

# Effects of Contaminant Radioactivity on Results of $^{125}\text{I}$ -Radioligand Assay

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Diagnostic radionuclide imaging procedures are often used in patients whose sera are later assayed by use of  $^{125}\text{I}$ -labeled radioligands; thus, it is important to identify those assays that potentially may be affected by contaminating radioactivity. Results obtained with assays in which specific separation methods (solid-phase primary antibody, second antibody) or small (10–25  $\mu\text{L}$ ) sample volumes are used are little affected by the presence of such contaminating radioactivity. Less-specific techniques (polyethylene glycol, charcoal, ion-exchange resin) segregate some of the contaminant activity into the bound fraction. The degree to which such activity is protein bound and the concentration of endogenous ligand then contribute to the resulting error in dose estimation. Samples for these assays should be screened for radioactivity before the assay is begun. Inclusion of nonspecific binding tubes for patients' samples when contamination is present permits the contaminating radioactivity to be evaluated and the patient's dose concentration to be more accurately estimated.

Contamination of patients' samples with radioactivity administered for diagnostic studies constitutes a potential source of error in radioassay procedures. The magnitude of this problem is determined by the amount of contaminant activity present relative to the assay total activity and its distribution into the antibody bound and free fractions.

Errors have been described in assays for vitamin B<sub>12</sub> and folate (1) and DNA (2). We have investigated the effects of contaminant [ $^{99\text{m}}\text{Tc}$ ]pertechnetate,  $^{99\text{m}}\text{Tc}$ -labeled albumin, and  $^{67}\text{Ga}$  citrate on radioligand assays in which  $^{125}\text{I}$  is used as the label. Six commonly used separation methods were examined.

## Methods

To determine the magnitude of contaminant activity, we obtained blood from five patients 15 to 30 min after they received 20 mCi of [ $^{99\text{m}}\text{Tc}$ ]pertechnetate or pyrophosphate intravenously for brain or myocardial scans, and from five other patients 15 to 30 min after they received 3 mCi of  $^{67}\text{Ga}$  citrate intravenously for diagnostic studies. The counting rates in a 15- to 75-keV window were measured and were found to be substantially affected by system efficiency (Table 1). To allow convenient study of contaminated samples, we added [ $^{99\text{m}}\text{Tc}$ ]pertechnetate,  $^{99\text{m}}\text{Tc}$ -labeled albumin, or  $^{67}\text{Ga}$  citrate to serum pools such that the counting rates approximated those found in patients' samples, and incubated the pools for 1 h at room temperature before beginning an assay. Because of the small volume of sample used in assays involving am-

monium sulfate, 10-fold more radioactive contaminant was added to the serum pools used in the thyroxine assay to determine percent distribution between bound and free fractions.

Table 2 shows the separation techniques studied and the assays chosen as representative of those separation techniques.

With the following exceptions, all assays were performed according to the manufacturer's instructions. A choriogonadotropin-free serum pool was substituted for the manufacturer's standard diluent. Radioactivity was measured in the bound and free fractions of all assays. Aliquots of the serum pools were assayed in duplicate. Apparent nonspecific binding (specific antibody omitted and replaced with an equal volume of assay buffer) was measured for each replicate, except in the solid-phase primary antibody assay. All dose estimations were based on the bound fraction counts.

## Results

Table 3 shows the effects on unknown dose estimations when contaminating radioactivity was deliberately added. Almost all added radioactivity was segregated into the free fraction by both solid-phase primary-antibody and second-antibody methods, and results for these two assays are therefore not shown. Dose estimates based on the bound-fraction counts were not interfered with by contaminating radioactivity for these two separation methods.

In all assays, the added activity found in the bound fraction was quantitatively similar to that appearing in the nonspecific binding tubes. Quantitation of the nonspecifically bound

**Table 1. Characteristics of Counting Rates Observed in Various Scintillation Counters for a Single Patient's Serum Containing Either  $^{67}\text{Ga}$  or  $^{99\text{m}}\text{Tc}$**

Instrument	Detector size	$^{99\text{m}}\text{Tc}$	
		$^{99\text{m}}\text{Tc}$	$^{67}\text{Ga}$
		cpm/100 $\mu\text{L}$	
Nuclear Medical Lab., Inc., Auto In-V-Tron 4000, Model 4003A	4.445 cm diam. X 5.08 cm deep 3.81 cm well	11 000	4 100
Packard Instrument Co., Inc., Model 5160	7.62 cm diam X 7.62 cm deep through hole	29 000	3 900
Packard Instrument Co., Inc., Model 5110	7.62 cm diam. through hole	17 500	2 400
Packard Instrument Co., Inc., Dual-channel Model 511	Dual 7.62 cm diam. detector with center hole	29 000	20 100

