

Claims

- 5 1. A method of detecting and/or quantitating an analyte of interest in a plurality of biological liquid samples, said method comprising a sample-specific process part, followed by a generic process part;

said sample-specific process part comprising the following steps:

- 10 - separately providing, in any order, a plurality of different biological liquid samples suspected of containing an analyte of interest, and a plurality of differently labelled subsets of porous microparticles, wherein, in said plurality of subsets, each of said subsets is separate from the other subsets;
- 15 - specifically labelling each of said different biological samples by separately exposing each of said separate subsets of microparticles to one biological liquid sample each, thus allowing each sample to be taken up by one specifically labelled subset of porous microparticles in an aqueous environment;
- 20 - separately transferring each subset of porous microparticles from said aqueous environment to a non-aqueous environment;

said generic process part comprising the steps:

- 25 - mixing together the differently labelled subsets of porous microparticles in said non-aqueous environment and thus generating a suspension of a plurality of differently labelled subsets of porous microparticles therein;
- 30 - performing a detection reaction for detecting said analyte of interest, on said suspension of said plurality of differently labelled subsets of porous microparticles; and
- detecting and/or quantitating said analyte of interest, if present, in any of said differently labelled subsets of suspended microparticles.

2. The method according to claim 1, wherein, in said step of separately exposing each of said separate subsets of microparticles to one biological liquid sample each, each of said separate subsets of microparticles is exposed to, in any

order, one biological sample each and a detection composition for performing a chemical or biochemical detection reaction, such that each subset of microparticles takes up the respective biological sample and the detection composition to which it has been exposed.

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3. A method of detecting and/or quantitating an analyte of interest in a plurality of biological liquid samples, in particular according to any of claims 1 and 2, said method comprising the following steps:

- 10 a. a step of separately providing, in any order, a plurality of separate biological liquid samples suspected of containing an analyte of interest, and a plurality of porous microparticles; each of said porous microparticles having a porous matrix and being configured to receive a volume of liquid in said porous matrix; wherein, in said plurality of porous microparticles, there are different subsets of microparticles provided, each subset of
15 microparticles being characterized by a specific label component that is attached to, contained in or otherwise associated with the respective subset; wherein in said step of providing, the number of different subsets of microparticles provided is at least as big as the number of separate biological liquid samples provided, and wherein furthermore in said step of
20 providing, the different subsets of microparticles are provided separate from each other; wherein, optionally, said different separate subsets of microparticles contain in their respective porous matrix a detection composition comprising reagents for performing a chemical or biochemical detection reaction of an analyte;
- 25 b. a step of exposing each separate subset of microparticles to exactly one separate biological liquid sample, thereby allowing each separate subset of microparticles to incubate with a volume of exactly one separate biological liquid sample and to take up such sample or a portion thereof, and, optionally, to accumulate analyte, if present in said sample, in or on the
30 matrix of the microparticle(s); and, further optionally, in case that said different separate subsets of microparticles, when provided in step a), do not yet comprise reagents for performing a chemical or biochemical detection reaction of an analyte, a step of exposing each separate subset of microparticles to a detection composition comprising reagents for
35 performing a chemical or biochemical detection reaction of an analyte,

thereby allowing each separate subset of microparticles to receive said reagents;

- 5 c. transferring each subset of microparticles separately into a non-aqueous phase and removing some or all of the aqueous phase surrounding the individual prefabricated microparticle(s) of said subset(s), thereby creating a plurality of separate subsets of insulated reaction spaces for detecting said analyte, which reaction spaces comprise an aqueous phase including sample and said reagents for performing a chemical or biochemical detection reaction of an analyte, and which reaction spaces are confined to said void volume(s) of said microparticles;
- 10 d. mixing the separate different subsets of microparticles in said non-aqueous phase, such that all of said different subsets of microparticles form a suspension of different microparticles in said non-aqueous phase; subjecting said mixed different subsets of microparticles to conditions required for performing a chemical or biochemical detection reaction of an analyte; performing such detection reaction of said analyte of interest; and detecting and/or quantitating said analyte of interest, if present, in any of said different subsets of microparticles, by means of a signal generated in said detection reaction in a respective subset of microparticles, if said analyte of interest is present in said respective subset.
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4. The method according to claim 3, said method further comprising the step
- 25 e) determining which sample(s) of said plurality of samples provided in step a) does contain said analyte of interest by determining the identity of the subset(s) of microparticles in which said analyte of interest is detected in step d), wherein, preferably the identity of the subset(s) of microparticles in step e) is determined by means of the specific label component that is attached to, contained in or otherwise associated with the respective subset of microparticle(s).
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5. The method according to any of claims 3 - 4, wherein step b) further comprises a substep of generating a first record of correlation indicating which separate subset of microparticles is or has been exposed to which sample, and step d) comprises a substep of generating a second record of
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correlation indicating in which subset of microparticles a signal has been generated in said detection reaction.

