An Improved Technique for Reducing the Number of Particles in a Technetium-99m Macroaggregated Albumin Injection

Denise M. Bolstad, Timothy B. Valley, Mark E. Wilson, and Joseph C. Hung

Nuclear Medicine, Department of Diagnostic Radiology, Mayo Clinic, Rochester, Minnesota

For patients with right-to-left cardiac shunts and pediatric lung scan patients who will receive technetium-99m macroaggregated albumin (^{99m}Tc-MAA), it is necessary to reduce the number of MAA particles given. A previous method for reducing the number of ^{99m}Tc-MAA particles limits the injected volume to 0.1 ml, which is too small for any practical use. In addition, if the volume injected is increased, the excessive dilution of the stannous chloride results in a poor labeling. By initially incubating a small volume of sodium [^{99m}Tc]pertechnetate in a predivided MAA vial, our proposed method is able not only to achieve a greater binding efficiency, but also a more practical injected volume (0.5–1.0 ml) compared to the previous method. The ^{99m}Tc-MAA particle size, number, and stability over time were all within an acceptable range.

For patients with right-to-left cardiac shunts and pediatric lung scan patients who will receive technetium-99m macroaggregated albumin (^{99m}Tc-MAA), it is optimal to reduce the number of MAA particles given (*1*). In shunt patients, some MAA particles can bypass the lungs and may localize in the cerebral or renal capillaries (*2,3*). The average adult has 600 $\times$ 10^6 alveoli (*4*), 200–300 $\times$ 10^6 precapillary arterioles, and 280 $\times$ 10^9 capillaries (*5*). There is an approximate 2:1 ratio between alveoli and precapillary arterioles (*4,5*). Since children of age four have ~257 $\times$ 10^6 alveoli (*4*) and newborns have 20 $\times$ 10^6 alveoli (*4*), 4-yr olds should have ~125 $\times$ 10^6 precapillary arterioles and newborns should have ~10 $\times$ 10^6 precapillary arterioles. Since children have considerably less capillaries and arterioles in which particles may localize, it is necessary to reduce the number of particles given, especially to those under the age of three (*6*).

The concept for reducing the number of MAA particles by splitting a single MAA cold kit was originally proposed by Davis and Taube in 1979 (*7*). Later, Levine et al. introduced a more detailed method for reducing the number of particles contained in an MAA kit (*8*). The reduction in the number of MAA particles is achieved by labeling the portion of solution containing the lesser number of particles from the prediluted MAA cold kit. The number of mCi and the volume of sodium [^{99m}Tc]pertechnetate can easily be calculated using two simple proportional formulas proposed by Levine et al. (*7*).

However, the primary disadvantage of using this method is that the volume to be injected is limited to 0.1 ml, which is too small for any practical use. According to the methodology described by Levine et al. (*7*), if the injected volume is increased to a more acceptable amount (e.g., 0.5–1.0 ml) and the ^{99m}Tc-MAA dose is not increased, this would require that the final volume of solution in the kit be further increased in order to yield the same number of ^{99m}Tc-MAA particles as in the recommended 0.1-ml volume dose (*7*). Consequently, the tin (II) in the predivided MAA kit would be diluted to a level that may not be sufficient to reduce the sodium [^{99m}Tc] pertechnetate and still allow for a good labeling efficiency (LE $\geq$ 90%). Since the vendor of the kit was not specified (*8*), this speculation cannot be verified.

To avoid any dilution effect on the LE of a ^{99m}Tc-MAA preparation, we used a different approach. We allowed the prediluted MAA kit to be reconstituted with a smaller volume (e.g., 0.5 or 1 ml) of sodium [^{99m}Tc]pertechnetate so that a more concentrated Sn^{2+} was available, thus assuring a complete binding. Once the LE of ^{99m}Tc-MAA reaches an acceptable limit (≥90%), 0.9% NaCl solution can be added to the predivided MAA vial to bring the final solution to a desirable volume.

In order to attain images with a uniform distribution of activity in the lungs, at least 60,000 ^{99m}Tc-MAA particles should be injected, if the lungs are mature (*9*). We have chosen 100,000 particles/1.0 ml as the target dose in our experiment for adult shunt patients. By using 100,000 particles, even when there is a 40% shunt (a right-to-left shunt of greater than 39% is considered to be large), there will still be an adequate number of particles to obtain a uniform image that should be statistically satisfactory. It has been recommended that the number of particles of ^{99m}Tc-MAA for use in a newborn should not exceed 50,000, or 165,000 in children up to 1-yr old (*6,10*). We have chosen to use 40,000 particles/0.5-ml dose in our experiment as an example for the pediatric patient.

