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(54) **Device, method, and system for the quantitative detection of the presence of multiple target analytes.**

(57) The present invention relates to a device, method and system for the quantitative detection of the presence or absence of two or more target analytes in a sample consisting of less than 200 µl liquid, said method inter alia comprising the steps of contacting an analyte containing sample in a reaction chamber with a population of magnetic and/or paramagnetic particles, said population comprising two or more pools, each pool being capable of capturing a specific and mutually different analyte, and each pool having a different mean of the ratio

of surface area to weight, separating the population of magnetic and/or paramagnetic particles comprising the captured analytes into said at least two pools by displacing a magnetic field relative to a detector part of the chamber, removing the magnetic field, and finally detecting the presence or absence of the target analytes in each pool of magnetic and/or paramagnetic material using conventional detection means.

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Description

Technical field

[0001] The present invention relates to a device for the quantitative detection of the presence or absence of two or more target analytes in a liquid sample, and to uses thereof.

[0002] The invention further relates to a method for the quantitative detection of the presence or absence of two or more target analytes in a liquid sample having a volume of less than 200 μ l.

[0003] The invention further relates to a system for the quantitative detection of the presence or absence of two or more target analytes in a liquid sample having a volume of less than 200 μ l.

Background

[0004] The use of microfluidic systems of reduced dimension and containing magnetic or paramagnetic particles for analytical applications are well-known, and magnetic or paramagnetic nano- and microparticles have become a hot topic in several technical fields of research. Functional nano- and microparticles ('beads') offer a large specific surface for chemical binding, and a polymer colloid or microsphere solution has a low viscosity compared to solutions having the same amount of solid, giving it special properties. Thus, such small particles can be advantageously used as a 'mobile substrate' for bioassays. One of the most prominent advantages of magnetic beads however, lies in the fact that the particles can be magnetically probed and manipulated using permanent magnets or electromagnets, independent of normal chemical or biological processes.

[0005] Some of the classical applications, like magnetic separation, have already found their way down to miniaturized fluidic or 'lab-on-a-chip' systems that strongly limit the consumption of samples and reagents; in such systems, magnetic beads effectively provide a chemically active substrate with a large surface-to-volume ratio. The booming area of miniaturized applications of magnetic beads offers many exciting possibilities for future developments. It is a highly multidisciplinary area, requiring contributions from inorganic chemistry involved in the preparation of the magnetic beads, through biochemistry and medical science to allow for their functionalization and, of course, the basic physics of magnetism and magnetic materials. Nearly all important functions in a bioassay can be realized using magnetic beads: sample purification, providing a solid substrate to the sample, mixing, labelling, manipulation and transport and, finally, separation.

[0006] Magnetic or paramagnetic beads coated with labels or capture molecules are widely used for the detection of biomarkers and biological molecules, and the magnetic particle-based assay has become a standard in modern chemical and biological diagnostics. The de-

tection of biological molecules is usually accomplished using biomolecular recognition between the target molecule (analyte) and a specific receptor (e.g. an antibody) that is tagged with a label. The label may for example be a radioisotope, enzyme, fluorescent molecule or a charged molecule or any combination thereof. Recently, also magnetic beads have been used as labels for biosensing. These magnetic and paramagnetic beads are manipulated by use of means generating a magnetic field.

[0007] In general, the devices and methods disclosed in the field have not been adapted to the quantitative detection of multiple analytes in one single test. An example of a prior art device suitable for manipulating magnetic and paramagnetic beads according to the present invention is disclosed e.g. in PCT/EP2008/066272, PCT/EP2008/066273 PCT/EP2008/066274 and PCT/EP2009/067863. However, the devices disclosed are not immediately suited for the use according to the present invention, as the paramagnetic material disclosed in these devices is not capable of targeting two or more analytes. Accordingly, when testing for more than one analyte, a test must be run for each specific analyte to be examined for. This situation is inconvenient for several reasons. First, more sample is needed, which naturally is troublesome and not always available. Also, it is time-labour- and material-consuming to run several tests, and hence expensive.

[0008] Accordingly, there is a need in the art for devices and methods for the detection of multiple analytes in a single, small volume sample analysed in a single test.

[0009] Thus, one object of the invention is to provide methods and devices to enable the detection of the presence or absence of multiple target analytes in a liquid sample of less than 200 μ l.

