissociated from the protein by TCA treatment. For the compromised kit containing normal HSA, analysis filter disc and USP centrifuge assay indicates that a substantial portion of the radioactivity was dissociated from the particles (Table 3) as $^{99m}TcO_4^-$, Tc-99m Sn colloid, and Tc-99m HSA. The TCA centrifuge assay (*2*) indicates that only a small amount of radioactivity (as $^{99m}TcO_4^-$ and Tc-99m Sn colloid) was left in the supernatant, and therefore a substantial proportion of the radioactivity in the supernatant from the USP centrifuge assay would have been associated with a soluble protein.

The kit that did not contain normal HSA gave paper-methanol chromatograms indicating the presence of high levels of $^{99m}TcO_4^-$. This situation, however, was not indicated by the Whatman 542, USP centrifuge assay, or by chromatography with HSA-loaded paper and saline (*7*), all of which indicated that little, if any, $^{99m}TcO_4^-$ was present. The preparation was subjected to a mouse bioassay (*10*) and the results from four mice, killed 10 min after injection, indicated that >95% of the radioactivity was present in the lungs, whereas the sum of radioactivity in the kidneys, liver, and gut was <3%. The bioassay confirmed the radiochemical assays, which indicated that the labeling efficiency of the preparation remained intact. It was not the intention of this study to analyze the effect of extremely adverse storage conditions on the labeling efficiency of MAA kits; rather it was to determine whether the assay procedures could detect any change in labeling efficiency that was produced by the adverse treatment. The inability of paper-methanol chromatography to confirm the results given by the other radiochemical test indicate that this method may lack sensitivity and may not be a reliable index of labeling efficiency.

The correlation coefficient for the Whatman 542 filter-disc and the USP centrifuge assay procedures was (+) 0.993, indicating that these methods measure the same group of radiochemical impurities and that a change in labeling efficiency signaled by one of these procedures would invariably be reflected by the other. The correlation coefficient for Whatman 542 and paper-methanol procedure was (−) 0.08. At $p = 0.01$ the (+) 0.993 is a significant correlation.

The analysis of variance of Table 2 results indicates that variation reflects significant effects related both to the company and the assay procedure. The difference between procedures 1 + 3 and 4 (Table 2) accounts for most of the significant assay effect.

The Whatman 542 and GF/C filter discs gave essentially the same results, indicating that very little reduced Tc-99m is present in any of the kits. Therefore, 542 or GF/C discs could be used for this rapid assay, but because reduced Tc-99m shows nonspecific adsorption to the GF/C discs it is recommended that Whatman 542 filters be used for maximum assay sensitivity.

FOOTNOTES

* Millipore XX10-02500, Millipore Corp., Bedford, Mass.
† GF/C Whatman, or Gelman, Gelman Instrument Co., Ann Arbor, Mich.
‡ NEN kit, North Billerica, Mass.

REFERENCES

1. DARTE L, PERSSON BR, SODERBOM L: Quality control and testing of ^{99m}Tc-macroaggregated albumin. *Nucl Med (Stuttg)* 15: 80–85, 1976
2. MCLEAN JR, WISE P: Impurities in a ^{99m}Tc lung imaging kit. *J Nucl Med Tech* 5: 28–31, 1977
3. MCLEAN JR, WELSH WJ: Radiochemical impurities in Tc-MAA preparations. *Nucl Med (Stuttg)* 16: 143, 1977
4. USP (XIX), USP Convention Inc., Rockville, Md, 1975, p 488
5. USP (XIX), USP Convention Inc., Rockville, Md. 1975, pp 488, 636–637
6. THOMAS J, WIENER SN: ^{99m}Tc- or ^{113m}In-labeled stannous hydroxide as a potential multipurpose scanning agent. *J Nucl Med* 12: 467–468, 1971 (Abst)
7. LIN MS, KRUSE SL, GOODWIN DA, et al: Albumin-loading effect: A pitfall in saline paper analysis of ^{99m}Tc-albumin. *J Nucl Med* 15: 1018–1020, 1974
8. ISPEN J, FEIGL P: *Bancroft's Introduction to Biostatistics*, New York, Harper and Row, 1970, pp 88–95
9. GUENTHER WC: *Analysis of Variance*, New Jersey, Prentice-Hall, 1964, pp 99–136
10. USP (XIX), 1975, pp 488–489