For reprints contact: Denise M. Bolstad, CNMT, Nuclear Medicine, Department of Diagnostic Radiology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905.

The primary goal of this experiment was to reduce the number of particles contained in a workable injected volume of ^{99m}Tc-MAA, i.e., ~40,000 particles/0.5 ml per 0.5-mCi (18.5-MBq) dose for a pediatric patient and ~100,000 particles/1.0 ml per 4.0-mCi (148-MBq) dose for a shunt patient. The radiochemical purity (RCP), particle size, and labeled particle stability of ^{99m}Tc-MAA over time were also evaluated.

MATERIALS AND METHODS

A MAA commercial kit was used for this study (Techne-Scan® MAA, Mallinckrodt Medical, Inc., St. Louis, MO), containing 2 mg of aggregated human albumin, 0.5 mg of human albumin, 120 μg of stannous chloride ($SnCl_2 \cdot 2H_2O$), 80 mg of lactose, 24 mg of succinic acid, and 1.4 mg of sodium acetate. Each vial contained ~8 ± 4 × 10^6 MAA particles (*11*).

An initial reduction of MAA particles was accomplished by adding 5 ml of 0.9% sodium chloride injection, USP to the MAA kit, withdrawing 1 ml, and adding it to a 30-ml sterile empty vial. This reduced the number of MAA particles to ~1.6 × 10^6.

The final total volume in the 30-ml vial was calculated using the following proportional formula.

$$V_1 = \frac{N_1 \times V_2}{N_2} \qquad \text{Eq. 1}$$

V_1 equals the total final volume (ml) of solution in the vial, V_2 equals the volume (ml) of solution per unit dose, N_1 equals the total number of particles in the vial, and N_2 equals the number of particles in a unit dose.

The total activity, A, of sodium [^{99m}Tc]pertechnetate to be added to the vial was determined by multiplying the desired specific concentration of ^{99m}Tc-MAA, B, contained in a unit dose syringe by the total volume of solution in the vial, V_1.

$$A = B\ (\text{mCi/ml}) \times V_1 \qquad \text{Eq. 2}$$

The calculated total activity of sodium [^{99m}Tc]pertechnetate in either 0.5 ml or 1 ml was then added to the 30-ml vial.

RCP was performed 5-min postreconstitution, using Whatman 31ET CHR paper (Whatman International, Ltd., Maidstone, UK), with acetone as the developing solvent. RCP determinations were performed every 5 min thereafter until results of ≥90% LE were achieved. Physiological saline (0.9% sodium chloride injection, USP) was then added to the ^{99m}Tc-MAA solution to achieve the total final volume.

RCP was then performed at 5-min and 1-hr postdilution. The number and size of ^{99m}Tc-MAA particles were also checked using a light microscope (Model CHT, Olympus Corporation, Lake Success, NY) and a hemacytometer counting chamber (Fisher Scientific, Pittsburgh, PA) (Fig. 1). No less than 90% of the observed aggregated particles must have a diameter between 10 and 90 μm, and none of the observed particles may have a diameter >150 μm (*12*).

The following equation was used to calculate the total number of injected ^{99m}Tc-MAA particles

$$N_2 = \frac{N_3}{V_3} \times \frac{1{,}000\ \text{mm}^3}{1\ \text{ml}} \times V_2 \qquad \text{Eq. 3}$$

N_3 equals the number of particles observed and V_3 equals the volume (ml) under the central square of the hemacytometer. To determine the V_3, a Vernier caliper (Model No. 579-1, Brown and Sharp, Kingston, RI) was used to measure the distance between the ruled surface of the hemacytometer and a cover glass. The measured distance was determined to be 0.10 ± 0.02 mm (n = 10), therefore, the V_3 was calculated using this distance, multiplied by the central square area (Fig. 1).

