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(57) Abstract

Liposomal immunoassays and liposomal reagents for assaying antigens and serum antibody. The liposomal reagents for assaying serum antibody are composed of liposomes having an outer surface to which anti-idiotypic antibody is linked. On the other hand, the liposomal reagents for assaying antigen are composed of liposomes having an outer surface to which antigen specific antibody is linked.

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LIPOSOMAL IMMONOASSAY FOR ANTIGENS AND FOR ANTIGEN SPECIFIC IMMUNOGLOBULINS

RELATED APPLICATIONS

This is a continuation of U.S. Applications Serial No. 07/014,996, filed February 17, 1987 and Serial No. 07/015,049, filed February 17, 1987, whose disclosures are, by reference, incorporated herein.

Background of the Invention

The invention relates to diagnostic immunoassays. More particularly, the invention relates to liposomal immunoassays for detecting antigens or haptens and for detecting antigen or hapten specific immunoglobulins.

Monroe (American Clinical Products Review, December 1986, pp 34-41) discloses two antigen competitive inhibition liposomal immunoassays for detecting sample antigen. The two immunoassays are similar in that the presence or absence of antigen in a sample is determined by the lysis of liposomes and the release of label. However, the two immunoassays are different in that different agents are employed to lyse the liposomes.

Monroe's first antigen competitive inhibition liposomal immunoassay employs free antibody, antigen-cytolysin conjugates, and simple labeled liposomes. The antigen-cytolysin conjugates have the special property that their activity is inhibited when the antigen portion of the conjugate is bound by antibody. However, in the presence of antigen, the binding of free antibody to the

antigen-cytolysin conjugate is inhibited. Consequently, when labeled liposomes are added to a mixture containing sample antigen, free antibody, and antigen-cytolysin conjugate, the activity of the conjugate is relatively uninhibited and lysis of the labeled liposomes occurs. Lysis of the labeled liposomes is detected by the release of the label. Conversely, in the absence of antigen, the binding of free antibody to the antigen-cytolysin conjugate is uninhibited. Thus the activity of the conjugate becomes inhibited and little or no lysis results.

Monroe's second antigen competitive inhibition liposomal immunoassay employs free antibody, labeled liposomes with surface bound antigen, and complement. In the presence of sample antigen, the binding of free antibody to the surface bound antigen on the labeled liposomes is inhibited. Consequently, when complement is added, there is relatively less antibody bound to the liposomes and therefore less lysis. Conversely, in the absence of antigen, the binding of free antibody to the liposomes is uninhibited and complement can efficiently lyse the liposomes so as to release the label.

Both of Monroe's liposomal immunoassays are dependent on special characteristics of the sample antigen. Monroe's first liposomal immunoassay can be employed only if the activity of the antigen-cytolysin conjugate is inhibited when the antigen portion of the conjugate is bound by antibody. Not all antigen-cytolysin conjugates necessarily exhibit such inhibition. Monroe's second liposomal immunoassay requires the use of labeled liposomes having

surface bound antigen. More particularly, the manner in which the antigen is attached to the surface of the liposome must be such as to enable the triggering of the complement cascade when antibody attaches to the surface bound antigen and must be such as to cause complement lysis following complement activation. Not all antigens can necessarily be made to be surface bound upon liposomes so as to satisfy these conditions.

Kinsky (Biochim. Biophys, Acta, vol. 265, pp 1-23. 1972: "Antibody-complement Interaction with Lipid Model Membrane") has shown that liposomes having surface bound antigen will agglutinate in the presence of antibody and that the addition of complement to the mixture of antibody and liposomes causes liposomal lysis. Liposomal lysis can be detected by the release of marker molecules from the lumen of the liposome. Kinsky teaches that complement induced liposomal lysis is an indication of the presence of surface bound antigens.

Willoughby (European Journal of Immunology, vol. 8, pp 628-634, 1978) discloses that liposomals having surface bound alloantigenic epitopes can agglutinate in the presence of antigen specific alloantisera. Agglutination can be used as an indication of the presence of the surface bound alloantigenic epitopes on the liposomes.

Engelhard (Proc. National Academy of Science USA, vol. 75, No. 7, pp 3230-3234) discloses that liposomes having surface bound antigens (HLA-A and HLA-B) can bind to antibody (anti-beta(2)-microglobulin IgG) which is coupled and immobilized onto Sepharose 4B beads. Antibody mediated

binding and immobilization of such liposomes to the Sepharose beads can be used as a measure of the antigen which is surface bound to the liposome.