- 5 6. The method according to claims 4 - 5, wherein step e) is performed by reference to said first and second records of correlation and by linking said records, thus allowing to determine which sample(s) of said plurality of samples provided in step a) does(do) contain said analyte of interest.
- 10 7. The method according to any of the foregoing claims, wherein each of said porous microparticles has a porous matrix which allows to accumulate analyte of interest by binding to analyte through:
- (i) a polymer or polymer mixture that forms or is said porous matrix;
or
 - 15 (ii) at least one ionisable group, or a plurality of ionisable groups, immobilized on said porous matrix, said ionisable group(s) being capable of changing its(their) charge(s) according to ambient conditions surrounding said microparticle(s); or
 - (iii) at least one charged group, or a plurality of charged groups
20 immobilized on said porous matrix; or
 - (iv) a combination of any of (i) – (iii).
- 25 8. The method according to any of claims 1 – 6 or 7, wherein each of said porous microparticles has a porous matrix and comprises an analyte-specific reagent (ASR) that is attached to said porous matrix or contained by said microparticle, such analyte-specific reagent allowing an enrichment of an analyte of interest and/or allowing a specific signal or target amplification reaction involving said analyte; wherein said analyte-specific reagent is capable of specifically binding to an analyte of interest,
- 30 wherein, preferably the analyte-specific reagent is selected from nucleic acids, including aptamers, Spiegelmers; antibodies or antibody fragments; non—antibody proteins capable of specifically binding an analyte or analyte complex, such as receptors, receptor fragments, and affinity proteins; wherein, preferably, said analyte-specific reagent is selected
- 35 from nucleic acids, in particular nucleic acid oligomers and nucleic acid primers.

5 9. The method according to claim 8, wherein each of said porous microparticles contains or has the same analyte-specific reagent attached to its porous matrix.

10 10. The method according to claim 9, wherein, in said plurality of porous microparticles, there are different subsets of microparticles,

with each subset

- having its distinct label component attached to, contained in or otherwise associated with said microparticles of said subset; and

all of said different subsets having

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- the same analyte-specific reagent attached to or contained in said microparticles of said subsets, said analyte-specific reagent being specific for one analyte of interest;

20 such that said different subsets of microparticles are identical in terms of the analyte-specific reagent attached or contained, but differ by

- the respective label component attached to, contained in or otherwise associated with said microparticles of each subset;

with each subset being unambiguously defined and identifiable by said respective label component.

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30 11. The method according to any of claims 1 – 7, or according to any of claims 8 – 10, wherein said method is a method of detecting and/or quantitating one analyte of interest in a plurality of biological liquid samples, wherein the number of different subsets of microparticles provided, preferably provided in step a), equals the number of separate biological liquid samples provided.

35 12. The method according to claim 8, wherein, in said plurality of porous microparticles, there are several different analyte-specific reagents attached to or contained in said microparticles.

13. The method according to claim 12, wherein there are different subsets of microparticles,

5 with each subset

- having its distinct label component attached to, contained in or otherwise associated with said microparticles of said subset; and

wherein furthermore, in said plurality of porous microparticles, there are different classes of subsets of microparticles with each class of subsets

- having a different analyte-specific reagent attached to the porous matrix of said microparticles or contained in said microparticles; wherein, preferably, there are at least two different classes of subsets of microparticles, more preferably at least three or more different classes of subsets of microparticles;

such that said different subsets of microparticles differ by the respective label component attached to, contained in or otherwise associated with said microparticles of each subset; and each subset of microparticles forms part of one class of subsets of microparticles; with each subset being unambiguously defined and identifiable by the respective label component and the respective analyte-specific reagent; and

such that said different classes of subsets of microparticles differ by the respective analyte-specific reagent attached or contained; and each of said different classes comprises several subsets of microparticles, all of which subsets have the same analyte-specific reagent attached or contained.

14. The method according to any of claims 12- 13, wherein said method is a method of detecting and/or quantitating more than one analyte of interest in a plurality of biological liquid samples, wherein, the number of different subsets of microparticles provided, preferably provided in step a), equals the number of separate biological liquid samples provided, multiplied by the number of analytes of interest to be detected, and

wherein there are as many classes of subsets of microparticles provided, preferably provided in step a), as the number of analytes of interest to be detected.

