

## IMMUNOASSAY INVOLVING SOLUBLE COMPLEX OF SECOND ANTIBODY AND LABELED BINDING PROTEIN

### BACKGROUND OF THE INVENTION

The present invention relates to a novel immunoassay. More particularly, the invention relates to an immunoassay which employs a labeled binding protein that nonspecifically binds with the  $F_c$  portion of the IgG of several species and classes of immunoglobulins.

Immunoassays involving the specific interactions between antibodies, antigens, haptens, and other binding proteins have been widely employed for various analytical procedures, particularly in clinical laboratories. The sensitivity and specificity of immunological reactions permit their use in analytical procedures that require the differentiation of small concentrations of substances that are very similar in chemical structure. These assays are widely used in clinical situations for determining concentrations of enzymes, hormones, antibodies, antigens, and the like in biological fluids, for the diagnosis of numerous diseases and disorders. They are also employed for monitoring the concentrations of therapeutic drugs or for determining the presence of illicit drugs in fluids such as blood or urine.

The reaction between an antibody and an antigen or hapten (hereinafter collectively called a ligand) is generally not directly measurable. That is, the reaction product does not exhibit any physical or chemical properties that permit it to be distinguished from the unreacted materials, using conventional procedures. Therefore, one of the reactants is usually labeled in a way that permits its detection conveniently. For example, antibodies or ligands have been labeled with radioactive isotopes, such as  $^{125}\text{I}$ , tritium,  $^{14}\text{C}$  and the like for use in radioimmunoassays. An important group of immunoassays is based on the use of an enzyme as a labeling substance. Enzymes can be covalently attached to an antibody or ligand in such a way that their enzyme activity is retained. The presence of an enzyme-labeled substance can be determined by the action of the enzyme on a substrate. Preferred enzyme substrates are those which, upon reaction with the enzyme, release a chromophore, i.e., a substance that absorbs visual or ultraviolet light or which fluoresces, and thus can be accurately measured using conventional analytical equipment. In addition to radioactive labels and enzyme labels, antibodies and ligands can also be labeled directly with chromogenic or fluorescent substances (e.g., fluorescein), as is known in the art.

A requirement that is common to each of these types of immunoassays is that the unbound labeled reactant be separated from the bound labeled reactant after the specific binding reaction has taken place. This separation is typically accomplished by fixing one or more of the reactants to a solid support. By this technique, the unbound labeled reactant can be eliminated from the system simply by washing it away from the solid support. Labeled reactant on the support or in the solution can then be determined quantitatively.

Immunoassays fall into two main categories: indirect or competitive assays and direct or sandwich assays. In a competitive assay, an analyte and a known concentration of a labeled analyte compete for a limited number of binding sites present on a solid surface which has been coated with the binding partner of the analyte. The amount of labeled reactant that binds to the solid

surface is, therefore, inversely proportional to the concentration of analyte in the unknown sample. In a direct or sandwich assay, again, one reactant of the binding reaction is immobilized on a solid support. The other reactant, which is the analyte of interest, is exposed to the support under binding conditions, and therefore becomes immobilized by reaction with the bound material. A labeled material which is capable of binding to the immobilized analyte is then contacted with the solid surface and binds to the immobilized analyte in an amount directly proportional to the concentration of that analyte on the solid support.

A variety of solid supports have been devised for immunoassays. Such supports include the surfaces of plastic test tubes, polyacrylamide beads, plastic finned devices, latex particles, cellulose or glass fiber pads, and many others.

Essential elements of the above-described reactions are the labeled reactants. The preparation of these reactants is often a tedious and expensive procedure, and adds to the cost of the analytical system. Generally, the reactant to be labeled must be purified and reacted with the labeling substance by a relatively complex chemical reaction. Moreover, each labeled reactant is usually specific for a single test.

The requirement for a specific labeled reagent for each immunochemical test has been partially alleviated by the discovery of bacterial binding proteins that are capable of binding to the  $F_c$  portion of immunoglobulins. The most widely used of such proteins is so-called protein A which is derived from the microorganism, *Staphylococcus aureus*. (See U.S. Pat. No. 3,966,898, Sjoquist, et al.) This protein has been found to bind to the  $F_c$  or heavy chain portion of IgG immunoglobulins from a variety of species. The binding affinity of the protein is somewhat species selective, and within a species, can be selective for certain subclasses of IgG. For example, protein A has a very high affinity for subclasses 1, 2 and 4 of human IgG, a high affinity for all subclasses of rabbit IgG, a weak affinity for the IgG of goat, rat, sheep and mouse and negligible affinity for the IgG of chicken. As used herein, the term binding protein is meant to include protein A and proteins derived from microbial or other sources, having similar activities, as well as fragments of such proteins or polypeptides that have an affinity for the  $F_c$  portion of certain IgG immunoglobulins, without affecting the specificity of the binding affinity of the  $F(ab')_2$  portion of these immunoglobulins.

The specific binding affinity of such binding proteins permits those materials to be labeled and used in a wide variety of immunoassay systems, thereby eliminating the necessity for specifically labeled antibodies or ligands for each test. For example, Sjoquist, et al., supra describe direct immunoassays employing protein A labeled with a radioactive isotope or an enzyme. The assays involve first, binding an antigen to a solid support, then exposing the solid support to an IgG antibody for the antigen and finally exposing the immobilized antigen-antibody complex to labeled protein A. The test can be designed such that the amount of bound labeled protein A can be correlated to either the amount of antigen originally bound to the support or to the amount of antibody. A competitive type of immunoassay employing labeled protein A is described by John J. Langone, et al. (*Journal of Immunological Methods*, 18, 281-283 (1977); 24, 269-285 (1978); and 34, 93-106