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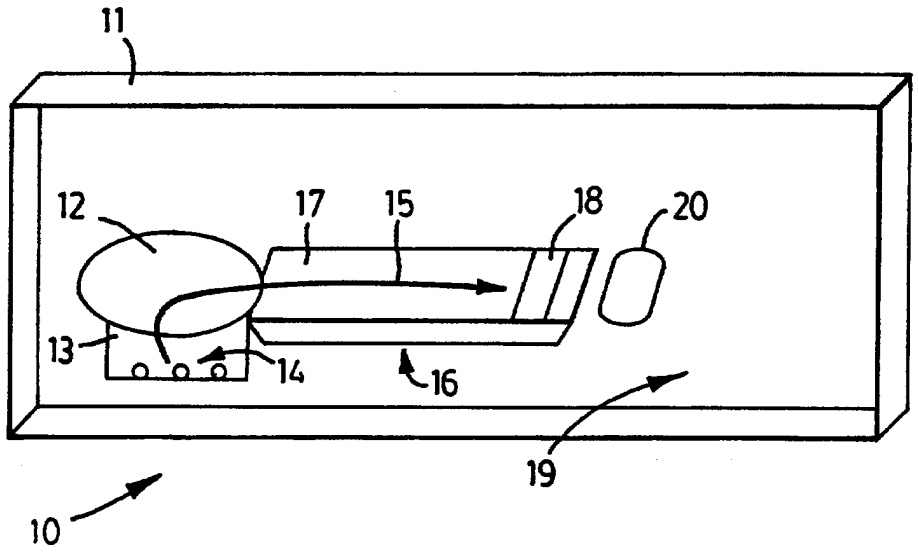
(54) Title: METHOD FOR CONDUCTING CHEMILUMINESCENT BINDING ASSAY



(57) Abstract: A method for conducting a receptor-ligand binding reaction of a solution containing or suspected of containing the target analyte. The method comprises the steps of bonding the first binding partner to the surface of a paramagnetic particle, conjugating a second binding partner to a calcium-sensitive luminescent compound; contacting the first and second binding partners with the solution to be tested, immobilizing the paramagnetic particles along a capture strip that has a transverse stripe containing streptavidin and containing a caged calcium compound, exposing the transverse stripe to a pulse of ultraviolet light to effect the release of calcium from the caged calcium compound, and measuring luminescence emitted by the calcium-sensitive luminescent material. The method may be used in the testing of blood. An apparatus is also disclosed.

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TITLE:
METHOD FOR CONDUCTING CHEMILUMINESCENT BINDING ASSAY

Field of the Invention

The present invention relates to a method for conducting a binding assay, and in particular to an immunoassay method that may be conducted on a Point Of Care (POC) device or an autoanalyzer.

Background to the Invention

The on-going needs to detect and quantify biomolecules (analytes) in various body fluids have resulted in the introduction of new and more accurate analytical techniques that can be adapted for measuring a wide spectrum of different analytes. Most of these detection methods have been introduced into the clinical diagnostic field in recent years. Currently, a broad expansion in both the variety of analytes that may be readily and accurately determined as well as the methods for the determination have been witnessed. However, convenient, reliable, non-hazardous, highly sensitive and technically less challenging methods for detecting the presence of low concentrations of analytes in liquids are still desired, especially when the analyte may be present in body fluids in very low concentrations.

Several methods for the detection and quantification of substances of biological origin in fluid samples are currently employed. Bioanalytical assays, such as immunoassays and nucleic acid hybridization assays, which are based on the specific binding between ligands and one or more members of specific binding pairs are widely used to determine the presence and quantity of analytes of interest, for example chemical constituents or substances of a sample. In particular, immunoassays are widely employed detection and quantification methods in the clinical laboratory.

In a typical procedure of a sandwich immunoassay, an antibody against a particular antigen, known as a capture antibody, is immobilized to a solid surface. The sample under investigation is contacted with the solid

surface under conditions that allow antigen in the sample to bind to the capture antibody. Another antibody known as a detector antibody is added. In the direct immunoassay format, the detector antibody is directly conjugated with a signal generating mechanism that allows the amount of the detector antibody to be quantified. In the indirect format, after the binding of the detector antibody to the antigen, another antibody against the detector antibody or another specific binding reaction that involves the detector antibody is utilized. This so-called anti-detector antibody is directly conjugated with a signal generating mechanism. The binding reaction and therefore the antigen level in the sample is quantified by quantifying the signal produced by the signal generating mechanism.

Several types of labeling material have been utilized for signal generation in the receptor-ligand binding assays. Radioactive atoms, such as ^{125}I, ^{131}I, ^{3}H and ^{14}C were commonly utilized as the label. Although radioactive labels for immunoassays are sensitive, they suffer commonly recognized disadvantages, including safety and the stringent regulatory requirements resulting in a relatively short reagent shelf life. Several alternative labeling methods are currently utilized in binding bioassays including colorimetric enzyme reactions, fluorescence and chemiluminescence reactions. Enzymes commonly utilized as labels are horseradish peroxidase, alkaline phosphatase, B-galactosidase and glucose oxidase. Although enzymes have an advantage over radioactive labels in that they are very stable and need no special facilities and instrumentation, enzyme immunoassays are generally slower, laborious and less sensitive. Luminescent labels, including fluorescent and chemiluminescent labels, have been utilized as an alternative for radioactive or enzyme labels as they possess the ease of use advantage of radiolabels and the reagent stability advantage of enzymes. Fluorescence detection can be used with a much wider variety of enzymes. However, due to the difficulty of conventional fluorescence detection in discriminating between specific and nonspecific signals and therefore the practical assay detection limit, fluorescence assays lack the sensitivity of either radioactive or enzyme

labels, making them seldom the assay method of choice for both research and clinical applications.

Chemiluminescent reactions as label of signal generation are the most sensitive and have been around for decades. Recent advances in DNA technologies have expanded the utilization of these labels as signal generators, but due to the limited number of known reactions that form chemiluminescent products, the luminescence assay method is currently under utilized. Also, luminescent reactions need one or more chemical activation steps, and automation of these reactions is difficult, although needing less complex instrumentation than fluorescence. Even though a large number of luminescence meters viz. luminometers, of various formats and sizes are available, automation of luminescence is complicated and fully-automated luminometers for carrying out binding assays are not available, at least in convenient, small-size analyzers.

The most common luminescence method utilized as a label for signal generation in binding assays is chemiluminescence. This may be classified according to the method utilized for generating the luminescent signal viz. chemiluminescent and bioluminescent labels. Bioluminescence refers to the emission of light by biological molecules and utilizes bioluminescent proteins which can be true enzymes. Examples are luciferases that catalyze the oxidation of luciferin with release of oxyluciferin and emit light, and photoproteins that catalyze the oxidation of luciferin to emit light but do not release the oxidized substrate.

The calcium-sensitive photoproteins, including Aequorin, Obeln, Mnemiopsin, Berovin, Pholasin, Luciferases and photoproteins isolated from Pelagia, Cypridina and ostracods were widely researched and employed in binding assays. Furthermore, the genes of some of them have been cloned, permitting the production of large quantities. Aequorin is the most commonly studied and employed member of this group of calcium-sensitive photoproteins.

Native aequorin, isolated from jellyfish (Aequorea), has been purified and utilized as a label in varieties of monitoring systems. Native aequorin

consists of a single polypeptide chain of MW 21,000 Daltons (called apoaequorin), containing one mole each of tightly bound coelenterate luciferin and oxygen. This complex is stable in the absence of calcium ions. Aequorin can also be produced by recombinant DNA techniques, for example as discussed by Cormier, M. J., U.S. Patent 5,162,227 and Zenno. S. et al. in U.S. Patent 5,288,623. Furthermore, modified forms of aequorin with enhanced bioluminescence properties have also been produced by recombinant DNA procedures, as disclosed by Prasher, D. in U.S. Patent 5,360,728.

The mechanism of photon emission of aequorin is well understood. Aequorin has a high-affinity for calcium ions. In the presence of excess calcium ions, aequorin catalyzes the oxidation of luciferin to oxyluciferin in a single turnover event with the generation of a glow-type "flash reaction" which persists for approximately 10 seconds with a relatively high quantum yield. Although peak light emission is initiated upon binding of three moles of calcium ions per mole of aequorin, binding of aequorin with trace of amount of free calcium results in partial oxidation of coelenterazine and yields apoaequorin, coelenteramide, CO and light.

As aequorin can be detected at the attomole level and the wavelength of its luminescence is very narrow and may be detected using commercially available luminometers, luminescence of aequorin offers many advantages including speed, high sensitivity and accuracy with a low background. Therefore, aequorin has proven useful as a label in binding assays. Furthermore, stable conjugates of aequorin with various binding reagents such as receptors, hormones, lectins, antibodies, antigens, DNA, RNA, oligonucleotides, and glycoproteins have been developed and a large number of such conjugates are commercially available.

When utilized in combination with streptavidin, biotinylated derivative of aequorin demonstrates the ability to detect nanogram to subnanogram amounts of the target analyte, including proteins and DNA, immobilized onto the wells of microtiter plates or nitrocellulose membranes. Marketed luminometers that employ aequorin are designed with injectors to inject

calcium at a particular moment. Although several clinical testing assays that utilize aequorin have been introduced, the luminometers are not automated and tanks of solutions of calcium have to be included. This makes them awkward to use by non-specialized personnel. A luminescent binding assay that utilizes aequorin and whole blood is disclosed by Pankratz et al in U.S. Patent No. 5,876,935.

