

TABLE III-continued

Ferritin Standard (ng/ml)	Bound Protein A (I-125) on Supported Rabbit Anti-Ferritin F(ab) ₂		
	CPM (Avg.)		
	Rabbit anti-ferritin IgG/PA-I ¹²⁵ (Molar)*		
	597/1	119/1	60/1
6.0	731	869	953
20.0	830	1102	1132
60.0	1613	2136	1970
200.0	4208	4778	4622
600.0	8003	8655	7212

*A constant Protein A-I¹²⁵ concentration (8.8 ng/ml) was used throughout. Rabbit anti-ferritin IgG concentrations were 20 ug/ml, 4.0 ug/ml and 2.0 ug/ml. These concentrations correspond to molar IgG/Protein A¹²⁵ ratios of 597/1, 119/1 and 60/1, respectively.

TABLE IV

Ferritin Standards (ng/ml)	Bound Protein A-Alkaline Phosphatase on Supported Rabbit Anti-Ferritin F(ab) ₂		
	OD ₄₀₅		
	Rabbit Anti-Ferritin IgG (ug/ml)		
	20.0	10.0	2.0
0	.026	.018	.021
6	.026	.028	.022
20	.035	.053	.041
60	.116	.115	.111
200	.389	.381	.410
600	.907	1.11	.910

We claim:

1. A method for quantitatively determining the presence of polyvalent ligand in an aqueous fluid which comprises:

- (a) incubating said fluid with a solid support having a first antibody to said ligand bound thereto to form an immobilized antibody-ligand complex;
- (b) incubating said immobilized antibody-ligand complex with a solution containing a soluble complex of a second antibody to said ligand and a labeled binding protein specifically bound to the F_c portion of said second antibody;
- (c) washing unbound antibody and labeled binding protein from the solid support; and
- (d) determining the presence of labeled binding protein bound to said solid support or remaining in solution, as a measure of the concentration of the ligand in the aqueous fluid;

wherein said method is further characterized in that the solution of second antibody-labeled binding protein complex contains an excess concentration of second antibody to provide a substantially linear relationship between the concentration of ligand and the amount of labeled binding protein bound to the solid support, while maintaining analytically acceptable low levels of background interference.

2. The method of claim 1, wherein the molar ratio of soluble second antibody to labeled binding protein is from about 250:1 to about 5,000:1.

3. The method of claim 1, wherein the molar ratio of soluble second antibody to labeled binding protein is from about 50:1 to about 24,000:1.

4. The method of claim 3, wherein the labeled binding protein is protein A obtained from *Staphylococcus aureus*.

5. The method of claim 4, wherein the ligand is ferritin.

6. The method of claim 4, wherein the ligand is selected from the group consisting of ferritin, thyroid stimulating hormone, creatinine phosphokinase, prostatic acid-phosphatase, and human chorionic gonadotropin, alpha fetoprotein, parathyroid hormone, insulin and C-peptide.

7. The method of claim 6, wherein the first antibody is an intact antibody.

8. The method of claim 5 or 6, wherein the labeled binding protein is selected from the group consisting of ¹²⁵I-Protein A, ³H-Protein A and ¹⁴C-Protein A.

9. The method of claim 5 or 6, wherein the labeled binding protein is enzyme-labeled Protein A.

10. The method of claim 5 or 6, wherein the labeled binding protein is protein A labeled with a fluorescent substance.

11. An immunoassay test kit for determining the presence of a ligand in an aqueous fluid comprising a solid support coated with an antibody to said ligand and, in a separate container, a solution containing a soluble complex of a second antibody to said ligand and a labeled binding protein, wherein the concentrations of second antibody and labeled binding protein in said solution are in a molar ratio of from about 50:1 to about 24,000:1 respectively.

12. The test kit of claim 11, wherein the molar ratio of second antibody to labeled binding protein is from about 250:1 to about 5,000:1.

13. The test kit of claim 12, wherein the labeled binding protein is radiolabeled Protein A.

14. The test kit of claim 12, wherein the labeled binding protein is enzyme-labeled Protein A, and said test kit further comprises a solution of a chromogenic substrate for said enzyme.

15. A method for quantitatively determining the presence of polyvalent ligand in an aqueous fluid which comprises:

- (a) incubating said fluid with a solid support having a first antibody to said ligand bound thereto to form an immobilized antibody-ligand complex;
- (b) incubating said immobilized antibody-ligand complex with a solution containing a soluble complex of a second antibody to said ligand and a labeled binding protein specifically bound to the F_c portion of said second antibody;
- (c) washing unbound antibody and labeled binding protein from the solid support; and
- (d) determining the presence of labeled binding protein bound to said solid support or remaining in solution, as a measure of the concentration of the ligand in the aqueous fluid;

wherein said method is characterized in that the first antibody is an IgG immunoglobulin which has been enzymatically digested to remove the F_c portion.

16. The method of claim 15, wherein the molar ratio of soluble second antibody to labeled binding protein is from about 500:1 to about 1:1.

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