

then extruded through polycarbonate membranes and coupled to reduced antibody according to Example I.

Thus, in accordance with the invention, a method for fluorescence immunoassay includes use of a tracer having a fluorescent dye occluded in the nonaqueous portion of a sac. Because the dyes are occluded in the nonaqueous portion of the sac, water insoluble dyes may be used, thereby greatly extending the range of usable dyes. Further, because there is no direct covalent linkage between the dye and the sac, no functional groups are needed in the dye, and, consequently, the dye can be used as is without any chemical modification. As some of the water-insoluble fluorescent dyes are rather resistant to self-quenching, it is possible to prepare labeled, bio-specific molecules with a high signal-to-molecule ratio ("high specific activity"). The method of the invention, unlike most others, makes it possible to select a fluorescent dye based only on its spectral characteristics. This flexibility opens up the way to the use of dyes which have their maximum emission in the near IR in assays of reduced background using inexpensive lasers in cell sorters and use of modern, inexpensive, solid-state light detectors.

What is claimed is:

1. A method for determining an unknown quantity of an analyte in a liquid comprising:

- (a) contacting a solid support having affixed thereto an antianalyte specific for an analyte with a liquid suspected of containing said analyte and with a tracer comprising a sac with aqueous and nonaqueous portions, said sac including a substantially water insoluble fluorescent lanthanide chelate occluded substantially in the nonaqueous portion of said sac whereby said analyte binds to said antianalyte and said tracer binds to one of said analyte and said antianalyte to give a bound fraction on said support;
- (b) separating said support having bound fraction thereon from said liquid, said bound fraction including an intact sac with said lanthanide chelate therein;
- (c) exciting lanthanide chelate in said intact sac by applying electromagnetic radiation thereto;
- (d) detecting fluorescence from said lanthanide chelate; and
- (e) determining the quantity of said analyte in said liquid by comparing the magnitude of said fluorescence with the magnitude of fluorescence established for a known quantity of the analyte.

2. The method in accordance with claim 1 wherein said tracer binds to said antianalyte and further comprises said analyte having said sac conjugated thereto.

3. The method in accordance with claim 1 wherein said tracer binds to said analyte and further comprises a second antianalyte having said sac conjugated thereto.

4. The method in accordance with claim 1 wherein said solid support further comprises an inert protein which fills binding sites of the support unoccupied by antianalyte.

5. The method in accordance with claim 1 wherein said analyte is selected from the group consisting of an antigen, an antibody and a hapten.

6. The method in accordance with claim 1 wherein said antianalyte is selected from the group consisting of an antigen, an antibody and an antibody complex.

7. The method in accordance with claim 1 wherein said lanthanide chelate includes an ion selected from the

group consisting of europium, terbium, and samarium, said ion being chelated to a  $\beta$ -diketone.

8. The method in accordance with claim 1 wherein said electromagnetic radiation is applied as a pulse and said detecting is performed by time resolved fluorescence.

9. The method in accordance with claim 1 further comprising a water soluble lanthanide chelate encapsulated substantially in the aqueous compartment of said sac.

10. The method in accordance with claim 10 further comprising exciting said water soluble dye and detecting fluorescence from said water soluble dye.

11. A method for determining an unknown quantity of analyte present in a liquid sample comprising:

- (a) preparing a mixture by contacting an antianalyte attached to the surface of a solid support with a liquid containing an unknown quantity of an analyte and a tracer, said tracer comprising a liposome occluding a substantially water insoluble fluorescent lanthanide chelate substantially in the lipid portion thereof, said liposome being conjugated to one of said analyte and a ligand specific to said analyte;
- (b) incubating said mixture to give a bound fraction on said support;
- (c) separating said support having bound fraction thereon from said mixture, said bound fraction including an intact liposome with said chelate therein;
- (d) exciting chelate in said intact liposome by applying electromagnetic radiation thereto;
- (e) detecting time resolved fluorescence from said chelate; and
- (f) determining the quantity of said analyte in said sample by comparing the magnitude of said fluorescence with the magnitude of fluorescence detected when a mixture containing a known quantity of said analyte is determined in accordance with steps (a) to (e).

12. The method in accordance with claim 11 wherein said ligand is selected from the group consisting of an antigen, an antibody and bound antigen-antibody complex.

13. A kit of materials for performing an assay for an unknown quantity of an analyte in a liquid comprising (1) a solid support having attached thereto an antianalyte specific to an analyte and (2) a tracer for said analyte, said tracer comprising a substantially water insoluble fluorescent lanthanide chelate occluded in a nonaqueous portion of a sac having aqueous and nonaqueous portions, said tracer being conjugated to one of said analyte and a ligand specific to said analyte.

14. The kit in accordance with claim 13 wherein said ligand is selected from the group consisting of an antigen, antibody and a bound antigen-antibody complex.

15. The kit in accordance with claim 13 further comprising a reagent selected from the group consisting of a buffer and saline.

16. The kit in accordance with claim 13 further comprising at least one liquid containing analyte of known concentration.

17. The kit in accordance with claim 13 further comprising a liquid substantially free of analyte.

18. The kit in accordance with claim 13 further comprising a water soluble lanthanide chelate encapsulated substantially in an aqueous compartment of said medium.

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