In the cell of an organism, calcium (Ca) is an important intracellular second messenger for a wide variety of processes, which have physiological, biochemical and pathophysiological significance such as muscle contraction, neurotransmitter release, ion channel gating and exocytosis. Attempts to understand and measure the rapid changes and release of intracellular calcium have resulted into the introduction of a class of calcium-sensitive compounds called calcium-caging compounds. Calcium-caging compounds have the ability to be loaded with calcium and to unload their calcium upon stimulation. Unloading of the encased calcium may be induced by several methods, one of which is through exposure to light. Light-stimulation release of calcium from the caged compounds (called photolysis) is usually done by illumination for fractions of a second with laser pulses typically in the UV 350-400 nm region of the spectrum. Two different classes of Ca-caging compounds have been introduced; the BAPTA derivative such as the nitr-5 and nitr-7 and the EDTA or EGTA derivatives such as DM-nitrophen and nitrophenyl-EGTA. BAPTA is 1,2-bis(ortho-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid. Nitr-7 is cis-1-(2-bis(carboxymethyl)amino-5-(1-hydroxy-1-(2-nitro-4,5-methylenedioxyphenyl)methyl)phenoxy)-2-(2-bis(carboxymethyl)amino-5-methylphenoxy)cyclopentane. Nitr-5 is 1-[2-Amino-5-(1-hydroxy-1-[2-nitro-4,5-methylenedioxyphenyl]methyl)phenoxy]-2-)2'-amino-5'methylphenoxy)ethane-N,N,N',N'-tetraacetic acid. DM-nitrophen is 1-(4,5 dimethoxy-2-nitrophenyl)-1,2 diaminoethane-N, N, N', N'-tetraacetic acid and nitrophenyl-EGTA i.e. nitrophenyl ethylenebis(oxyethylenenitrilo) tetraacetic acid. The latter class was designed to produce photosensitive derivatives of chelators with known high affinity for calcium, see US Patent 5,446,186 and U.S. Patent 4,981,985. The DM-nitrophen and nitrophenyl-

EGTA calcium-caging compounds offer the advantage of calcium-selectivity. On irradiation, the chelated calcium cleaves with the subsequent cleaved remainders having a substantially lower affinity for the released calcium. Thus, large mounts of calcium are rapidly released. These photosensitive calcium-caging compounds are commercially available.

A binding assay e.g. immunoassay or nucleic acid binding assay, that utilizes photosensitive calcium-caging compounds would be useful.

Summary of the Invention

A method has now been found for conducting a receptor-ligand binding assay utilizing calcium caging compounds and calcium-sensitive luminescent compounds.

Accordingly, one aspect of the present invention provides a method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting a first binding partner with said solution, said first binding partner being conjugated to a calcium-sensitive chemiluminescent material;

(b) after a period of time, mobilizing the first binding partner in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the first binding partner with a stripe transversely located on said capture strip, said transverse stripe having immobilized second binding partner and containing a calcium-caging compound,

(c) allowing a period of time sufficient for the first binding partner to contact said second binding partner immobilized onto said transverse stripe,

(d) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect the release of calcium from the calcium caging compound; and

(e) measuring luminescence emitted by the calcium-sensitive luminescent material.

Another aspect of the present invention provides a method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting said solution with a first binding partner of a binding reaction, said first binding partner being immobilized on a solid surface, said solid surface being paramagnetic particles and said first binding partner being conjugated to calcium-sensitive luminescent material;

(b) after a period of time, mobilizing the paramagnetic particles in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the particles with a stripe of a second binding partner transversely located on said capture strip, said capture strip having the second binding partner immobilized onto said transverse stripe, said transverse stripe additionally containing a calcium-caging compound,

(c) allowing a period of time sufficient for the paramagnetic particles to contact said second binding partner immobilized onto said transverse stripe,

(d) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect the release of calcium from the calcium caging compound; and

(e) measuring luminescence emitted by the calcium-sensitive luminescent material.

In preferred embodiments of the invention, the method is an immunoassay for detecting and quantifying an antigen, an immunoassay for detecting and quantifying an antibody, or a nucleic acid hybridization assay for detection and quantifying a particular sequence of nucleic acid.

In another embodiment, the solution is pretreated prior to contacting the calcium sensitive chemiluminescent material in step (a), especially filtered to remove calcium, the filter containing an agent for removal of calcium.

In another embodiment, the solution is whole blood, said whole blood being pretreated by filtering prior to being contacted with the paramagnetic particles.

In a further embodiment, the luminescent material is calcium-sensitive luminescent material, especially aequorin, Obeln, Mnemiopsin, Berovin, Pholasin, Luciferases or photoproteins isolated from Pelagia, Cypridina and ostracods.

In still further embodiments, the ultraviolet light is in the form of a pulse of light in the range of 250-400 nm, and the luminescence is measured by a photomultiplier. In particular, the calcium-sensitive luminescent material is aequorin and photomultiplier detects light of 400-600 nm and is protected from the magnetic field.

In other embodiments, the elongated capture strip is formed of nitrocellulose, polyacrylamide or other natural or synthetic polymer and has a transverse stripe with immobilized second binding partner and impregnated with a calcium caging compound.

In a further embodiment, the calcium-caging compound is loaded with calcium in excess of the stoichiometric amount for said calcium-sensitive luminescent material. Preferably, the calcium-caging compound is nitr-5, nitr-7, DM-nitrophen or nitrophenyl-EGTA.

A further aspect of the invention provides a method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) immobilizing a first binding partner of a binding reaction onto a solid surface, said solid surface being paramagnetic particles, said first binding partner being biotinylated;

(b) contacting said first binding partner with said solution;

(c) contacting the solution with a second binding partner, said second binding partner being conjugated to a calcium-sensitive luminescent material;

(d) after a period of time, mobilizing the paramagnetic particles in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the particles with a stripe transversely located on said capture strip, said capture strip having streptavidin immobilized onto said

transverse stripe, said transverse stripe additionally contain a calcium-caging compound,

(e) allowing a period of time sufficient for the paramagnetic particles to contact said streptavidin immobilized onto said transverse stripe,

(f) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect the release of calcium from the calcium caging compound; and

(g) measuring luminescence emitted by the calcium-sensitive luminescent material.

In an embodiment, steps (b) and (c) are carried out simultaneously.

Yet another aspect of the invention provides a method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting a first binding partner with said solution, said first binding partner being biotinylated;

(b) after a period of time, contacting the solution with a second binding partner, said second binding partner being conjugated to a calcium-sensitive luminescent material;

(c) after a further period of time, mobilizing the binding partners in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the binding partners with a stripe transversely located on said capture strip, said capture strip having streptavidin immobilized onto said transverse stripe, said transverse stripe additionally contain a calcium-caging compound,

(d) allowing a period of time sufficient for the binding partners to contact said streptavidin immobilized onto said transverse stripe,

(e) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect the release of calcium from the calcium caging compound; and

(f) measuring luminescence emitted by the calcium-sensitive luminescent material.

In an embodiment, steps (a) and (b) are carried out simultaneously.

In a further embodiment, the elongated capture strip has a transverse stripe impregnated with streptavidin and a calcium-caging compound.

In a still further aspect, the present invention provides an elongated capture strip for binding assays, said strip having a transverse section thereof impregnated with a binding partner and a caged calcium compound.

In preferred embodiments, the capture strip is formed from nitrocellulose, polyacrylamide, polyamide or any other synthetic or naturally occurring polymer.

In another embodiment, the capture strip is in a housing, especially within a support as a single use testing cartridge.

In a further embodiment, the binding partner is streptavidin.

A further embodiment of the invention provides a plastic cartridge comprising:

a housing with a receptacle for receipt of a sample, a reservoir containing biotinylated first binding partner immobilized onto paramagnetic particles and a second binding partner conjugated to calcium-sensitive chemiluminescence material, an elongated capture strip within the housing and in fluid communication with the reservoir, said capture strip having a transverse section thereof impregnated with streptavidin and a calcium-caging compound, said transverse section being protected with a light barrier.

In a preferred embodiment, there is a filter between the receptacle and the reservoir, especially a filter containing an agent for removal of calcium.

A further embodiment provides apparatus for carrying out a binding assay comprising a housing enclosing (a) a receptacle to receive the said plastic cartridge; (b) a means for removing the light protective layer over the transverse stripe; (c) a electromagnet to provide a magnetic field; (e) a ultraviolet light source to project light on a pre-selected portion of the capture strip, and (f) a photomultiplier disposed to receive light emitted by the pre-selected portion of the capture strip.

Brief Description of the Drawings

The present invention is illustrated by the embodiment shown in the drawings, in which:

Fig. 1 is a schematic representation of a capture strip of the present invention;

Fig. 2 is a schematic representation of the cartridge of the present invention;

Fig. 3 is a schematic representation of apparatus of the present invention;

Fig. 4 is a graphical representation of photoemission from a sample in Example I;

Fig. 5 is a graphical representation of photoemission from a sample in Example I, after photolysis with ultraviolet light;

Fig. 6 is a graphical representation of the combined graphs of Fig.s 5 and 6;

Fig.s 7 and 8 are graphical representations of photoemission from samples in Example II.

