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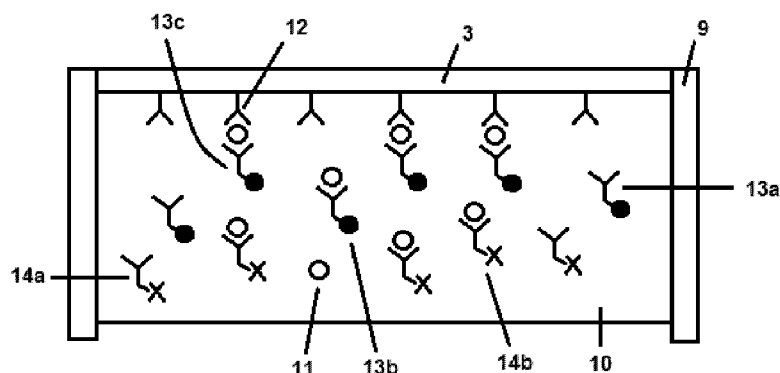


Fig. 2

(57) Abstract: This invention provides a method for detecting an analyte in a sample comprising: providing a device having a sample chamber, a radiation source adapted to generate a series of pulses of electromagnetic radiation, a transducer having a pyroelectric or piezoelectric element and electrodes, which is capable of transducing energy generated by non-radiative decay into an electrical signal, a detector which is capable of detecting the electrical signal, a controller for controlling the source of electromagnetic radiation and the detector, and a capture reagent proximal to the transducer surface, the capture reagent having a binding site which is capable of binding a labelled reagent proportionally to the concentration of the analyte in the sample, wherein the labelled reagent has a first label which is capable of absorbing the electromagnetic radiation to generate energy by non-radiative decay, in which the detector is arranged to detect only the electrical signal corresponding to non-radiative decay occurring proximal to the transducer; exposing the sample to the transducer; introducing a virtual- diluent reagent, the virtual-diluent reagent having a second label and a binding site which is capable of binding the analyte, wherein the second label is capable of retarding movement of the virtual-diluent reagent towards the transducer and/or is incapable of absorbing the electromagnetic radiation to generate energy by non-radiative decay; and irradiating the sample with electromagnetic radiation and detecting the electrical signal.

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A method for detecting an analyte

The present invention relates to a method for detecting an analyte, and particularly to dilution methods for performing assays.

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Immunoassays are often used for measuring the concentration of an analyte in a sample. However, they are limited in terms of the lower limit of detection, the upper limit of detection and the dynamic range.

10 In a typical sandwich immunoassay, an antibody specific for an antigen of interest is attached to a polymeric support such as a sheet of polyvinylchloride or polystyrene. A drop of cell extract or a sample of serum or urine is laid on the sheet, which is washed after formation of the antibody-antigen complex. Antibody specific for a different site on the antigen is then added, and the sheet is again washed. This second antibody carries a label so that it can be
15 detected with high sensitivity. The amount of second antibody bound to the sheet is proportional to the quantity of antigen in the sample. This assay and other variations on this type of assay are well known, see, for example, "The Immunoassay Handbook, 2nd Ed." David Wild, Ed., Nature Publishing Group, 2001.

20 The upper limit of detection in an immunoassay is usually limited by the binding affinities and the total quantity of reagents used. The amount of antibody that can be immobilised on a support is limited by the finite surface area of the support. When excess analyte is present in the sample, the system can become saturated, as all of the antibody binding sites become blocked with analyte. The excess analyte is removed during subsequent wash steps.
25 Therefore, the system is unable to discriminate between concentrations of analyte above saturation, typically at concentrations between 1 μ M and 100 mM.

In a homogenous sandwich assay, the first antibody, the analyte and the second antibody are all incubated simultaneously and there are no wash steps. As the analyte concentration
30 increases, the detected signal increases, until saturation is reached. At concentrations of analyte above saturation, the analyte binds separately to the first and second antibodies, preventing sandwich formation. Consequently, the amount of second antibody bound to the sheet is now no longer proportional to the quantity of antigen in the sample and the detected signal decreases – an effect known as "high-dose hook".

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In a competitive assay, a limited amount of antibody is present and the analyte of interest competes for the limited binding sites present on the antibody with a derivative of the analyte. Although competitive assays do not suffer from "high-dose hook", they are limited by their narrow dynamic range owing to the limited amount of antibody present.

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To overcome the problems associated with high concentrations of analyte, samples are commonly diluted. However, this is not always practical, especially in point-of-care assays or assays with only dry reagents.

- 5 Hyttiä et al., Anal. Biochem 2014, 446, 82-86 discloses the use of an additional reagent in an immunoassay which binds to the analyte and lowers the effective concentration of the analyte in the sample. However, the additional reagent-analyte complex was found to interfere with the detection system. Adding additional reagents to effect the “dilution” of a sample also requires reagents with particular epitope specificity, which may not be available for a wide
10 range of assays. Simply adding additional unlabelled capture or reporter antibody leads to complications of unwanted sandwich formation in solution.

Therefore, there exists a need for the provision of a solution to the problem of high analyte concentration which does not suffer from the above-described disadvantages.

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Accordingly, the present invention provides a method for detecting an analyte in a sample comprising:

providing a device having

a sample chamber,

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a radiation source adapted to generate a series of pulses of electromagnetic radiation,

a transducer having a pyroelectric or piezoelectric element and electrodes, which is capable of transducing energy generated by non-radiative decay into an electrical signal,

a detector which is capable of detecting the electrical signal,

a controller for controlling the source of electromagnetic radiation and the detector, and

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a capture reagent proximal to the transducer surface, the capture reagent having a binding site which is capable of binding a labelled reagent proportionally to the concentration of the analyte in the sample, wherein the labelled reagent has a first label which is capable of absorbing the electromagnetic radiation to generate energy by non-radiative decay,

in which the detector is arranged to detect only the electrical signal corresponding to non-

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radiative decay occurring proximal to the transducer;

exposing the sample to the transducer;

introducing a virtual-diluent reagent, the virtual-diluent reagent having a second label and a binding site which is capable of binding the analyte, wherein the second label is capable of retarding movement of the virtual-diluent reagent towards the transducer and/or is incapable

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of absorbing the electromagnetic radiation to generate energy by non-radiative decay; and

irradiating the sample with electromagnetic radiation and detecting the electrical signal.

Thus, the present invention provides a method for detecting an analyte in which the effective concentration of the analyte can be reduced through the provision of a virtual-diluent reagent,

which binds to the analyte and the resultant complex does not interfere with the detection system.

