

# IMMUNOASSAY WHEREIN LABELED ANTIBODY IS DISPLACED FROM IMMOBILIZED ANALYTE-ANALOGUE

## BACKGROUND OF THE INVENTION

### 1. Field of the Invention

This invention relates to heterogeneous immunoassays for analytes having antigenic or haptenic properties, and more specifically to a heterogeneous immunoassay in which an analyte in a sample preferentially binds to and displaces labeled antibody bound to an analyte-analogue immobilized on a solid phase.

### 2. Description of Background Art

A large and expanding market exists for clinical laboratory diagnostic tests which can be used for the rapid and accurate determination of the concentrations of various organic analytes present in biological fluids, frequently present at micromolar concentrations or less.

In recent years, a number of immunoassay techniques have been developed for the measurement of such analytes. These immunoassays are based on the haptenic or antigenic property of the analytes, i.e., the ability to elicit specific anti-analyte antibody when injected into an animal host. A typical competitive binding immunoassay utilizes a labeled analyte, unlabeled analyte and an anti-analyte antibody, all of which participate in competitive binding reactions to produce two species of the labeled analyte, a bound-species and a free-species. The relative amounts of bound-species and free-species are a function of the analyte concentration in the test sample.

If the labeled analyte in the bound-species and that in the free-species are essentially indistinguishable by the means used to measure the label, the bound-species and free-species must be physically separated. The type of assay in which a separation step is required is referred to as a heterogeneous assay.

Two widely used heterogeneous immunoassays are the radioimmunoassay (RIA) and the enzyme-linked immunosorbent assay (ELISA). In the RIA, a sample containing an unknown amount of unlabeled analyte is mixed with a known amount of radiolabeled analyte and anti-analyte antibody. The system is allowed to react to near-equilibrium and then a separation step is employed to separate the antibody-bound analyte from the free analyte. Since unlabeled analyte and labeled analyte compete for a limited number of antibody binding sites, the more unlabeled analyte, the less labeled analyte will be detected in the bound fraction (or the more in the free fraction). This process is generally time-consuming (1-3 hours) and labor intensive.

Recently, the RIA has been automated by immobilizing the antibody on a porous support. After the sample containing unlabeled analyte is mixed with a known amount of labeled analyte, the sample is percolated through a column containing a limited number of immobilized antibody binding sites. Either the free or bound label can be quantified. One disadvantage of this method is that it requires very precise column synthesis in order to insure accurate and reproducible measurements of analyte concentration.

ELISA is similar in principle to RIA except that the labeling substance is an enzyme such as  $\beta$ -galactosidase or alkaline phosphatase rather than a radioisotope.

In addition to enzymes and isotopes, numerous other labeling substances have been described in the literature. These include fluorophores (e.g., fluorescein, rhodamine), coenzymes (e.g., FAD), bio- and chemi-

luminescent materials (e.g., luciferin), enzyme inhibitors (e.g., phosphonates), etc.

The use of an affinity column to effect the separation step has been described in French Patent Appl. No. 79,15992, published Jan. 9, 1981. It discloses the use of a gel having coupled to it a ligand which has affinity for the labeling substance and which additionally has molecular sieving properties. The use of a gel having affinity for the ligand of interest rather than for the labeling substance and having molecular sieving properties is also contemplated.

U.S. Pat. No. 3,654,090, issued Apr. 4, 1972, to Schurrs et al., describes a noncompetitive heterogeneous immunoassay for human chorionic gonadotropin (HCG) which uses an excess of enzyme-labeled divalent antibody and a column having immobilized HCG to accomplish the separation step. This assay is limited in sensitivity by the fact that one cannot distinguish between an antibody with one molecule of HCG bound and an antibody with no HCG bound. Both species will be retained by the affinity column.

Girma et al., Brit. J. Haematol., Volume 47, 269 (1981), describe a two-site radioimmunoassay (IRMA) for coagulation factor VIII in which monovalent Fab fragments of antibodies are used. Their results indicate that a ten-fold higher sensitivity can be attained using monovalent rather than divalent antibodies.

U.S. Pat. No. 4,298,593, issued Nov. 3, 1981 to Ling, discloses a method for immunochemically assaying a member of an antigen-antibody binding pair which utilizes labeled antigen having a plurality of antigenic binding sites immunochemically bound to labeled Fab as the indicating reagent. This assay suffers from the disadvantage that haptens (having only a single antigenic binding site) cannot be used.

U.S. Pat. No. 4,200,436, issued Apr. 29, 1980 to Mochida et al., discloses an immunochemical measuring process using a labeled, monovalent antibody and an insolubilized antigen. This assay suffers from the disadvantage that sample containing analyte must be pre-incubated with the anti-analyte/label conjugate. Pre-incubation steps are time-consuming and difficult to automate.

Cocola et al., *Analyt. Biochem.*, Volume 99, 121 (1979), describe a release radioimmunoassay for the determination of human chorionic somatomammotropin wherein a radiolabeled antigen is released from a labeled antigen/antibody complex-coated cellulose disk in an amount proportional to the amount of antigen present in the test sample. This method suffers from the disadvantage that the affinity of the immobilized antibody for labeled and unlabeled antigen is the same. Thus, in order to get substantial release of the label, long incubation times (18 hours) or inclusion of a chaotropic agent (4M urea) are desirable.

There is a need for a heterogeneous immunoassay which does not require a pre-incubation step and which is readily adaptable to automated or semi-automated instrumentation.

## SUMMARY OF THE INVENTION

The method of this invention comprises the following steps:

- (1) contacting a liquid sample suspected of containing analyte with a solid phase having immobilized thereon an analyte-analogue to which there is displaceably bound a labeled, anti-analyte antibody in