Brief disclosure of the invention

[0010] In the experimental development leading to the present invention, the inventors found that obtaining a highly sensitive, reproducible and fully quantitative assay for quantitatively detecting the presence or absence of multiple analytes in small liquid samples required the presence and separability of different pools of magnetic material, each pool being capable of capturing specific and mutual different analytes. Furthermore, these different pools of magnetic or paramagnetic material should be allowed to be detected separately.

[0011] Surprisingly, the problem of the invention was solved by providing two or more pools of magnetic particles in a chamber device according to the prior art such as described in PCT/EP2008/066272, PCT/EP2008/066273 PCT/EP2008/066274 PCT/EP2009/067863, wherein the pools differ from one another in the amount of magnetic material relative to the surface area. Each pool of magnetic particles is capable of capturing a specific and mutually different analyte, thus providing an assay for multiple target analysis. The inventors of the

present invention found that they could separate these pools of different particles by displacing an external magnet in a rectilinear movement across the chamber device comprising the magnetic particles. The motion of the external magnet allows the different pools of particles containing different amounts of magnetic material relative to the surface area in the chamber device to be released from the magnetic field generated by the external magnet at different times, thus falling to the bottom part of the chamber device in distinct pools. In this way, the different pools are separated in distinct piles at the bottom of the detector part of the chamber device. The amount of analyte can be detected by scanning the area of the detector part of the chamber with suitable means for detection.

[0012] Accordingly, the invention relates to a method for the quantitative detection of the presence or absence of two or more target analytes in a sample consisting of less than 200 μ l liquid, comprising the steps of:

- a) providing an analyte containing liquid sample consisting of less than 200 μ l liquid;
- b) supplying the liquid sample to a reaction chamber;
- c) contacting the sample in the reaction chamber with a population of magnetic and/or paramagnetic material, said population comprising two or more pools, each pool being capable of capturing a specific and mutually different analyte, and each pool having a different mean of the ratio of surface area to weight, the weight being the weight of the magnetic or paramagnetic material present in the particle;
- d) immobilising the population of magnetic and/or paramagnetic material comprising the captured analytes;
- e) washing the population of magnetic and/or paramagnetic material comprising the captured analytes with a washing solution;
- f) transferring the population of magnetic and/or paramagnetic material comprising the captured analytes to a detector part of the chamber;
- g) separating the population of magnetic and/or paramagnetic material comprising the captured analytes into said at least two pools by displacing an external magnet in a rectilinear movement, and;
- h) removing the magnetic field and detecting the presence or absence of the target analytes in each pool of magnetic and/or paramagnetic material using conventional detection means.

[0013] Further, the invention relates to a chamber device for the quantitative detection of the presence or absence of two or more target analytes in a liquid sample having a volume of less than 200 μ l, the device comprising a reaction chamber comprising a population of magnetic and/or paramagnetic material, said population comprising two or more pools, each pool capable of capturing a specific and mutually different analyte and each pool having a different mean of the ratio of surface area to weight, the weight being the weight of the magnetic or

paramagnetic material present in the particle.

[0014] Further, the invention relates to a system for the quantitative detection of the presence or absence of two or more target analytes in a sample consisting of less than 200 μ l liquid, said system comprising a magnet device and a chamber device, said magnet device being capable of moving a magnetic field along side said chamber device so that the magnetic and/or paramagnetic material in the chamber is subjected to the magnetic field.

[0015] The invention further relates to the use of a population of magnetic and/or paramagnetic material, said population comprising two or more pools, each pool capable of capturing a specific and mutually different analyte, and each pool having a different mean of the ratio of surface area to weight, the weight being the weight of the magnetic or paramagnetic material present in the particle for the quantitative detection of the presence or absence of two or more target analytes in a liquid sample consisting of less than 200 μ l liquid.

Definitions:

[0016] In the context of the present invention, the term "unimodal" has the conventional mathematical meaning of unimodality, i.e. distributions having only one mode. A function $f(x)$ between two ordered sets is unimodal if for some value m (the mode), it is monotonically increasing for $x \leq m$ and monotonically decreasing for $x \geq m$. In that case, the maximum value of $f(x)$ is $f(m)$, and there are no other local maxima.

[0017] In the context of the present invention, the term "bimodal" has the conventional mathematical meaning of bimodality, i.e. distributions having two modes. Generally, bimodal distributions are a mixture of two different unimodal distributions.

[0018] In the context of the present invention, the term "magnetic material or particles" includes paramagnetic material or particles.