Methods for non-immunologically linking antibody to liposomes are described in Liposome Technology, vol. III "Targeted Drug Delivery and Biological Interaction," ed. by Gregory Gregoriadis, CRC Press (1984) by Leserman et al. (Chapter 2: "Covalent Coupling of Monoclonal Antibodies and Protein A to Liposomes: Specific Interaction with Cells in Vitro and in Vivo" pp 29-40), by Hashimoto et al. (Chapter 3: "Chemical Methods for the Modification of Liposomes with Proteins or Antibodies" pp 41-49), and by Huang et al. (Chapter 4: "Coupling of Antibodies with Liposomes" pp 51-62). Leserman, Hashimoto, and Huang disclose liposomal reagents having non-immunologically bound antibody which is targeted against the cell surface of various cell types. These liposomal reagents bind to their targeted cell surface and are subsequently endocytosed into the cell. Leserman shows that, if the liposomal reagent is loaded with a fluorescent dye, target cells which endocytose the liposomal reagent subsequently become fluorescent. Hashimoto shows that liposomal reagents having non-immunologically bound antigens will agglutinate in the presence of the corresponding serum antibody. Hashimoto further shows that, if such liposomal reagents are radioactively labeled, then this agglutination in the presence of serum antibody can be used as a quantitative assay of serum antibody. However, these authors do not teach that liposomal reagents having non-immunologically

found antibody can be employed in liposomal immunoassays. Since these liposomal reagents are targeted against cells, none of these authors suggest that liposomal reagents can be fabricated which can react with soluble antigens or that such liposomal reagents can agglutinate in the presence of antigen or can form immobilized complexes in the presence of antigen and immobilized antibody.

Vander-Mallie (U.S. Patent No. 4,536,479 - Aug. 1985) discloses that anti-idiotypic antibody can substitute for antigen and haptens in various immunoassays of the prior art. However, Vander-Mallie does not disclose that anti-idiotypic antibody can be employed in liposomal immunoassays. Monoclonal anti-idiotypic antibody is particularly advantageous because of its reproducibility and consistancy. Additionally, as compared to antigens derived from pathogenic materials, anti-idiotypic antibody is much safer to manufacture and handle.

What is needed are liposomal immunoassays which employ liposomal reagents having surface bound antibody for assaying antigens and antigen specific immunoglobulins.

Summary of the Invention

The invention is a liposomal immunoassay which employs liposomal reagents for detecting the presence of an antigen or an antigen specific immunoglobulin within a sample fluid. The liposomal reagent for assaying antigens includes a liposome, antibodies having a specifity against the antigen, a linkage member for linking the antibody to the liposome, and a label carried by the liposome. Similarly, the liposomal reagent for assaying antigen

specific immunoglobulin includes a liposome, anti-idiotypic antibodies having a specifity against the antigen specific immunoglobulin, a linkage member for linking the anti-idiotypic antibody to the liposome, and a label carried by the liposome. The linkage member is chosen so that the linkage between the antibody or anti-idiotypic antibody and the liposome does not interfere with the ability of the antibody or anti-idiotypic antibody to bind to its respective antigen or antigen specific immunoglobulin in solution. The antibody or anti-idiotypic antibody may be whole antibody or antibody fragments or derivatives. However, the antibody fragments or derivatives must be capable of linkage to the liposome by means of a linkage member. The specificity of the liposomal immunoassay is determined by the specificity of the antibody or anti-idiotypic antibody.

There are two basic liposomal immunoassay protocols, viz. an immobilization protocol and an agglutination protocol. The immobilization protocol involves the use of a solid phase; the agglutination protocol dispenses with the the solid phase and depends upon the agglutination of the liposomal reagents. However, within each protocol, there are slight differences with respect to the precise methodology for assaying antigen and for assaying antigen specific immunoglobulin.

