# Quality Control of Technetium-99m DTPA: Correlation of Analytic Tests with In Vivo Protein Binding in Man

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When [$^{99m}$Tc]DTPA is administered, a small fraction of the activity (presumably an impurity) is bound to plasma proteins. This causes an error in the calculation of glomerular filtration rate from plasma clearance. This paper presents two methods of laboratory quality control for measuring the fraction that binds to plasma proteins. One method involves in vitro binding to human serum albumin followed by gel filtration. The other method involves descending paper chromatography on wet pre-equilibrated anion exchange paper. In a series of 80 patients, correlation was demonstrated between laboratory characteristics and actual clinical performance of the [$^{99m}$Tc]DTPA preparation. Both laboratory methods appear suitable for routine quality control.

J Nucl Med 27:560–562, 1986

When technetium-99m diethylenetriaminepentaacetic acid ([$^{99m}$Tc]DTPA) is administered intravenously, a small fraction of the activity is bound to plasma proteins (*1,2*). The protein-bound fraction is thought to represent an impurity and not true [$^{99m}$Tc]DTPA. Since it remains in circulation while unbound activity is excreted, it can lead to significant errors in the calculation of glomerular filtration rate (GFR) from plasma clearance (*2,3*). Accurate GFR measurement is possible in the face of significant protein binding, if the binding is measured for each individual patient and the appropriate correction made; however, this is cumbersome. It would be better to have laboratory tests capable of predicting the extent of protein binding, in order to select lots of [$^{99m}$Tc]DTPA for which protein binding was within acceptable limits. Until now, no laboratory tests have been available that have the proven ability to predict protein binding in man. In this paper, we compare the results of two analytic quality control methods with protein binding in 80 patients. Both of the analytic methods were found to correlate with in vivo protein binding, and thus appear suitable for screening [$^{99m}$Tc]DTPA kits for GFR measurement.

Received June 26, 1985; revision accepted Nov. 7, 1985.

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## MATERIALS AND METHODS

Plasma samples were obtained from 80 patients who were undergoing [$^{99m}$Tc]DTPA renal function studies for routine clinical indications. The GFR was estimated from the activity in a single plasma sample obtained 3 hr after i.v. administration (*4*). The sample was collected in a standard ethylenediaminetetraacetic acid-containing vacuum tube and the protein-bound fraction was measured using two methods, ultrafiltration and gel filtration, as described elsewhere (*2*). In brief, for ultrafiltration, the plasma sample was passed through a membrane filter that retained plasma proteins, and the activity in the protein-free ultra-filtrate was compared with that in the original plasma. For gel filtration, the sample was applied to a conventional dextran gel size-exclusion column, and the activity in the high-molecular-weight fraction that eluted at the void volume was compared with the activity in the original plasma.

To compare different formulations of [$^{99m}$Tc]DTPA, four different commercial formulations were employed. For three of these, kits from at least two different lot numbers were evaluated. Each lot was subjected to two different in vitro quality control tests, one using ion-exchange paper chromatography (*2*) and the other using gel filtration after mixing with human serum albumin. The method for in vitro measurement of binding to human serum albumin was as follows. One microliter of the dose was added to 1 ml of a solution containing 1% human serum albumin, 0.15*M* NaCl, 0.002*M* $NaH_2PO_4$, 0.002% chlorhexidene as bacteriostat, with pH adjusted to 7.4 with NaOH. This was allowed to stand for 15 min at room temperature and then the bound fraction was

