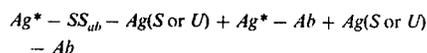
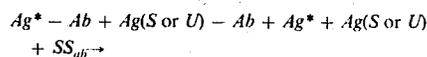


Antibody to T<sub>3</sub> conjugated to cyanogen bromide activated dextran coated Zipax was added to fill the chamber compartment. The upper support was a single Teflon felt disc.

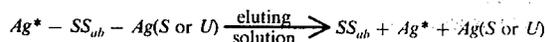
Assays were conducted using the automated equipment as described in United States application Ser. No. 565,850, filed Apr. 7, 1975, assigned to the same assignee, and whose disclosure is incorporated herein by reference. The buffer pump speed was 1.00 ml/min.

In practice, assays involve generation of data from standard solutions using a specific antibody chamber, followed by assays of unknowns using the same chamber and reagents and process times used for the standards. The sequence involves adding to each 1.0 ml of standard solution (prepared above) in a polystyrene sample cup, 0.1 ml of diluted (1:1,000) T<sub>3</sub> antisera, prepared as described. The total volume of the sample cup was 2 ml. The mixture was incubated, covered, and in the dark for at least three hours at room temperature.

Unknowns were prepared for analysis by diluting 40



The bound fraction is pumped to the detector for counting and thereafter the free fraction is eluted from the antibody covalently bound to the solid support, as indicated in the following:



The released antigen is also pumped to the detector for counting.

Using the above described procedures, four clinical samples were run along with two sets of commercial serum control samples. Data from standards were also generated, as shown in the following table.

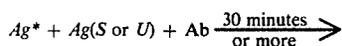
T <sub>3</sub> DATA						
	Free	Bound	Total	% Bound(7)	Assay pg/ml(8)	Serum pg/ml
Blank (1)	436	527				
Blank (2)	4082	2805	5924	38.5		
Standard - 3.9 (3)	3964	2908	5909	40.3		
Standard - 7.8	3712	3084	5833	43.8		
Standard - 15.6	3713	3297	6047	45.8		
Standard - 31.2	3216	3646	5899	52.9		
Standard - 62	2684	4302	6023	62.7		
Standard - 125	2194	4573	5804	69.7		
Standard - 250	1688	5183	5908	78.8		
Standard - 500	1496	5417	5950	82.2		
Standard - 1000	1306	5364	5707	84.8		
Blank (1)	529	568				
Blank (2)	4267	2781	6085	37.0		
Clinical (High) (4)	3060	2851	5959	56.0	40	1040
Clinical (Low)	3491	3628	6156	50.4	24	624
Clinical (High)	2950	4186	6173	59.3	50	1300
Clinical (Low)	3567	3680	6284	50.2	24	624
Commercial (Low)	2995	3046	6078	41.4	5	130
Commercial (Normal)	3494	3570	6101	49.9	21	546
Commercial (High)	2605	4536	6178	64.9	76	1976
Commercial (5)	3385	3848	6270	53.0	32	832
Commercial (6)	2283	4701	6021	69.3	110	2860

microliters of unknown serum with 1.0 ml of sample buffer in a serum cup, to which was added 0.1 ml of diluted T<sub>3</sub> antisera, prepared as described. The dilution of the unknown was 26 fold while the dilution of the standard was 21 fold. Following incubation of the unknown with the antibody, the free and bound antigen may be separated and counted using the automated equipment.

The labelled antigen is present in the sample buffer in a known amount. The reactions during an assay of a standard or unknown may be understood using the following symbols:

Ag\* is labelled antigen; Ag(S or U) is the standard or unknown unlabelled antigen; Ab is the antibody; and SS<sub>ab</sub> is the immunoabsorbent. The sequence may be represented as follows:

In the sample cup (preincubation):



In the presence of the immobilized immunoabsorbent, the mixture resulting from preincubation forms two fractions, a free fraction (underlined) and a bound fraction, as follows:

(1) The blank contained no radioisotope but merely the components of the sample buffer less the labelled antigen. This blank provided background counts.

(2) This blank was the sample buffer without unlabelled T<sub>3</sub>.

(3) Standard concentration expressed in picograms of T<sub>3</sub>/ml.

(4) Clinical samples were of unknown concentration, but in the general ranges indicated.

(5) Commercial sample of a concentration of 700 pg T<sub>3</sub>/ml ± 200.

(6) Commercial sample of a concentration of 2,500 pg T<sub>3</sub>/ml ± 400.

(7) Calculated by subtracting the free and bound background counts from the free and bound net counts.

(8) Expressed in terms of percentage of antigen bound to the antibody covalently coupled to the solid support.

From the above data, it can be seen that there is good correlation between the determination made in accordance with the present invention, by automated equipment, and the actual concentration.

The above data also indicate the sequence used for generation of data from the standards, all of which are processed by the same procedure, and the same procedure used for the unknowns which include both the clinical and commercial samples.