The present invention will now be described with reference to the drawings, in which:

- 5 Fig. 1 shows a schematic representation of the chemical sensing device of WO 2004/090512 which is used with the present invention;
Fig. 2 shows a sandwich immunoassay using the device of the present invention;
Fig. 3 shows a cartridge which may be used in the present invention; and
Fig. 4 shows a TSH assay in whole blood using magnetic beads and without using magnetic
10 beads.

The method of the present invention is used for detecting an analyte in a sample (which may be via the detection of a derivative of the analyte). The method provides a device having: a radiation source adapted to generate a series of pulses of electromagnetic radiation; a
15 transducer having a pyroelectric or piezoelectric element and electrodes, which is capable of transducing energy generated by non-radiative decay into an electrical signal; and a detector which is capable of detecting the electrical signal generated by the transducer. The device of the present invention is based on the device described in WO 2004/090512.

- 20 Fig. 1 shows a chemical sensing device 1 for use in accordance with the present invention which relies on heat generation in a label 2 on irradiation of the label 2 with electromagnetic radiation. For the sake of simplicity, only the label is shown in Fig. 1 (the remaining components of the device of the present invention will be described in further detail hereinbelow). Fig. 1 shows the chemical sensing device 1 in the presence of a label 2. The
25 device 1 comprises a pyroelectric or piezoelectric transducer 3 having electrode coatings 4,5. The transducer 3 is preferably a poled polyvinylidene fluoride film. The electrode coatings 4,5 are preferably transparent and most preferably formed from indium tin oxide. The electrodes preferably have a thickness of about 35 nm, although almost any thickness is possible from a lower limit of 1 nm below which the electrical conductivity is too low and an upper limit of 100
30 nm above which the optical transmission is too low (it should not be less than 80%T). Preferably the transducer is an indium tin oxide-coated polyvinylidene fluoride film.

- The label 2 is held proximal to the transducer 3 by a binding event. A preferred feature of the present invention is that the label 2 generates heat when irradiated by a source of
35 electromagnetic radiation (typically termed "light") 6, preferably visible light. The light source may be, for example, an LED. The light source 6 illuminates the label 2 with light of the appropriate wavelength (e.g. a complementary colour). Although not wishing to be bound by theory, it is believed that the label 2 absorbs the light to generate an excited state which then undergoes non-radiative decay thereby generating energy, indicated by the curved lines in
40 Fig. 1. This energy is primarily in the form of heat (i.e. thermal motion in the environment)

although other forms of energy, e.g. a shock wave, may also be generated. The energy is, however, detected by the transducer and converted into an electrical signal. The device of the present invention is calibrated for the particular analyte and label being measured and hence the precise form of the energy generated by the non-radiative decay does not need to be determined. Unless otherwise specified the term "heat" is used herein to mean the energy generated by non-radiative decay. The light source 6 is positioned so as to illuminate the label 2. Preferably, the light source 6 is positioned opposite the transducer 3 and electrodes 4,5 and the label 2 is illuminated through the transducer 3 and electrodes 4,5. The light source may be an internal light source within the transducer in which the light source is a guided wave system. The wave guide may be the transducer itself or the wave guide may be an additional layer attached to the transducer. The wavelength of illumination depends on the label used and the sample used. For example, the preferred wavelength for making measurements in whole blood is 690 nm, to avoid interfering signal from erythrocytes.

The energy generated by the label 2 is detected by the transducer 3 and converted into an electrical signal. The electrical signal is detected by a detector 7. The light source 6 and the detector 7 are both under the control of the controller 8. The light source 6 generates a series of pulses of light (the term "light" used herein means any form of electromagnetic radiation unless a specific wavelength is mentioned) which is termed "chopped light". In principle, a single flash of light, i.e. one pulse of electromagnetic radiation, would suffice to generate a signal from the transducer 3. However, in order to obtain a reproducible signal, a plurality of flashes of light are used which in practice requires chopped light. The frequency at which the pulses of electromagnetic radiation are applied may be varied. At the lower limit, the time delay between the pulses must be sufficient for the time delay between each pulse and the generation of an electrical signal to be determined. At the upper limit, the time delay between each pulse must not be so large that the period taken to record the data becomes unreasonably extended. Preferably, the frequency of the pulses is from 1-50 Hz, more preferably 1-10 Hz and most preferably 2 Hz. This corresponds to a time delay between pulses of 20-1,000 ms, 100-1,000 ms and 500 ms, respectively. In addition, the so-called "mark-space" ratio, i.e. the ratio of on signal to off signal is preferably one although other ratios may be used without deleterious effect. There are some benefits to using a shorter on pulse with a longer off signal, in order to allow the system to approach thermal equilibrium before the next pulse perturbs the system. In one embodiment, a light pulse of 1-50 ms, preferably 8 ms, followed by a relaxation time of 10-500 ms, preferably 250 ms allows a more precise measurement of particles bound directly to the surface. Sources of electromagnetic radiation which produce chopped light with different frequencies of chopping or different mark-space ratios are known in the art. The detector 7 determines the magnitude of the electrical signal as a function of the time delay between each pulse of light from light source 6 and the corresponding electrical signal detected by detector 7 from transducer 3. The applicant has

found that this time delay is a function of the distance, d . The signal is preferably measured from 2-7 ms.

Any method for determining the time delay between each pulse of light and the corresponding electrical signal which provides reproducible results may be used. Preferably, the time delay is measured from the start of each pulse of light to the point at which a maximum in the electrical signal corresponding to the absorption of heat from bound label is detected as by detector 7.

The finding that the label 2 may be separated from the transducer surface and that a signal may still be detected was surprising since the skilled person would have expected the heat to be dispersed into the surrounding medium and hence be undetectable by the transducer 3 or at least for no meaningful signal to be received by the transducer. It was found, surprisingly, that not only was the signal detectable through an intervening medium capable of transmitting energy to the transducer 3, but that different distances, d , may be distinguished (this has been termed "depth profiling") and that the intensity of the signal received is proportional to the concentration of the label 2 at the particular distance, d , from the surface of the transducer 3. Moreover, it was found that the nature of the medium itself influences the time delay and the magnitude of the signal at a given time delay. In the present invention, the detector is arranged to detect only the electrical signal corresponding to non-radiative decay occurring proximal to the transducer, and hence detect the binding effect.