Detailed Description of the Invention

While the present invention may be used for detection and quantification of a binding partner of a binding reaction, it will be described herein with particular reference to a sandwich immunoassay for the detection and quantification of antigen that additionally employs a biotin-streptavidin reaction and paramagnetic particles, which is preferred.

Fig. 1 shows a capture strip, generally indicated by 1. Capture strip 1 has an elongated matrix 2. Elongated matrix 2 is formed from a matrix composition that will permit the paramagnetic particles with associated immune complex thereon to pass along the capture strip under the influence of a magnetic field. Examples of the matrix composition include nitrocellulose, polyacrylamide, polyamide or other synthetic or naturally-occurring polymer. In other embodiments, the matrix is in the form of a microfluidic channel, especially a channel etched into the capture strip. In this embodiment, a

matrix composition would not be required. The capture strip must be formed of a clear material, especially in the location of the transverse stripe 3, to permit passage of light. Examples of such materials include acrylic polymers, polystyrene, acrylonitrile-butadiene-styrene (ABS), polycarbonate and other transparent polymers.

Elongated matrix 2 has transverse stripe 3 located towards one end, such end being opposed to inlet end 4. Transverse stripe 3 contains both streptavidin and calcium-loaded calcium caging compounds 5 or such other compounds as are disclosed herein.

Fig. 2 shows a plastic cartridge for carrying out the immunoassay reaction, generally indicated by 10. Plastic cartridge 10 has cartridge housing 11. Cartridge housing 11 has a sample receiving receptacle that contains a filter 12, a reservoir 13 for housing the paramagnetic particles 14 and the second binding partner thereon, and particle path 15. Particle path 15 is in fluid communication with the reservoir 13 and leads from reservoir 13 into capture strip 16, where particle path 15 extends along elongated path 17 of capture strip 16 to transverse stripe 18. Filter 12, reservoir 13 and capture strip 16 are all located within a holder 19 that forms part of plastic cartridge 10. It is to be understood that at least transverse stripe 18 would have a peelable protective light barrier thereon which would be removed before use, i.e. before exposure to light from the light source. Additionally, the elongated capture strip is in communication with a discharge reservoir 20 at the opposite end of the sample receiving receptacle for receiving reagents that pass from the transverse stripe 18.

Fig. 3 shows a testing platform apparatus, generally indicated by 30. Testing platform apparatus 30 has housing 31. Within the housing 31 are plastic cartridge 32, electromagnet 33, ultraviolet light source 34 and photomultiplier 35. Plastic cartridge 32 has been described previously, and could be accommodated within the receptacle of the housing 31 of the platform apparatus 30. Electromagnet 33 extends for the length of plastic cartridge 32. Electromagnet 33 is preferably comprised of sectional pre-determined magnetic fields that facilitate mobilization of paramagnetic beads

(particles) along the elongated path 17 into capture strip 16. Ultraviolet light source 34 is directed at plastic cartridge 32 and, in particular, at transverse stripe 18 of plastic cartridge 32, which has been described previously. Photomultiplier 35 is also directed at transverse stripe 18.

Testing platform apparatus 30 additionally has display 36, which would typically be an LCD display. Housing 31 would also contain appropriate controls and associated computer hardware and software to permit appropriate interpretation of the results obtained.

In use, a sample containing an antigen e.g. blood, is placed on filter 12. Liquid containing the target analyte passes through filter 12 into reservoir 13, where it contacts the paramagnetic particles which has the biotinylated first binding partner (capture partner) immobilized onto it and the second binding partner conjugated to a calcium-sensitive luminescent label (detector partner). In addition, it is understood that when the cartridge is designed to detect and quantify an antigen, the first and second binding partners are antibodies. On the other hand, when the cartridge is designed for detecting an antibody, the first binding partner is an antigen while the second binding partner is an antibody.

The plastic cartridge 10, 32 is then placed in the testing platform 30 if it is not already located within the platform. It is understood that at least transverse stripe 18 of capture strip 16 of plastic cartridge 10 would need to be protected from light. Such protection could be removed within testing platform 30, in a light-tight manner. Such removal could be automatic.

After allowing appropriate time for the binding reaction, the magnetic field is applied, using electromagnet 33. Then, the paramagnetic particles and attached immune complexes move along particle path 15 and into capture strip 16. The particles then pass along capture strip 16 until transverse stripe 18 is reached. At that time, the particles become bound to streptavidin, already located in transverse stripe 18, through the biotinylated binding partner immobilized onto the particles. Transverse stripe 18 additionally contains a calcium caging compound.

After an appropriate time, which would depend in particular on the dimensions of the capture strip 16, but which conveniently could be 4-6 minutes, ultraviolet light source 34 is activated and sends a pulse of light onto transverse stripe 18. The light causes the release of calcium from the calcium-loaded calcium caging compound, which occurs essentially instantaneously. The calcium contacts the calcium-sensitive chemiluminescent material, which then glows for a short period of time in the range of 4-10 seconds. The light that is emitted is detected by the photomultiplier 35, and the amount of light emitted is interpreted and is displayed on display 36. The rate of emission of light depends on the energy of the ultraviolet light source. High energy levels will cause a high emission rate i.e. a sharp peak of emitted light, but it is preferred that lower energy levels be used such that the emitted light is a broader band. This will lead to more accurate recording of the amount of light by the photomultiplier, especially if emission of light commences prior to completion of the re-setting of the photomultiplier to its zero or null point, as discussed herein.

Some examples of the source of the solution containing or suspected of containing the target analyte that is subjected to the method of the present invention are blood or blood products, saliva, or any other body fluids. Other solutions could be tested.

Utilizing calcium-sensitive luminescent material as the signal generating label in binding assays requires that the solutions that will be contacting the calcium-sensitive luminescent conjugate have to be calcium-free before the moment of generating the light emission. Calcium in the solution will react with the calcium-sensitive luminescent conjugate. In particular, the solution should contain less than 20 nanomolar of calcium. Furthermore, when the goal is to determine the presence of an analyte in whole blood, the sample of blood normally must be pretreated to remove cellular components and hemoglobin, which can interfere with the specific signal of the binding assay. Filters impregnated in calcium-chelating agents would achieve both functions of removing the cellular components as well as calcium from the solutions that contain the target analyte.

The method of the invention disclosed herein utilizes any calcium-sensitive luminescent material for the signal generation in binding assays including, but not limited to, aequorin, mitrocomin, clytin, obelin, mnemiopsin, berovin, halistaurin and phialidin. In case of utilizing a calcium-sensitive luminescent photoprotein, other than aequorin, the optimal wavelength, other than 469 nm that is the optimal wavelength for detecting the aequorin signal, of the photomultiplier has to be adapted accordingly. For example, the wavelength may be 400-600 nm.

Photolysis of the calcium caging compounds may be achieved by many light sources generating light within a wavelength from 250-400 nm. Such light sources are referred to herein as ultraviolet light sources. One such source is a laser source, which is a convenient source to accurately deliver light for less than 1 millisecond at a wavelength of 300-350 nm. Upon the release of calcium from the caging compound when light-triggered and upon binding of three moles of calcium ions per mole of aequorin, the light emission is initiated with a flash of blue light that persists for approximately ten seconds. The generated light could then be measured with a suitable photomultiplier both as peak light or total photon counting.

The method of the present invention preferably utilizes a time-resolved mechanism, particularly time-resolved chemiluminescence. In this method, there is a short period of time between the flash emitted by the ultraviolet light source and the emission of light by the calcium-sensitive luminescent material. The calcium-sensitive luminescent material is selected to obtain such a period of time. The photomultiplier records the stray light after the flash from the ultraviolet source followed by a period of zero or substantially zero light, which is then followed by the emission of light. During the period of zero light, the reading on the photomultiplier can be re-set to its zero or null point, thereby permitting a more accurate reading of the emission of light. The period between the pulse of light and the emission of light is short, but such time is sufficient to reset the photomultiplier to a zero baseline.

The ultraviolet light source should be generally shielded from the capture strip, with the light being focussed on the transverse stripe, e.g. using

coated quartz lenses. As discussed herein, the intensity of the light source may be varied, but one embodiment is at least 150 mJ.

Native calcium-sensitive luminescent photoproteins are particularly useful as a label in the method of the present invention of carrying out binding assays. Other modified recombinant DNA-driven forms of these photoproteins with enhanced luminescence, due to either the ability of regeneration or a higher affinity for calcium, are also compatible with the method of the invention.

Although encasing compounds such as in light sensitive liposomes have been extensively researched, the recent introduction of cation-specific caging compounds is particularly useful in carrying out the method of the invention. The recently introduced two classes of calcium-caging compounds which are derivatives of chelating agents are particularly useful as they are more stable and the mechanism(s) of their triggering is well defined. In particular, the breakdown derivatives of DM-nitrophen derivative of EDTA (ethylenedinitrilo tetraacetic acid, disodium salt) and nitrophenyl-EGTA (ethylenebis(oxyethylenenitrilo) tetraacetic acid) have a very low affinity for calcium once light-triggered. Also, the wavelength of fluoroescence of the cleaved compounds is much different than that of the calcium-sensitive luminescent photoproteins and lasts for a very brief period of time. These photosensitive calcium-caging compounds are commercially available.

The detector materials on the transverse stripe may be located and immobilized on glass beads, which provides a high surface area of detector material.

Combining aequorin, which can be detected at the attomol level, together with exploiting the high affinity of biotin/streptavidin reaction offers a very high sensitivity of the method of the invention to measure analytes at a subnanogram level of detection. Furthermore, modified forms of streptavidin are also compatible with the method of the invention and both streptavidin and its derivatives could be easily immobilized onto the lateral transverse stripe of the capture matrix strip.

