with 42 μl of 79.6mM MBS (dissolved in tetrahydrofuran) for 60 minutes at 25° C. The reaction is stopped and reaction products are removed by desalting the mixture on a Sephadex G-25 column (1.5×40 cm) equilibrated in phosphate buffered saline. The derivatized melittin fractions which elute from the column are combined with the Fab'-SH fraction, the pH of the solution readjusted to pH 7.5, and allowed to incubate with mixing at 4° C. After 16–20 hours the Fab'-melittin conjugate is purified by column chromatography using Sephadex G-100.

EXAMPLE 3b

Heterogeneous Assay

In the preferred mode, the heterogeneous assay using the antibody-cytolysin conjugate is performed as follows. A known volume of patient sample, usually 5 μl to 500 μl, containing an unknown amount of analyte is mixed with a solution containing an amount of monovalent antibody-cytolysin conjugate known to be in excess over analyte. Usually the conjugate will be present in approximately 100-fold molar excess over analyte. Analyte and conjugate are preincubated for a specific length of time, usually at least 5 minutes and not more than 30 minutes, at a fixed temperature between 4° C. and 45° C., usually 22°–25° C. A known volume (usually 5 μl to 500 μl) of this solution containing analyte and conjugate is passed through a column, preferably of dimensions 2 mm×10 mm, consisting of analyte or analyte-analog immobilized on a porous support. Sufficient analyte-coupled (or analyte-analogue-coupled) support must be used to bind preferably all of the free conjugate. The column is eluted at a flow rate of 0.2–5.0 ml per minuted with a suitable buffer, usually 1–5 ml total volume. The fraction which elutes from the column contains conjugate complexed with analyte from patient serum.

A suspension of vesicles containing marker material is added to this fraction. As the cytolysins reacts with the lipid vesicle, the permeability of the vesicles will increase, thereby releasing the marker material into the external milieu, thereby allowing the marker material to be detected.

It will be apparent that the instant specification and examples are set forth by way of illustration and not limitation, and that various modifications and changes may be made without departing from the spirit and scope of the present invention.

What is claimed is:

1. 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) at least one analyte molecule.

2. The conjugate of claim **1** wherein the cytolysin is selected from the group consisting of aerolysin, amphotericin B, alamethicin, A-23187 (calcium ionophore), ATP translocase, cereolysin, diptheria toxin, filipin, gramicidin, melittin, nigericin, nystatin, polymyxin B, *Staphylococcus aureus* toxin α, β, γ, or δ, Streptolysin O, Streptolysin S, tubulin and valinomycin.

3. The conjugate of claim **1** wherein the cytolysin is melittin.

4. The conjugate of claim **3** wherein the attachment of analyte derivative is through an amino acid residue selected from the group consisting of lysine, serine, threonine, N-terminal glycine, and C-terminal glutamine.

5. The conjugate of claim **1** wherein the analyte derivative 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.

6. The conjugate of claim **5** wherein the analyte derivative is a drug.

7. The conjugate of claim **6** wherein the drug is ouabain.

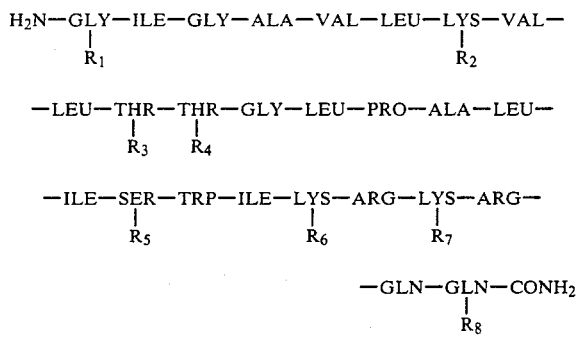
8. The conjugate of claim **4** wherein at least one ouabain molecule is attached to melittin through an ε-amino group of a lysine residue.

9. The conjugate of claim **5** wherein the analyte derivative is a vitamin.

10. The conjugate of claim **9** wherein the vitamin is biotin.

11. The conjugate of claim **4** wherein at least one biotin derivative molecule is attached to melittin through an ε-amino group of a lysine residue.

12. An analyte-cytolysin conjugate comprising a substituted melittin molecule of the formula:



wherein at least one of R_1 through R_8 is an analyte derivative molecule selected from the group consisting of drug, vitamin, hormone, metabolite, steroid, pesticide, environmental pollutant, food toxin, protein, microbial surface marker, cancer cell marker, fungus, protozoan, cell or tissue antigen, provided that when at least one of R_2, R_6, or R_7 is said analyte derivative, attachment is through an ε-amino group of the amino acid side chain; when at least one of R_3, R_4, or R_5 is said analyte derivative, attachment is through a hydroxyl group of said amino acid side chain; when R_1 is said analyte derivative, attachment is through the N-terminal amino group; and when R_8 is said analyte derivative, attachment is through the C-terminal carboxyl group.

13. The conjugate of claim **12** wherein at least one of R_2, R_6, or R_7 is a ouabain derivative.

14. The conjugate of claim **12** wherein at least one of R_2, R_6, or R_7 is a biotin derivative.

15. A method for determining the amount of an analyte in liquid medium comprising the steps of:

(A) forming a reaction system by contacting said liquid medium with

(1) analyte specific binding agent;

(2) analyte-cytolysin conjugate; and

(3) vesicles containing marker material sequestered within the vesicle

in proportions such that the interaction of unbound analyte-cytolysin conjugate with said vesicles results in the release of said marker material; and