The method of the present invention includes exposing the sample to the transducer; introducing a virtual-diluent reagent, the virtual-diluent reagent having a second label and a binding site which is capable of binding the analyte, wherein the second label is capable of retarding movement of the virtual-diluent reagent towards the transducer and/or is incapable of absorbing the electromagnetic radiation to generate energy by non-radiative decay; and irradiating the sample with electromagnetic radiation and detecting the electrical signal.

By way of an explanation of the principle underlying the present invention, Fig. 2 shows a typical capture antibody assay using the device of the present invention. The device includes a transducer 3, a sample chamber 9 for holding a sample 10 containing an analyte 11 present therein and a capture reagent 12 proximal to the transducer surface. The transducer has a capture reagent 12, in the figure, an antibody, attached thereto. This attachment of the capture reagent 12 may be via a covalent bond or non-covalent adsorption onto the surface, such as by hydrogen bonding. An additional layer may separate the capture reagent 12 and the transducer 3, such as a parylene polymer layer, or the antibody could be attached to inert particles and the inert particles are then attached to the transducer 3. Alternatively, the capture reagent 12 could be entrapped within a gel layer which is coated onto the surface of the transducer 3.

In use, the sample chamber is filled with sample 10 containing an analyte 11. The sample chamber also contains an unbound labelled reagent 13a and an unbound virtual-diluent reagent 14a, both of which have a binding site which is capable of binding the analyte 11. A fraction of the analyte binds to the unbound labelled reagent 13a to form a complex containing bound labelled reagent 13b, and another fraction of the analyte binds to the unbound virtual-diluent reagent 14a to form a complex containing bound virtual-diluent reagent 14b, such that the analyte is partitioned across the two reagents 13a and 14a. Subsequently, the complex containing bound labelled reagent 13b can diffuse and bind to the capture reagent 12, leading to the formation of a so-called "sandwich" complex between the capture reagent 12, the analyte 11 and the surface-bound labelled reagent 13c. In this manner, the unbound virtual-diluent reagent 14a has the effect of diluting the sample without actually diluting the sample (hence the term "virtual").

Depending on the initial concentrations of all reagents, at any point in time the sample can contain a mixture of any or all of the following: the unbound analyte 11; the unbound labelled reagent 13a; the unbound virtual-diluent reagent 14a; the complex containing bound labelled reagent 13b; the complex containing bound virtual-diluent reagent 14b; and the sandwich complex between the capture reagent 12, the analyte 11 and the surface-bound labelled reagent 13c.

During or following formation of the sandwich complex, the sample is irradiated using a series of pulses of electromagnetic radiation, such as light. The time delay between each pulse and the generation of an electrical signal by the transducer 3 is detected by a detector. The appropriate time delay is selected to measure primarily the heat generated by the surface-bound labelled reagent 13c. Since the time delay is a function of the distance of the label from the transducer 3, the surface-bound labelled reagent 13c may be distinguished from the unbound labelled reagent 13a and the complex containing bound labelled reagent 13b. This provides a significant advantage over the conventional sandwich immunoassay in that it removes the need for washing steps. In a conventional sandwich immunoassay, the unbound labelled reagent 13a and the complex containing bound labelled reagent 13b must be separated from the surface-bound labelled reagent 13c before any measurement is taken since the unbound labelled reagent 13a and the complex containing bound labelled reagent 13b interfere with the signal generated by the surface-bound labelled reagent 13c. However, on account of the "depth profiling" provided by the present invention, surface-bound labelled reagent 13c may be distinguished from unbound labelled reagent 13a and the complex containing bound labelled reagent 13b. Indeed, the ability to distinguish between labels proximal to the transducer (i.e. bound to the surface) and labels in the bulk solution (i.e. unbound to the surface) is a particular advantage of the present invention.

If a molecule is sufficiently small that formation of an antibody sandwich is not achievable, different types of assay need to be considered. One class of assay for small molecules is the “competitive assay”, in which the analyte of interest competes with another component in the system to prevent binding. In competitive assays the signal is inversely related to the analyte concentration. One particular type of assay is presented in which an analogue of the analyte is immobilised on the transducer and a labelled antibody is introduced into the sample. The analyte and the analogue of the analyte then “compete” for the antibody. In the absence of analyte, the labelled antibody will bind to the analogue of the analyte at the maximum possible rate. However, in the presence of analyte, the labelled antibody becomes populated with analyte and the rate of binding of the analogue of the analyte is diminished. The present invention has applicability to such assays in which the virtual-diluent reagent reduces the effective concentration of the analyte in the sample.

The capture reagent has a binding site which is capable of binding a labelled reagent proportionally to the concentration of the analyte in the sample. The proportionality is important for the functioning of the assay since the binding must be dependent on the concentration of the analyte for any meaningful measure of the concentration of the analyte to be determined. The binding may be directly proportional or indirectly proportional to the concentration of the analyte depending on the type of assay being performed. In the case of a non-competitive assay, e.g. a sandwich assay, the binding is directly proportional to the concentration of the analyte, but for a competitive assay, the binding is indirectly proportional to the concentration of the analyte.

The capture reagent may be adapted to bind to the analyte, or a derivative of the analyte, in which case the labelled reagent will bind to the capture reagent in the presence of the analyte or the derivative of the analyte. In this case, the capture reagent has a binding site which is capable of binding to the labelled reagent in the presence of the analyte or the derivative of the analyte. The binding is, however, still proportional to the concentration of the analyte.

Alternatively, the capture reagent may itself be an analogue of the analyte and the labelled reagent binds directly to the capture reagent (it is an analogue because it is bound to the transducer surface either through covalent bonding or non-covalent interactions). In this case, the capture reagent will compete with the unbound analyte, or an derivative of the analyte, for the binding of the labelled reagent. Accordingly, the capture reagent will simply be capable of binding to the labelled reagent.

Determining the extent of binding of the labelled reagent to the capture reagent (either directly or mediated by the analyte or the derivative of the analyte) provides a measurement of the concentration of the analyte in the sample.

The assay also requires the presence of a labelled reagent. By “labelled” reagent is meant a reagent which is attached to a first label, which first label being capable of absorbing the electromagnetic radiation to generate energy by non-radiative decay. It is this non-radiative decay which is transduced into an electrical signal by the transducer.

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In this way, the labelled reagent can be thought of as “active”, as the labelled reagent can be detected by the detector when proximal to the transducer. In contrast, the virtual-diluent reagent can be thought of as “inactive” or “silent”, as the virtual-diluent reagent cannot be detected by the detector, either by avoiding proximity to the transducer, or by not interacting with the electromagnetic radiation even when proximal to the transducer.