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**Table 2. Separation Techniques and Assays Studied**

Technique	Analyte	Manufacturer
Solid-phase primary antibody (glass bead)	triiodothyronine	Corning Medical Diagnostics
Second antibody (solid phase)	cortisol	Beckman Instruments, Inc.
Ammonium sulfate	thyroxine	Nuclear Medical Lab., Inc.
Ion-exchange resin (Amberlite)	gastrin	Becton, Dickinson and Co.
Charcoal (pellet)	digoxin	Nuclear Medical Lab., Inc.
Polyethylene glycol	choriogonadotropin	Serono Lab., Inc.

counts in a radioactivity-contaminated sample permitted quantitation of the spuriously increased counts in the bound fraction and correction of the dose estimate.

Figure 1 illustrates the effects of adding a constant amount of contaminating radioactivity to samples having different concentrations of endogenous ligand.

### Discussion

Because diagnostic nuclide-imaging and radioassay pro-

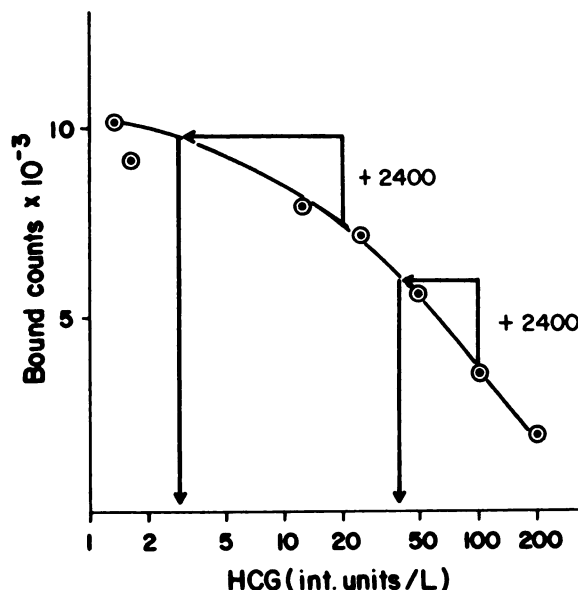


Fig. 1. Effect of adding 2400 cpm on dose estimation when 100 and 20 int. units of endogenous ligand was present per liter. In both samples, the dose was underestimated, the magnitude and significance of the error depending on the concentration of endogenous ligand.

cedures are frequently ordered for the same patient, substantial problems with radioactive contaminants in patients' sera can result.  $^{99m}\text{Tc}$  is by far the most widely used pharmaceutical label; it is rapidly cleared from the blood in most forms and its 6-h physical half-life decreases the amount of contamination that it produces. However, concurrent use of

**Table 3. Separation Methods and Assay Results**

	TA, <sup>a</sup> cpm	MB, <sup>b</sup> cpm (%TA)	Added acty., cpm (%TA)	Distrib. of added acty.		Nonspec. binding cpm	Bound	Dose estimate (from bound- fraction counts)
				%B <sup>c</sup>	%F <sup>d</sup>			
<i>Separation with ammonium sulfate</i>								
Thyroxine, 10- $\mu\text{L}$ sample								
Serum pool	108 400	66 800 (62)	—	—	—	10 000	35 200	$\mu\text{g/L}$ 84
Pool + $^{99m}\text{TcO}_4^-$			590 (5.5)	7 <sup>e</sup>	93	10 040	35 200	84
Pool + $^{67}\text{Ga}$			650 (6.0)	14 <sup>e</sup>	86	10 090	35 300	84
Pool + $^{99m}\text{Tc-Alb}$			490 (4.5)	14 <sup>e</sup>	86	10 070	35 300	84
<i>Separation on Amberlite ion-exchange resin</i>								
Gastrin, 100- $\mu\text{L}$ sample								
Serum pool	8 400	4 000 (48)	—	—	—	170	1 700	299
Pool + $^{99m}\text{TcO}_4^-$			5700 (68)	13	87	880	2 400	136
Pool + $^{67}\text{Ga}$			6700 (68)	30	70	2 200	3 800	17
Pool + $^{99m}\text{Tc-Alb}$			5400 (64)	82	18	4 600	6 100	>MB
<i>Separation on charcoal</i>								
Digoxin, 50- $\mu\text{L}$ sample								
Serum pool	24 800	17 000 (69)	—	—	—	1 440	9 850	2.1
Pool + $^{99m}\text{TcO}_4^-$			3900 (16)	43	57	2 840	11 500	1.6
Pool + $^{67}\text{Ga}$			3100 (13)	97	3	4 590	13 000	1.2
Pool + $^{99m}\text{Tc-Alb}$			3800 (15)	81	19	4 390	12 900	1.2
<i>Separation with polyethylene glycol</i>								
Choriogonadotropin, 100- $\mu\text{L}$ sample								
Serum pool	20 000	7 700 (39)	—	—	—	2 200	4 300	72
Pool + $^{99m}\text{TcO}_4^-$			4600 (23)	15	85	3 000	5 000	43
Pool + $^{67}\text{Ga}$			6300 (31)	12	88	3 300	5 000	41
Pool + $^{99m}\text{Tc-Alb}$			4400 (22)	22	78	3 600	5 300	34

