

which the antibodies are attached by co-valent bonds. A mass of such beads with attached antibodies is supported on a screen in a tube or other hollow column through which the antigen bearing sample and other reagents are flowed sequentially. Co-valent coupling of antibodies or antigens to said carrier is known. (It is disclosed for instance in U.S. Pat. No. 3,652,761).

In accordance with this invention, antibodies, specific to the antigen under analysis, are co-valently coupled to a solid support such as beads, a mass of the beads is immobilized, an antigen bearing solution is flowed therepast, the percentage of free labelled antigen remaining in the solution is detected, the bound antigen is released simultaneously with regeneration of the antibody mass and the percentage of released antigen is also detected. The percentages are determined with reference to the total antigen in the incoming sample. As noted, detection is of the tracer or labelled antigen.

Release of bound antigen and concomitant regeneration or reactivation of the antibody mass is effected by rinsing it with a solvent or eluting solution that breaks the bond between antigen and antibody, but does not break the co-valent bond between the antibody and its support. Moreover, the solvent must not alter either the flow characteristic or antigen affinity of the antibody mass.

Another type of immobilized antibody mass can be formed by encapsulating antibodies in a semi-permeable membrane. A quantity of such microcapsules is then held in place in a column through which the antigen-bearing solution flows. The membranes are selected to block the outward passage of antibodies while permitting the free passage of antigens and their solvent in both directions.

In the case of membrane capsules, since there is no covalent bond, there is no need to be concerned with its destruction by the regenerating solvent. However, the limitation must still be observed that the solvent not adversely affect the flow characteristic or adsorption efficiency of the antibody. It is possible to increase efficiency of the membranes by coupling the antibodies to a soluble polymer thereby increasing the molecular weight of the combined antibody thus enabling the use of a relatively more permeable membrane.

In connection with flow characteristics in either system, the solvent should not cause swelling of the antibody support or the membrane wall to the point where flow of relatively small antigens is blocked or the loss of relatively larger antibodies through the wall occurs.

In summary then, the method of the invention comprises the steps of providing a mass of immobilized specific antibodies to selected antigen, holding such mass in a liquid flow path, preparing an antigen bearing sample by adding a known quantity of labelled antigen to a sample containing an unknown amount of the same antigen, flowing such sample along said liquid flow path over and in contact with said immobilized antibody, thereafter detecting the quantity of free labelled antigen remaining in the sample and/or that which is bound to the antibody.

The quantity of antigen bound to the antibody is determined by rinsing the antibody with a particular solvent to release bound antigens then detecting the antigens in the rinse solution.

The solvents found to meet the requirements of this invention have been hydrophobic in character. Typical

solvents are methyl, ethyl and isopropyl alcohols as well as dimethylformamide.

In its most essential form, the apparatus invention comprises a contact chamber having an inlet and outlet and adapted to accommodate the flow of a liquid stream therethrough, a mass of antibodies, means for immobilizing said antibodies and holding them in position in said contact chamber in the path of antigen flow therethrough, a detection chamber having an inlet and outlet, a detector associated with said chamber for determining the quantity of a given tracer flowing through said chamber, conduit means for conducting liquid from the outlet of said contact chamber to the inlet of said detection chamber, a source of sample solution containing both labelled and unlabelled antigen, a source of regenerating solvent, means for supplying separately and sequentially to the inlet of said contact chamber for flow therethrough a quantity of said sample and of said solvent, and means for controlling the flow of said sample and solvent through said contact chamber and said detection chamber. Means are also provided for introducing a so-called scintillation cocktail into the system between the contact chamber and detector to flow through the latter and therein to convert radioactive decay impulses to light or photons for detection by a photo multiplier detector.

The required flow rates through the contact chamber and detector are determined empirically and are controlled by metering pumps. The flow sequence is controlled by valves. By proper timing the flow through the system may be continuous with the rates being selected to provide sufficient time at each station to achieve the results, such as binding, releasing or counting, sought at each station.

In order that the invention may be more readily understood and carried into effect, reference is made to the accompanying drawing and the description thereof which are offered by way of illustration and not in limitation of the invention, the scope of which is defined by the appended claims and equivalents thereof rather than by any illustrative description.

BRIEF DESCRIPTION OF THE DRAWINGS AND DESCRIPTION OF PREFERRED EMBODIMENT

The FIGURE is a schematic diagram of apparatus embodying the invention.

As illustrated, the system comprises a sample source 10, a source 11 of buffer rinse, a source 12 of regenerating solvent, a source 13 of scintillation cocktail, a rinse water reservoir 14, a contact chamber 16 filled with immobilized antibodies (immunoabsorbent) a mixing chamber 17, and a detector chamber or coil 18 with detector 19 adjacent thereto. The foregoing components are connected together by a series of conduits and flow directing valves which, together with metering pumps and a timing mechanism, regulate flow through the system.

Specifically, the sample source 10 connects via a conduit 21 to a two-position valve 22 which in one position connects to a sample loop 23. The sample loop terminates in a two-position valve 24 which in one position connects to a conduit 26 leading to another two-position valve 27 which in one position connects the conduit 26 and sample loop 23 to an aspirator pump 28 which discharges to waste. A rinse water inlet conduit 29 also connects to the pump 28 via the alternate posi-