According to the method of the invention for carrying out a binding assay, separation of the bound from free luminescent label is effected by applying a magnetic field. It will be recognized that the force on suspended magnetic particle subjected to a magnetic field urges the particle to move to stronger field regions, typically towards the pole of a magnet, and that the strength of the force depends both on the field gradient and magnetism induced in the particle by the field. Thus, for rapid separation, a strong separator and a highly magnetizable particle appear preferable. Furthermore, the electromagnet is capable of producing several field gradients in pre-determined optimized directions.

Microscopic magnetic particles ranging from 0.7-1.5 microns are compatible with the method of the invention and may be used as they provide a large surface area for coating with proteins, for example, those disclosed in U.S. Patents No.s 3,970,518; 4,018,886; 4,230,685; 4,267,234; 4,452,773; 4,554,088; and 4,659,678. However, smaller size paramagnetic particles of the size 0.03 to 10, especially 0.5-1.0 micrometers, as described in the US Patent No. 5,736,349 are more suitable as large size particles of magnetic material tend to adhere to one another after removal of the magnetic field, due to residual magnetism. Suitable magnetic materials include ferromagnetic, ferrimagnetic and superparamagnetic materials. Other suitable magnetic materials include oxides, such as, for example, ferrites, perovskites, chromites and magnetoplumbites. Nickel particles may also be used.

The magnetic separation apparatus/method used for separating of target analyte-bearing magnetic particles from test media will depend on the nature and size of the magnetic particle. The micron-size magnetic particles suitable in the invention are readily removed from solution by means of commercially available magnetic separation devices. These devices employ a single relatively inexpensive permanent magnet located external to a container holding the test medium. Examples of such magnetic separators are the MAIA Magnetic Separator manufactured by Serono Diagnostics, Norwell, Mass., the DYNAL MPC-1 manufactured by DYNAL, Inc., Great Neck, N.Y. and the BioMag Separator, manufactured by Advanced Magnetics, Inc.,

Cambridge, Mass. The preferred magnetic separator for the present invention would have several aligned field gradients. In particular, multiple magnets could be used to effect stirring of the paramagnetic particles, and then to successively move the particles out of the vessel onto and along the capture strip. The magnets could be operated independently and/or in a coordinated sequence so as to effect stirring and then the movement of the particles along a pre-determined path e.g. to the capture strip and then to the transverse stripe. An example of the use of magnets in the stirring of magnetic particles is disclosed in US Patent No. 5,835,329.

In developing a bioassay, there are many considerations for the assay to attain value in the clinical laboratory. One consideration is the signal response to changes in the concentration of analyte. A second consideration is the ease with which the protocol for the assay may be carried out. A third consideration is the variation in interference from sample to sample. Also, ease of preparation and purification of the reagents, availability of equipment, ease of automation and interaction with material of interest are some of the additional considerations in developing a useful assay.

The method of the invention for carrying out a binding assay offers improvement in such consideration. The invention offers the high sensitivity of luminescence, the availability, sensitivity and high quantum yield of calcium-sensitive luminescent material, particularly aequorin, the physical characteristic of calcium-sensitive luminescent material to response to changes in calcium without having to manually inject calcium, the availability of commercial luminometers with photomultipliers that could detect the generated photons without the interference of the magnetic field, and the development of solid chromatographic capturing matrices that offer the convenience of point of care testing. Most important, the large difference in the wave length of exciting the caged calcium (240-400 nm) and the wavelength of measuring the generated photons (450-500 nm) facilitates detection of emitted light without interference from the incident light from the ultraviolet light source or due to fluorescence of the medium. Thus, a time-resolved chemiluminescence is used, as described herein.

The present invention of carrying receptor-ligand binding reaction utilizing a calcium-sensitive chemiluminescent label has been described herein with reference to the paramagnetic particle having the biotinylated first binding partner immobilized onto its surface, the chemiluminescent material conjugated to the second binding partner, and with the calcium caging compound being associated with the streptavidin to carry out a full sandwich immunoassay for detecting and quantifying an antigen as the preferred embodiment of the method invention. However, it is to be understood that the method of the invention is as equally beneficial in detecting an antibody as well as a nucleic acid as the target analyte of a receptor-ligand binding reaction. Also, it is to be understood that the method of the invention could be carried out with the first binding partner conjugated to a calcium-sensitive luminescent material and immobilized onto paramagnetic particles and the second binding partner immobilized in the transverse stripe of the capture strip together with the calcium caging compound.

The present invention is illustrated by the following examples.

EXAMPLE I

5 µg of aequorin in 10 µl was added to a 200 µl solution of buffered 1-(4,5 dimethoxy-2-nitrophenyl)-1,2 diaminoethane-N, N, N', N'-tetraacetic acid (DM-NP) containing calcium chloride. The solution contained 80 mM of 4-morpholine propane sulphonic acid (MOPS) buffer and 20 mM of KCl, with the pH of the solution adjusted to 7.2. The DM-NP was loaded with calcium up to 75% i.e. 2mM DM_NP + 1.5 mM $CaCl_2$.

Photoemission from the solution was monitored for 30 seconds at a wavelength of 470 nm. The results obtained shown in Fig. 4, shown minimal emission. The solution was then photolysed using an ultraviolet light of a wavelength of 347 nm. The pulse of light was 100 mJ.

The results obtained are shown in Fig. 5, and the results of Fig.s 4 and 5 are combined in Fig. 6.

The results show that the pulse of ultraviolet light caused release of sufficient calcium to trigger photoemission from aequorin. Prior to the pulse of

ultraviolet light (Fig. 4), the caged calcium did not trigger emission from aequorin. Emission of light was complete within 30 seconds.

EXAMPLE II

The procedure of Example I was repeated using solutions of 5 µg of aequorin. In separate experiments, 1mM of $CaCl_2$ and 500 µM of $CaCl_2$ were added. The total photon count after the pulse of ultraviolet light was monitored at 470 nm for 30 seconds.

The results are shown in Fig.s 7 and 8, respectively. Although the peak heights are different, the total amount of photons emitted is the same. Thus, the total amount of photons can be used to monitor the reaction. Under more controlled conditions i.e. not the manual addition of these examples, peak intensity could also be used.

CLAIMS:

1. A method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting a first binding partner with said solution, said first binding partner being conjugated to a calcium-sensitive chemiluminescent material;

(b) after a period of time, mobilizing the first binding partner in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the first binding partner with a stripe transversely located on said capture strip, said transverse stripe having immobilized second binding partner and containing a calcium-caging compound,

(c) allowing a period of time sufficient for the first binding partner to contact said second binding partner immobilized onto said transverse stripe,

(d) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect the release of calcium from the caged calcium compound; and

(e) measuring luminescence emitted by the calcium-sensitive luminescent material.

2. The method of Claim 1 for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting said solution with a first binding partner of a binding reaction, said first binding partner being immobilized on a solid surface, said solid surface being paramagnetic particles and said first binding partner being conjugated to calcium-sensitive luminescent material;

(b) after a period of time, mobilizing the paramagnetic particles in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the particles with a stripe of a second binding partner transversely located on said capture strip, said capture strip having the second binding partner immobilized onto said transverse stripe, said transverse stripe additionally containing a calcium-caging compound,

(c) allowing a period of time sufficient for the paramagnetic particles to contact said second binding partner immobilized onto said transverse stripe,

(d) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect the release of calcium from the calcium caging compound; and

(e) measuring luminescence emitted by the calcium-sensitive luminescent material.

3. The method of Claim 2 in which the method is an immunoassay for detecting and quantifying an antigen, an immunoassay for detecting and quantifying an antibody, or a nucleic acid hybridization assay for detection and quantifying a particular sequence of nucleic acid.

4. The method of any one of Claims 1-3 in which the solution is pretreated prior to contacting the calcium sensitive luminescent material in step (a).

5. The method of Claim 4 in which the solution is filtered to remove calcium, the filter containing an agent for removal of calcium.

6. The method of any one of Claims 1-5 in which the solution is whole blood, said whole blood being pretreated by filtering prior to being contacted with the calcium sensitive luminescent material.

7. The method of any one of Claims 1-6 in which the calcium-sensitive luminescent material is aequorin, Obeln, Mnemiopsin, Berovin, Pholasin, Luciferases or photoproteins isolated from Pelagia, Cypridina and ostracods.

8. The method of any one of Claims 1-7 in which the ultraviolet light is in the form of a pulse of light in the range of 250-400 nm, and the luminescence is measured by a photomultiplier.

9. The method of Claim 8 in which the calcium-sensitive luminescent material is aequorin and in which the photomultiplier detects light of 400-600 nm and is protected from the magnetic field.

10. The method of any one of Claims 1-9 in which the elongated capture strip is formed of nitrocellulose, polyacrylamide or any other natural or synthetic polymer.

11. The method of Claim 10 in which the elongated capture strip has a transverse stripe with immobilized second binding partner and impregnated with a calcium caging compound.

12. The method of any one of Claims 1-11 in which the calcium caging compound is loaded with calcium in excess of the stoichiometric amount for said calcium-sensitive luminescent material.

