

c. Preparation of the test device

The same procedure as described in Example 3 was followed.

d. Performance of the assay

The same procedure as described in Example 3 was followed except that the columns were equilibrated with 10 ml of PBS buffer containing 0.1% Tween 20, a detergent available from the Atlas Powder Company, Wilmington, Delaware, and that PBS buffer containing 0.1% Tween 20 was used in place of PBS buffer. The standard digitoxin solutions were prepared to the following concentrations: 5 ng/ml, 10 ng/ml, 20 ng/ml, 30 ng/ml, 40 ng/ml, and 50 ng/ml.

The results were as follows (with % bound being calculated based on setting 100% bound as the % bound of the control):

Digitoxin Concentration ng/ml	% Bound
0.0	100
5.0	83
10.0	73.5
20.0	64
30.0	56.6
40.0	50.2
50.0	42

Unknown samples could be assayed using the standard curve plotted from these data, as described in Example 1c.

EXAMPLE 5

This Example relates to the use of the present method and device in obtaining dose response curves for the determination of Vitamin B₁₂ wherein an equilibrium method is followed.

a. Preparation of the test device

Intrinsic factor (17), a Vitamin B₁₂ binder available from Nutritional Biochemicals Corp., Cleveland, Ohio, was covalently coupled to CNBr-activated Sepharose 4B, available from Pharmacia AB, Uppsala, Sweden, according to the method described in *Scand. J. Clin. Lab. Invest.* 27:151 (1971). The intrinsic factor (17)-coupled Sepharose 4B was diluted with plain uncoupled Sepharose 4B to a point where 40-60% of the radioactive labeled Vitamin B₁₂ was retained in the column following procedure *b.* below where the fluid sample contained no Vitamin B₁₂. Radioactive Vitamin B₁₂ (⁵⁷Co cyanocobalamin), available from Amersham/Searle, Arlington Heights, Illinois, was used. The diluted mixture was slurried with 0.9% saline and added to 3 ml plastic syringes to give 1 ml packed Sepharose columns as in Example 1b.

b. Performance of the assay

Standard Vitamin B₁₂ solutions were prepared for the following concentrations: 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 63 pg/ml and 31 pg/ml. A control containing no Vitamin B₁₂ and the seven Vitamin B₁₂ standards were respectively mixed in 0.5 ml volumes with the radioactive labeled Vitamin B₁₂ solution to give final volumes of 1 ml. These mixtures were added to columns prepared as in procedure *a.* above and allowed to incubate for 90 minutes. The columns were then washed with 6 ml. volumes of assay buffer (prepared as described in *Scand. J. Clin. Lab. Invest.* 27: 151 [1971]) and counted using a Gammacord gamma

counter. The results were as follows (with % bound being calculated based on setting 100% bound as the % bound of the control):

Vitamin B ₁₂ Concentration pg/ml	% Bound
0	100.0
31	94.9
63	87.8
125	79.8
250	65.6
500	44.7
1000	24.2
2000	13.6

Unknown samples could be assayed using the standard curve plotted from these data, as described in Example 1c.

EXAMPLE 6

This Example relates to the use of the present method and device in obtaining dose response curves for the determination of human placental lactogen (HPL) wherein the labeled HPL is in an enzyme labeled form.

a. Preparation of enzyme labeled HPL

HPL was labeled with peroxidase according to the method described in *Immunochemistry* 6: 43(1969) with the following modifications: 10 mg (HPL and 10 mg horseradish peroxidase were dissolved in 1.4 ml of 0.05M carbonate buffer (pH 9), 0.05 ml of 3% glutaraldehyde solution was added to the mixture. The mixture was then rotated at ambient temperature for 90 minutes and then dialyzed against 128 ml of 0.1M borate buffer (pH 8) with several buffer changes.

b. Preparation of the test device

The same procedure as in Example 1b was followed.

c. Performance of the assay

The packed columns were equilibrated by adding 20 ml portions of a borate buffer (pH 9) to the columns and allowing them to drain. The borate buffer was prepared as in Example 1c. HPL standards were prepared for the following HPL concentrations 1000 ng/ml and 62 ng/ml. A control containing no HPL and the two HPL standards were added to columns, prepared according to procedure *b* above in 0.5 ml volumes and allowed to incubate for 25 minutes. The reference sample containing peroxidase labeled HPL, prepared as in procedure *a.* above, was then added to each column in 0.5 ml portions and allowed to incubate for 25 minutes. The columns were washed with 10 ml volumes of 0.1M borate buffer (pH 8) followed by 3 ml volumes of 0.1M phosphate buffer (pH 6). A substrate-indicator solution, prepared by mixing 0.28 ml of 0.03% H₂O₂ in water, 0.1 ml of 0.25% 3,3'-dimethylbenzidine, and 0.12 ml of 0.1M phosphate buffer (pH 6), was added to each column and allowed to incubate for 10 minutes. The columns were each washed with a 3 ml of volume of 0.1N HCl and the optical density (OD) of the washings were read at 460 nm with the following results:

HPL Concentration ng/ml	OD ₄₆₀	% Color Bound
0	1.2	100
62	0.96	63