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The first label may be composed of any material which is capable of interacting with the electromagnetic radiation in this manner. Preferably the label is selected from, but not limited to, a carbon particle, a coloured-polymer particle (e.g. coloured latex), a dye molecule, an enzyme, a fluorescent molecule, a metal (e.g. gold) particle, a haemoglobin molecule, a red blood cell, a magnetic particle, a nanoparticle having a non-conducting core material and at least one metal shell layer, a particle composed of polypyrrole or a derivative thereof, and combinations thereof. Preferably, the first label is a carbon particle or a gold particle and most preferably a carbon particle.

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In the case of a magnetic particle, the electromagnetic radiation is radio frequency radiation. All of the other labels mentioned hereinabove employ light, which can include IR or UV radiation. Gold particles are commercially available or may be prepared using known methods (see for example G. Frens, *Nature*, 241, 20-22 (1973)). For a more detailed explanation of the nanoparticle label see US 6,344,272 and WO 2007/141581.

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Preferably, the present invention uses a particle having a particle size of 20 to 1,000 nm, more preferably 100 to 500 nm. By particle size is meant the diameter of the particle at its widest point. The density of the particle will depend on the type of assay. Where the assay is diffusion-controlled, the particle preferably has a density of 0.5 to 3.0 g/mL, more preferably 1.5-2.0 g/mL and most preferably 1.8 g/mL. In this assay type, the particle is a carbon particle having the aforementioned particle size and density. Where the assay is gravity-assisted, the particle preferably has a density of 1.5 to 23 g/mL, more preferably 15-20 g/mL and most preferably 19 g/mL. In this assay type, the particle is a gold particle having the aforementioned particle size and density.

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The first label is proximal to the transducer when the binding event has occurred. That is, the first label is sufficiently close to the surface of the transducer for the transducer to be able to detect the energy generated by the first label on irradiation of the sample. The actual distance between the first label and the surface of the transducer will, however, depend on a

number of variables, such as the size and nature of the label, the size and nature of the antibodies and the analyte, the nature of the sample medium, and the nature of the electromagnetic radiation and the corresponding settings of the detector. The device of the present invention may include a radiation source which is adapted to generate a series of pulses of electromagnetic radiation and the detector is adapted to determine the time delay between each pulse of electromagnetic radiation from the radiation source and the generation of the electric signal thereby allowing a precise determination of the position of the first label with respect to the transducer as discussed with reference to Fig. 1.

The maximum observable signal is the maximum signal that can be achieved when monitoring the first label binding to a surface. In the absence of alternative mass transport phenomena (e.g. convection, magnetic movement, buoyancy, sedimentation, etc.), the binding of particles to the transducer is governed by the diffusion rate of the analyte and labelled reagent which is, in turn, governed largely by the hydrodynamic radius of these components and the viscosity/temperature of the sample.

The assay also requires the presence of a virtual-diluent reagent. The virtual-diluent reagent has a binding site which is capable of binding the analyte and a second label, wherein the second label is capable of retarding movement of the virtual-diluent reagent towards the transducer and/or is incapable of absorbing the electromagnetic radiation to generate energy by non-radiative decay.

The formation of a complex between the virtual-diluent reagent and the analyte translates into an effective reduction of the concentration of the analyte. The actual concentration of the analyte present in the sample remains unchanged as all of the analyte remains in the sample but the concentration of analyte available to promote binding of the labelled reagent to the sensor surface is reduced. Owing to the presence of the second label on the virtual-diluent reagent, the movement of the complex through the sample medium may be hindered and preferably the complex moves away from the transducer. In this case, the complex is prevented from binding to the capture reagent and blocking binding sites on the capture reagent that would otherwise be free to bind analyte. In one embodiment, the second label may be capable of absorbing the electromagnetic radiation to generate energy by non-radiative decay but owing to its distance from the transducer, the detector is not arranged to detect the electrical signal corresponding to non-radiative decay from the second label.

Alternatively, in the event that the complex formed from the virtual-diluent reagent and the unbound analyte binds to the capture reagent, the second label is incapable of absorbing the electromagnetic radiation to generate energy by non-radiative decay. Accordingly the second label on the virtual-diluent reagent is "silent" and is undetected by the detector.

In a further embodiment, the second label may prevent the complex formed from the virtual-diluent reagent and the analyte from binding to the capture reagent by steric hindrance. In another embodiment, the second label may prevent the complex formed from the virtual-diluent reagent and the analyte from binding to the capture reagent by encapsulation of the analyte.

The "virtual dilution" of the sample can be controlled in a variety of alternative ways, including the choice of the first and second labels, the relative affinities of the labelled reagent and the virtual-diluent reagent and the order in which the reagents are added to the sample.

Preferably the second label has a density that causes the second label to move away from the transducer. For example, the first label is a carbon particle and the second label is a silica particle. A virtual-diluent reagent having a silica particle will sink to the bottom of the sample chamber, providing the virtual-diluent reagent is denser than the sample. If the transducer forms the upper surface of the sample chamber, the virtual-diluent reagent having the silica particle will be on the lower surface of the sample chamber and thus be far removed from the transducer.

Alternatively, the second label is a magnetic particle, such as an iron oxide particle, and the method further comprises the step of applying a magnetic field to the sample chamber to attract the magnetic particles away from the transducer. For example, the first label is a carbon particle and the second label is a magnetic particle. If the transducer forms the lower surface of the sample chamber and a magnetic field is applied to the upper surface of the sample chamber, the magnetic particles will be attracted to the upper surface of the sample chamber and will not be proximal to the transducer. Any heat generated in the magnetic particle upon irradiation will not be detected by the transducer owing to the distance of the magnetic particle from the transducer.

In a further alternative, the second label is larger than the first label such that the virtual-diluent reagent diffuses through the sample more slowly than the labelled reagent. For example, the first label is a carbon particle and the second label is a large latex particle. The diameter of the carbon particle is approximately 100 nm and the diameter of the large latex particle is approximately 500-1,000 nm. Therefore, the movement of the large latex particle through the sample is more hindered and the binding of the large latex particle to form a sandwich complex with the analyte or derivative of the analyte and the capture reagent is more sterically hindered than the carbon particle. Therefore, the rate of sandwich complex formation for the large latex particle is lower than for the carbon particle. In a particularly preferred embodiment, the large latex particle is colourless or white so that in the unlikely event of the virtual-diluent reagent binding to the capture reagent, either directly or indirectly via the analyte, no signal corresponding to the second label will be detected by the detector.