[0019] In the context of the present invention, the term "paramagnetic material" means material which is magnetic in the presence of an externally applied magnetic field, but are not magnetic prior to being subjected to a magnetic field. The paramagnetic material according to the invention is preferable paramagnetic particles capable of binding and immobilizing a particular analyte species of interest, e.g. by being coated with a receptor or antibody.

[0020] In the context of the present invention, by "displacing an external magnet in a rectilinear movement" is meant that one or more magnets are moved relative to the chamber device so that the external magnet applies a magnetic field strong enough to influence or attract the magnetic particles present in the chamber device. The movement is preferable unidirectional and may be stepwise alternating between acceleration and deceleration relative to the chamber device. The preferably unidirectional movement may also be at a substantially constant velocity relative to the chamber device. During the dis-

placement, the magnetic influence attracts the magnetic particles present in the chamber device, but due to different size of magnetic material (by weight) the beads move at different velocity through the liquid, and is thus separated.

[0021] In the context of the present invention, by "scanning the area of the detector part of the chamber" is meant that a suitable detector for detecting a signal derived from the captured analytes is arranged in a suitable manner, which enables the discriminated detection of signal derived from the different pools (modus) of magnetic and/or paramagnetic material.

[0022] In the context of the present invention, by "point of care system" is meant a relatively small transportable, portable, and/or handheld instrument or system. The point of care system or instrument is preferably located at or near the site of patient care, e.g. in the doctors clinic, or located at the hospital such as in the intensive care unit instead of in a central laboratory.

Disclosure of the invention

[0023] An object of the present invention is to develop methods and devices for the quantitative detection of the presence or absence of even very small quantities of multiple target analytes in a sample consisting of less than 200 μ l liquid, preferably applying the methods and devices in relatively small handheld "point of care" instruments.

[0024] Surprisingly, it was found that the problem of targeting multiple analytes in one single test in a microfluidic system could be solved by providing pools of magnetic particles wherein the amount (by weight) of magnetic material differs and wherein the different pools of particles are capable of capturing a specific analyte present in the liquid. It was found that it was possible to separate the different pools of magnetic particles through a suitable manipulation by means of an externally applied magnetic field so that the particles are grouped in their distinct pools in the detection part of a chamber device. Thus separated, the different pools of magnetic particles can be analyzed for the presence or absence of the analyte by suitable detection means.

[0025] By combining microfluid- and magnetic particle technology in a special constellation, the present inventors found that it was possible to fulfil the critical parameters, and at the same time apply the constellation in a relative small handheld instrument (below 500 grams), capable of analysing samples of less than 200 μ l.

[0026] Accordingly, the invention relates in one embodiment to a method for the quantitative detection of the presence or absence of two or more target analytes in a sample consisting of less than 200 μ l liquid, comprising the steps of:

- a) providing an analyte containing liquid sample consisting of less than 200 μ l liquid;
- b) supplying the liquid sample to a reaction chamber;

c) contacting the sample in the reaction chamber with a population of magnetic and/or paramagnetic material, said population comprising two or more pools, each pool being capable of capturing a specific and mutually different analyte, and each pool having a different mean of the ratio of surface area to weight, wherein the weight is calculated on the basis of the magnetic of paramagnetic material present in the particle;

d) immobilising the population of magnetic and/or paramagnetic material comprising the captured analytes;

e) washing the population of magnetic and/or paramagnetic material comprising the captured analytes with a washing solution;

f) transferring the population of magnetic and/or paramagnetic material comprising the captured analytes to a detector part of the chamber;

g) separating the population of magnetic and/or paramagnetic material comprising the captured analytes into said at least two pools by displacing an external magnet in a rectilinear movement relative to the chamber device, and;

h) removing the magnetic field detecting the presence or absence of the target analytes in each pool of magnetic and/or paramagnetic material using conventional detection means.

[0027] According to the present invention, the method provides a physical separation, in a microfluidic system, of the steps of reacting, immobilising and purifying the analytes (steps a) to e)) from the steps of detecting the analytes (steps f) to h)), thus minimizing the unspecific background signal relating to the first steps a) to e) of the method.

[0028] The liquid sample of step a) can be any kind of liquid containing analytes to be detected. It is preferred that the liquid is from a mammal, preferably a human. The liquid may be any liquid deriving from the body, such as blood, saliva, urine, peritoneal fluid, gastric juice or the like.