13. The method of any one of Claims 1-12 in which the calcium-caging compound is selected from the group consisting of cis-1-(2-bis(carboxymethyl)amino-5-(1-hydroxy-1-(2-nitro-4,5-methylenedioxyphenyl)methyl)phenoxy)-2-(2-bis(carboxymethyl)amino-5-methylphenoxy)cyclopentane, 1-[2-Amino-5-(1-hydroxy-1-[2-nitro-4,5-methylenedioxyphenyl]methyl)phenoxy]-2-)2'-amino-5'methylphenoxy)ethane-N,N,N',N'-tetraacetic acid, 1-(4,5 dimethoxy-2-nitrophenyl)-1,2 diaminoethane-N, N, N', N'-tetraacetic acid and nitrophenyl-ethylenebis(oxyethylenenitrilo) tetraacetic acid.

14. The method of any one of Claims 1-13 which is an immunoassay for detecting and quantifying an antigen.

15. The method of any one of Claims 1-13 which is an immunoassay for detecting and quantifying an antibody.

16. The method of any one of Claims 1-13 in which the binding assay is nucleic acid hybridization assay for detection and quantifying a particular sequence of nucleic acid.

17. The method of any one of Claims 1-16 in which the calcium-sensitive luminescent material is aequorin.

18. The method of any one of Claims 1-17 in which the ultraviolet light source emits a pulse of light in the range of 250-400 nm.

19. The method of any one of Claims 1-18 in which the luminescence is measured by a photomultiplier.

20. The method of any one of Claims 1-19 in which the calcium-sensitive luminescent material is aequorin and photomultiplier detects light of 400-600 nm and is protected from the magnetic field.

21. A method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) immobilizing a first binding partner of a binding reaction onto a solid surface, said solid surface being paramagnetic particles, said first binding partner being biotinylated;

(b) contacting said first binding partner with said solution;

(c) contacting the solution with a second binding partner, said second binding partner being conjugated to a calcium-sensitive luminescent material;

(d) after a period of time, mobilizing the paramagnetic particles in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the particles with a stripe transversely located on said capture strip, said capture strip having streptavidin immobilized onto said transverse stripe, said transverse stripe additionally contain a calcium-caging compound,

(e) allowing a period of time sufficient for the paramagnetic particles to contact said streptavidin immobilized onto said transverse stripe,

(f) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect the release of calcium from the calcium caging compound; and

(g) measuring luminescence emitted by the calcium-sensitive luminescent material.

22. The method of Claim 21 in which steps (b) and (c) are carried out simultaneously.

23. A method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting a first binding partner with said solution, said first binding partner being biotinylated;

(b) after a period of time, contacting the solution with a second binding partner, said second binding partner being conjugated to a calcium-sensitive luminescent material;

(c) after a further period of time, mobilizing the binding partners in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the binding partners with a stripe transversely located on said capture strip, said capture strip having streptavidin immobilized onto said transverse stripe, said transverse stripe additionally contain a calcium-caging compound,

(d) allowing a period of time sufficient for the binding partners to contact said streptavidin immobilized onto said transverse stripe,

(e) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect the release of calcium from the calcium caging compound; and

(f) measuring luminescence emitted by the calcium-sensitive luminescent material.

24. The method of Claim 23 in which steps (a) and (b) are carried out simultaneously.

25. The method of any one of Claims 21-24 in which the elongated capturing strip has a transverse section thereof impregnated with streptavidin and a calcium-caging compound.

26. The method of any one of Claims 1-25 in which the pulse of ultraviolet light and the detection of chemiluminescence are conducted in a time-resolved manner.

27. The method of any one of Claims 1-26 in which the solution contain less than 20 nanomolar of calcium before the pulse of ultraviolet light.

28. An elongated capture strip for binding assays, said strip having a transverse section thereof impregnated with streptavidin and a calcium caging compound.

29. The elongated capture strip of Claim 28 in which the capture strip is formed from nitrocellulose, polyacrylamide, polyamide or any other synthetic or naturally occurring polymer.

30. The elongated capture strip of Claim 28 or Claim 29 in which the capture strip is in a housing.

31. The elongated capture strip of Claim 30 in which the capture strip is housed within a support as a single use testing cartridge.

32. The elongated capture strip of any one of Claims 28-31 in which calcium-caging compound is selected from the group consisting of cis-1-(2-bis(carboxymethyl)amino-5-(1-hydroxy-1-(2-nitro-4,5-methylenedioxyphenyl)methyl)phenoxy)-2-(2-bis(carboxymethyl)amino-5-

methylphenoxy)cyclopentane, 1-[2-Amino-5-(1-hydroxy-1-[2-nitro-4,5-methylenedioxyphenyl]methyl)phenoxy]-2-)2'-amino-5'methylphenoxy)ethane-N,N,N',N'-tetraacetic acid, 1-(4,5 dimethoxy-2-nitrophenyl)-1,2 diaminoethane-N, N, N', N'-tetraacetic acid and nitrophenyl-ethylenebis(oxyethylenenitrilo) tetraacetic acid.

33. A plastic cartridge for conducting a binding assay to detect the presence of an analyte in a solution, comprising:
a housing with a receptacle for receipt of a sample, a reservoir containing biotinylated first binding partner immobilized onto paramagnetic particles and a second binding partner conjugated to calcium-sensitive chemiluminescence material, an elongated capture strip within the housing and in fluid communication with the reservoir, said capture strip having a transverse section thereof impregnated with a calcium-caging compound and streptavidin, said transverse section being protected with a light barrier.

34. The plastic cartridge of Claim 32 in which there is a filter between the receptacle and the reservoir.

35. The plastic cartridge of Claim 33 in which there is a filter containing an agent for removal of calcium.

36. The plastic cartridge of any one of Claims 33-35 in which the calcium-sensitive luminescent material is aequorin, Obeln, Mnemiopsin, Berovin, Pholasin, Luciferases or photoproteins isolated from Pelagia, Cypridina and ostracods.

37. Apparatus for carrying out a binding assay comprising a housing enclosing (a) a receptacle to receive the plastic cartridge of any one of Claims 33-36; (b) a means for removing the light protective layer over the transverse stripe; (c) an electromagnet to provide a magnetic field; (e) an ultraviolet light source to project light on a pre-selected portion of the capture strip, and (f) a

photomultiplier disposed to receive light emitted by the pre-selected portion of the capture strip.

38. The apparatus of Claim 37 in which the electromagnet projects multiple magnetic fields along the plastic cartridge.

39. The apparatus of Claim 37 or Claim 38 in which the ultraviolet light source provides light in the range of 250-400 nm.