The immobilization protocol of the liposomal immunoassay for detecting antigens employs an immobilized antibody, i.e. antibody which is immobilized onto a solid

phase. This immobilized antibody has a specificity for the antigen. During the immobilization protocol, the sample fluid is incubated with both the immobilized antibody and the liposomal reagent antibody under conditions which result in the formation of a complex between the liposomal reagent, the antigen, and the immobilized antibody. The formation of this complex is dependent upon the presence of antigen in the sample fluid. During the incubation, the sample antigen cross links the liposomal reagent with itself and with the immobilized antibody. The complex of liposomal reagent, which is bound to immobilized antibody on the solid phase, is then washed so as to remove unreacted components which are not bound to the solid phase. This results in a separation of bound and unbound components. After the separation step, label belonging either to the complex associated with the solid phase or to the unreacted liposomal reagent is then detected or measured. The presence or absence of antigen can then be correlated with the measurement or detection of such label.

The protocol of the immobilization protocol for assaying antigen specific immunoglobulins is similar to the above protocol for antigens except that the immobilized antibody and liposomal reagents employ anti-idiotypic antibody having a specific for the antigen specific immunogobulin instead of antibody having a specificity for antigen.

The agglutination protocol of the liposomal immunoassay for detecting antigens dispenses with the immobilized antibody, and, instead, relies upon an

agglutination reaction. During the agglutination protocol, the sample fluid is incubated with a multivalent liposomal reagent. The multivalent liposomal reagent is a liposome to which are attached multiple surface bound antibodies, each having a specificity for the antigen. The liposomal reagent agglutinates in the presence of high levels of antigen, i.e. an agglutinized complex results. During the agglutination process, the antigen causes the multivalent liposomal reagent to become cross linked. After the formation of the agglutinized complex, the complex is then separated from unreacted antigen and liposomal reagent and is then detected by the presence of label. If quantitative results are desired, quantitative measurements of the presence of label may be made and compared with measurements taken from liposomal immunoassays on standard samples of antigen. The amount of antigen in the sample fluid may then be quantitatively determined by interpolating or extrapolating the result with the standard samples of antigen.

The protocol of the agglutination protocol for assaying antigen specific immunoglobulins is similar to the above agglutination protocol for antigens except that the multivalent liposomal reagents employ anti-idiotypic antibody having a specific for the antigen specific immunogobulin instead of antibody having a specificity for antigen.

Detailed Description of the Invention:

Construction of the

Liposomal Reagent

There are four elements to the liposomal reagent, viz. the antibody, the liposome, the label contained by the liposome, and the linkage member between the antibody and the liposome. If the liposomal reagent is multivalent, then there are two or more antibodies attached to each liposome.

If the immunoassay is directed to the detection of antigens, the above antibody has a specificity for the antigen which is to be assayed. Either high affinity monoclonal antibodies or polyclonal antibodies are preferred. Methods for producing monoclonal antibodies are described by Kohler and Milstein (Nature, vol. 256, pp 495-498 (1975)). Polyclonal antibodies may be made by standard immunological techniques or may be obtained, for for a wide variety of specificities, from the American Type Culture Collection or from a number of commercial vendors.

On the other hand, if the immunoassay is directed to the detection of antigen specific immunoglobulins, the above antibody is an anti-idiotypic antibody and has a specificity for the antigen specific immunoglobulin which is to be assayed. High affinity monoclonal antibodies are preferred over polyclonal antibodies for use as the anti-idiotypic antibodies. Methods for producing both monoclonal and polyclonal anti-idiotypic antibodies are described by Vander-Mallie, i.e. U.S. Patent No. 4,536,479, which is incorporated herein by reference. It is assumed

that, for virtually every possible antigen specific immunoglobulin, it is possible to produce a corresponding anti-idiotypic antibody.

The construction and composition of the liposome may depend, in part, on the particular linkage member which is employed for binding the antibody or anti-idiotypic antibody to the surface of the liposome. Three equally preferred methods for making liposomes and for linking these liposomes to antibody are described in chapters 2, 3, and 4 of Liposome Technology, vol. III "Targeted Drug Delivery and Biological Interaction," ed. Gregory Gregoriadis, CRC Press (1984). In Chapter 2, viz. "Covalent Coupling of Monoclonal Antibodies and Protein A to Liposomes: Specific Interaction with Cells in Vitro and in Vivo" pp 29-40, Leserman et al. disclose a disulfide linkage member which links antibody to dipalmitoyl phosphatidylethanolamine (DPPE), which, in turn is incorporated into the membrane portion of the liposome. In Chapter 3, viz. "Chemical Methods for the Modification of Liposomes with Proteins or Antibodies" pp 41-49, Hashimoto et al. disclose linkage members which both specifically and nonspecifically link amino group-bearing liposomes to IgG or other proteins. In Chapter 4, viz. "Coupling of Antibodies with Liposomes" pp 51-62, Huang et al. disclose a linkage member which joins IgG to a fatty acid, i.e. palmitic acid. The antibody conjugate of palmitic acid is then incorporated into a liposome bilayer. The above three references are incorporated herein by reference.

