

IMMUNO-AGGLUTINATION PARTICLE SUSPENSIONS

The present invention relates to tests which employ antibodies to detect the presence of particular antigenic substances and more particularly to improved suspensions of antibody molecules bound to solid support particles which agglutinate in the presence of the particular antigen to which the antibody is reactive.

BACKGROUND OF THE INVENTION

Antibodies are large proteinaceous molecules that are produced by animals in response to the presence of a foreign substance for the purpose of neutralizing that substance. An antibody molecule may be highly specific, recognizing only a certain site of a particular molecule which is the antigen to that antibody. Because of their high specificities, antibodies are very useful in ascertaining the presence or absence of various antigenic substances, and a number of test procedures, such as radioimmunoassays, have been developed to take advantage of the specificity of the antibodies. Relatively recently, monoclonal antibodies have been developed, providing a practical method for assuring that an antibody fraction contains only a single type of antibody molecule. Monoclonal antibodies may be substituted for conventional antibody fractions in most diagnostic tests, providing greater accuracy and reliability than tests which utilize conventional antibody fractions.

One particular type of test which has been developed that is particularly useful for detecting the presence of large antigens having multiple antibody-recognition sites (antigenic determinants), is an agglutination test. Antibody molecules are bound to minute particles formed of a polymer, and the particle-bound antibody is suspended in a liquid medium. In the presence of the antigen, the particle-bound antibody attaches to the recognition sites on the antigen. If antibody molecules on more than one particle attach to the same antigen molecule, the particles become cross-linked, and as multiple particles cross-link, they agglutinate and precipitate from the solution. Agglutination and precipitation of the suspended particles is readily observable by the naked eye, providing a very simple and very certain test that a particular antigen is present.

One currently used method of attaching antibodies to polymer particles is described in Molday, R. S., W. J. Dreyer, A. Rembaum, and S. P. S. Yen; "New Immunolabel Spheres: Visual Makers of Antigens on Lymphocytes for Scanning Electron Microscopy", *J. Cell Biol* (1975) 64, pp. 75-88. Carboxylate derivatized latex particles are reacted with the antibody in the presence of a carbodiimide, coupling amino groups on the antibody to the carboxyl groups on the peptide. This procedure is useful for binding relatively small antibody or immunoglobulin molecules, such as IgG, but does not work in acceptable fashion for binding larger antibody molecules such as IgM. Furthermore, this method provides no method for controlling the amount of antibody that binds to the particle. If excessive amounts of antibody bind to the particles, the particles may tend to agglutinate prematurely, i.e., before introduction of the antigen.

Another method of binding antibodies to latex particles is through adsorption of antibodies to the surface of latex particles, as is described in Carel J. Van Oss and J. M. Singer; "The Binding of Immune Globulins and

Other proteins by Polystyrene Latex Particles", *J. Reticuloendothelial Soc.* (1966) 3, pp. 29-40. The success of this procedure depends to a great extent on the exact lot of the latex, different lots having vastly different adsorptive properties and stabilities. Because of the unpredictability of results, this procedure is used largely for larger antibody molecules, such as IgM.

It would be desirable to provide latex particles having bound antibody which can be more reproducibly manufactured, irrespective of the type of antibody and irrespective of the adsorptive properties of the particular lot of latex particles. It would be further desirable to control the number of antibody molecules binding to the particles to assure that premature agglutination will not occur due to the particles having excessive bound antibody molecules and yet assure that there are sufficient bound antibody molecules to agglutinate the particles rapidly in the presence of the antigen.

SUMMARY OF THE INVENTION

The invention provides a diagnostic composition comprising a suspension of carboxylate-derivatized latex particles bound to antibody molecules through avidin-biotin bridges. The latex particles, having multiple free carboxyl groups on their surfaces, are bound to avidin using a carbodiimide intermediate. Biotin is covalently bound to the antibody. A mixture of biotin and biotinylated antibody in a predetermined molar ratio are reacted with the latex-bound avidin, linking antibody moieties to a selected portion of the avidin binding sites. The number of antibody molecules attached through avidin-biotin bridges to the latex is controlled to assure that the particles remain in suspension until they cross-link and agglutinate in the presence of an antigen to the antibody.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In accordance with the invention, diagnostic particles are provided in which antibodies are attached to carboxylate-derivatized latex particles through avidin-biotin bridges. The diagnostic particles comprise carboxylate-derivatized polymeric core particles, avidin moieties linked through amide bonds to the core particles, biotin moieties complexed to the avidin moieties and antibody (immunoglobulin) molecules linked to the biotin moieties through amide bonds. The diagnostic particles are suspended in a liquid medium to form a latex composition that is useful for diagnosing the presence of an antigen to which the bound antibody is specific. The diagnostic particles suspended in the liquid medium agglutinate by cross-linking through antigen molecules that have multiple antigenic determinants that are recognized by the antibody, and the cross-linked diagnostic particles precipitate from the liquid medium.

To form the diagnostic particles, carboxylate-derivatized latex core particles are attached to avidin through an amide bond formed between the surface carboxyl groups of the polymeric core particle and primary amino groups on the avidin. An antibody of interest is attached to biotin through an amide bond formed by the carboxyl group of biotinic acid and an amine group of the antibody. Avidin has a very strong affinity for biotin, and the antibody-bound biotin moiety readily complexes to an avidin moiety linked to the core particle. To assure that the diagnostic particles will be stable as a latex suspension until agglutination testing, the number