

SOLID PHASE ASSAY

This invention relates to an assay for a ligand and to products used in such assay. More particularly, this invention relates to a solid phase assay.

Immunoassay methods, in general, are based on the competition between a specific analyte, the amount of which is to be determined in a sample, and a known amount of tracer, which is generally the analyte or appropriate analog thereof in labeled form, with the analyte and tracer competing for a limited number of available binding sites on a binder which is specific towards the analyte and tracer.

If the concentration of tracer and binder is fixed and the only variable is the level of analyte, it is possible to establish an assay system for measuring the unknown level of analyte by determining the amount of bound and/or free tracer in the system. The values determined in the assay are compared with the values given by a range of known amounts of the analyte treated in the same manner, and by such comparison, it is possible to determine the amount of analyte in the sample.

In one such procedure, the binder is supported on a solid support, whereby the bound and free components of the assay, after incubation, may be easily separated by separation of the sample and the solid support.

In general, the tracers used in such assays require either instrumentation and/or treatment of the tracer in order to determine the tracer in the bound and/or free portion of the assay as a measure of analyte. Thus, for example, in an assay in which an enzyme is used as the label or marker for the tracer, the enzyme must be developed with a suitable developer. When the label or marker is a fluorescent material, the tracer in the bound and/or free portion is determined by the use of appropriate instrumentation for determining fluorescence.

Although such assays are effective for determining analyte in a sample, there is always a need for increasing the sensitivity of the assay and for facilitating the overall assay procedure.

In accordance with one aspect of the present invention, there is provided a method and product for determining analyte wherein a binder for at least one of the analyte and tracer to be used in the assay is supported on a test area located on the surface of a solid support wherein the binder is supported on a test area of the solid support in a concentration of at least $1 \mu\text{g}/\text{cm}^2$. The tracer used in the assay is a ligand labeled with a particulate label which contains a detectable marker which is not visible under assay conditions, and wherein the ligand is bound by either the binder or analyte.

More particularly, the solid support which is used in the assay is one which has a surface area (area/unit weight of material) such that the binder can be supported on the support in a concentration (weight/unit area) of at least $1 \mu\text{g}/\text{cm}^2$.

The term "not visible" when referring to the detectable marker as used herein means that the marker cannot be seen without further treatment and/or without the use of instrumentation. thus, for example, a fluorescent marker requires excitation and an enzyme marker requires developing.

In accordance with still another aspect of the present invention, there is provided a method and product for determination analytes which are present in test samples in low concentrations wherein the analyte is detected on a test area located on the surface of a solid support

by use of a tracer, wherein the solid support has a surface area such that the binder is supported on the test area in a concentration of at least $1 \mu\text{g}/\text{cm}^2$. The tracer is a ligand labeled with a particulate label which includes a detectable marker which is not visible, and wherein the ligand is bound by either the binder or the analyte.

The solid support which is employed in the assay is generally a cellulose ester with nitrocellulose giving exceptionally good results. It is to be understood that the term "nitrocellulose" refers to nitric acid esters of cellulose, which may be nitrocellulose alone, or a mixed ester of nitric acid and other acids, and in particular, aliphatic carboxylic acids having from one to seven carbon atoms, with acetic acid being preferred. Such solid supports which are formed from cellulose esterified with nitric acid alone, or a mixture of nitric acid and another acid such as acetic acid, are often referred to as nitrocellulose paper.

Although nitrocellulose is a preferred material for producing the solid support, it is to be understood that other materials, having a surface area sufficient for supporting the binder in a concentration as hereinabove described may also be employed for producing such solid supports.

In general, the support which is used in the assay has a surface area such that it is capable of supporting binder in a concentration of at least $1 \mu\text{g}/\text{cm}^2$, (most generally in a concentration of at least $10 \mu\text{g}/\text{cm}^2$) and preferably at least $40 \mu\text{g}/\text{cm}^2$.

In accordance with a particularly preferred embodiment, the pore size of the solid support is such that the tracer (ligand labeled with a particulate label), when bound to the binder or to the analyte bound to the binder, remains on the surface of the support. Thus, for example, particularly good results have been obtained with a nitrocellulose support having a pore size of from 0.2 to 0.45μ .

Applicant has found that the sensitivity of the assay can be increased by increasing the concentration of binder on the support and, accordingly, supports having high surface areas (such as nitrocellulose) are particularly preferred in that the binder may be supported on such supports in a high concentration. It is to be understood, however, that the concentration of binder which is actually used is dependent in part on the binding affinity of the binder. Accordingly, the scope of the invention is not limited to a particular concentration of binder on the support.

Applicant has further found that it is possible to determine the detectable marker included in the particulate label, such as a sac, without releasing the marker from the sac.

The binder which is supported on the solid support, as hereinabove indicated, is either a binder for both the analyte and tracer, or a binder for only one of the analyte and tracer, with the type of binder which is employed being dependent upon the assay which is to be used for determining the analyte. Thus, for example, if the assay is a competition type of assay, then the binder supported on the solid support would be a binder for both the tracer and analyte, whereby both tracer and analyte would compete for a limited number of binding sites on the binder.

If the assay is a so-called "sandwich" type of assay, then the binder which is supported on the solid support is a binder for only the analyte. In such an assay, the tracer is a tracer which is specific for the analyte,