[0029] The reaction chamber of b) may be part of a chamber device suited for a microfluidic system, preferably a point-of-care system. The reaction chamber comprises one or more capillary channels having a volume of less than 200 μ l, a sample inlet for the introduction of a liquid sample containing analytes, and a discharge outlet for the discharge of waste products.

[0030] In step c), the liquid sample within the reaction chamber is contacted with a population of magnetic and/or paramagnetic material. The population of magnetic and/or paramagnetic material comprises two or more pools of magnetic and/or paramagnetic material, each being capable of capturing a specific and mutually different analyte. By having two or more pools targeted at different analytes, the method enables the analysis of multiple analytes in the same sample.

[0031] In a preferred aspect of the invention, each pool

(being capable of capturing a specific and mutually different analyte) refers to a subpopulation of particles that is essentially normally distributed around a mean in terms of the ratio of surface area to weight, wherein the weight is calculated on the basis of the weight of magnetic or paramagnetic material present in the particle. The population of particles according to the invention may comprise two or more pools or subpopulations of particles, each pool having different surface areas and different weights in terms of weight of magnetic or paramagnetic material. In one preferred aspect of the invention, the population of particles according to the invention comprises two or more pools of particles made from the same magnetic or paramagnetic material, but differing in mean size.

[0032] Alternatively, the population of particles according to the invention may have essentially the same weight in terms of magnetic or paramagnetic material, but comprise two or more pools or subpopulations of particles in terms of surface area, wherein each pool or subpopulation has a normally distributed surface area.

[0033] Alternatively, the population of particles according to the invention may have essentially the same surface area but comprise two or more pools or subpopulations of particles in terms of weight of magnetic or paramagnetic material, wherein each pool or subpopulation has a normally distributed weight in terms of weight of magnetic or paramagnetic material.

[0034] In another preferred alternative, the population of particles according to the invention may have essentially the same weight in terms of magnetic or paramagnetic material but comprise two or more pools or subpopulations of particles in terms of surface area, wherein each pool or subpopulation has a normally distributed surface area.

[0035] In one aspect of the invention, the population of magnetic and/or paramagnetic material is at least bimodally distributed in terms of weight of magnetic and/or paramagnetic material. Preferably, each pool constitutes a single modus. By providing magnetic material, wherein the amount of magnetic material is the same within the pool, but is different from the amount in the other pools, the different pools of magnetic material are influenced differently by an externally applied magnetic field and behave differently in a liquid environment, which can be an advantage in the separation step.

[0036] In one aspect of the invention, the magnetic and/or paramagnetic material according to the invention is selected from the group comprising magnetic particles, paramagnetic particles, magnetic nanoparticles, paramagnetic nanoparticles or superparamagnetic particles.

[0037] In one aspect of the invention, the population of magnetic material may differ in terms of surface area so that the population of magnetic and/or paramagnetic material is at least bimodally distributed in terms of surface area. Preferably, each pool constitutes a single modus. Hereby, a population of magnetic material, preferably in the form of particles, is provided, wherein the

surface area of the material is significantly different, each pool containing material of the overall same surface area, but differing from the surface area of the material in the other pools. This means that pools are provided, wherein the magnetic material of each pool behave differently in a liquid environment. The viscous resistance is small for material with small surface areas and greater for material with larger surface areas. Material with smaller viscous resistance can, all other things being equal, be carried a longer distance in the liquid by the magnetic force applied by an external magnet than material with greater viscous resistance. This is advantageous because it enables a separation of the pools by the displacement of an external magnet relative to the chamber device.

[0038] Another advantage of applying pools of material with different surface areas is that great differences in the amount of the different analytes to be detected in the sample may be balanced by using material with a greater surface area to capture the analyte present in trace or small amounts, whereas analytes present in greater amount may be captured by magnetic material with a smaller surface area. The signal derived from the trace or small amount analyte may be seen as being amplified in this way so that the signal from both pools can be detected within the same detection window.

[0039] The separation of the pools of material may be further aided by providing pools wherein the material not only differ in terms of surface area but also in terms of amount by weight of magnetic material. Accordingly, a first pool may contain material with a small surface area and large amount by weight of magnetic material, and a second pool may contain material with a greater surface area and smaller amount by weight of magnetic material compared to the first pool. By applying a moving magnetic field upon the chamber device, the material of the first pool will travel a longer way in the liquid in the direction of the moving magnetic field compared to material of the second pool, and the material can thus be separated because the material of the first pool has a smaller viscous resistance and a larger magnetic force than the material of the second pool.