Preferably the binding sites, which are capable of binding the analyte, of the labelled reagent and the virtual-diluent reagent are the same. In this case, the labelled reagent and the virtual-diluent reagent have the same binding affinity to the analyte or derivative of the analyte. This leads to a linear dilution of the sample as a function of analyte concentration i.e. the ratio of analyte binding to the virtual-diluent reagent and the labelled reagent is independent of concentration.

However, the virtual-diluent reagent may have a greater affinity for the analogue or derivative of the analogue than the labelled reagent. This may lead to non-linear dilution of the sample as a function of analyte or derivative of analogue concentration. In this respect, most of the analyte or derivative of the analyte binds to the virtual-diluent reagent at low concentration of the analyte or derivative of the analyte. As the concentration of the analyte or derivative of the analyte increases, the virtual-diluent reagent may become saturated and the analyte or derivative of the analyte would only then bind to the labelled reagent. This could be advantageous in controlling assay precision around a cut-off value.

The order of addition of the virtual-diluent reagent and the labelled reagent may be controlled. In one embodiment, the virtual-diluent and the labelled reagent are added simultaneously. In a preferred embodiment, the virtual-diluent reagent is added prior to the labelled reagent. For example, the virtual-diluent reagent is added to the sample first and incubated for a fixed time. In this way, the virtual-diluent reagent binds most of the unbound analyte or derivative of the analyte. The labelled reagent is then added subsequently to the sample. This may also lead to non-linear dilution of the sample as a function of analyte or derivative of analogue concentration, depending upon the relative affinities of the reagents, the dissociation rates, the incubation times and the quantities of the virtual-diluent and labelled reagents used.

The virtual-diluent reagent may be added to the sample before the sample is exposed to the transducer and the complex formed between the virtual-diluent reagent and the analyte does not enter the sample chamber. In one embodiment, the second label on the virtual-diluent reagent is a magnetic particle and a magnetic field is applied to the sample containing the virtual-diluent reagent prior to the sample entering the sample chamber. In another embodiment, the second label on the virtual-diluent reagent is a large particle and the sample containing the virtual-diluent reagent is filtered prior to the sample entering the sample chamber. In this case, the filter is selected to trap only the complex formed between the virtual-diluent reagent and the analyte thus preventing the complexed analyte from binding with the capture reagent.

The virtual-diluent reagent may be formed in situ. In this embodiment, a bifunctional reagent is introduced which contains two binding sites, the first being capable of binding a second

label such as a red or white blood cell and the second being capable of binding the analyte or the derivative of the analyte.

5 The nature of the capture reagent and the labelled reagent will depend on the nature of the analyte, but they are preferably antibodies. In a preferred embodiment, the capture reagent is an antibody and the analyte is an antigen. The capture antibody is raised to the antigen. In an alternative preferred embodiment, the capture reagent is an analogue of an antigen. The labelled reagent is a labelled antibody raised to the antigen or analogue of the antigen. In principle, a single molecule could be used for each reagent, but in practice, the capture
10 reagent and the labelled reagents are a population of molecules. The term "antibody" preferably includes within its scope a Fab fragment, a single-chain variable fragment (scFv), and a recombinant binding fragment.

15 As alternatives to antibody-antigen reactions, the reagents and analyte may be a first and second nucleic acid where the first and second nucleic acids are complementary, or a reagent containing avidin or derivatives thereof and an analyte containing biotin or derivatives thereof, or vice versa. The reagents may also be aptamers. The system is also not limited to biological assays and may be applied, for example, to the detection of heavy metals in water. The system also need not be limited to liquids and any fluid system may be used, e.g. the
20 detection of enzymes, cells and viruses etc. in the air.

The analyte may be a macromolecule or a small molecule. The macromolecule is typically a protein, such as a protein-based hormone, and may also be part of a larger particle, such as a virus, a bacterium, a cell (e.g. a red blood cell) or a prion. The small molecule may be a drug.
25

The term "small molecule" used herein is a term of the art and is used to distinguish the molecule from macromolecules such as proteins and nucleic acids. A small molecule is often referred to in the field of immunoassays as a "hapten", being a small molecule which, when attached to a large carrier molecule such as a protein, can elicit an immune response and
30 includes molecules such as hormones and synthetic drugs. A small molecule of this type will typically have a molecular weight of 2,000 or less, often 1,000 or less and even 500 or less. The capture reagent may be adapted to bind to the analyte itself, although the analyte can undergo a chemical reaction or initial complexing event before binding to the first reagent. For example, the analyte might be protonated/deprotonated in the pH of the assay conditions.
35 Thus, the analyte which is bound to the first reagent may be analyte itself or a derivative of the analyte; both are included within the scope of the present invention.

The device of the present invention is not restricted to detecting only one analyte and different analytes may be detected by employing different capture reagents which selectively bind
40 each analyte, or a derivative of the analyte, being detected. Multiple tests can be carried out

using only one electrical connection to the transducer, by illuminating different locations of the transducer sequentially and interrogating the outputs sequentially. In a preferred embodiment, the present invention may be used to detect the presence of a small molecule and a macromolecule in the same sample at the same time. That is, the sample includes at least two analytes, one being a small molecule and one being a macromolecule. At least two capture reagents are used, one to bind to the small molecule in a competitive assay and one to bind to the macromolecule in a sandwich assay. The virtual-diluent reagent could be used to lower the effective concentration of either or both analytes being measured.

Preferably the method is carried out without removing the sample from the transducer between the steps of exposing the sample to the transducer and irradiating the sample, i.e. the method is a homogeneous assay.

The sample which is suspected of containing the analyte of interest will generally be a fluid sample, e.g. a liquid sample, and usually a biological sample, such as a bodily fluid, e.g. blood, plasma, saliva, serum or urine. The sample may contain suspended particles and may even be whole blood. An advantage of the method of the present invention is that the assay may be performed on a sample which does contain suspended particles without unduly influencing the results of the assay. The sample will typically be in the order of microlitres (e.g. 1-100 μ L, preferably 1-10 μ L). In order to hold a fluid sample, the transducer is preferably located in a sample chamber having one or more side walls, an upper surface and a lower surface. Accordingly, the device of the present invention preferably further comprises a chamber for holding a liquid sample containing the analyte or the derivative of the analyte in contact with the transducer. In a preferred embodiment, the transducer is integral with the chamber, i.e. it forms one of the side walls, or upper or lower surface which define the chamber.