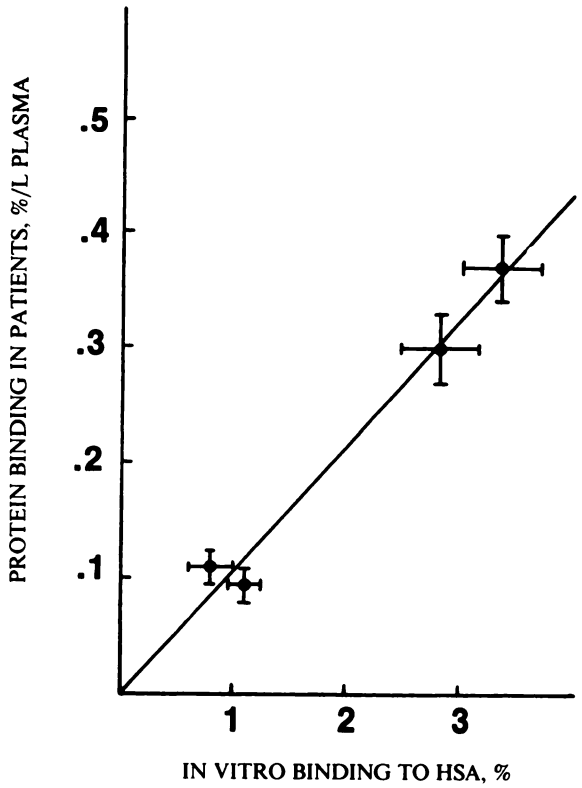


**FIGURE 1**
Correlation between in vivo and in vitro binding measurements, mean ± s.e.e. for four different formulations

determined by gel filtration, using the method described for plasma samples (*2*).

The commercial [$^{99m}$Tc]DTPA kits were formulated as follows.

A. 20.6 mg $CaNa_3DTPA$, 0.21 mg $SnCl_2$, reconstituted to 1–8 ml.
B. 5 mg "sodium salt of DTPA" 0.25 mg $SnCl_2$, reconstituted to 2–8 ml.
C. 10 mg $CaNa_3DTPA$, 0.50 mg $SnCl_2$, reconstituted to no more than 5 ml.
D. 3.0 mg $CaNa_3DTPA$, 0.15 mg $SnCl_2$, reconstituted to 1.3 ml as unit dose.

All measurements were made in duplicate and averaged, using at least nine vials from each lot.

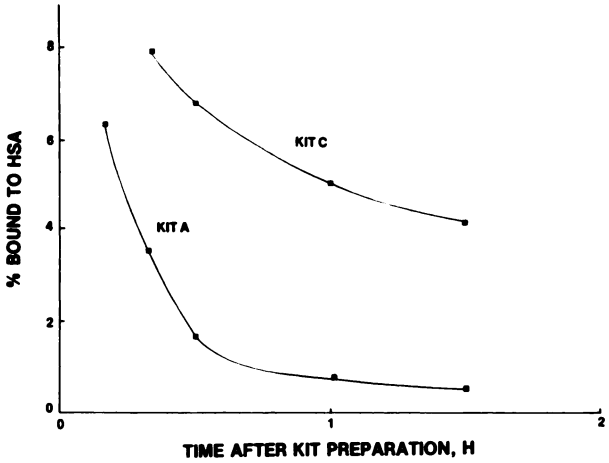


**FIGURE 2**
Change in [$^{99m}$Tc]DTPA kits with time after preparation

## RESULTS

The two different analytic tests agreed with each other and with the in vivo protein binding. This is presented graphically in Fig. 1 for one of the analytic tests. The numerical results for both analytic tests are presented in Table 1.

Kits with two separate lot numbers were tested for formulations A through C, but no statistically significant lot-to-lot variation was found. Technetium generators from three different manufacturers were used, also without significant differences. However, preliminary data showed that the time interval between compounding and use was important (Fig. 2). This variable was not studied in detail. Instead, its effect was limited by administering the dose and beginning analytic measurements at the fixed time of 30–60 min. All measurements in Table 1 and Fig. 1 were made in this manner.

