

quently formed chelate, resulting in dissociation of the ion. Dissociation is a particular problem when the labeled protein in the aqueous phase of the assay is in very low concentration. It is toward the solution of these problems that this invention is directed.

### SUMMARY OF THE INVENTION

One aspect of the present invention comprises a method for fluorescence immunoassay of an analyte in a fluid. An antianalyte affixed to a solid support is contacted with the fluid and a tracer having an attached medium which occludes a fluorescent dye. After the analyte binds to the antianalyte, bound and free fractions are separated, and the dye in an intact medium is excited. Fluorescence is measured and compared with the magnitude of emission measured when one or more known quantities of analyte is assayed under essentially identical conditions.

The preferred dye is a fluorescent chelated lanthanide ion which is substantially water insoluble and which is occluded in the nonaqueous portion of a sac, most preferably in the lipid portion of a liposome. The most preferred dye is a lanthanide ion chelated with a  $\beta$ -diketone, wherein measurement of fluorescence is performed by time resolution.

In a particularly preferred embodiment of the method, substantially all of the analyte binds to both the antianalyte and the tracer in a sandwich assay. In another preferred embodiment of the method, the tracer and the analyte compete for an insufficient number of antianalyte binding sites in a competitive assay.

Another aspect of the invention includes a kit of materials for performing the method of the invention.

In accordance with the method of the invention, fluorescent dyes which are substantially insoluble in water but substantially soluble in solvents of low polarity are occluded in the lipid bilayer of the sacs. No chemical attachment of the dye to the sac is involved. The dyes do not leak out of the lipid phase of the sac and thus, when the sacs are conjugated by standard methods to a ligand, such as an antigen or antibody, they may be used in an immunoassay for an analyte. In contrast to conventional liposome-based assays, the method of the invention does not include rupture of the sac. Instead, measurement of fluorescence is carried out subsequent to excitation of the dye in the intact liposome by electromagnetic radiation of the appropriate wavelength.

Among the many advantages of the method of the invention are elimination of any chemical manipulation of the dye so that dyes, as for example, Rhodamine 800 (Raue et al., *Heterocycles* 21, 167 (1984)) may be used in spite of their water insolubility and lack of a functional group suitable for conjugation. Further, fluorescent, water-insoluble lanthanide chelates, such as the  $\beta$ -diketone chelates, can be taken up into liposomes and used in an assay directly in contrast to prior art methods in which water soluble lanthanide chelates of low fluorescence are taken up into the aqueous phase of a liposome, followed by liposome lysis and a fluorescence enhancement step. The method of the present invention may also be compared with prior art methods in which europium ions are conjugated directly to an assay ligand by complex chemical synthesis giving tracers having low ratios of europium ion to ligand.

### BRIEF DESCRIPTION OF THE DRAWING

The Figure is a plot of concentration versus fluorescence for assay of human chorionic gonadotropin in accordance with the method of the invention.

### DETAILED DESCRIPTION OF THE INVENTION

While this invention is satisfied by embodiments in many different forms, there will herein be described in detail preferred embodiments of the invention, with the understanding that the present disclosure is to be considered as exemplary of the principles of the invention and is not intended to limit the invention to the embodiments illustrated and described. The scope of the invention will be measured by the appended claims and their equivalents.

In accordance with the method of the invention, a substance suspected to be present in a fluid may be detected or its concentration determined by means of an immunological reaction. The substance, hereinafter referred to as the analyte, may be an antigen, a hapten such as a drug or hormone, or an antibody, and may be present in any suitable fluid. For example, the fluid may be a buffer, saline, or a body fluid such as serum or urine. In some cases, the analyte may be isolated from a body fluid and subsequently be introduced into a different fluid, such as buffer, for determination. The preferred analyte is an antigen, for example, a bacterial or viral antigen or an antigenic marker on a cell surface.

By the term "immunological reaction," as used herein, is meant a specific binding reaction of an antigen and an antibody, a hapten and an antibody, or, any appropriate analogue of an antigen, an antibody, or a hapten which also binds specifically. Antibodies useful in this invention may be either polyclonal or monoclonal.

The immunological reaction of the method of the invention is carried out either in solution or preferably, on the surface of a solid support. As known in the art, the solid support may be any support which does not interfere with the assay. Exemplary of solid supports which may be used are glass and polymeric materials, such as polyethylene, polystyrene and the like. Such supports may be fabricated into any suitable shape, such as sheets, plates, wells, strips or tubes. In particularly preferred embodiments of the invention, the immunological reaction is carried out on microtiter strips, in the wells of a microtiter plate or on the inside walls and bottom of a tube, preferably a plastic tube with one closed end.

An antianalyte is attached to the surface of the solid support. The antianalyte may be an antigen, an antibody, or an antibody complex having from two to about ten antibodies, which reacts specifically with the analyte, or it may be any appropriate analogue thereof which reacts specifically with the analyte. Attachment of the antianalyte to the solid support may be carried out by a conventional procedure, such as, for example, absorption or covalent bonding. These procedures are well-known in the art, and no further details in these respects are deemed necessary for a complete understanding of the invention.

Subsequent to attachment of the antianalyte to the solid support, any remaining binding sites on the support may preferably be filled with an inert protein, such as, for example, bovine serum albumin, (BSA). Attach-