Multivalent liposomal reagents are preferred over monovalent liposomal reagents. Multivalent liposomal reagents are liposomal reagents which have, on the average, more than one antibody or anti-idiotypic antibody bound to the surface of each liposome. Multivalency allows the average liposome to bind more than one antigen or antigen specific immunoglobulin and allows antigen or antigen specific immunoglobulin to cross link and agglutinate the liposomal reagent. Highly multivalent liposomal reagents are preferred over low multivalent liposomal reagents. Low multivalent liposomal reagents are liposomal reagents which have, on the average, approximately two antibodies or anti-idiotypic antibodies bound to the surface of each liposome. Highly multivalent liposomal reagents are liposomal reagents which have significantly more surface bound antibodies or anti-idiotypic antibodies than low multivalent liposomal reagents and which, under identical conditions, are capable of agglutination in the presence of a lower level of antigen or antigen specific immunoglobulin than the minimum level of antigen or antigen specific immunoglobulin required for the agglutination of low multivalent liposomal reagent. An agglutination protocol which can easily be adapted for this test is provided by Hashimoto (supra). The use of high multivalent liposomal reagents enhances the sensitivity of liposomal immunoassays.

The sensitivity of the liposomal immunoassay is also determined by the label. The label is contained by the liposome. Leserman, Hashimoto, and Huang disclose the

encapsulation of various labels. Leserman discloses the encapsulation of a water soluble fluorescent dye, viz. carboxyfluorescein (CF). At high concentrations within the lumen of the liposome, the fluorescence of CF is self quenching. However, upon dilution, after fusion or lysis of the liposome, the CF becomes highly fluorescent. Hashimoto discloses methods for radiolabeling the membrane portion of liposomes, e.g. by incorporation of cholesteryl [1-(14)C]oleate. And finally, Huang discloses the lumenal encapsulation of lactate dehydrogenase. The presence of lactate dehydrogenase can be detected by lysing the liposomal reagent, adding substrate, and observing the formation of enzyme product. Similar enzyme labels are used extensively in the field of enzyme immunoassays and may be adapted to liposomal immunoassays. The invention is not limited to the above labels, but may generally include the use of any label employable in the field of liposomal immunoassays. However, since the sensitivity of the liposomal immunoassay is enhanced by the employment of highly sensitive labels, the use of such labels is preferred.

The sensitivity of the liposomal immunoassay can also be enhanced by increasing the quantity of lumenally included label. Hence, increasing the size of the liposome and increasing the concentration of the lumenally included label can enhance the sensitivity of the liposomal immunoassay. However, the operability of this effect is limited. The increase in the size of the liposome and the lumenal concentration of the label is limited by the

requirement that the liposome should be relatively stable against leakage and spontaneous lysis.

Construction of the

Immobilized Antibody

The immobilization protocol of the liposomal immunoassay employs an immobilized antibody, i.e. antibody which is attached to a solid phase. The immobilized antibody serves as an anchor and participant in the formation of an immobilized complex. The immobilized antibody is antigen specific if the immunoassay is directed to such antigen. The immobilized antibody is an anti-idiotypic antibody having a specificity for antigen specific immunoglobulins if the immunoassay is directed to such antigen specific immunoglobulins. Preferred methods for making immobilized antibody which is antigen specific are described by David et al. (U.S. Patent No. 4,376,110 - Mar. 1983). David discloses a method for binding monoclonal antibody to agarose particles and to microtiter plates. The David reference is incorporated herein by reference. Preferred methods for making immobilized anti-idiotypic antibody are described by Vander-Mallie (U.S. Patent No. 4,536,479 - Aug. 1985) and again by David et al. (U.S. Patent No. 4,376,110 - Mar. 1983). Vander-Mallie discloses a method for coating microtiter plates with anti-idiotypic antibody. The Vander-Mallie reference is incorporated herein by reference. However, prior art homogeneous immunoassays employ a number of other methods for making immobilized antibody, many of which may be adapted to the present invention.