The present invention may be supplied as a part of a kit comprising the device, the labelled reagent and the virtual-diluent reagent. In one embodiment, the present invention provides a kit comprising the device and the virtual-diluent reagent as defined hereinabove in relation to the method of the present invention. Preferably, the kit further comprises the labelled reagent as defined hereinabove in relation to the method of the present invention. In a preferred embodiment, the kit further comprises a chamber for storing the labelled reagent and the virtual-diluent reagent.

A potential additional source of background interference is the settling of suspended particles on to the surface of the piezo/pyroelectric transducer, including labelled reagent and cellular components of the sample. This source of interference may be reduced by positioning the transducer above the bulk solution, e.g. on the upper surface of the reaction chamber. Thus, if any settling occurs, it will not interfere with the transducer. Alternatively, the particles could

be less dense than the medium and hence float to the surface of the bulk solution rather than settling on the surface of the transducer. This and other modifications are included in the scope of the present invention.

5 In a preferred embodiment, the device of the present invention consists essentially of the above-described features. By “essentially” is meant that no other features are required to perform the assay. The device may take the form of a separate reader and cartridge, or an integrated device. In the former, the device is formed of a reader and a cartridge, in which the cartridge is releasably engageable with the reader, and in which the reader incorporates the
10 radiation source and the detector, and the cartridge incorporates the transducer and the capture reagent. The reader is preferably a portable reader. The present invention also provides the cartridge comprising the transducer and the capture reagent as defined herein. The cartridge is preferably a disposable cartridge.

15 Fig. 3 shows a cartridge assembly which may be used in the present invention containing an entry channel to collect a sample, a mixing channel where reagents can be dried down, and three chambers for measuring the concentration of an analyte in a sample. One chamber is used for measuring the concentration of analyte in the sample and the other two chambers are used for control measurements.

20

The present invention will now be described with reference to the following example which is not intended to be limiting.

Example

25

PVDF film

A poled piezo/pyroelectric polyvinylidene fluoride (PVDF) bimorph film, coated in indium tin oxide was used as the sensing device. The indium tin oxide surface was coated with a layer
30 of parylene (of approximate thickness 1 micron) by a vapour phase gas deposition process. This method involved the sublimation and subsequent pyrolysis of a paracyclophane precursor, followed by a free-radical polymerisation on the surface. See WO 2009/141637 for further details. The resulting parylene layer was then coated with a layer of biotinylated bovine serum albumin (10 µg/mL in 10 mM phosphate buffer) by passive adsorption over the
35 course of two hours. The surface was washed and then coated in polystreptavidin solution (10 µg/mL in PBS-10 mmol/L phosphate buffer containing 2.7 mmol/L KCl, 137 mmol/L NaCl and 0.05% Tween) by incubation at room temperature overnight. Polystreptavidin was prepared as described by Tischer et al (U.S. Pat. No. 5,061,640).

40

Materials

Monoclonal antibodies were raised essentially as described in "Monoclonal Antibodies: Properties, Manufacture and Applications" by J. R. Birch and E. S. Lennox, Wiley-Blackwell, 1995, and biotinylated by methods known in the art. Carbon-labelled reporter conjugates were prepared essentially as described by Van Doorn et al. (U.S. Pat. No. 5,641,689).

Preparation of the cartridge

Strips of PVDF pyroelectric polymer film were coated in three separate areas with a universal streptavidin coating, as described above. The three areas were separated by an adhesive spacer attached to the surface of the sensor, allowing subsequent incubation of reagents onto each area without cross-contamination of the surfaces. The three surfaces (labelled spot 1, spot 2 and spot 3) were coated with three different biotinylated reagents, washed and then dried in the presence of sucrose stabiliser.

A cartridge was fabricated to perform the assay, as shown in Fig. 3. The cartridge was fabricated from an antibody-coated piezo/pyrofilm supported on a stiffener. A pressure sensitive adhesive-coated polyester film die-cut to form three sample chambers was applied to the surface. Provision was made to allow for electrical connections to the top and bottom surfaces of the piezo/pyrofilm in order to detect the charge generated. The cartridge was then formed by sandwiching the above components between a top cover, to which a label was applied, and a core, seal and bottom cover.

'Virtual' dilution assays

The concept of virtual dilution was demonstrated using an assay for TSH (thyroid stimulating hormone). The results are shown in Fig. 4.

The assay used the three measurement chambers within the cartridge, denoted spot 1 (negative control), spot 2 (sandwich assay/test) and spot 3 (positive control). Spot 1 was uncoated but washed with a protein and blocker solution, spot 2 was coated with a biotinylated anti-TSH 5409 Medix antibody at a concentration of 2 µg/ml and finally spot 3 was coated with a fixed positive control, biotinylated Abcam goat anti-mouse IgG at a concentration of 1 µg/ml.

Carbon particles coated in a matching anti-TSH antibody, Medix 5407, were used as the label in the system. 0.2% w/v of carbon particles were suspended in deionized water and coated passively with antibody at 200 µg / mL. Dynabeads® MyOne magnetic Tosylactivated beads were also coated in Medix 5407 antibody (10% w/v of beads were coated with antibody at 40

mg / mL). Two experiments were conducted; the first containing the magnetic beads and the second in the absence of magnetic beads. When including the beads into the first experiment, these were added simultaneously to the carbon particles with no incubation time included prior to mixing with the sample.

5

Assays were carried out by charging the sample chambers with the sample through the capillary channel in the core.

10 Samples of whole donor blood were utilised, each containing increasing concentrations of TSH up to 2500 ng/ml. These samples were pre-mixed with the labelled carbon particles and/or the antibody-coated magnetic beads prior to being loaded into the cartridge to fill the three measurement wells. After the measurement chambers had filled, they were then irradiated sequentially with chopped LED light. For each LED pulse, a voltage was measured across the piezo/pyrofilm using an amplifier and analogue to digital (ADC) converter. The
15 change in the ADC signal is calculated over time. The final output from each spot is calculated as the rate of change of signal from the ADC as a function of time. For each cartridge, the final output from spot 1 was subtracted from the outputs in both spot 2 and spot 3 (i.e. both measurements were baseline corrected). Then the baseline corrected measurement in spot 2 was divided by the baseline corrected measurement in spot 3. The
20 mean final assay count (and standard deviation) was then calculated from the cartridges run at each TSH concentration, with and without antibody-coated magnetic beads and plotted accordingly.

