

METHOD AND APPARATUS FOR RADIOIMMUNOASSAY WITH REGENERATION OF IMMUNOADSORBENT

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates generally to the analytical technique known as radioimmunoassay in which antigens are bound to specific antibodies and, through the use of tracers (labels) and predetermined behavior standards the concentration of antigen in a sample is determined. More particularly, the invention relates to improved methods and apparatus for radioimmunoassay in which a short-cycle time, hence more rapid analysis is achieved, the antibody mass (immuno-adsorbent) is regenerated to be reused indefinitely rather than being wastes and the entire operation is automated.

2. 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 work on 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 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 antigens 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 immuno-adsorbent may also be similarly counted. Then, from the standard curve, the concentration of antigen in the original sample is determined. Afterwards, the antibody or immuno-adsorbent 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 charcoal is allowed to adsorb 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 immuno-adsorbent by binding the antibodies onto an insoluble cross-linked dextran. The immuno-adsorbent 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 immuno-adsorbent 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 (immuno-adsorbent) 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.

SUMMARY OF THE INVENTION

The present invention provides improved method and apparatus for carrying out radioimmunoassay. In accordance with the invention the immuno-adsorbent (antibody) is repeatedly and rapidly regenerated thus obviating the need and therefore the time and cost of constant replacement. According to the invention, steps are incorporated by which the method may be carried on continuously in constantly repeating cycles thereby eliminating the expensive time-consuming batch operation. A novel equipment arrangement for automating the method is also provided.

The invention is predicated on the discoveries that (1) by forming the immuno-adsorbent mass as an immobilized mass of antibodies through which the antigen sample and other reagents may flow the procedure may be sequentially carried out without resort to the use of several manual steps and (2) the immobilized immuno-adsorbent may be regenerated for repeated reuse by the rinsing with a solvent or eluting solution having particular characteristics. That is to say, bound antigens are released so that the antibody may be washed clean of bound antigen and thereby regenerated for reuse without affecting the essential characteristic of the antibody mass such as its ability to permit repeated flow of antigen solution therethrough and its antigen-binding efficiency during a large number of cycles.

As used herein, the term immobilized antibody mass refers to a mass of antibodies held in place in a liquid stream flow path in such a manner that the stream may flow over or through the mass while the latter remains in place.

A suitable antibody mass may be formed from solid surfaces such as glass or water insoluble polymers to