The specificity of the antibody employed in the immobilized antibody should correspond to the specificity of the antibody employed in the above liposomal reagent, i.e. both antibodies should have specificities for the same antigen or antigen specific immunoglobulin.

In the case of immobilized antibody employed in liposomal immunoassays directed to the detection of antigens, the immobilized antibody may be monoclonal or polyclonal. However, if the antibody of the liposomal reagent and of the solid phase are monoclonal, then, it may be desirable to employ two or more monoclonal antibodies, each having non-interfering epitopic specificities such that antibody binding at one epitope does not interfer with antibody binding at an other epitope. This insures that more than one antibody can simultaneously bind with the antigen so as to form a three part complex during the immobilization protocol of the liposomal immunoassay.

In the case of immobilized anti-idiotypic antibody employed in liposomal immunoassays directed to the detection of antigen specific immunoglobulins, the specificity of the immobilized anti-idiotypic antibody may be the same as the specificity of the anti-idiotypic antibody employed in the liposomal reagent, since the target immunoglobulin is usually divalent or multivalent. High affinity monoclonal antibody is preferred.

Protocol for the Immobilization Method of the Liposomal Immunoassay:

There are three different methods for practicing the immobilization protocol of the liposomal immunoassay. Each method employs the liposomal reagent and the immobilized anti-idiotypic antibody described above or their functional equivalent. Each of the three different methods can in turn be adapted to the detection of antigens or of antigen specific immunoglobulins.

In the first protocol for detecting antigens, a sample fluid, potentially containing the antigen, is first combined and incubated with liposomal reagent under conditions which enable either binding or cross linking between antigen and the antibody. The liposomal reagent is constructed to have a specificity for the antigen, as described in the above section. Cross linking is promoted by the use of highly multivalent liposomal reagents. The conditions for cross linking can be empirically observed by the occurance of agglutination as measured by the method of Hashimoto (supra). The conditions for binding include the conditions for cross linking. After this first incubation step, the mixture is then combined with the corresponding immobilized antibody. The immobilized antibody is constructed to have a specificity for antigen, as desribed in the above section. The combined components are then allowed to incubate for a second period. In the presence of antigen, an immobilized complex will form which includes the antibody, the immobilized antibody, and the liposomal reagent. If cross linking has occured, the immobilized

complex will include several liposomal reagents cross linked by antigen for each immobilized antibody. If the antigen in the fluid sample is particularly dilute, the above mixture can be sedimented during the second incubation period in order to accelarate the formation of the immobilized complex. If this sedimention step is employed, the immobilized antibody should take the form of a coated microtiter plate or tube. After this second incubation period, the immobilized antibody is then washed so as to remove and separate unreacted components of the sample fluid and liposomal reagent from the immobilized complex. The detection step follows and is described below.

The above first method for the detection of antigens can be easily adapted to formulate a method for the detection of antigen specific immunoglobulins. In order to detect antigen specific immunoglobulins, both the immobilized antibody and the liposomal reagents are constructed with anti-idiotypic antibody having a specificity for the antigen specific immunoglobulin, as described in the prior two sections.

In the second protocol for detecting antigen, a sample fluid is first combined and incubated with the immobilized antibody under conditions which enable binding between antigen and the immobilized antibody. After this first incubation step, the mixture is then combined with the corresponding liposomal reagent and allowed to incubate for a second period. During this second incubation period, the liposomal reagent will bind to the immobilized antibody to

form the immobilized complex. An optional sedimentation step be added after the second incubation step to promote the formation of the immobilized complex. After this second incubation period, the immobilized antibody is washed and the presence of label is detected as described below.

In the third protocol for detecting antigen, the sample fluid is simultaneously combined and incubated with both the immobilized antibody and the liposomal reagent under conditions which enable the formation of an immobilized complex. Like the first protocol, the use of highly multivalent liposomal reagents to promote cross linking is preferred. An optional sedimentation step may also be added during this incubation step to promote the formation of the immobilized complex. After the incubation step, the immobilized complex is washed and the presence of label is detected as described below.

Each of the above second and third methods for the detection of antigens can be easily converted to formulate a method for the detection of antigen specific immunoglobulins. Like the conversion of the first protocol, the immobilized antibody and the liposomal reagents are each modified so that they are constructed with anti-idiotypic antibody having a specificity for the antigen specific immunoglobulin instead of antibody having a specificity for antigen.

