

AUTOMATED DIRECT SERUM RADIOIMMUNOASSAY

BACKGROUND OF THE INVENTION

This invention relates to radioimmunoassay and more specifically to an improved method for the direct assay of antigens such as triiodothyronine (T_3), in serum by the use of an immobilized immunoadsorbent.

STATE OF THE ART

Radioimmunoassay is an analytical technique which depends upon the competition (affinity) of antigen for antigen-binding sites on antibody molecules. In practice, standard curves are constructed from data gathered from a plurality of samples each containing (a) the same known concentration of labelled antigen, and (b) various, but known, concentrations of unlabelled antigen. Antigens are labelled with a radioactive isotope tracer. The mixture is incubated in contact with an antibody, the free antigen is separated from the antibody and the antigen bound thereto, and then, by use of a suitable detector, such as a gamma or beta radiation detector, the percent of either the bound or free labelled antigen or both is determined. This procedure is repeated for a number of samples containing various known concentrations of unlabelled antigens and the results plotted. The percent of bound tracer antigens is plotted as a function of the antigen concentration. Typically, as the total antigen concentration increases the relative amount of the tracer antigen bound to the antibody decreases. After the standard graph is prepared, it is thereafter used to determine the concentration of antigen in samples undergoing analysis.

In actual analysis, the sample in which the concentration of antigen is to be determined is mixed with a known amount of tracer antigen. Tracer antigen is the same antigen known to be in the sample but which has been labelled with a suitable radioactive isotope. The sample with tracer is then incubated in contact with the antibody. Thereafter, it may be counted in a suitable detector which counts the free antigen remaining in the sample. The antigen bound to the antibody or immunoadsorbent may also be similarly counted. Then, from the standard curve, the concentration of antigen in the original sample is determined. Afterwards, the antibody or immunoadsorbent mass is discarded.

In order to detect the percentage of antigen that is bound to the antibody (bound antigen) and/or the percentage that remains free or unbound it is necessary to first separate the sample into a fraction containing bound antigen and one containing only free antigen. One common method for doing this is to add a dextran coated charcoal to the mixture. The dextran permits the unbound antigen, of lower molecular weight than the bound antigen, to pass through the dextran and the charcoal adsorbs the free antigen. The charcoal with adsorbed free antigen is then separated from the antibody (and bound antigen) by centrifugation.

Another known procedure is to add to the mixture another antibody which selectively precipitates the first antibody (with the bound antigen) thus leaving in solution only free antigen. Classification into appropriate free and bound fractions is then effected by separating the precipitate from the supernatant by centrifugation or other suitable means. Some workers have resorted to the technique of binding the antibody to the inner walls of a plastic vessel, filling the vessel with the antigen

bearing sample, allowing it to stand for an incubation period that typically ranges from 4 to 72 hours and then separating free antigen from bound antigen by draining and rinsing the vessel leaving therein only the antibody and bound antigen. A more recently developed technique is to prepare the immunoadsorbent by binding the antibodies onto an insoluble cross-linked dextran. The immunoadsorbent and antigen bearing sample are incubated then the dextran with bound antigen is separated from the solution by suitable means.

In all of the foregoing procedures, the percentage of labelled antigen in either or both the bound or free fractions is determined and the standard curve used to determine the antigen concentration. Thereafter, the immunoadsorbent is discarded.

Although the foregoing radioimmunoassay techniques have proven to be valuable tools and have gained widespread acceptance, they are still not all that are to be desired because the antibody (immunoadsorbent) is consumed with each analysis hence must be discarded. Moreover, prior practice is batch type and the several reagents are added to the antibody in test tubes in which the separate steps, such as incubation, rinsing and the like, are performed, thus resulting in a slow and costly operation.

An improvement of the above described prior procedure is set forth in U.S. Pat. No. 3,896,217 of July 22, 1975, assigned to the same assignee. There, an immobilized immunoadsorbent having covalently bound antibodies is used to bind antigen, followed by release of antigen by flowing an eluting solution through the immunoadsorbent. By this system, the immunoadsorbent may be reused repeatedly.

An automated apparatus is described in U.S. Ser. No. 565,850, filed Apr. 7, 1975, as a continuation-in-part of U.S. Pat. No. 3,896,217 setting forth in more detail the various aspects of the equipment for automated radioimmunoassay by the use of a reusable immobilized immunoadsorbent.

Application Ser. No. 565,848, filed Apr. 7, 1975, describes an improved immunoadsorbent and methods of making and using the same. As there described, the immunoadsorbent includes a particulate substrate having bonded thereto by covalent bonds the antibody specific to the antigen.

Since the systems of the above patents and applications relate principally to a flow-through type of system, one of the practical aspects of the technology is the flow-through character of the various mixtures which pass into and through the immobilized immunoadsorbent. More particularly, if the materials flowing through the immobilized immunoadsorbent, usually placed in a chamber, tend to plug up the flow passages, the use and reuse of the chamber is adversely affected. Such adverse effects are manifest in several different ways including physically plugging to prevent flow and thus interference with subsequent tests by trapping labelled and unlabelled antigen in the column, or by slowing the flow through the chamber sufficiently to extend unreasonably the time for what should be a relatively rapid automative assay, or both.

As will be appreciated, plugging is a characteristic only of those immunoadsorbents which are reusable. With single use immunoadsorbents, to be described, the flow through and repeated flow through of material is not of major consequence.

One source of plugging are the proteins, other than the antigen, which may be present in a sample. While