40. The apparatus of any one of Claims 37-39 in which the photomultiplier detects light in the range of 400-600 nm.

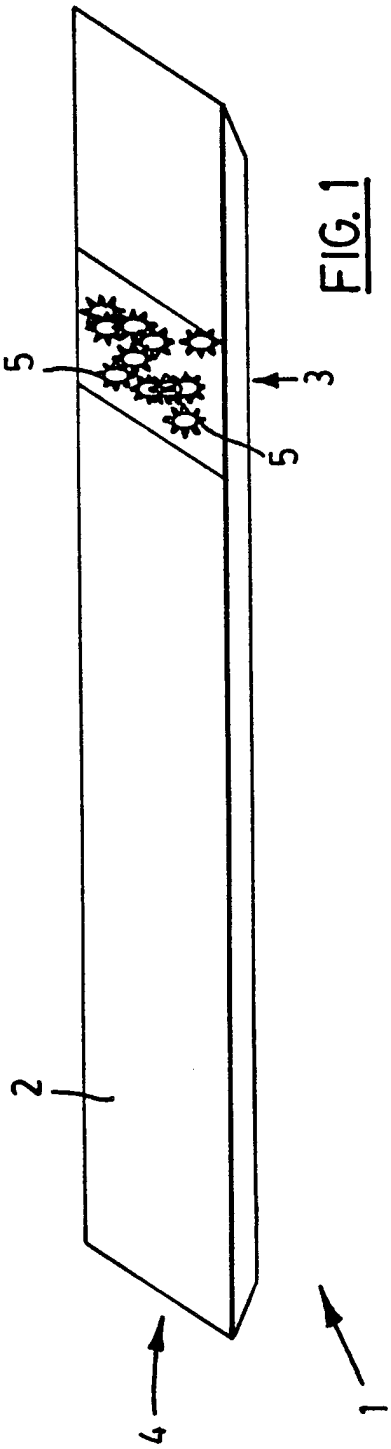

FIG. 1

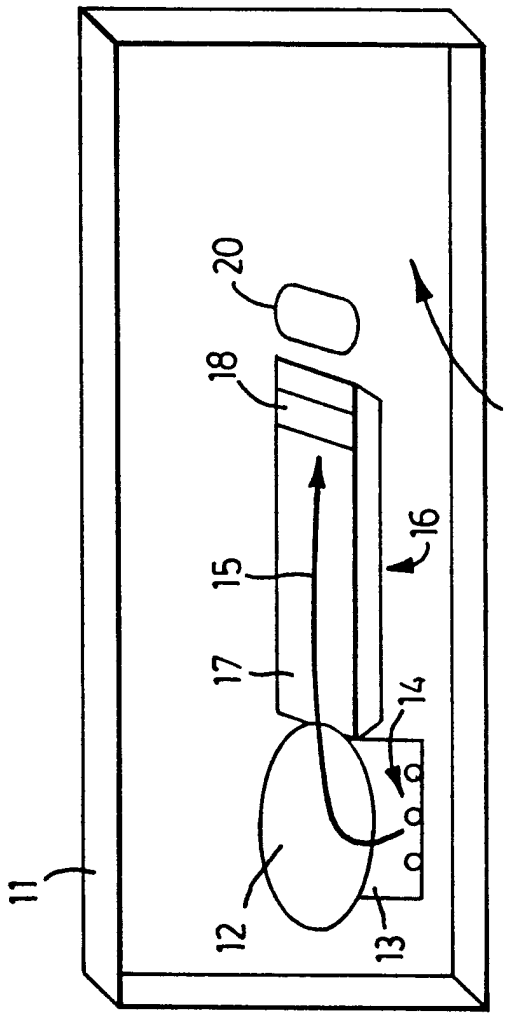

FIG. 2

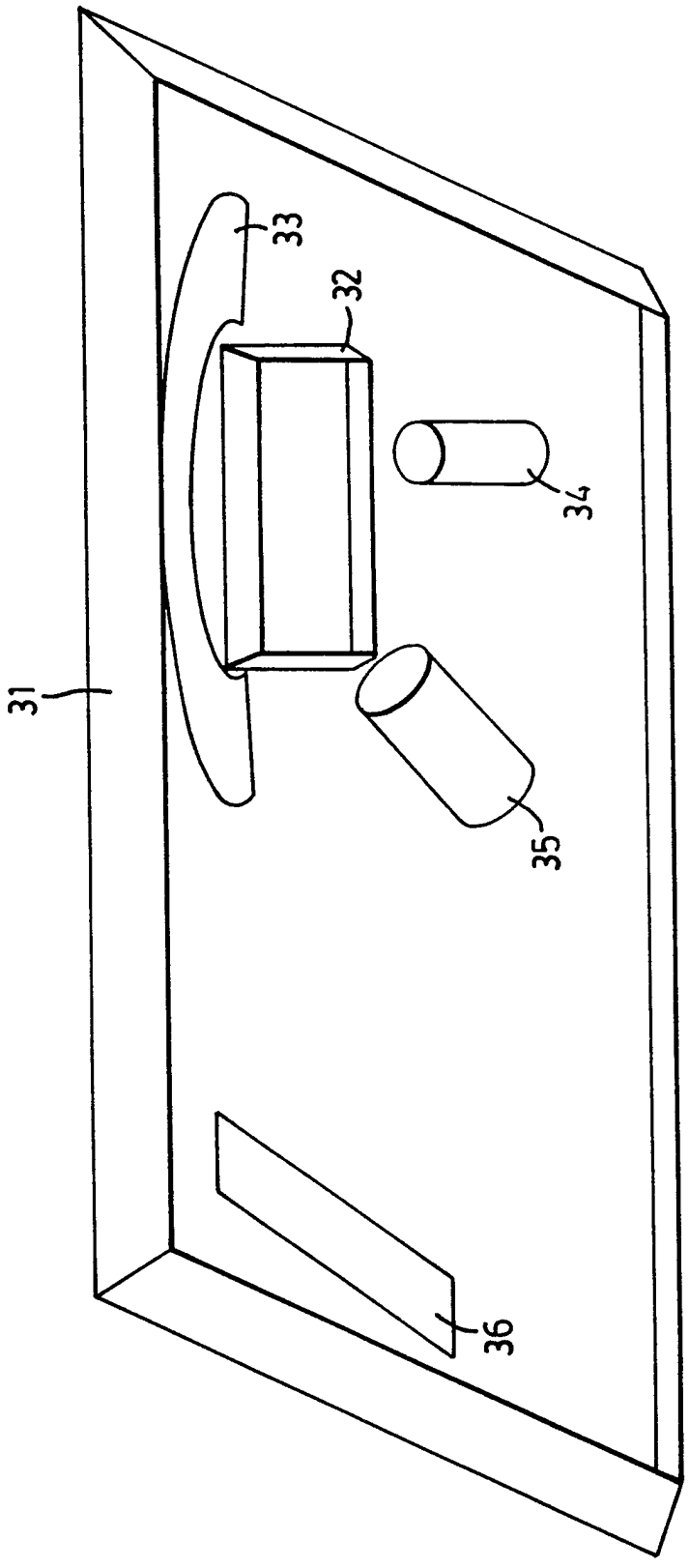

FIG. 3

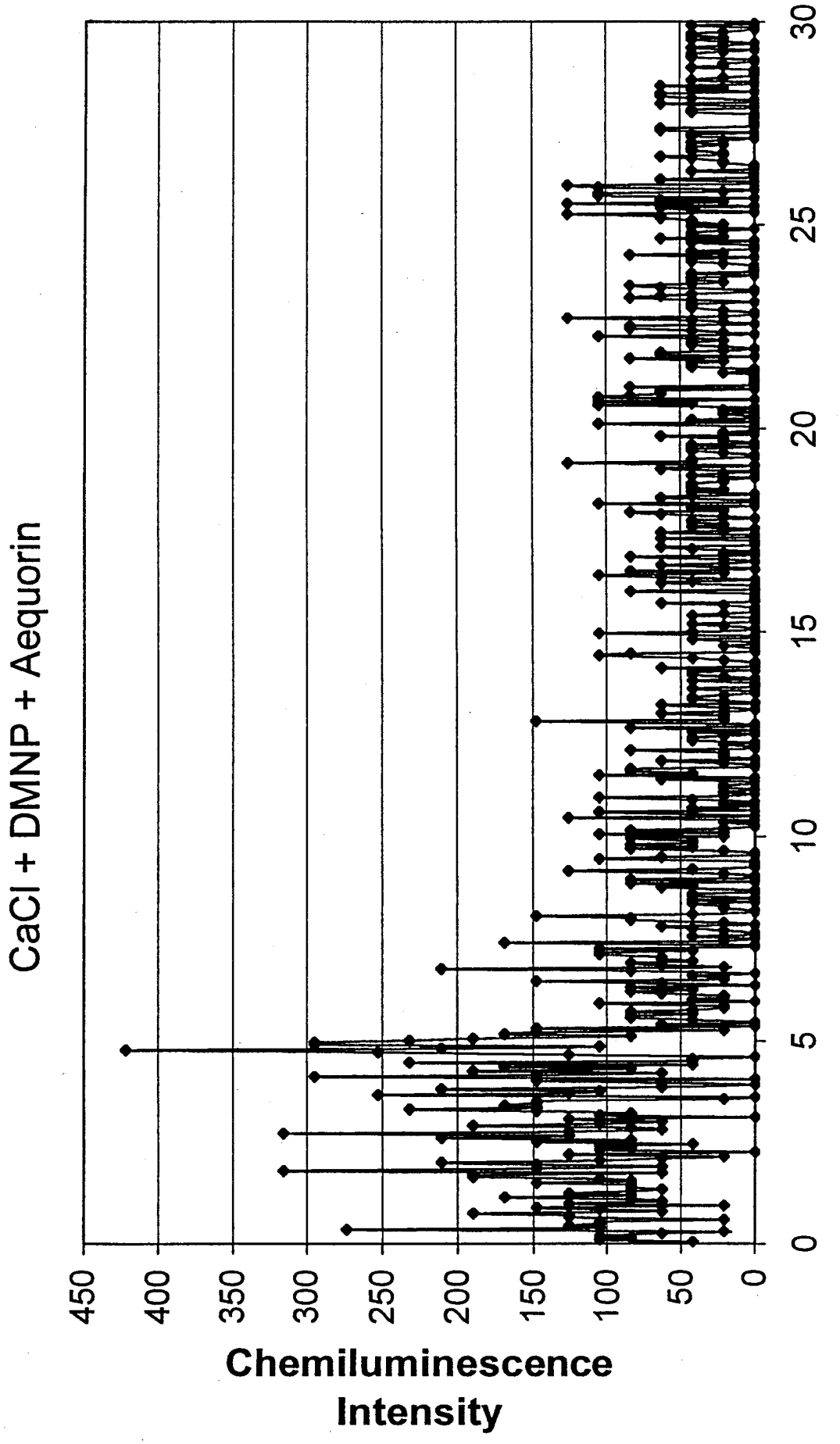
CaCl + DMNP + Aequorin
Chemiluminescence Intensity
450
400
350
300
250
200
150
100
50
0
0
5
10
15
20
25
30
Time (s)