The immobilized complex is detected by means of the label contained by the liposomal reagent. There are several different labels which may be employed. Monroe

(supra) describes the use and detection of liposomally entrapped dyes (Arsenazo III) and enzyme markers, e.g. horseradish peroxidase, alkaline phosphatase, and glucose oxidase. Leserman describes the use and detection of liposomally entrapped fluorescent dyes, e.g. carboxyfluorescein. Hashimoto describes the use and detection of liposomally contained radiolabels [(14)C]DPPE and (125)I. Any of the detection methods employed in the prior art for liposomal immunoassays can be employed for detecting the immobilized complex.

For any given label, the sensitivity of the liposomal immunoassy can be modulated by using liposomal reagents having a range of different quantities of label. For example, a first liposomal reagent having twice the quantity of label as compared to a second liposomal reagent has approximately twice the sensitivity.

Once the immobilized complex is formed, the presence or absence of antigen or of antigen specific immunoglobulin in the sample fluid can be determined by measuring or detecting the presence of label either retained or lost by the immobilized complex after the separation step. The detection step may focus on the immobilized complex, the wash eluant, or both. In order to detect or measure label trapped in the immobilized complex, it may be necessary to release the label from the liposomal reagent. Several methods for releasing trapped label are described in Monroe. If a semi-quantitative measurement of the level of antigen in a sample fluid is desired, a quantitative measurement of label from a liposomal immunoassay for the

sample fluid is compared with the similar quantitative measurements taken for fluids having known concentrations of antigen or antigen specific immunoglobulins. The level of antigen in the sample fluid is then calculated by interpolation or extrapolation with the standard antigens or antigen specific immunoglobulins.

Protocol for the Agglutination Method of the Liposomal Immunoassay

The agglutination method for practicing the liposomal immunoassay is only a slight modification of the immobilized method. In the agglutination method, the extent of cross linking of liposomal reagents induced by antigen must be sufficient to induce agglutination. The agglutination method requires the use of multivalent liposomal reagents. Preferrably, highly multivalent liposomal reagents should be employed. The agglutination method is appropriately employed when there are high levels of antigen in the sample fluid.

In the agglutination method, the sample fluid is combined with highly mutlivalent liposomal reagent and allowed to incubate under conditions which promote binding and cross linking between the antigen and the liposomal reagent. Such cross linking forms an agglutinized complex. The agglutinized complex may then be separted from unreacted components by a filtration procedure described by Hashimoto (supra pp 46-47). Alternatively, the agglutinized complex may be separated from unreacted components by sedimentation. The formation of the agglutinized complex is an indication of the presence of

antigen. Measurement of the presence of label in the agglutinized complex may be made by the method of Hashimoto.

The agglutination method may be employed to detect either antigens or antigen specific immunoglobulins. To detect antigens, a multivalent liposomal reagent with antibody having a specificity for the antigen is employed. To detect antigen specific immunoglobulins, a multivalent liposomal reagent with antibody having a specificity for the antigen specific immunoglobulins, is employed.

What is claimed is:

1. A liposomal immunoassay for detecting the presence of an antigen within a fluid sample comprising the following steps:

Step 1: simultaneously contacting the fluid sample with a solid phase antibody and a liposomal reagent, the solid phase antibody including a first antibody which has a specificity for the antigen and which is immobilized onto a solid phase, the liposomal reagent including a liposome which contains a label and which has an outer surface to which is attached a second antibody having a specificity for the antigen, the contacting of said Step 1 causing, when in the presence of the antigen, the formation of a complex which includes the antigen, the solid phase antibody, and the liposomal reagent; then

Step 2: separating the complex from the fluid sample and unreacted liposomal reagent; and then

Step 3: detecting the label of the liposomal reagent for assaying the separated complex and correlating the separated complex with the presence of antigen within the fluid sample.

2. A liposomal immunoassay for detecting the presence of an antigen specific immunoglobulin within a fluid sample comprising the following steps:

Step 1: simultaneously contacting the fluid sample with a solid phase anti-idiotypic antibody and a liposomal reagent, the solid phase anti-idiotypic antibody including a first anti-idiotypic antibody which has a specificity for the antigen specific immunoglobulin and which is immobilized onto a solid phase, the liposomal reagent including a liposome which contains a label and which has an outer surface to which is attached a second anti-idiotypic antibody having a specificity for the antigen specific immunoglobulin, the contacting of said Step 1 causing, when in the presence of the antigen specific immunoglobulin, the formation of a complex which includes the antigen specific immunoglobulin, the solid phase anti-idiotypic antibody, and the liposomal reagent; then

Step 2: separating the complex from the fluid sample and unreacted liposomal reagent; and then

Step 3: detecting the label of the liposomal reagent for assaying the separated complex and correlating the separated complex with the presence of antigen specific immunoglobulin within the fluid sample.