## DISCUSSION

Because the fraction of [$^{99m}$Tc]DTPA that remains immobile on anion exchange paper agrees quantitatively with the fraction that binds to human serum albumin in vitro (Table 1), we shall assume for the present that these two tests measure the same impurity

**TABLE 1**
Comparison of In Vitro with In Vivo Measurements for Four Different [$^{99m}$Tc]DTPA Formulations*

| | In vitro | | In vivo | |
|---|---|---|---|---|
| Formulation | HSA (% bound) | Ion exchange (% at origin) | Gel filtration (% bound/ml) | Membrane filtration (% bound/ml) |
| A | 0.79 ± 0.19 | 0.65 ± 0.08 | 0.111 ± 0.015 | 0.095 ± 0.026 |
| B | 1.09 ± 0.17 | 1.01 ± 0.19 | 0.093 ± 0.015 | 0.078 ± 0.030 |
| C | 3.3 ± 0.3 | 4.4 ± 0.5 | 0.37 ± 0.03 | 0.41 ± 0.04 |
| D | 2.8 ± 0.4 | 3.5 ± 0.4 | 0.30 ± 0.03 | 0.31 ± 0.05 |

* Mean ± s.e.e. for N samples, where N = 26 formulation A, 19 for B, 21 for C, and 9 for D.

or combination of impurities. The apparent volume of distribution of this impurity is 11.7 l, calculated from the 3-hr plasma samples (dividing administered dose of impurity by the bound plasma activity per unit volume). This volume is substantially larger than plasma volume, so that the complex with plasma proteins must either penetrate the extravascular space or else slowly dissociate. Attributing protein binding to an impurity rather than to [$^{99m}$Tc]DTPA itself (*2,5,6*) explains why binding depends so strongly on such details of preparation as the source of the kits and the time interval after compounding.

What level of purity is required for use in GFR measurement? Suppose that GFR is to be estimated from a single 3-hr plasma sample. Using the observed 3-hr distribution volume of 11.7 l for the bound fraction together with an equation that relates GFR to plasma activity (*4*) leads to the following error estimates: An impurity level of 1% of the dose will cause an error of 1.2 ml/min in the GFR when the GFR is 5 ml/min, an error of 1.9 ml/min when the GFR is 40 ml/min, and an error of 6.7 ml/min when the GFR is 150 ml/min. These errors, when compared with other errors inherent in the procedure (*4*), are negligible when GFR is low and acceptable when GFR is high. A 1% impurity level is therefore acceptable when GFR is to be measured from a single 3-hr plasma sample. Inspection of Table 1 shows that this standard can be met in practice.

We have demonstrated the validity of two methods of quality control. We suspect that some readers will prefer to draw inferences from our data instead of applying these methods in their own environment. Ideally, quality control measurements should be repeated for each separate lot of radiopharmaceutical and under the actual conditions of use.

## CONCLUSION

Two analytic methods have been presented that predict protein binding of [$^{99m}$Tc]DTPA. These can be used to screen preparations intended for GFR measurements. If GFR is to be estimated from a single 3-hr plasma sample, the impurity that binds to plasma proteins should not exceed 1% of the dose.

## ACKNOWLEDGMENTS

The authors thank Ms. Dorothea Ballard for help with this manuscript. This work was supported by the Birmingham Veterans Administration Medical Center, Birmingham, Al.

## REFERENCES

1. Klopper JF, Hauser W, Atkins HL, et al: Evaluation of $^{99m}$Tc-DTPA for the measurement of glomerular filtration rate. *J Nucl Med* 13:107–110, 1972
2. Russell CD, Bischoff PG, Rowell KL, et al: Quality control of Tc-99m-DTPA for measurement of glomerular filtration: Concise communication. *J Nucl Med* 24:722–727, 1983
3. Carlsen JE, Moller ML, Lund JO, et al: Comparison of four commercial Tc-99m (Sn) DTPA preparations used for the measurement of glomerular filtration rate: Concise communication. *J Nucl Med* 21:126–129, 1980
4. Russell CD, Bischoff PG, Kontzen FN, et al: Measurement of GFR: Single injection plasma clearance method without urine collection. *J Nucl Med* 26:1243–1247, 1985
5. Kempi V, Persson RBR: Tc-99m-DTPA(Sn)—dry-kit preparation. Quality control and clearance studies. *Nucl Med* (Stuttgart) 13:387–388, 1974
6. McAfee JG, Gagne G, Atkins HL, et al: Biological distribution and excretion of DTPA labeled with Tc-99m and In-111. *J Nucl Med* 20:1273–1278, 1979