

FLUORESCENT ASSAYS, INCLUDING IMMUNOASSAYS, WITH FEATURE OF FLOWING SAMPLE

This invention relates to immunoassay, and more particularly to fluorescent immunoassay wherein the evanescent wave produced by total internal reflection is used to restrict the observed volume to a lamina, thereby avoiding a separation or wash step while also reducing the effects of interfering, immunologically non-reacting background substances in the sample.

The use of total internal reflection techniques to reduce the effects of immunologically non-reactive substances in immunoassays has been the subject of a number of investigations. For instance, in U.S. Pat. No. 3,939,350, the evanescent wave produced by total internal reflection at the interface between a totally internally reflecting substrate, such as a plate, and the sample is used to induce fluorescence in a fluorescently tagged portion of the sample immunologically bound to the plate, the portion of the sample beyond the evanescent zone not being excited. In this way, an assay may be performed without the necessity of removing the unreacted sample and reagent. In effect, the evanescent wave functions as a separation mechanism.

As taught in U.S. application Ser. No. 406,324, filed Aug. 9, 1982, U.S. Pat. No. 4,582,809, and assigned to the assignee of the present application, greater optical efficiency may be achieved by observing the fluorescence, excited by the evanescent wave, that reenters (tunnels back into) the optically denser medium and propagates therein by total internal reflection. An improved apparatus of this type and the corresponding method of immunoassay is taught in U.S. Pat. No. 4,447,546, where it is shown that both the amount of sample and the reaction end point may be automatically controlled by surrounding a controlled area of the immunologically activated totally reflecting element (e.g. a known length of an optical fiber of known diameter) with a capillary tube of known dimensions. In this manner, rapid, simple, and accurate non-ballistic assays may be performed.

The measurement time required for incubation in the capillary surrounded fiber apparatus is proportional to the square of the mean diffusion distance. The latter is of the order of the distance between the fiber and the capillary wall. By making this distance smaller, the time required to reach the reaction end point, and hence the speed of a non-ballistic assay may be dramatically improved. However, such a reduction also reduces the sampled volume and thus the total signal per unit length of fiber, and thus deleteriously affects sensitivity. Such loss of sensitivity can only be partially regained by increasing the fiber length due to signal attenuation in an unclad fiber, as required by the present invention.

Accordingly, it is an object of the present invention to provide apparatus and methods for a more rapid, enclosed-fiber, fluorescent immunoassay wherein the sensitivity of the assay is not deleteriously affected by the increased speed.

It is another object of the present invention to provide apparatus and methods for an increased sensitivity fluorescent immunoassay.

These and other objects are met in the present invention of a total internal reflection fluorescence immunoassay wherein the sample is caused to flow along the length of a totally internally reflecting substrate having

an active surface region surrounded by and spaced from an enclosure to provide a fixed volume, the sample flow rate being adjusted to make the dwell time of the sample opposite the active region similar to the time required to scavenge the volume between the substrate and enclosure. In a preferred embodiment, the substrate is an optical fiber and the enclosure is a tube dimensioned and disposed so that the distance between the internal wall of the tube and the fiber surface is of capillary dimensions.

With this structure, a large sample volume may be sampled in relatively short time, with but a small part of the sample being illuminated by the evanescent wave at any one time. The binding to the activated portion of the fiber is cumulative, depending on the titre of the unknown in the sample and the volume scavenged.

Other objects of the invention will in part be obvious and will in part appear hereinafter. The invention accordingly comprises the apparatus possessing the construction, combination of elements, and arrangement of of parts and the method comprising the several steps and the relation of one or more of such steps with respect to each of the others which are exemplified in the following detailed disclosure and the scope of the application of which will be indicated in the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

For a fuller understanding of the nature and objects of the present invention, reference should be to the following detailed description taken in connection with the accompanying drawings wherein:

FIG. 1 is a diagrammatic representation of the apparatus of the present invention as connected to known apparatus for the purpose of this invention;

FIG. 2 is longitudinal cross-sectional view of an immunoassay kit which forms a preferred embodiment of a portion of the apparatus of the present invention; and

FIG. 3 is a stylized view of a portion of the apparatus of FIG. 2, illustrating a typical immunochemical reaction in the realization of the present invention.

In the figures, like index numbers refer to like elements. It should also be noted that the representation in the figures is diagrammatic and no attempt has been made to indicate actual scales or ratios.

With reference to terminology, it will be noted in the detailed description of the apparatus of this invention that portions of the apparatus are referred to as "upper" and "lower" portions. This is done wholly for convenience and to relate the description to the diagrammatic representations in the drawings. It will be appreciated that the apparatus can function in any position or orientation and it is within the scope of this invention to have it do so.

The present invention operates by total reflection fluorescence, coupled with tunneling of the radiation, as described in copending U.S. application Ser. No. 406,324, filed Aug. 9, 1982, and assigned to the assignee of the present application, and which is incorporated herein by reference for further details particularly of the optical mode of operation of the apparatus.

FIG. 1 illustrates exemplary apparatus useful to practice of the present invention, which apparatus comprises kit 6, fluorometer 7, syringe 8, syringe pump 9, and container 10.

Referring to FIG. 2, there may be seen a longitudinal cross-sectional view of an immunoassay kit 6 made in accordance with the principles of the present invention.