3. A liposomal immunoassay as described in claim 1 and/or 2 comprising the following further step between said Step 1 and said Step 2:

Step 1.1: sedimenting the fluid sample and liposomal reagent.

4. A liposomal immunoassay for detecting the presence of an antigen within a fluid sample comprising the following steps:

Step 1: contacting the fluid sample with a solid phase antibody, the solid phase antibody including a first antibody which has a specificity for the antigen and which is immobilized onto a solid phase, the contacting of said Step 1 causing, when in the presence of the antigen, the formation of a first complex which includes the antigen and the solid phase antibody; then

Step 2: contacting the first complex with a liposomal reagent, the liposomal reagent including a liposome which contains a label and which has an outer surface to which is attached a second antibody having a specificity for the antigen, the contacting of said Step 2 causing the formation of a second complex which includes the first complex and the liposomal reagent; then

Step 3: separating the second complex from the fluid sample and unreacted liposomal reagent; and then

Step 4: detecting the label of the liposomal reagent for assaying the separated second complex and correlating the separated second complex with the presence of antigen within the fluid sample.

5. A liposomal immunoassay for detecting the presence of an antigen specific immunoglobulin within a fluid sample comprising the following steps:

Step 1: contacting the fluid sample with the solid phase anti-idiotypic antibody, the solid phase anti-idiotypic antibody including a first anti-idiotypic antibody which has a specificity for the antigen specific immunoglobulin and which is immobilized onto a solid phase, the contacting of said Step 1 causing, when in the presence of the antigen specific immunoglobulin, the formation of a first complex which includes the antigen specific immunoglobulin and the solid phase anti-idiotypic antibody; then

Step 2: contacting the first complex with a liposomal reagent, the liposomal reagent including a liposome which contains a label and which has an outer surface to which is attached a second anti-idiotypic antibody having a specificity for the antigen specific immunoglobulin, the contacting of said Step 2 causing the formation of a second complex which includes the first complex and the liposomal reagent; then

Step 3: separating the second complex from the fluid sample and unreacted liposomal reagent; and then

Step 4: detecting the label of the liposomal reagent for assaying the separated second complex and correlating the separated second complex with the presence of antigen specific immunoglobulin within the fluid sample.

6. A liposomal immunoassay as described in claim 4 and/or 5 comprising the following further step between said Step 2 and said Step 3:

Step 2.1: sedimenting the fluid sample and liposomal reagent.

7. A liposomal immunoassay for detecting the presence of an antigen within a fluid sample comprising the following steps:

Step 1: contacting the fluid sample with a liposomal reagent, the liposomal reagent including a liposome which contains a label and which has an outer surface to which is attached a first antibody having a specificity for the antigen, the contacting of said Step 1 causing, when in the presence of the antigen, the formation of a first complex which includes the antigen and the liposomal reagent; then

Step 2: contacting the first complex with a solid phase antibody, the solid phase antibody including a second antibody which has a specificity for the antigen and which is immobilized onto a solid phase, the contacting of said Step 2 causing the formation of a second complex which includes the first complex and the solid phase antibody; then

Step 3: separating the second complex from the fluid sample and unreacted liposomal reagent; and then

Step 4: detecting the label of the liposomal reagent for assaying the separated second complex and correlating the separated second complex with the presence of antigen within the fluid sample.

8. A liposomal immunoassay for detecting the presence of an antigen specific immunoglobulin within a fluid sample comprising the following steps:

Step 1: contacting the fluid sample with a liposomal reagent, the liposomal reagent including a liposome which contains a label and which has an outer surface to which is attached a first anti-idiotypic antibody having a specificity for the antigen specific immunoglobulin, the contacting of said Step 1 causing, when in the presence of the antigen specific immunoglobulin, the formation of a first complex which includes the antigen specific immunoglobulin and the liposomal reagent; then

Step 2: contacting the first complex with the solid phase anti-idiotypic antibody, the solid phase anti-idiotypic antibody including a second anti-idiotypic antibody which has a specificity for the antigen specific immunoglobulin and which is immobilized onto a solid phase, the contacting of said Step 2 causing the formation of a second complex which includes the first complex and the solid phase anti-idiotypic antibody; then

Step 3: separating the second complex from the fluid sample and unreacted liposomal reagent; and then

Step 4: detecting the label of the liposomal reagent for assaying the separated second complex and correlating the separated second complex with the presence of antigen specific immunoglobulin within the fluid sample.