FIG. 4

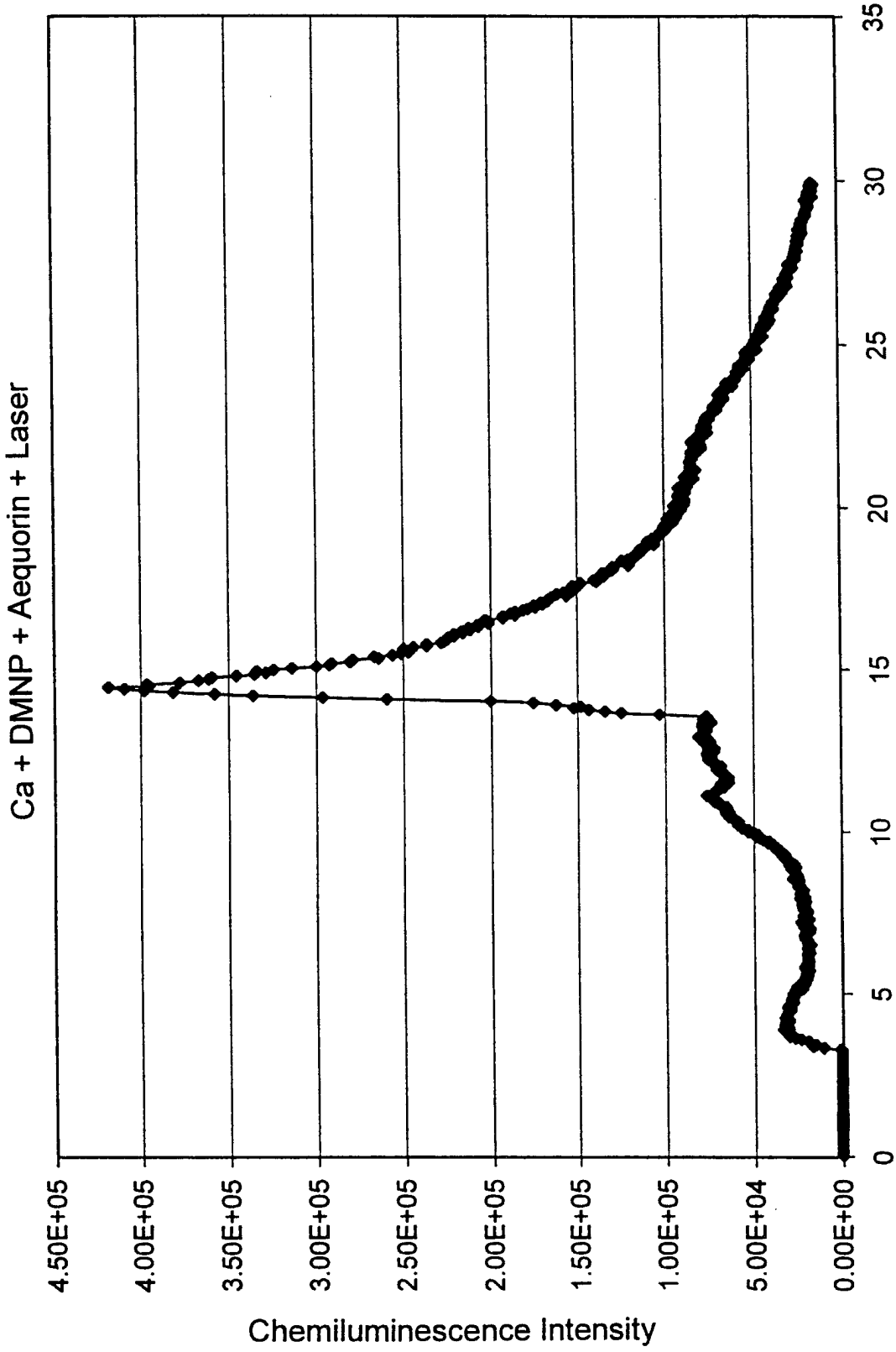
Ca + DMNP + Aequorin + Laser
Chemiluminescence Intensity
4.50E+05
4.00E+05
3.50E+05
3.00E+05
2.50E+05
2.00E+05
1.50E+05
1.00E+05
5.00E+04
0.00E+00
Time (s)
0
5
10
15
20
25
30
35

FIG. 5

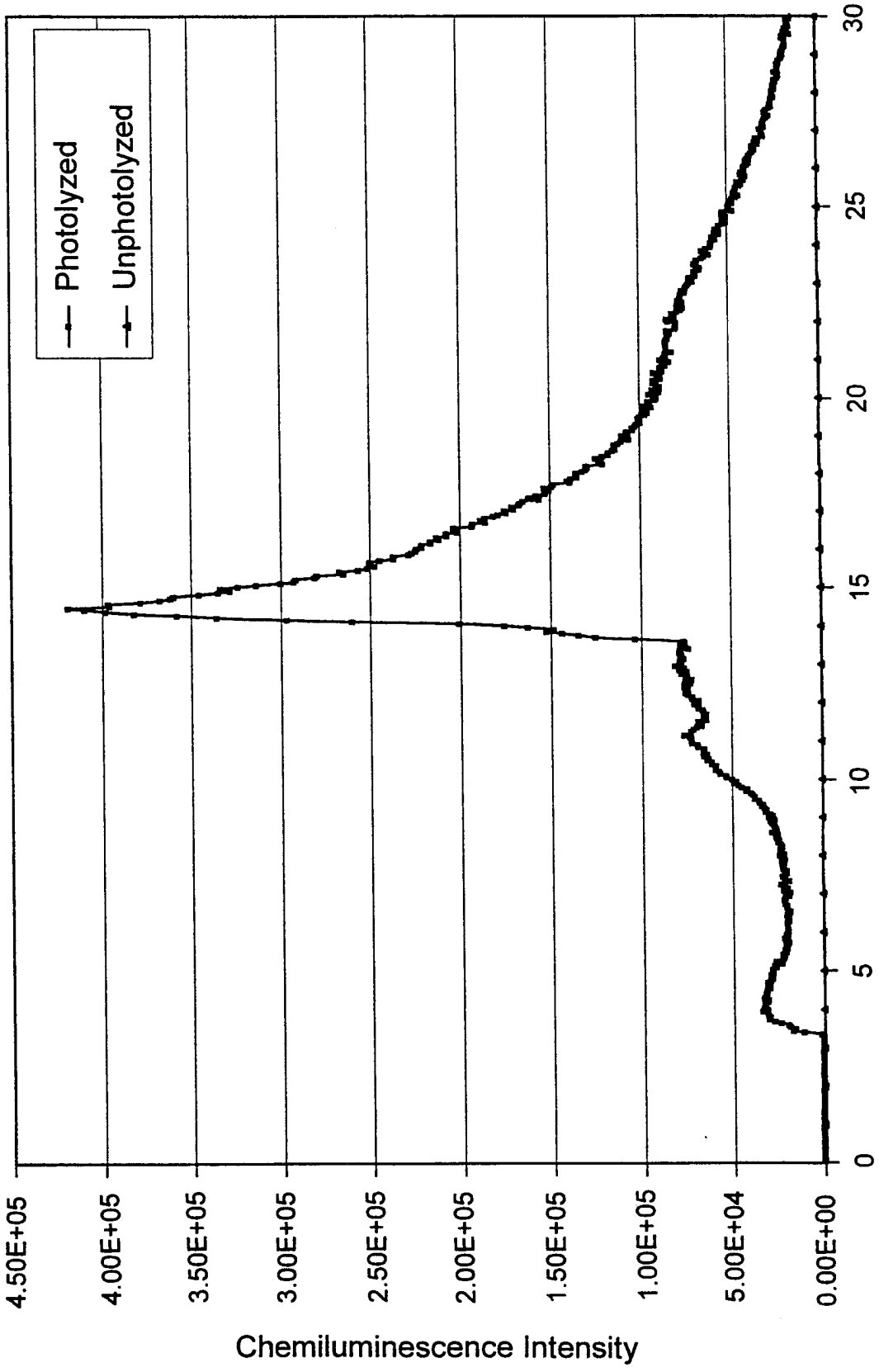
Photolyzed
Unphotolyzed
4.50E+05
4.00E+05
3.50E+05
3.00E+05
2.50E+05
2.00E+05
1.50E+05
1.00E+05
5.00E+04
0.00E+00
Chemiluminescence Intensity
0
5
10
15
20
25
30
Time (s)

FIG. 6

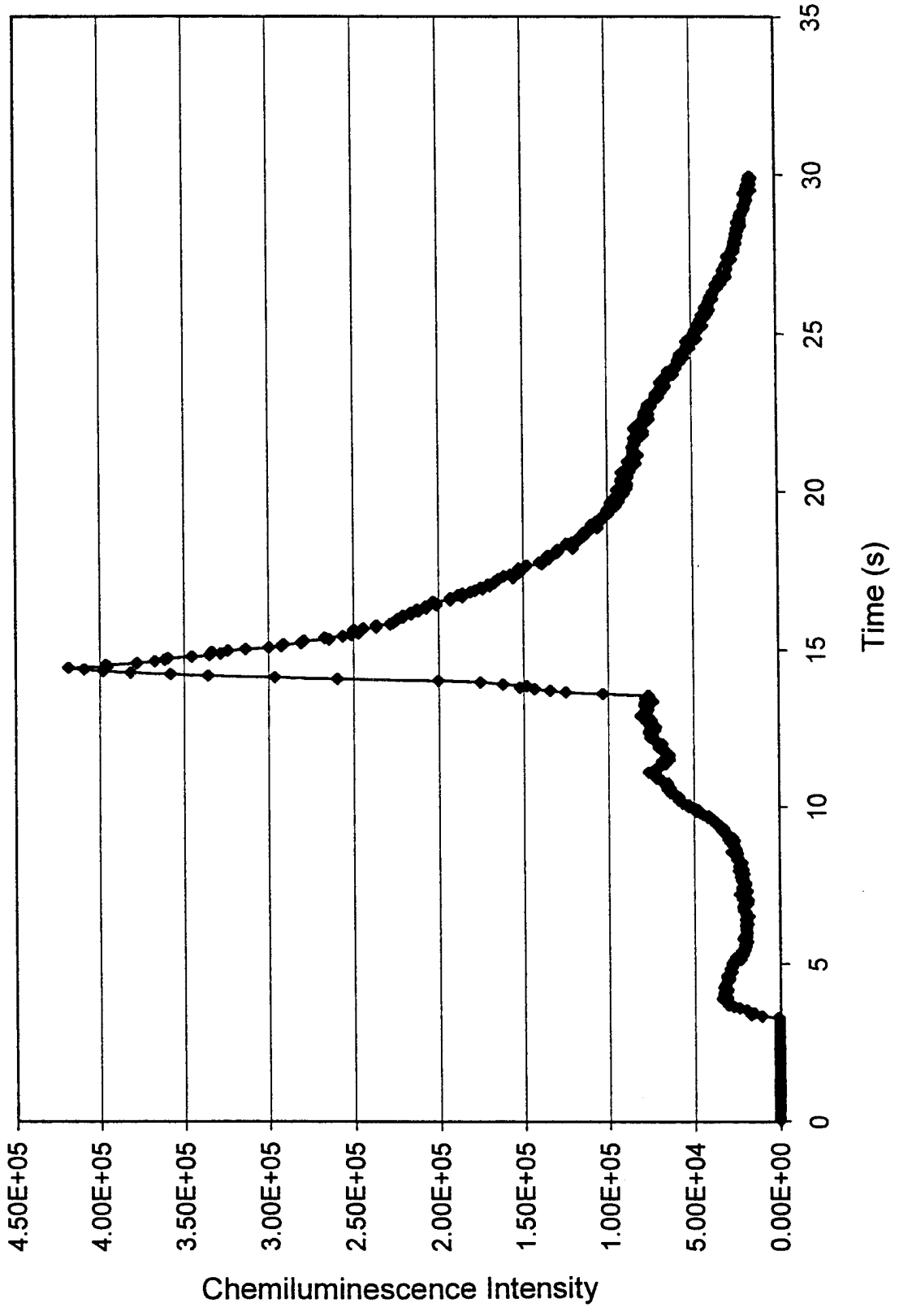
4.50E+05
4.00E+05
3.50E+05
3.00E+05
2.50E+05
2.00E+05
1.50E+05
1.00E+05
5.00E+04
0.00E+00
Chemiluminescence Intensity
0
5
10
15
20
25
30
35
Time (s)