Pediatric Portion

Our goal was to obtain a pediatric unit-dose of 0.5 mCi (18.5 MBq) of ^{99m}Tc-MAA in 0.5 ml, containing ~40,000 particles. After the initial particle reduction, there were ~1.6 × 10^6 particles. Assuming a 0.5-mCi (18.5-MBq) unit-dose, Equation 1 was used to calculate the final volume in the 30-ml vial

$$V_1 = \frac{1{,}600{,}000\ \text{particles} \times 0.5\ \text{ml}}{40{,}000\ \text{particles}} = 20\ \text{ml}$$

The total ^{99m}Tc activity added to the vial was determined by Equation 2.

$$A = \frac{0.5\ \text{mCi}}{0.5\ \text{ml}} \times 20\ \text{ml} = 20\ \text{mCi}\ (740\ \text{MBq})$$

Next, 20 mCi (740 MBq) of sodium [^{99m}Tc]pertechnetate in 0.5 ml was added. The vial contained 1.5 ml (1-ml diluted

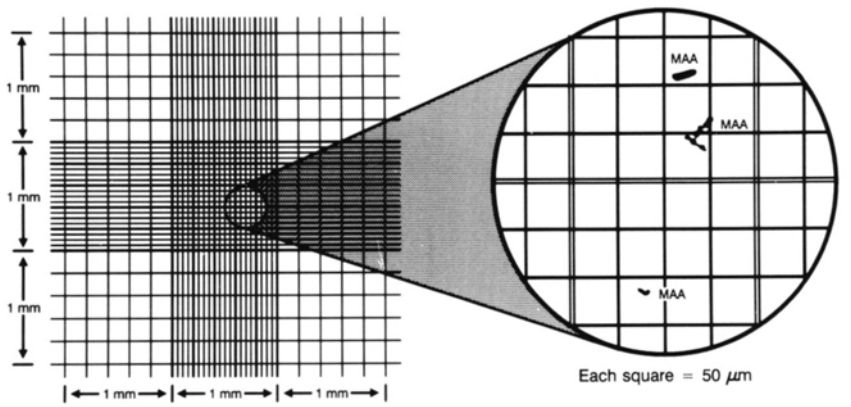

FIG. 1. Estimating particle size and number of a diluted ^{99m}Tc-MAA preparation with a hemacytometer grid.

MAA solution and 0.5-ml ^{99m}Tc) of diluted ^{99m}Tc-MAA solution. We then added 18.5 ml of 0.9% sodium chloride injection, USP, bringing the total volume to 20 ml.

Shunt Portion

Our goal was to obtain an adult shunt dose of 4 mCi (148 MBq) of ^{99m}Tc-MAA in 1 ml, containing ~100,000 particles. After the initial reduction of particles, there were ~1.6×10^6 particles. Assuming a 4-mCi (148 MBq) unit-dose, Equation 1 was used to calculate the final volume in the 30-ml vial

$$V_1 = \frac{1{,}600{,}000 \text{ particles} \times 1 \text{ ml}}{100{,}000 \text{ particles}} = 16 \text{ ml}$$

The total sodium [^{99m}Tc]pertechnetate activity added to the vial was determined by Equation 2.

$$A = \frac{4 \text{ mCi}}{1 \text{ ml}} \times 16 \text{ ml} = 64 \text{ mCi (2,368 MBq)}$$

Next, 64 mCi (2.368 MBq) of sodium [^{99m}Tc]pertechnetate in 1 ml was added. The vial contained 2 ml (1-ml diluted MAA solution and 1-ml ^{99m}Tc) of diluted ^{99m}Tc-MAA solution. We then added 14 ml of 0.9% sodium chloride injection, USP, bringing the total volume to 16 ml.

RESULTS

These procedures were used with nine kits (n = 20) for the pediatric portion and with eight kits (n = 16) for the shunt portion. As indicated in Tables 1 and 2, both groups of predivided ^{99m}Tc-MAA kits achieved acceptable LEs within 10 min after reconstitution with sodium [^{99m}Tc]pertechnetate. The average LE at 5-min postdilution was 97.8 ± 1.7% for shunt kits and 97.4 ± 2.4% for pediatric kits (Tables 1 and 2). The LEs of these two groups of kits maintained similar levels over 1 hr.

TABLE 1. Labeling Efficiencies of the ^{99m}Tc-MAA Shunt Kits

Incubation Time	LE (%)	Postdilution Time	LE (%)
5 min	91.3 ± 6.4	5 min	97.8 ± 1.7
10 min	96.3 ± 3.4	60 min	97.9 ± 1.5

n = 16.

TABLE 2. Labeling Efficiencies of the ^{99m}Tc-MAA Pediatric Kits

Incubation Time	LE (%)	Postdilution Time	LE (%)
5 min	89.9 ± 8.8	5 min	97.4 ± 2.4
10 min	95.6 ± 4.1	60 min	97.8 ± 2.1

n = 20.

