

The advantage of the present invention is the ability to provide relatively rapid and accurate determinations directly from serum for antigens present in relatively small amounts by the use of automated equipment. The preincubation sequence not only reduces on machine time, but permits dilution of the serum so as to reduce substantially the concentration of plugging proteins present in serum. By use of preincubation, the sensitivity of the assay is increased substantially to the point where the antigens present in small amounts may be assayed accurately by a flow through system, as described.

On aspect of the present invention is the provision of an immunoabsorbent in which the amount of covalently bound antibody is in excess of the free unbound labelled and unlabelled antigen which is not bound during the preincubation sequence. Unlike prior disclosed procedures using a solid immunoabsorbent in which only a portion of the antigen (labelled and unlabelled) is bound by the immobilized antibody on the substrate, the preferred procedure of the present invention involves binding to the substrate substantially all of the labelled and unlabelled antigen which is not bound to the antibody during the preincubation sequence. Thus, the binding activity of the solid immunoabsorbent is such that it is at least 80% or more based on the amount of antigen in the sample to be assayed.

For example, based on the data given in the Table, the immunoabsorbent should have sufficient binding activity to bind 2850 pg/ml of T_3 . Unlike the immobilized immunoabsorbents previously disclosed, the immobilized immunoabsorbent useable in accordance with the present invention has a binding activity which is quite high based on the anticipated antigen concentration in the sample. As a practical matter, the binding activity of the immunoabsorbent may be several times the anticipated concentration of the samples in order to provide assurance that substantially all of the labelled and unlabelled antigen not bound in the preincubation sequence are bound during flow through the immunoabsorbent.

It has been noted that antigens such as triiodothyronine and tetraiodothyronine tend to deposit on the surfaces of the various flow lines through which they flow, especially if the flow lines are of plastic material. The accumulation of T_3 or T_4 in the system may adversely affect the sensitivity of the procedure. Thus, in accordance with this invention, a rinse solution is used, after each assay, which releases any T_3 or T_4 which may tend to deposit.

A typical material useable as a rinse solution is 2% solution (by weight) of dimethylammonium linear dodecyl benzene sulfonate (DMS) in a 30% solution (by volume) of methanol in water or 1% DMS in water.

While the above invention has been described with reference to T_3 , it will be understood that the procedures herein described have applicability to those instances in which the antigen is present in relatively small amounts and with proteins which tend to create flow problems through a solid substrate immunoabsorbent, especially if the immunoabsorbent is rinsed.

It will also be apparent to those skilled in the art that various modifications and alternatives may be made without departing from the spirit and scope of the invention as set forth in the appended claims.

I claim:

1. In a process for automated radioimmunoassay of an antigen sample by flowing the sample through a cham-

ber containing an immobilized immunoabsorbent composed of particulate substrate having antibodies specific to the antigen covalently bound thereto, and wherein the sample contains proteins tending to plug up the particulate substrate, the improvement comprising:

5 diluting the sample to a consistency for free unplugged flow through the immobilized immunoabsorbent,

preincubating the sample with an antibody specific to the antigen in said sample being assayed together with a known amount of a radioactive labelled antigen to form a mixture including

- (a) unlabelled antigen bound to the antibody,
- (b) labelled antigen bound to the antibody,
- (c) free unbound unlabelled antigen, and
- (d) free unbound labelled antigen;

providing a chamber containing an immobilized immunoabsorbent including a solid particulate substrate having covalently bound thereto an antibody specific to said antigen and in an amount in excess of the sum of the free unbound labelled and unlabelled antigen,

flowing said mixture through said chamber to form two fractions, one of which is the free unbound labelled and unlabelled antigen bound to the antibody on said substrate and the second being the labelled and unlabelled antigen bound to the antibody during the preincubation step, whereby said second fraction passes through said chamber and said first fraction binds to the immobilized immunoabsorbent,

flowing an eluting solution through said chamber to effect release of said first fraction,

counting the radioactivity of at least one or both of the fractions leaving said chamber as a function of the quantity of the antigen sought to be assayed in said sample and rinsing the chamber for subsequent assays of other samples of the same antigen.

2. In a process as set forth in claim 1 wherein said sample is serum containing proteins tending to plug said chamber.

3. In a process as set forth in claim 1 wherein the binding activity of said immobilized immunoabsorbent is not less than 80% of the concentration of the antigen in the sample.

4. In a process as set forth in claim 2 wherein the steps are carried out at room temperature.

5. In a process as set forth in claim 1 wherein said preincubation is conducted for a period of time sufficient to establish an equilibrium between the labelled and unlabelled antigen bound to the antibody and the free and unbound labelled and unlabelled antigen.

6. A process for the direct radioimmunoassay of T_3 antigen in a serum sample comprising the steps of preincubating an antibody specific to T_3 antigen in the presence of radioactive labelled T_3 antigen and unlabelled T_3 antigen sample to form a mixture including

- (a) unlabelled antigen bound to the antibody,
- (b) labelled antigen bound to the antibody,
- (c) free unbound unlabelled antigen, and (d) free unbound labelled antigen;

passing said mixture through a chamber containing an immobilized immunoabsorbent including a solid particulate substrate having an amount of antibodies covalently bound to said support which is in excess of the amount of the sum of the free unbound labelled and free unbound unlabelled anti-