9. A liposomal immunoassay as described in claim 7 and/or 8 comprising the following further step between said Step 2 and said Step 3:

Step 2.1: sedimenting the fluid sample and liposomal reagent.

10. A liposomal immunoassay for detecting the presence of a soluble antigen within a fluid sample comprising the following steps:

Step 1: contacting the fluid sample with a multivalent liposomal reagent, the multivalent liposomal reagent including a liposome which contains a label and which has an outer surface to which is attached two or more first antibodies having specificity for the soluble antigen, the contacting of said Step 1 causing, when in the presence of the soluble antigen, an agglutination of the multivalent liposomal reagent so as to form an agglutinized complex with the soluble antigen; then

Step 2: separating the agglutinized complex from the fluid sample and unreacted multivalent liposomal reagent; and then

Step 3: detecting the label of the multivalent liposomal reagent for assaying the separated agglutinized complex and correlating the separated agglutinized complex with the presence of soluble antigen within the fluid sample.

11. A liposomal immunoassay for detecting the presence of an antigen specific immunoglobulin within a fluid sample comprising the following steps:

Step 1: contacting the fluid sample with a multivalent liposomal reagent, the multivalent liposomal reagent including a liposome which contains a label and which has an outer surface to which is attached two or more first anti-idiotypic antibodies having a specificity for the antigen specific immunoglobulin, the contacting of said Step 1 causing, when in the presence of the antigen specific immunoglobulin, an agglutination of the multivalent liposomal reagent so as to form an agglutinized complex with the antigen specific immunoglobulin;

Step 2: separating the agglutinized complex from the fluid sample and unreacted multivalent liposomal reagent; and then

Step 3: detecting the label of the multivalent liposomal reagent for assaying the separated agglutinized complex and correlating the separated agglutinized complex with the presence of antigen specific immunoglobulin within the fluid sample.

12. A liposomal immunoassay as described in claim 10 and/or 11 wherein the multivalent liposomal reagent is a highly multivalent liposomal reagent.

13. A liposomal reagent for use in a liposomal immunoassay for detecting a soluble antigen within a liquid sample, the liposomal reagent comprising:

a liposome,

highly multiple antibodies with at least two of said highly multiple antibodies having different epitopic specificities for the soluble antigen,

linkage members which link each of said highly multiple antibodies to the outer surface of said liposome in a fashion which leaves said antibodies free to bind the soluble antigen within the liquid sample, and

a label contained by said liposome.

14. A liposomal reagent for use in a liposomal immunoassay for detecting an antigen specific immunoglobulin within a liquid sample, the liposomal reagent comprising:

a liposome,

multiple anti-idiotypic antibodies, each of said multiple anti-idiotypic antibodies having a specificity for the antigen specific immunoglobulin,

linkage members which link each of said anti-idiotypic antibodies to the outer surface of said liposome in a fashion which leaves said antibody free to bind the antigen specific immunoglobulin within the liquid sample, and

a label contained by said liposome.

15. A liposomal reagent as described in claim 14 wherein:

said multiple antibodies are highly multiple and

said multiple linkage members are highly multiple for rendering the liposomal reagent highly multivalent.

16. A liposomal immunoassy kit for detecting antigen comprising:

a liposomal reagent including a liposome, a label contained by the liposome, and a first antibody attached to the outer surface of the liposome, the first antibody having a specificity for the antigen, and

a solid phase antibody which includes a second antibody which has a specificity for the antigen and which is immobilized onto a solid phase.

17. A liposomal immunoassy kit for detecting antigen specific immunoglobulin comprising:

a liposomal reagent including a liposome, a label contained by the liposome, and a first antibody attached to the outer surface of the liposome, the first antibody having a specificity for the antigen specific immunoglobulin, and

a solid phase antibody which includes a second antibody which has a specificity for the antigen specific immunoglobulin and which is immobilized onto a solid phase.