[0040] In yet another embodiment, the population of magnetic and/or paramagnetic material within the chamber device is unimodal in terms of surface area, but bimodal in terms of weight of the magnetic or paramagnetic material. This could e.g. be achieved by using pools of material, preferably in the form of particles, wherein each particle within a certain pool is composed of different layers or compartments consisting of different materials. Hereby, a population of magnetic material, preferably in the form of particles, is provided, wherein the surface area of the material in the population is substantially equal, and wherein the amount of the magnetic material in terms of weight differs between the pools of the population. This is advantageous in a number of applications, especially when quantitatively comparing the ratio between the presences of two or more analytes in a sample. A further advantage is that material of substantially equal

size is particularly well mixed in liquids such as e.g. plasma.

[0041] In one embodiment, the means for capturing the analytes is provided by coating the magnetic and/or paramagnetic material with a biological material capable of capturing a specific analyte. The biological material comprises but is not limited to monoclonal antibodies, polyclonal antibodies, antigens, receptors, ligands, enzymes, proteins, peptides and nucleic acids. It is preferred that it is antibodies. Each pool of particles is coated with the same biological material so that each pool is specific for a single analyte in the sample. Accordingly, by providing at least two pools of material, the method is capable of detecting the presence or absence of at least two different analytes.

[0042] In step c), the magnetic particles are optionally thoroughly mixed in the liquid containing the analytes by means of a changing magnetic field causing the particles to be stirred, thus facilitating the capturing of analytes by the particles. The changing magnetic field may be generated by displacing an external magnet relative to the reaction chamber.

[0043] In another embodiment, the method further comprises a reaction step wherein the population of magnetic and/or paramagnetic material comprising the captured analytes is contacted with a second biological material linked to a marker, said material being capable of binding to the specific analytes targeted by the coated particles. Hereby, the analytes are captured in a sandwich between two biological materials specific for the analyte. It is preferred that the marker of the second biological material is selected from the group comprising HRP, ALP, fluorophores and biotin or any combination thereof. The marker is capable of transmitting a signal either by itself or after having reacted with a substrate which may be provided in a subsequent step. Accordingly, in another aspect of the invention, the method further comprises a step of contacting the population of magnetic and/or paramagnetic material comprising the captured analytes with a substance capable of reacting with the marker. The substrate is chosen so that it is a substrate that reacts with the marker to emit some kind of signal that can be quantitatively detected. Since the marker is coupled to a biological material that specifically binds to the analytes to be detected, the amount of analytes can be detected by quantitatively detecting the signal emitted from marker/substrate.

[0044] In one aspect of the invention, the reaction of the substance with the marker emits light which is detectable by a suitable light detector. Hereby, the quantity of bound analyte can be calculated from the amount of emitted light from each pool of material.

[0045] It is preferred that the second biological material is selected from the group comprising monoclonal antibodies, polyclonal antibodies, antigens, receptors, ligands, enzymes, proteins, peptides and nucleic acids, preferably it is mono- or polyclonal antibodies.

[0046] In step d), the population of magnetic and/or par-

amagnetic material comprising the captured analytes is immobilized by means of a magnetic field which may be generated by an external magnet. The magnetic field attracts the material, thus gathering the material and allowing for the subsequent purification of magnetic material, e.g. by moving the material from one part of the reaction chamber to another part. This may be achieved by means of a moving magnetic field, e.g. by displacing an external magnet relative to the chamber device.

[0047] The reaction chamber is preferably divided into a "dirty zone" and a "clean zone", the dirty zone comprising the liquid sample comprising analytes as well as unbound, contaminating material, and the "clean zone" being substantially free from unbound, contaminating material. In d), the magnetic material is preferably moved from the dirty zone into the clean zone, thus minimizing the contamination from unspecific material in the liquid sample. The preferred separation of the chamber device into a dirty zone and a clean zone provides for a minimal unspecific background signal relating to the unspecific material from the liquid sample.

[0048] In e), the population of magnetic material, preferably within the clean zone, is washed with a washing solution. This step may be repeated several times. Hereby, any contaminating unbound material is removed from the material so that only specifically bound analyte is present at the surface of the magnetic material.

