

- (B) measuring said marker material thereby released which is related to the amount of said analyte initially present in said liquid medium.
16. The method of claim 15 wherein the analyte is a drug, metabolite, hormone, steroid, pesticide, environmental pollutant, food toxin, vitamin, protein, microbial surface marker, cancer cell marker, fungus, protozoan, virus, cell or tissue antigen.
17. The method of claim 15 wherein the analyte is a drug.
18. The method of claim 17 wherein the drug is digoxin.
19. The method of claim 15 wherein the analyte is a vitamin.
20. The method of claim 19 wherein the vitamin is biotin.
21. The method of claim 15 wherein the analyte binding agent is selected from the group consisting of antibody, hormone-receptor, lectin, and specific binding protein.
22. The method of claim 21 wherein the binding agent is an antibody.
23. The method of claim 22 wherein the antibody is specific for digoxin.
24. The method of claim 21 wherein the binding agent is a specific binding protein.
25. The method of claim 24 wherein the specific binding protein is avidin.
26. The method of claim 15 wherein the analyte-cytolysin conjugate is ouabain-melittin.
27. The method of claim 15 wherein the analyte-cytolysin conjugate is biotin-melittin.
28. The method of claim 15 wherein the vesicle utilized to sequester marker material is selected from the group consisting of lipid vesicle, red blood cell, and red blood cell ghost.
29. The method of claim 28 wherein the vesicle is a lipid vesicle.
30. The method of claim 15 wherein the marker material is a substance selected from the group consisting of enzyme, cofactor, chromophore, fluorophore, spin label, and ion.
31. The method of claim 29 wherein the marker material is an enzyme.
32. The method of claim 30 wherein the enzyme is alkaline phosphatase.
33. The method of claim 30 wherein the enzyme is  $\beta$ -galactosidase.
34. The method of claim 30 wherein the fluorophore is 6-carboxyfluorescein.
35. A method for determining the amount of digoxin in liquid medium comprising the steps of:  
 (A) forming a reaction system by contacting said liquid medium with

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- (1) digoxin specific antibody;  
 (2) ouabain-melittin conjugate;  
 (3) lipid vesicles containing sequestered alkaline phosphatase; and  
 (4) p-nitrophenyl phosphate  
 in proportions such that the interaction of unbound ouabain-melittin conjugate and said lipid vesicles results in the conversion of said p-nitrophenyl phosphate by said alkaline phosphatase; and
- (B) measuring the concentration of p-nitrophenolate anion produced which is related to the amount of said digoxin initially present in said liquid medium.
36. A conjugate for the detection of an analyte in a test sample, comprising in combination:  
 (1) cytolysin means for altering the permeability of a membrane of a vesicle, said means being conjugated to  
 (2) an antibody molecule capable of binding to the analyte.
37. The conjugate of claim 36 wherein the antibody molecule is a monovalent antibody molecule selected from the group consisting of Fab, Fab', and half-molecules.
38. The conjugate of claim 36 wherein the cytolysin is selected from the group consisting of aerolysin, amphotericin B, alamethicin, A-23187 (calcium ionophore), ATP translocase, cerolysin, diphtheria toxin, filipin, gramicidin, melittin, nigericin, nystatin, polymyxin B, *Staphylococcus aureus* toxin  $\alpha$ ,  $\beta$ ,  $\gamma$ , or  $\delta$ , Streptolysin O, Streptolysin S, tubulin and valinomycin.
39. The conjugate of claim 8 wherein the cytolysin is melittin.
40. A heterogeneous immunoassay for detecting an analyte in a liquid sample, comprising:  
 (1) forming a reaction mixture by contacting the sample with a cytolysin/anti-analyte antibody conjugate in immunochemical excess over the analyte, whereby a fraction of said conjugate binds to analyte to form a complex and a fraction of said conjugate remains free,  
 (2) contacting the reaction mixture with a solid phase having analyte or analyte-analog bound thereto to separate the complex from the free conjugate, and  
 (3) contacting either the complex or the free conjugate with vesicles having marker material sequestered therein, whereby the permeability of the vesicle increases, thereby releasing marker material,  
 (4) measuring the amount of released marker material, and  
 (5) relating the amount of released marker material to the amount of analyte initially present in the test sample.

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