25 It can be observed that the dose-response curve of the system in the absence of the virtual-diluent reagent rises sharply and saturates at a TSH concentration of around 200 ng / mL. Hence, the assay only has an effective range up to 200 ng / mL. In contrast, the dose-response curve in the presence of the virtual-diluent reagent rises less steeply and that concentrations of TSH up to 2000 ng / mL can now be distinguished. The effective upper limit of the assay range has been extended by a factor of ten.

30

Claims

1. A method for detecting an analyte in a sample comprising:
providing a device having
 - 5 a sample chamber,
 - a radiation source adapted to generate a series of pulses of electromagnetic radiation,
 - a transducer having a pyroelectric or piezoelectric element and electrodes, which is capable of transducing energy generated by non-radiative decay into an electrical signal,
 - a detector which is capable of detecting the electrical signal,
 - 10 a controller for controlling the source of electromagnetic radiation and the detector, and
 - a capture reagent proximal to the transducer surface, the capture reagent having a binding site which is capable of binding a labelled reagent proportionally to the concentration of the analyte in the sample, wherein the labelled reagent has a first label which is capable of absorbing the electromagnetic radiation to generate energy by non-radiative decay,
 - 15 in which the detector is arranged to detect only the electrical signal corresponding to non-radiative decay occurring proximal to the transducer;
 - exposing the sample to the transducer;
 - introducing a virtual-diluent reagent, the virtual-diluent reagent having a second label and a binding site which is capable of binding the analyte, wherein the second label is capable of
 - 20 retarding movement of the virtual-diluent reagent towards the transducer and/or is incapable of absorbing the electromagnetic radiation to generate energy by non-radiative decay; and
 - irradiating the sample with electromagnetic radiation and detecting the electrical signal.
2. A method as claimed in claim 1, wherein the labelled reagent and the virtual-diluent reagent have the same binding affinity to the analyte.
- 25 3. A method as claimed in claim 1 or 2, wherein the second label has a density that causes the second label to move away from the transducer.
4. A method as claimed in claim 1 or 2, wherein the second label is a magnetic particle and the method further comprises the step of applying a magnetic field to attract the magnetic particles away from the transducer.
- 30 5. A method as claimed in claim 1 or 2, wherein the second label is larger than the first label such that the virtual-diluent reagent diffuses through the sample more slowly than the labelled reagent.
6. A method as claimed in any preceding claim, wherein the capture reagent is an antibody and the analyte is an antigen.

7. A method as claimed in claims 1-5, wherein the capture reagent is an antigen.
8. A method as claimed in any preceding claim, wherein the labelled reagent is a labelled antibody.
9. A method as claimed in any preceding claim, wherein the first and/or second label is
5 selected from a dye molecule, a gold particle, a coloured-polymer particle, a fluorescent molecule, an enzyme, a red blood cell, a haemoglobin molecule, a magnetic particle and a carbon particle.
10. A method as claimed in any preceding claim, wherein the method is carried out without removing the sample from the transducer between the steps of exposing the sample to the
10 transducer and irradiating the sample.
11. A method as claimed in any preceding claim, wherein the virtual-diluent reagent is added prior to the labelled reagent.
12. A kit comprising the device and the virtual-diluent reagent, as defined in any preceding claim.
- 15 13. A kit as claimed in claim 12, wherein the kit further comprises the labelled reagent, as defined in any of claims 1-11.