[0049] In f), the population of magnetic material is transferred to a detection chamber of the chamber device, said detection chamber comprising a detection window. The material may be moved by means of displacing a magnetic field relative to the chamber device. The detection chamber is preferably arranged in a suitable manner to allow a detector to receive the emitted marker/substrate signal relating to the bound analytes. As the signal is proportional to the amount of bound analytes, the quantity of bound analyte can be calculated from the signal intensity.

[0050] Subsequently, in g) the population of magnetic material is separated into the distinct pools by displacing a magnetic field relative to the detection chamber. Due to the fact that the pools of magnetic material are differing in terms of weight and/or surface area of magnetic material, this displacement of the magnetic field will cause the magnetic material containing substantially the same amount by weight and/or substantially equal surface area of magnetic material, and thus having the same magnetic and viscous characteristics, to be released from the magnetic field at substantially the same time, thus causing the material of one pool to fall to the bottom of the chamber in substantially one group. Material with a greater content by weight of magnetic material has a greater magnetic force and will thus be carried further in the liquid in the direction of the moving magnetic field compared to material having less content by weight of magnetic material and thus less magnetic force, which will travel a shorter way. Accordingly, due to the different magnetic characteristics of the pools of magnetic material, the

pools can be separated in the chamber device by applying a moving magnetic field to the chamber device.

[0051] As mentioned above, the magnetic material of each pool may alternatively differ in surface area, with or without a difference in amount by weight of magnetic material. As the surface area affects the viscous resistance of the magnetic material in a liquid, such a difference can also be used to separate the pools of magnetic material. Material with a small surface area will travel a longer way in the liquid in the direction of the moving magnetic field than material with a larger surface area provided that the material contains substantially the same magnetic characteristics. Material with a different surface area may be even better separated if the material with a small surface area further contains a larger amount by weight of magnetic material than the magnetic material containing the larger surface area. The characteristics of such material will thus be small viscous resistance and high magnetic force and will travel further in liquid in direction of the moving magnetic field compared to the material of a different pool containing a larger surface area and less magnetic material by weight.

[0052] In one aspect of the invention, the magnetic field is applied so that the magnetic material is attracted to the top part of the detection chamber. When the material is released from the magnetic field, the material consequently falls from the top part of the chamber device through the liquid and reaches the bottom part of the detection chamber. As explained above, the material is released from the magnetic field at different times depending on the magnetic and viscous characteristics and thus, the different pools of material will be separated at the bottom part of the detection chamber. It may be that the pools overlap in the detection chamber, but significant parts of the pools are separated so that the signal from one pool can be discriminated from the signal relating to another pool.

[0053] The displacement of the magnetic field in g) may be obtained by the displacement of an external magnet relative to the chamber device.

[0054] In one embodiment of the invention, the magnetic field is displaced in a rectilinear movement relative to the chamber device preferably by moving an external magnet in a single unidirectional movement. The magnetic field may be moved stepwise alternating between acceleration and deceleration.

[0055] In one embodiment, the single unidirectional movement is performed at a substantially constant velocity, such as between 1 mm/sec and 50 mm/sec, preferably between 3 and 20 mm/sec.

[0056] In yet another embodiment, the magnetic field is displaced by moving a magnetic field in an angle of between 0 and 90 degrees, such as an angle between 0 and 80 degrees, or such as an angle between 0 and 70 degrees, or such as an angle between 0 and 60 degrees, or such as an angle between 0 and 50 degrees, or such as an angle between 0 and 40 degrees, or such as an angle between 0 and 30 degrees, or such as an angle

between 0 and 20 degrees, or such as an angle between 0 and 10 degrees relative to the surface of the detection chamber, so that the magnetic field is moved further away from the chamber as the magnetic field passes over the detection chamber, thus reducing the magnetic force/impact on the magnetic material as the magnetic field passes the detection chamber.

[0057] In another aspect of the invention, the displacement of the magnetic field as described above may be at an increasing velocity, preferably the displacement is performed in a linearly increasing velocity.

[0058] The direction of the moving magnetic field is preferably arranged so that the magnetic material upon release from the magnetic field is sedimented in the detection part of the chamber device.

[0059] In the detection step h), the emitted signal derived from the marker linked to the second biological material, and if relevant, which marker is reacted with a substrate to emit said signal, is detected using conventional detection means. In order to discriminate signals from the separate pools of magnetic material relating to different analytes, the detection is performed by scanning the area of the detection part of the chamber with suitable means for detection. The scanning of the detection chamber may be performed by moving the chamber device relative to the detector. The signal intensity is proportional to the quantity of bound analyte and thus the quantity of analyte present in the liquid sample can be calculated from the signal intensity.