The average number of ^{99m}Tc-MAA particles that we obtained for the pediatric portion of the experiment was 48,500 ± 8.217 particles (n = 20), with a range in particle size of 10–90 μm. For the shunt portion, the average particle number obtained was 103,750 ± 10,247 particles (n = 16), with a range in particle size of 10–70 μm.

DISCUSSION

The main objective of this work is to provide a feasible methodology for ^{99m}Tc-MAA particle reduction so that a safe and effective lung perfusion study can be offered to neonates, young children, and patients with right-to-left cardiac shunts. There is a concern about the administration of the ^{99m}Tc-MAA particles to patients with pulmonary hypertension (*1, 13–15*) because the pulmonary vessels of some of these patients are already attenuated, in addition to the fact that they have fewer vessels. Although there have been incidents of death in these patients with the administration of Tc-MAA, it is a very rare occurrence, and this issue should be addressed only in cases of severe primary pulmonary hypertension. In any event, patients with pulmonary hypertension should also receive a lower number of ^{99m}Tc-MAA particles.

Our method of ^{99m}Tc-MAA particle reduction involves adding a small volume (0.5–1.0 ml) of sodium [^{99m}Tc]pertechnetate to a prediluted 1.0 ml Mallinckrodt TechneScan MAA vial, incubating for 10 min, and then diluting the ^{99m}Tc-MAA to the desired volume, once ≥90% LE has been achieved. This method allows us to give ~40,000 particles to pediatric patients and ~100,000 particles to shunt patients. We are also able to increase the injected amount of ^{99m}Tc-MAA to a more practical volume of 0.5 ml for pediatric patients and 1.0 ml for shunt patients.

The specific concentration of sodium [^{99m}Tc]pertechnetate that we used (64 mCi/ml; 2,368 MBq/ml) for the shunt portion should be workable with most molybdenum-99 (^{99}Mo)/^{99m}Tc generators used in nuclear medicine laboratories. Ideally, the volume of sodium [^{99m}Tc]pertechnetate should be kept as low as possible to enhance the LE of ^{99m}Tc-MAA, since the level of tin has been reduced in the divided MAA kit.

In this experiment, only one type of commercial MAA kit was tested (TechneScan MAA). Our results are valid only for this particular kit. For other commercially available MAA kits (Table 3), similar calculations and evaluation studies must be performed based upon the kit's specifications.

It would be ideal to select an MAA kit which has a narrow range for number of particles, as this would allow for a more consistent number of particles in the final patient dose. In addition, the LE would be greatly enhanced by selecting the kit that has the largest tin content. It is hypothesized that the Pulmolite* (E. I. du Pont de Nemours & Co., Billerica, MA) kit may give the most consistent results because the particle number range ($3.6–6.5 \times 10^6$ particles) is the smallest among the kits presented. However, if the Pulmolite kit were selected, the actual tin content (20–120 μg) might not be sufficient to allow for good binding using our proposed method. Other

TABLE 3. Comparison of Commercially Available MAA Kits

Vendor	Particle Number	Tin Content
CIS-US	$12–15.2 \times 10^6$	95–210 μg
E. I. du Pont	$3.6–6.5 \times 10^6$	20–120 μg
Mallinckrodt	$8 \pm 4 \times 10^6$	120 μg
Medi-Physics	$4–8 \times 10^6$	60–110 μg
Squibb	$2–7 \times 10^6$	70–190 μg

commercially available MAA kits (Table 3) have a wide range in tin content, which may yield some inconsistent results.

For all commercial kits, it is optimal to count the number of particles before reconstitution with sodium [^{99m}Tc]pertechnetate since in many commercial MAA kits, there is a wide range in number of particles (Table 3). However, if the time is taken to count the particles before the addition of sodium [^{99m}Tc]pertechnetate to the MAA kit, there is a large chance that stannous chloride could be oxidized by the dissolved oxygen in physiological saline. To avoid the potential problem of oxidation, one may use physiological saline purged with nitrogen or use low dissolved oxygen saline.

