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(54) **Solid phase reagent for immunoassay**

(57) The invention relates to a reagent for use in immunoassays, comprising a solid phase support, a primary coating of antibody or antigen on the solid phase support, and a secondary coating of immunologically inert protein over the primary coating.

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SPECIFICATION

Solid phase reagent for immunoassay

This invention relates to a solid phase reagent for use in immunoassays.

Antibodies and antigens are commonly coupled to solid phase supports for use in immunoassays. Various water-insoluble carrier materials can be used as the solid phase support without substantial loss of biological activity. The solid phase immobilised reagent can be readily separated, e.g. by centrifugation, at an appropriate stage in the immunoassay procedure.

Any coupling procedure, for attaching the antibody or antigen to the solid phase support, is by necessity carried out in aqueous medium. However, for convenient handling and stability in storage, it is of value to present the chosen solid phase in a dry form. If a conventional solid phase immunological reagent is simply dried without any preliminary treatment, it is found that the immunological properties deteriorate to an unacceptable extent. An object of the present invention is accordingly to provide a stable, solid phase immunological reagent in dry form.

We have now found that stabilisation of antibody- or antigen-coated solid phase supports can be achieved by employing a proteinaceous coating procedure. Thus, the present invention provides a reagent for use in immunoassays, comprising a solid phase support, a primary coating of antibody or antigen on the solid phase support, and a secondary coating of immunologically inert protein over the primary coating.

In principle, any water-insoluble solid phase support can be used in the present invention. Preferred examples are polystyrene and glass beads. Both covalent and non-covalent bonding methods are known for such solid phase supports and these include the use of silane derivatives as an example of covalent bonding, or simple incubation with a solution containing the appropriate concentration of antibody or antigen as an example of non-covalent bonding.

The protein employed in the secondary coating should have certain characteristics such as; it must be immunologically inert with respect to the antibody or antigen in the primary coating; it must contain no proteolytic enzymes and it must be cheap and readily available in large quantity. Suitable proteins which have been examined include bovine serum albumin, gelatin and degraded gelatin. Such proteins can be bonded to the primary coating (including surfaces which are uncoated during the primary coating process) by simple incubation at room temperature.

Once the secondary coating of protein has been applied, the reagent is finally dried, preferably in a current of warm air or under vacuum. The product can then be conveniently stored at 4°C in a stoppered container and under such conditions it has been found to substantially retain its immunological properties.

The following examples illustrate the invention.

EXAMPLE 1

Anti serum containing antibody to hepatitis B surface antigen (anti HBs) was diluted 1:500 in 0.1M phosphate buffer, pH 7.5. This was added to polystyrene beads of 6.4 mm diameter, previously washed with ethanol. The coating process was allowed to proceed for approximately 10 hours at 4°C. The beads were then washed 3 (three) times with deionised water (DIW).

To obtain stable beads, DIW washed beads were placed in 1% aqueous, protein solution and incubated at room temperature for approximately 15 minutes and finally air dried. Proteins examined included gelatin bovine serum albumin BSA (fraction V).

Assay Procedure: Beads dried in the described manner were utilised in a simultaneous sandwich assay with radio-labelled antibody.

TABLE 1

	% Bound[1]	S/N[2]
Washed beads (wet)	14	44
BSA dried beads	15.1	47
Gelatin dried beads	13.5	43
Dried beads without addition	7.0	23

1. % bound refers to the percentage of total radioactivity bound to a positive control in the simultaneous assay.

2. S/N: Signal to noise ratio is the number of counts of the postive control divided by the counts of the negative control.

EXAMPLE 2

Beads of 6 mm diameter of semi-borosilicate glass had antibody to hepatitis B surface antigen coupled covalently via a known procedure involving silane derivatisation, glutaraldehyde activation and protein coupling. Glass beads having anti HBs thus coupled were dried in air following immersion in 1% BSA in 0.1M phosphate buffer pH 7.5 for approximately 16 hours at 4°C.

TABLE II

	% Bound	S/N
Washed beads	11.76	22.8
BSA dried beads	12.24	22.8
Dried without addition	7.2	10.1

Beads, both glass and polystyrene, dried by the described procedure have been shown to be stable for at least several months, i.e. their functionality in an immunoassay is retained, when stored at 4°C.

EXAMPLE 3

Antibody (anti HBs) purified by chromatographic techniques was adsorbed onto polystyrene beads previously washed with ethanol. The method of absorption was essentially that of Example 1. Antibody solution was incubated with polystyrene beads for approximately 10 hours at 4°C. The beads were then washed 3 (three) times with deionised water. Beads were then immersed in BSA or partially degraded gelatin for 15 minutes prior to drying in a current of air or under vacuum.

TABLE III

		% Bound	S/N
Without addition		11	203
BSA	air dried	21	389
Partially degraded gelatin	air dried	29	446
Partially degraded gelatin vaccum dried		34	502

CLAIMS

1. A reagent for use in immunoassays, comprising a solid phase support, a primary coating of antibody or antigen on the solid phase support, and a secondary coating of immunologically inert protein over the primary coating.

2. A reagent as claimed in claim 1, wherein the solid phase support comprises polystyrene beads or glass beads.

3. A reagent as claimed in claim 1 or 2, wherein the immunologically inert protein of the secondary coating is gelatin or bovine serum albumin or partially degraded gelatin.

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