[0060] If, for example, two pools of particles having bound analytes are present, the resulting signal will be bimodal when scanning the detector part of the chamber. A possible correction for overlapping pools may be needed in any given sample.

[0061] According to the invention, suitable means for detection may be selected from the group comprising surface acoustic wave (SAW) detectors, spectrophotometers, fluorometers, CCD sensor chip(s), CCOS sensor chip(s), PMT detector(s), or any other suitable detector.

[0062] In one embodiment, the method of the present invention is preferably performed in a point-of-care system. Hereby, the detection for the presence or absence of the target analytes, i.e. diagnostic testing, is brought conveniently and immediately to the patient. This increases the likelihood that the patient will receive the results in a timely manner.

[0063] The method according to the invention is preferably performed in a chamber device.

[0064] Thus, in one embodiment, the invention relates to a chamber device for the quantitative detection of the presence or absence of two or more target analytes in a liquid sample having a volume of less than 200 μ l, the device comprising a reaction chamber comprising a population of magnetic and/or paramagnetic material, said population comprising two or more pools, each pool being capable of capturing a specific and mutually different analyte and each pool having a different mean of the ratio of surface area to weight, the weight being the weight of

the magnetic of paramagnetic material present in the particle. By having two or more pools targeted at different analytes, the device is capable of detecting multiple analytes in the same sample at the same time.

[0065] In one embodiment, the chamber is a capillary channel. In a preferred embodiment, the chamber has a volume of less than 150 μl . In a more preferred embodiment the chamber has a volume of less than 100 μl . In an even more preferred embodiment the chamber has a volume of less than 90 μl , even more preferred less than 80 μl , even more preferred less than 70 μl , such as less than 60 μl , less than 50 μl or even less than 40 μl . In an even more preferred embodiment, the chamber has a volume of less than 30 μl , even more preferred less than 25 μl , even more preferred less than 20 μl , such as less than 15 μl , less than 10 μl or even less than 5 μl .

[0066] The method according to the invention is preferably performed in a system comprising a chamber device and a magnetic device. Thus, in one embodiment, the invention relates to a system for the quantitative detection of the presence or absence of two or more target analytes in a sample consisting of less than 200 μl liquid, said system comprising a magnet device and a chamber device according to the invention, said magnet device being capable of displacing one or more magnetic fields relative to the chamber device so that the magnetic and/or paramagnetic material in the chamber is subjected to the one or more magnetic fields.

[0067] In one embodiment, the system of the present invention is preferably a point-of-care system.

[0068] The invention further relates to the use of a chamber device according to the invention for the quantitative detection of the presence or absence of two or more target analytes.

[0069] Further, the invention relates to the use of a population of magnetic and/or paramagnetic material, said population comprising two or more pools, each pool being capable of capturing a specific and mutually different analyte, and each pool having a different mean of the ratio of surface area to weight, the weight being the weight of the magnetic of paramagnetic material present in the particle for the quantitative detection of the presence or absence of two or more target analytes in a liquid sample consisting of less than 200 μl liquid.

[0070] Further, the invention relates to the use of a population of magnetic and/or paramagnetic material as described above, wherein the population of magnetic and/or paramagnetic material is at least bimodally distributed in terms of weight of magnetic and/or paramagnetic material, each pool constituting a single modus.

[0071] Further, the invention relates to the use of a population of magnetic and/or paramagnetic material as described above, wherein the population of magnetic and/or paramagnetic material is at least bimodally distributed in terms of surface area, each pool constituting a single modus.

[0072] Further, the invention relates to the use of a population of magnetic and/or paramagnetic material as

described above, wherein the population is unimodal in terms of surface area.

Example 1

[0073] Two pools of paramagnetic particles were mixed to form a population of particles consisting of two pools. One pool had a mean particle diameter of 2.8 μm and the other a mean particle diameter of 1.0 μm . Both pools consisted of paramagnetic material. The population of particles was suspended in a buffer solution within a chamber device.

[0074] The population of paramagnetic particles was immobilised on the top surface of the capillary device using an external magnet. The magnet was subsequently displaced over the top surface of the capillary device by moving the magnet slowly at a substantially constant velocity in one direction. The population of particles was observed to be released from the magnetic field as the external magnet was displaced and the particles were observed to sediment into two distinct pools at the bottom of the chamber device.

