

IMMUNOASSAYS USING F(ab')₂ FRAGMENTS

This is a continuation of application Ser. No. 005,261, now abandoned filed Jan. 22, 1979.

This invention is concerned with immunoassays, more particularly with immunoassays involving the binding properties of immunoglobulins, and with certain reagents useful in such assays.

It is well known that antibodies will bind with the corresponding specific antigens or haptens (Ag), and use is made of this in many immunoassay procedures. For example, human sera can be assayed for the presence therein of a particular Ag by using a corresponding antibody, for example by competitive binding techniques or latex agglutination techniques. These and other techniques are well known in the art.

One problem which can arise in such immunoassay procedures is interference by other proteins present in the serum under assay. In particular, human serum contains rheumatoid factor (RF) and Clq (a component of complement), and both these substances bind with IgG antibodies. Moreover, the amounts of RF and Clq in human sera can vary widely and it is usually necessary, therefore, to treat the sera (prior to said assay) to inactivate or remove endogenous RF and Clq. If this is not done, the assay results (particularly any quantitative results) may be significantly in error.

We have now devised a method of immunoassay involving the binding property of antibodies for antigens (by which term we include haptens), which can be carried out in the presence of RF and/or Clq without interference therefrom. In particular, according to the present invention, the method of immunoassay utilises the F(ab')₂ fragments of the immunoglobulin rather than whole immunoglobulin. The F(ab')₂ fragments of, for example, IgG have the property of specific binding with antigen, but they do not bind with RF or Clq. (The F(c) fragment of IgG is responsible for the binding reaction of IgG with RF or Clq, but not for the binding reaction with a specific antigen.) It will be appreciated that by proceeding according to the method of the invention, the specificity of the immunoglobulin towards a particular Ag is maintained, but without the associated property of binding with RF and/or Clq.

The invention thus provides a method of immunoassay which comprises reacting an antigen in a fluid with the F(ab')₂ fragments of an immunoglobulin which is specific to said antigen, the reaction being effected in the substantial absence of the (whole) immunoglobulin and of F(c) fragments thereof.

The invention also provides a method of assaying a fluid for the presence and/or amount therein of an antigen, which comprises the steps of:

- (a) mixing a sample of the fluid with the F(ab')₂ fragments of an immunoglobulin which is specific to said antigen, to form a reaction mixture, the reaction mixture being substantially free from the whole immunoglobulin and the F(c) fragments thereof;
- (b) incubating the mixture to allow reaction between the said F(ab')₂ fragments and any said antigen present, and
- (c) determining the extent (if any) to which said reaction has occurred in the mixture and thereby the presence and/or amount of said antigen in said fluid sample.

Immunoglobulins such as IgG can be split into their constituent F(ab')₂ and F(c) fragments by methods known in the art, for example by enzymatic digestion using pepsin. The F(ab')₂ fragments can then be separated from the F(c) fragments and used in the method of the invention. One example of the preparation of F(ab')₂ is as follows. Whole IgG is mixed with pepsin (2 mg pepsin per 100 mg IgG) in a 0.1 M acetate buffer of pH 4.5. The mixture is incubated for 24 hours at 37° C. The F(ab')₂ formed is then separated on an Ultrogel AcA 4.4 column, yielding 80-90% of the theoretical yield of F(ab')₂.

The F(ab')₂ immunoglobulin fragments can be used in place of whole immunoglobulin in accordance with the invention in a variety of immunoassay procedures in which a specific reaction occurs between the immunoglobulin and an antigen, but in which no reaction is required between the immunoglobulin and RF or Clq. (It will be appreciated that there are known assays in which RF and Clq is added as a reagent to react with an immunoglobulin. The F(ab')₂ fragments cannot be used simply as replacements for whole immunoglobulin in such assays.) Thus, the F(ab')₂ fragments may be used in solution, in for example, certain competitive binding assays. It is often advantageous to use a radioactive atom or other label in such assays (and other assays) and the F(ab')₂ fragments may carry such a label, the extent of reaction between the antigen and the fragments then being determined utilising the label. In one example of a competitive binding assay of the invention, the antigen-containing fluid is mixed with both labelled and unlabelled F(ab')₂ fragments whereby a competitive binding reaction occurs between the said fragments and the said antigen; and wherein the amount of labelled fragments which either have reacted with the antigen or remain unreacted in the mixture, is analysed whereby the presence and/or amount of antigen in the fluid is determined.

The F(ab')₂ fragments may be used in insolubilised form, i.e. bound (by which we include both absorbed and covalently linked) to a water-insoluble substrate. The nature of the substrate can vary widely: for example it may be in sheet form, or in the form of a hollow tube, or it may be a particulate material. One preferred form or particulate material is magnetically attractable so that, after reaction between the antigen under assay and the fragments, the particulate material may be separated by using a magnetic field. Assays of this type are described in our Belgian Pat. no. 852327 to which reference should be made for further details.

Another preferred form of insolubilised F(ab')₂ fragments is a latex suspension, and such suspensions can be used in place of whole immunoglobulin in latex agglutination tests for antigens. In such tests, a sample of the fluid to be tested for an antigen (for example human serum) is mixed with latex particles which have a coating of an antibody to the said antigen. In the method of the invention, F(ab')₂ fragments are used in place of whole antibody in the coating. Specific binding between the coating and the antigen causes the latex particles to become agglutinated. The extent of agglutination can be observed visually (for qualitative results) or can be quantitated by counting (preferably by automatic counting of the agglutinated, or more preferably the unagglutinated particles).

In another aspect, therefore, the invention includes a method of quantitatively assaying an Ag in a fluid, wherein a sample of the fluid under assay is mixed with