<sup>a</sup> TA = total activity in an assay. <sup>b</sup> MB = zero standard or maximum binding. <sup>c</sup> %B = % in antibody-bound fraction. <sup>d</sup> %F = % in free fraction. <sup>e</sup> Based on adding 10-fold more radioactive contaminant to serum pool.

certain tests, such as functional cardiac blood pool imaging with  $^{99m}\text{Tc}$ -labeled human serum albumin or myocardial imaging with [ $^{99m}\text{Tc}$ ]pyrophosphate and the estimation of digoxin in serum by radioimmunoassay, illustrates that contamination with  $^{99m}\text{Tc}$  may be of more than theoretical interest.

Nuclides with longer biological and physical half-lives constitute a more serious problem. Substantial activity may persist in the serum for several days after  $^{67}\text{Ga}$  citrate is administered for diagnostic imaging procedures, or after  $^{125}\text{I}$ -labeled albumin is administered to measure plasma volume.

The amount of contaminating radioactivity detected in a radioassay procedure depends upon the amount of activity present in the specimen, the volume of the specimen taken for assay, and the time that has elapsed between specimen collection and measurement of sample radioactivity. The system efficiency of the counting instrument also is important (Table 1). Although a spectrometer window set to admit the primary and sum emissions of  $^{125}\text{I}$  (15 to 75 keV) excludes primary photopeaks of both  $^{99m}\text{Tc}$  and  $^{67}\text{Ga}$ , the number of scattered events detected results in substantial counting rates from either nuclide.

Separation of bound and free fractions by different methods resulted in various contaminations of the bound fraction. Ideally, the separation techniques used in radioligand assays would segregate only the antibody-bound ligand into the bound fraction. The separation techniques with the greatest specificity for the antibody-bound fraction were solid-phase primary antibody and secondary antibody. Ammonium sulfate was also found to segregate almost all contaminating radioactivity into the free fraction (Table 3). Less specificity was found for polyethylene glycol, Amberlite ion-exchange resin, and charcoal (Table 3). When the radiopharmaceutical was administered in a protein-bound form ( $^{99m}\text{Tc}$ -labeled albumin) or was endogenously protein bound ( $^{67}\text{Ga}$  citrate), adsorption of the free fraction (charcoal, ion-exchange resin) left contaminating radioactivity in the bound fraction (Table 3). The ion-exchange resin appeared to adsorb [ $^{99m}\text{Tc}$ ]pertechnetate to a greater degree than did charcoal.

All contaminant activity could be accounted for by examining apparent nonspecific binding in the patient's sample; there was no evidence suggesting association of  $^{67}\text{Ga}$  citrate, or [ $^{99m}\text{Tc}$ ]pertechnetate or  $^{99m}\text{Tc}$ -labeled albumin with the primary antibody or primary antibody-ligand complex. Because contaminating radioactivity is quantitated by measuring nonspecific binding, inclusion of these tubes when radioactive contamination of the sample is present permits quantitation and subtraction of the spurious counts from the bound fraction. The correct dose estimates can then be obtained by using the corrected bound fraction.

If the bound fraction is counted and contaminating radioactivity is present, the dose will be underestimated. Because a radioimmunoassay standard dose-response curve is not linear, the magnitude of this error will be determined by the concentration of endogenous ligand (Figure 1). If the concentration of contaminating radioactivity is such that counts in patients unknown assay tubes exceed those in the zero standard ( $B/B_0 > 100\%$ ), contaminating radioactivity should be suspected. If the non-antibody bound free fraction is counted, as is common for separants adsorbing the free fraction (i.e., charcoal), and the free fraction is contaminated, the dose will be overestimated.

Contaminating radioactivity may lead to dose concentration errors in assays involving nonspecific separation media or large sample volumes. Nonspecific binding tubes should be included for all patients' samples or samples should be screened for radioactive contamination before assay, to avoid such errors.

## References

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