

HETEROGENEOUS IMMUNOASSAY FOR DIGOXIN USING OUABAIN AS A SEPARATION MEANS

RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Ser. No. 538,772 filed Oct. 3, 1983, now abandoned.

TECHNICAL FIELD

This invention relates to an improved immunoassay for digoxin and, more specifically, to a noncompetitive heterogeneous immunoassay employing a labeled, monovalent or divalent anti-digoxin antibody as the indicator reagent and a column of immobilized ouabain as the means for effecting a separation.

BACKGROUND ART

A large and expanding market exists for clinical laboratory diagnostic tests capable of determining rapidly and accurately the concentration of digoxin present in biological fluids. Digoxin frequently is present at concentrations of nanomolar or less.

In recent years, a number of immunoassay techniques have been developed for the measurement of clinically important ligands. Typically, a competitive binding immunoassay consists of a conjugate of a labeling substance linked to a binding component which participates in a binding reaction to produce two species of the labeled conjugate, a bound species and a free species. The relative amounts of the labeled conjugate that result in the bound species and the free species are a function of the concentration of the ligand to be detected in the test sample.

Where the labeled conjugate in the bound species and that in the free species are essentially indistinguishable by the means used to measure the labeling substance, the bound species and the free species must be physically separated. This type of assay is referred to as heterogeneous.

The two most widely used heterogeneous immunoassays are the radioimmunoassay (RIA) and the enzyme linked immunosorbant assay (ELISA). In the RIA, a sample containing an unknown amount of antigen is mixed with a known amount of radiolabeled antigen and antibody. The system is allowed to react to near-equilibrium and then the antibody-bound antigen is separated from the unbound antigen. Since sample antigen competes with the labeled antigen for a limited number of antibody binding sites, the more the antigen in the sample, the less labeled antigen is in the bound fraction (or the more is in the unbound fraction). This process is generally time-consuming (1-3 hours) and labor intensive.

More recently, the RIA has been automated by immobilizing the antibody on a porous support. After the sample suspected of containing antigen is mixed with a known amount of labeled antigen, 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. Although rapid, this assay requires precise metering of antibody if it is to be reproducible.

RIA suffers from 2 major disadvantages: First, the labeling substance employed is a radioisotope which poses numerous problems associated with handling, storage, and disposal. Second, RIA is performed in a competitive mode (i.e., the analyte and the labeled ana-

lyte compete for a limited number of binding sites on the antibody), and therefore the antibody affinity constant limits the sensitivity of the assay, typically in the range of $10^{-8}M$ to $10^{-11}M$.

ELISA is similar in principle to RIA except that the labeling substance is an enzyme rather than a radioisotope. It will suffer from the limitation that sensitivity is a strict function of the antibody affinity constant.

Other labeling substances have been described in addition to isotopes and enzymes. These include fluorophores, coenzymes, bioluminescent materials, enzyme inhibitors, etc.

Various methods of effecting the separation step in heterogeneous immunoassays are known. These include filtration, centrifugation, chromatography, 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 describes 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 disclosed. The assay described can be performed in a competitive or noncompetitive mode.

U.S. Pat. No. 4,298,687, issued Nov. 3, 1981 to Moes, discloses a heterogeneous immunoassay in which the substance to be determined is reacted with a labeled primary binding partner and the amount of unreacted binding partner is then determined by absorption on a solid phase endowed with specific binding properties for the primary binding partner. The primary binding partner is present in limited amount.

U.S. Pat. No. 3,654,090, issued Apr. 4, 1972, to Schuurs et al., describes a noncompetitive heterogeneous immunoassay for human chorionic gonadotropin (HCG) which uses an excess of enzyme-labeled divalent antibody and an immobilized HCG column 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.

U.S. Pat. No. 4,134,792, issued Jan. 16, 1979 to Boguslaski et al., discloses a heterogeneous immunoassay in which the labeled specific binding partner for the ligand of interest is present in excess. The labeled specific binding partner is a divalent antibody and suffers from the same disadvantage cited above.

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

U.S. Pat. No. 4,200,436, issued Apr. 29, 1980 to Mochida et al., discloses an immunoassay employing a labeled monovalent antibody in which immobilized antigen (the same antigen as that to be measured) is used to separate the bound and free fractions. Since it is primarily the bound fraction which is measured, this assay is usually performed in a competitive mode. Hence, sensitivity is limited by the affinity constant of the antibody when the assay is performed according to the preferred mode.

In some cases it is possible to substitute an analyte analogue for analyte in an immunoassay. In general, the