

## IMMUNOASSAY PRODUCTS AND METHODS

### RELATED APPLICATION

This application is a continuation-in-part of application Ser. No. 117,864, filed Feb. 4, 1980, abandoned.

### BACKGROUND OF THE INVENTION

There has always been a need for high volume screening assays to identify the presence or absence of antigenic materials, antibodies and analytes in a large number of different sampling situations. Various test methods have been used in the past including gas chromatography, mass spectrometry, liquid chromatography and various bioassay methods. Often these methods are time consuming, expensive and cannot be applied to large scale screening programs in an efficient manner.

It has been suggested that immunoassay methods could be used for such screening since immunoassays are known to be easily designed to be specific, highly sensitive and simple to perform. Radioimmunoassays for example have found a large market and use in connection with clinical diagnostics. However, RIA procedures are often incompatible with large scale screening programs. Radiotracers used have inherently limited stability and special disposal and personnel screening procedures are often required. Sophisticated instrumentation is often necessary. For certain uses RIA may create potential hazards as in food processing environments.

Other techniques have been developed such as fluorescent or enzymatic immunoassay techniques which are useful in that potentially hazardous reagents are avoided. However, often these methods require separation by filtration or centrifugation steps in procedures used. Such separations make test procedures inherently slower and difficult to automate.

In a more recent development, enzyme labeled antigen is used which requires no bound-free separation and thus can be performed quickly with excellent sensitivity. Such a system can be automated for high volume assays as in EMIT system disclosed by Rosenthal, A. F., Vargas, M. G. and Klass, C. S. (1976) Clin Chem. 22, 1899. This system utilizes a mode of coupling antigen to enzyme which is quite critical and can result in the system being not readily adapted to different analyses without extensive development for each new system.

Recently, there have been reports of liposomes which can carry enzymes or substrates and be labeled with antigens or antibodies. Liposomes labeled with antigens at their external surface and containing an enzyme entrapped in their internal volume are reportedly mixed with cognate antibody and complement to determine whether or not the liposomes permit release of the entrapped enzyme. This determination is reported made by detecting enzymatic activity which is physically released from the liposomes after separating liposomes from surrounding medium. See Uemura, K. and Kinsky, S. C. (1972) Biochemistry, 11, 4085-4094 and Kataoka, T., Williamson, J. and Kinsky, S. (1973) Biochemics et Biophysica Acta 298, 158-179. However, there has been no recognition that such liposomes when suitably formed with suitable high signal to noise ratios can be useful for immunoassay procedures which avoid the use of separation steps and permit testing in homogeneous phase reactions. Moreover there are reported difficulties in preparing prior art immunospecific liposomes, G. H. Strejan, P. M. Smith, C. W. Grant and D. Surlan,

"Naturally Occurring Antibodies to Liposomes", The Journal of Immunology, Vol. 123, No. 1, July 1979, 370-378. Furthermore, it has long been established that diffusion of macromolecules such as enzymes through lesions produced by complement in bilayer membranes is very much slower than that of small molecules (Green, H., Barrow, P. and Goldberg, B. [1959] J. Exp. Med. 110, 699).

### SUMMARY OF THE INVENTION

It is an object of this invention to provide immunoassay products and methods for use in rapid and simplified testing procedures which can quantitatively and/or qualitatively determine the presence or absence of antigenic materials or antibodies.

It is another object of this invention to provide methods in accordance with the preceding object which can be carried out by relatively untrained personnel with test results determined in a single step with ease of resulting readout and without the need for any separation step after the test reaction.

It is another object of this invention to provide a homogeneous phase reaction in which antigen or antibody-tagged enzymeladen liposomes are immunospecifically caused to release enzyme in the presence of cognate antigen or antibody and active complement.

It is still another object of this invention to provide liposomes labeled with an antigen or antibody and carrying an enzyme yet having a signal to noise ratio no less than 10 and preferably having a stability of at least about 60 days when carried in a liquid.

According to the invention a liposome is labeled with an antigen or antibody and carries an enzyme, yet, has a signal to noise ratio of no less than 10. The enzyme is encapsulated within the liposome. Preferably the liposome is carried in a liquid media and is stable for a period of at least 60 days. Preferably the liposome signal to noise ratio is high and above 60. with stability over six months at 4° C. under inert gas atmosphere. In a kit form the liposome of this invention is sold along with vials of cognate antibodies of antigen which are immunospecific for the antigen or antibody attached to the surface of the liposome, and complement.

According to the method of this invention, an immunoassay method comprises forming a mixture of (a) liposomes labeled with an antigen or antibody carrying an enzyme and having a signal to noise ratio of no less than 10, (b) a substrate for said enzyme, (c) a test material to be tested for specific antigen or antibody activity and (d) complement. The mixture is observed and the presence of enzymatic activity detected as by color change visible to the eye, spectroscopic readout or the like. Preferably, additional cognate antigen or antibody as attached to the liposomes is admixed with the mixture and the test is carried out for the same antigen or antibody as is attached to the liposomes. If immunospecific antigen or antibody tested for, is present in the test material, the free antibody or antigen as the case may be, in the mixture reacts with that antigen or antibody leaving the liposome intact, thus preventing complement attack while if the cognate is not present in the test material the liposome label is reacted and enzyme activity becomes detectable. The amount of cognate in the test sample if present can permit some complement attack if insufficient to react with all of the free cognate in the test mixture, and a portion of the enzymatic activity can then be detected.