[0075] Although the example does not demonstrate the detection of analytes, it is easily conceivable that the surprising finding may be used according to the present invention.

Claims

1. A method for the quantitative detection of the presence or absence of two or more target analytes in a sample consisting of less than 200 μl liquid, comprising the steps of:
 - a) providing an analyte containing liquid sample consisting of less than 200 μl liquid;
 - b) supplying the liquid sample to a reaction chamber;
 - c) contacting the sample in the reaction chamber with a population of magnetic and/or paramagnetic material, said population comprising two or more pools, each pool being capable of capturing a specific and mutually different analyte, and each pool having a different mean of the ratio of surface area to weight, the weight being the weight of the magnetic of paramagnetic material present in the particle;
 - d) immobilising the population of magnetic and/or paramagnetic material comprising the captured analytes;
 - e) washing the population of magnetic and/or paramagnetic material comprising the captured analytes with a washing solution;
 - f) transferring the population of magnetic and/or paramagnetic material comprising the captured analytes to a detector part of the chamber;
 - g) separating the population of magnetic and/or

- paramagnetic material comprising the captured analytes into said at least two pools by displacing a magnetic field relative to the detector part of the chamber, and;
- h) removing the magnetic field and detecting the presence or absence of the target analytes in each pool of magnetic and/or paramagnetic material using conventional detection means.
2. The method according to claim 1, wherein the population of magnetic and/or paramagnetic material is at least bimodally distributed in terms of weight of magnetic and/or paramagnetic material. 10
 3. The method according to claim 1 or 2, wherein the population of magnetic and/or paramagnetic material is at least bimodally distributed in terms of surface area. 15
 4. The method according to claim 1 or 2, wherein the population is unimodal in terms of surface area. 20
 5. The method according to any of claims 1-4, wherein the step h) is performed by scanning the area of the detector part of the chamber with suitable means for detection. 25
 6. A chamber device for the quantitative detection of the presence or absence of two or more target analytes in a liquid sample having a volume of less than 200 μ l, the device comprising a reaction chamber comprising a population of magnetic and/or paramagnetic material, said population comprising two or more pools, each pool being capable of capturing a specific and mutually different analyte, and each pool having a different mean of the ratio of surface area to weight, the weight being the weight of the magnetic of paramagnetic material present in the particle. 30 35
 7. The chamber device according to claim 6, wherein the population of magnetic and/or paramagnetic material is at least bimodally distributed in terms of weight of magnetic and/or paramagnetic material. 40
 8. The chamber device according to claim 6 or 7, wherein the population of magnetic and/or paramagnetic material is at least bimodally distributed in terms of surface area. 45
 9. The chamber device according to claim 6 or 7, wherein the population is unimodal in terms of surface area. 50
 10. A System for the quantitative detection of the presence or absence of two or more target analytes in a sample consisting of less than 200 μ l liquid, said system comprising a magnet device and a chamber 55
- device according to any of claims 6-9, said magnet device being capable of displacing a magnetic field relative to said chamber device so that the magnetic and/or paramagnetic material in the chamber is subjected to the magnetic field.
11. Use of a chamber device according to any of claims 6-9 for the quantitative detection of the presence or absence of two or more target analytes.
 12. Use of a population of magnetic and/or paramagnetic material, said population comprising two or more pools, each pool capable of capturing a specific and mutually different analyte, and each pool having a different mean of the ratio of surface area to weight, the weight being the weight of the magnetic of paramagnetic material present in the particle for the quantitative detection of the presence or absence of two or more target analytes in a liquid sample consisting of less than 200 μ l liquid.
 13. Use according to claim 12, wherein the population of magnetic and/or paramagnetic material is at least bimodally distributed in terms of weight of magnetic and/or paramagnetic material.
 14. Use according to claim 13, wherein the population of magnetic and/or paramagnetic material is at least bimodally distributed in terms of surface area.
 15. Use according to claim 13, wherein the population is unimodal in terms of surface area.



EUROPEAN SEARCH REPORT

Application Number
EP 09 18 0620

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
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The present search report has been drawn up for all claims			
Place of search Munich		Date of completion of the search 26 April 2010	Examiner Behrens, Ralf
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

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**ANNEX TO THE EUROPEAN SEARCH REPORT
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