Since the number of particles varies between kits and different vendors, it is very important to perform the appropriate quality control. Counting the number of particles is especially important. We recommend that quality control testing (determination of RCP, particle size, and number of particles) be performed on every ^{99m}Tc-MAA preparation for particle reduction purposes. Methanol is recommended as the solvent of choice for ^{99m}Tc-MAA paper chromatography (*12*); however, this method requires a time period in excess of 30 min. Using acetone, we achieved similar results within 5 min. We performed quality control for only 1 hr because with the low number of pediatric and shunt patient studies we perform, the kit would most likely be used within 1 hr of preparation. If a kit were to be used beyond 1 hr from preparation time additional quality control testing would have to be performed to assure that the shelf life of ^{99m}Tc-MAA could be extended.

This proposed method deviates from the preparation procedures stated in the MAA package insert (*11*). However, because it is being changed in order to reduce the medical risk to some patients due to their medical condition, this deviation may be justified under the interim final rule issued by the Nuclear Regulatory Commission (NRC). The NRC interim final rule, however, requires that the written directive and record of the number of patient administrations performed under this departure be retained for a period of 5 yr (*20*).

In conclusion, by initially incubating a small volume of sodium [^{99m}Tc]pertechnetate with the reduced level of tin in a predivided MAA vial, we were able not only to achieve greater LE, but also to achieve a more practical volume and number of particles of ^{99m}Tc-MAA for administration to shunt and pediatric patients.

ACKNOWLEDGMENTS

The authors would like to thank Ms Vicki S. Krage and Mrs. Rose M. Busta for their assistance in preparing this manuscript. We are grateful to Heinz W. Wahner, MD and Brian P. Mullan, MD for their assistance in locating clinical information used in this paper.

This study was presented, in part, at the 39th Annual Meeting of The Society of Nuclear Medicine in Los Angeles, California on June 10, 1992.

REFERENCES

1. Kowalsky RJ. Safety and effectiveness considerations with particulate lung scanning agents. *J Nucl Med Technol* 1982;10:223–227.
2. Larcos CD. Shunts in and over the lungs. *Respiration* 1972;29:24–39.
3. Verzijlbergen F, van Tellingen C, Plokker HWM. Significance of the site of injection in unexpected right-to-left shunting. *J Nucl Med* 1984;25: 1103–1105.
4. Thurlbeck WM, Angus GE. Growth and aging of the normal human lung. *Chest* 1975;67:3S–7S.
5. Weibel ER. *Morphometry of the human lung.* Berlin: Springer-Verlag; 1963: 86.
6. Heyman S. Toxicity and safety factors associated with lung perfusion studies with radiolabeled particles. (Letter.) *J Nucl Med* 1979;20: 1098–1099.
7. Davis MA, Taube RA. (Reply to Letter.) *J Nucl Med* 1979;20:1099.
8. Levine EK, Perritt JS, Gordon L. Particle reduction of a macroaggregated albumin kit: simplified calculations. *J Nucl Med Technol* 1989;17: 143–144.
9. Heck LL, Duley JW. Statistical considerations in lung imaging with ^{99m}Tc albumin particles. *Radiology* 1974;113:675–679.
10. Treves ST, Harris GBC. Lung. In: Treves ST, ed. *Pediatric nuclear medicine.* New York: Springer-Verlag; 1985:289–330.
11. TechneScan® MAA package insert, R1/91. St. Louis, MO: Mallinckrodt Medical, Inc.; 1991.
12. Technetium Tc 99m albumin aggregated injection. *U.S. pharmacopeia,* XXII. Rockville, MD: United States Pharmacopeial Convention, Inc.; 1990:1313–1314.
13. Vincent WR, Goldberg SJ, Desilets D. Fatality immediately following rapid infusion of macroaggregates of ^{99m}Tc albumin (MAA) for lung scan. *Radiology* 1968;91:1180–1184.
14. Williams JO. Death following injection of lung scanning agent in a case of pulmonary hypertension. *Br J Radiol* 1974;47:61–63.
15. Powe JE, Palevsky HI, McCarty KE, Alavi A. Pulmonary arterial hypertension: Value of perfusion scintigraphy. *Radiology* 1987;164:727–730.
16. AN-MAA® package insert. Bedford, MA: CIS-US, Inc.; December 1988.
17. Pulmolite® package insert. Billerica, MA: E.I. du Pont de Nemours & Co.; October 1989.
18. MPI MAA kit package insert. Arlington Heights, IL: Medi-Physics, Inc.; October 1990.
19. Macrotec® package insert. Princeton, NJ: Squibb Diagnostics; 1989.
20. Authorization to prepare radiopharmaceutical reagent kits and elute radiopharmaceutical generators; use of radiopharmaceuticals for therapy. *Federal Register* 1990;55:34513–34518.