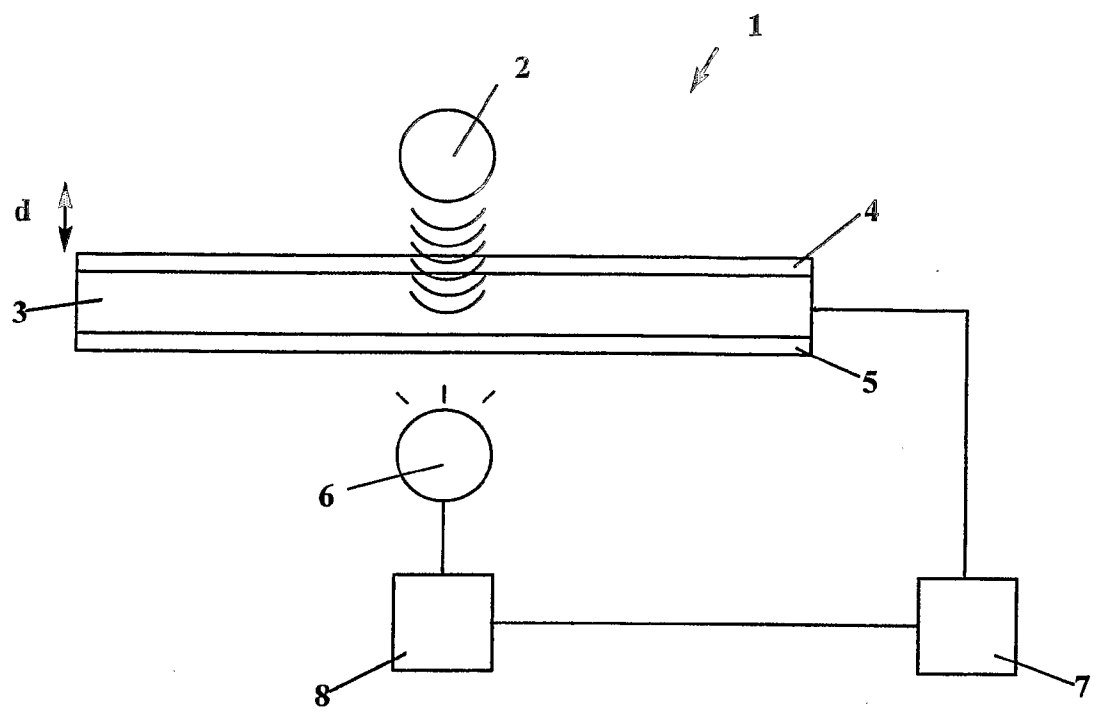


Fig. 1

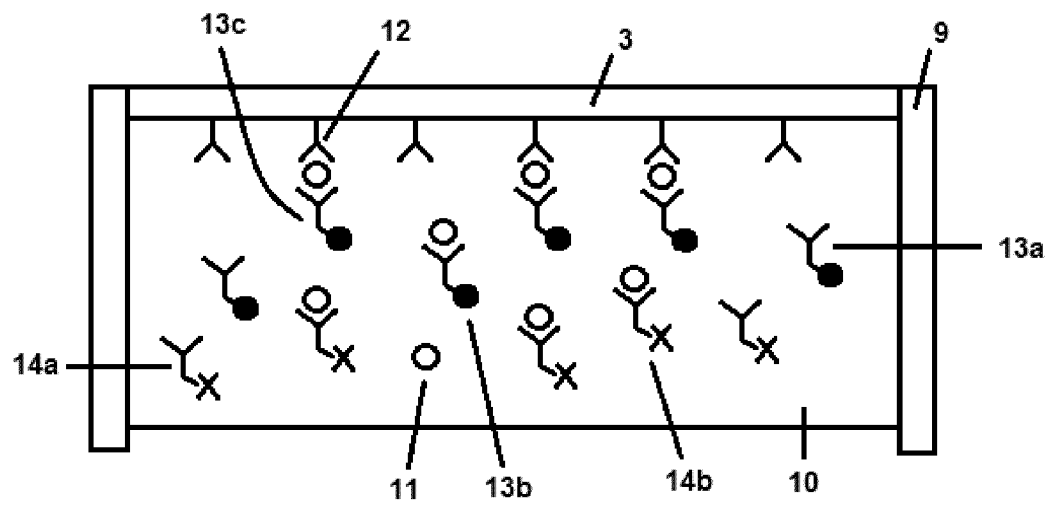


Fig. 2

2/3

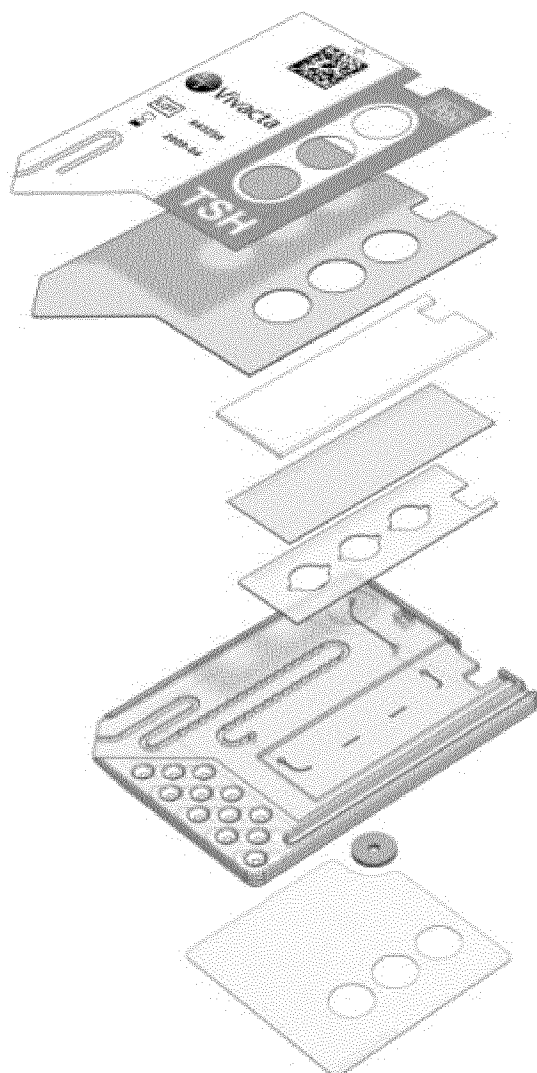


Fig. 3

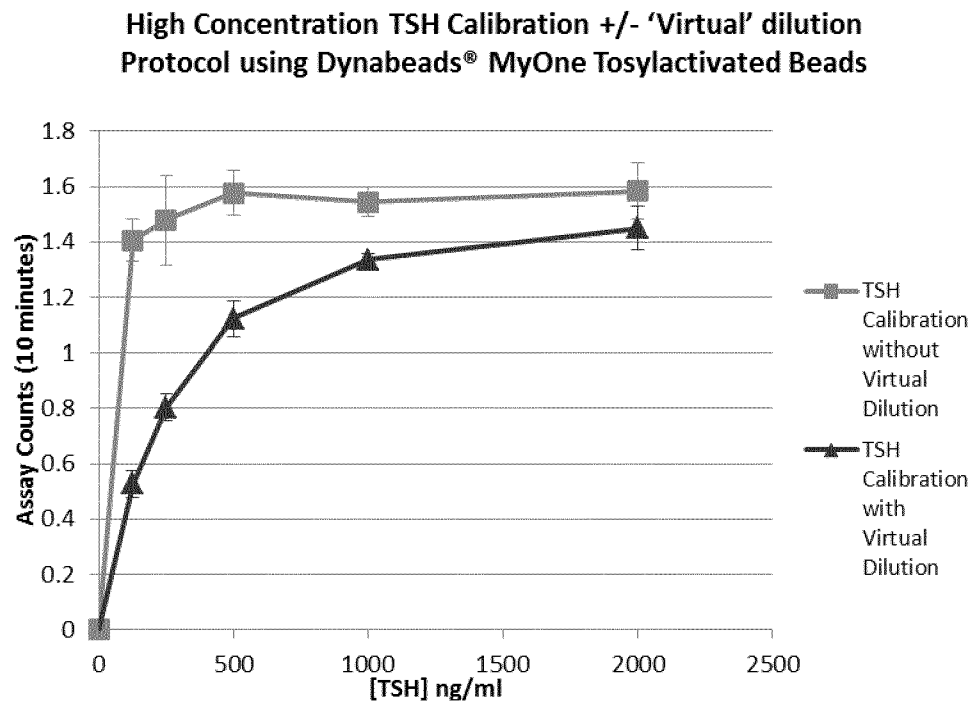


Fig. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/077007

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N29/24 G01N21/17 G01N33/543 G01N33/53
ADD.

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)

G01N

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 practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/137009 A1 (VIVACTA LTD [GB]; ROSS STEVE [GB]; RICHARDS JULIE [GB]; CARTER TIM [GB] 11 October 2012 (2012-10-11) p.2 last par., figure 9, p.13 par.3 -----	1-13
A	HYTTIÄ HEIDI ET AL: "Extension of dynamic range of sensitive nanoparticle-based immunoassays", ANALYTICAL BIOCHEMISTRY, ELSEVIER, AMSTERDAM, NL, vol. 446, 6 November 2013 (2013-11-06), pages 82-86, XP028671393, ISSN: 0003-2697, DOI: 10.1016/J.AB.2013.10.034 -----	1-13



Further documents are listed in the continuation of Box C.



See patent family 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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"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

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"&" document member of the same patent family

Date of the actual completion of the international search

21 December 2017

Date of mailing of the international search report

08/01/2018

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2017/077007

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