FIG. 7

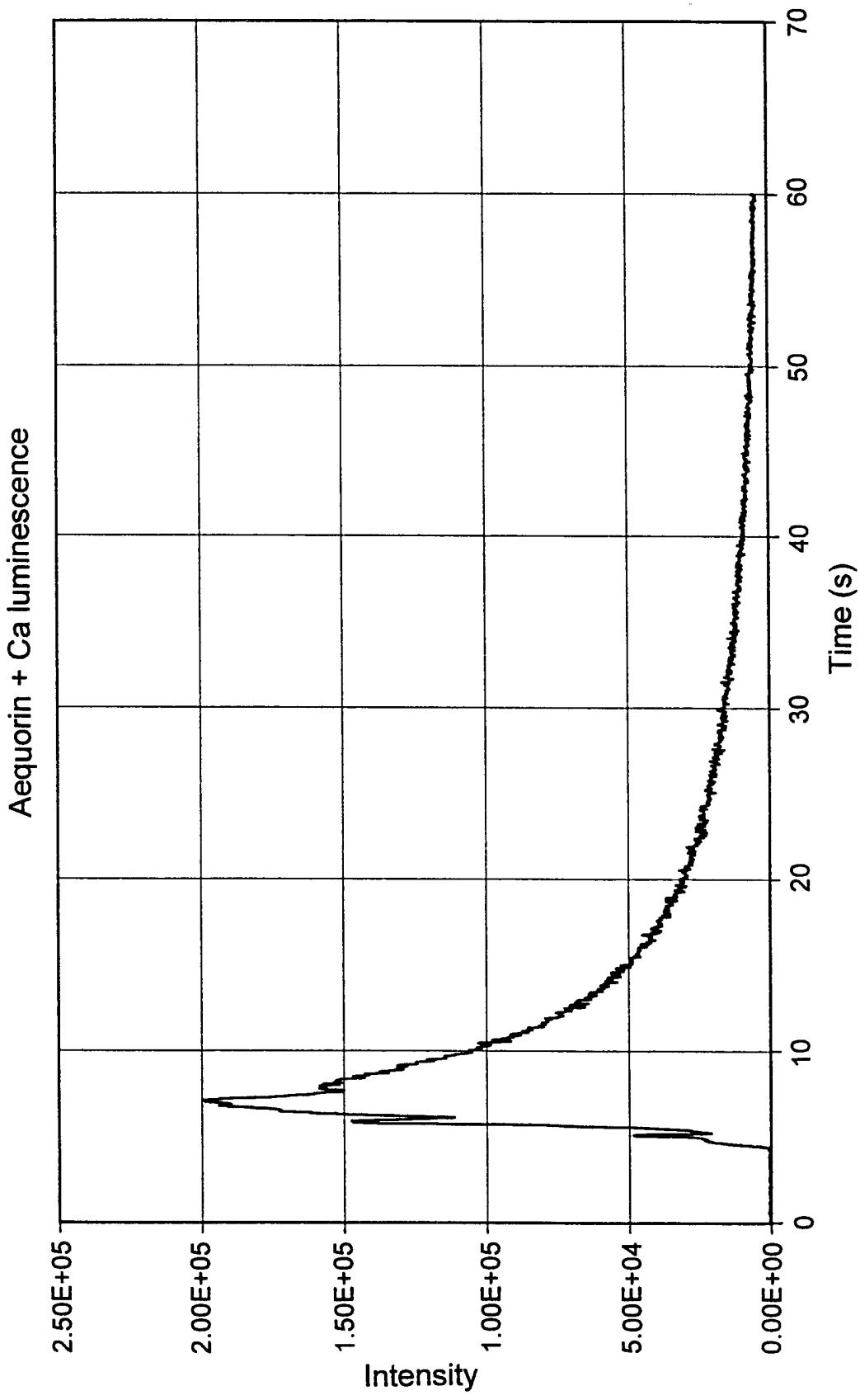
Aequorin + Ca luminescence
Intensity
2.50E+05
2.00E+05
1.50E+05
1.00E+05
5.00E+04
0.00E+00
Time (s)
0
10
20
30
40
50
60
70

FIG. 8

INTERNATIONAL SEARCH REPORT

Interna .l Application No
PCT/CA 00/00718

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/533 G01N33/58

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, FSTA, INSPEC, COMPENDEX, BIOSIS, CHEM ABS Data, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 30908 A (DADE BEHRING INC) 16 July 1998 (1998-07-16) page 3, line 30 -page 4, line 23 page 6, line 26 -page 9, line 21 examples 1,2 ---	1-8,10, 14-19, 21-24, 26,33-40
X	EP 0 437 013 A (ELA TECHNOLOGIES INC) 17 July 1991 (1991-07-17) column 1, line 29 - line 56 column 4, line 12 - line 47 column 7, line 32 - line 58 claims 1-7 --- -/--	1,4-8, 10,14-19

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 October 2000	19/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Menidjel, R

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna I Application No
PCT/CA 00/00718

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	KENDALL J M ET AL: "Aequorea victoria bioluminescence moves into an exciting new era" TRENDS IN BIOTECHNOLOGY,GB,ELSEVIER PUBLICATIONS, CAMBRIDGE, vol. 16, no. 5, 1 May 1998 (1998-05-01), pages 216-224, XP004117786 ISSN: 0167-7799 page 216, right-hand column, paragraph 4 -page 217, right-hand column, paragraph 2 ---	1,3-8, 10,11, 13-19, 33-40
A	US 5 486 455 A (STULTS NANCY L) 23 January 1996 (1996-01-23) column 2, line 41 - line 64 column 4, line 1 - line 44 ---	21-32
P,X	WO 99 38999 A (PACKARD INSTRUMENT CO INC) 5 August 1999 (1999-08-05) abstract page 3, line 16 -page 5, line 2 -----	1,3,4, 6-8, 10-16, 18,19

1

INTERNATIONAL SEARCH REPORT

mation on patent family members

Interna l Application No
PCT/CA 00/00718

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		MX 9401112 A	31-08-1994
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专利名称(译)	进行化学发光结合测定的方法		
公开(公告)号	EP1194781A1	公开(公告)日	2002-04-10
申请号	EP2000938417	申请日	2000-06-15
[标]申请(专利权)人(译)	CARDIOGENICS		
申请(专利权)人(译)	CARDIOGENICS INC.		
当前申请(专利权)人(译)	CARDIOGENICS INC.		
[标]发明人	GAWAD YAHIA		
发明人	GAWAD, YAHIA		
IPC分类号	C12M1/34 C12N15/09 C12Q1/66 C12Q1/68 G01N21/78 G01N33/533 G01N33/543 G01N33/558 G01N33/58		
CPC分类号	G01N33/558 G01N33/54326 G01N33/582		
优先权	60/139941 1999-06-18 US		
其他公开文献	EP1194781B1		
外部链接	Espacenet		

摘要(译)

一种用于进行含有或怀疑含有靶分析物的溶液的受体 - 配体结合反应的方法。该方法包括以下步骤：将第一结合配偶体结合到顺磁颗粒的表面，将第二结合配偶体与钙敏感的发光化合物结合;使第一和第二结合配偶体与待测溶液接触，使顺磁颗粒沿着捕获条固定，所述捕获条具有包含链霉抗生物素蛋白并含有笼状钙化合物的横条纹，将横条纹暴露于紫外光脉冲以实现释放来自笼状钙化合物的钙，并测量由钙敏感的发光材料发射的发光。该方法可用于血液测试。还公开了一种装置。