- 5 15. The method according to any of claims 3 - 14, wherein said porous matrix is a porous polymer matrix formed by a polymer or polymer mixture, wherein, preferably, said porous polymer matrix is composed of a polymer (or polymers) that is (are) not crosslinked, wherein, more preferably, said polymer or polymer mixture that forms said porous
10 polymeric matrix, is composed of agarose or a combination of agarose and gelatin, wherein, more preferably, in said combination of agarose and gelatin, said agarose is present in a range of from 0.1 %(w/v) to 4%(w/v), and said gelatin is present in a range of from 0.1%(w/v) to 20%(w/v), preferably from 0.5% (w/v) to 20%(w/v).
- 15 16. The method according to any of claims 3 - 15, wherein said analyte of interest is a nucleic acid, said detection reaction is a nucleic acid amplification, and said detection composition is a composition for performing a nucleic acid amplification which comprises a buffer, mono-nucleoside-triphosphates, an amplification enzyme, such as a suitable
20 nucleic acid polymerase, e.g. Taq polymerase, and a nucleic acid dye for the detection of an amplification product, such as an amplified nucleic acid, and, optionally, one or more pairs of amplification primers and, further optionally, respective molecular probes (e.g. TaqMan Probes,
25 molecular beacons, etc.), if such primers and/or probes are not already provided as analyte-specific reagent(s) (ASR) being attached to or contained in said microparticles; AND/OR
- 30 wherein said analyte of interest is a protein or other non-nucleic acid molecule, said detection reaction is an immunochemistry detection reaction, and said detection composition is a composition for performing such immunochemistry detection reaction and is provided in said method as two
35 separate components: wherein a first component of said detection composition comprises necessary reagents for performing an immunochemistry detection reaction, such as a buffer, and a secondary

antibody or secondary antibody fragment coupled to a suitable reporter enzyme and being specific for the same analyte as a primary antibody, antibody fragment, or non-antibody protein, used as analyte-specific reagent (ASR) in said immunochemistry detection reaction; and, optionally, a primary antibody, antibody fragment, or a non-antibody protein capable of specifically binding said protein analyte or other non-nucleic acid analyte, if such a primary antibody, antibody fragment, or non-antibody protein is not already provided as analyte-specific reagent(s) (ASR) being attached to or contained in said microparticles; and wherein a second component of said detection composition comprises, as a detection reagent, a suitable substrate for said suitable reporter enzyme which substrate upon having been reacted by said reporter enzyme, becomes detectable, preferably optically detectable, more preferably detectable by fluorescence.

17. The method according to any of the foregoing claims, wherein, in said step of detecting and quantitating said analyte of interest, quantitation of said analyte is performed by a method selected from:

- a) digital nucleic acid amplification, in particular digital polymerase chain reaction (PCR);
- b) real-time quantitative nucleic acid amplification, in particular real-time polymerase chain reaction (PCR);
- c) immunochemistry detection methods, in particular digital immunochemistry detection methods, such as digital immunoassays, e.g. digital enzyme-linked immunosorbent assays (ELISAs);
- d) immunochemistry detection methods, in particular digital immunochemistry detection methods, combined with nucleic acid amplification, such as immuno-polymerase chain reaction; in particular digital immune-PCR; and
- e) a combination of any of a) – d);

wherein quantitation is performed using any of methods a) or b), or a combination of a) and b), if the analyte of interest is a nucleic acid; and wherein quantitation is performed using any of methods c) or d), if the analyte is a protein, peptide or other non-nucleic acid analyte.

18. A kit for detecting an analyte of interest in a plurality of biological liquid samples, in particular for performing the method according to any of claims 1 – 17, said kit comprising:

- 5 - a plurality of containers comprising a plurality of microparticles, each container comprising a subset of said plurality of porous microparticles, with each of said porous microparticles within each subset having a porous matrix and being configured to receive a volume of liquid in said porous matrix; each subset of microparticles being characterized by a specific label
10 component that is attached to, contained in or otherwise associated with the respective subset, wherein, preferably, said microparticles are as defined in any of claims 1 - 17; optionally, a container comprising an aqueous washing reagent for washing said microparticles;
- 15 - a container comprising a detection composition for detecting an analyte of interest; said detection composition comprising reagents for performing a chemical or biochemical detection reaction of an analyte; wherein said detection composition is either a composition for performing a nucleic acid amplification, or is a composition for performing an immunochemistry
20 detection reaction;
- a container comprising a non-aqueous phase for transferring said different subsets of microparticles into a non-aqueous phase, once each subset has been exposed to a biological liquid sample, and for generating separate different suspensions of subsets of microparticles in a non-aqueous phase;
- 25 - a mixing container for mixing the separate different suspensions of subsets of microparticles in said non-aqueous phase together, such that all of said different suspensions of subsets of microparticles form a single suspension of different microparticles in said non-aqueous phase which is then subjected to a detection reaction;
- 30 - a container for performing a detection reaction.

19. The kit according to claim 18, wherein each of said porous microparticles has an analyte-specific reagent (ASR) attached to its porous matrix or contains an analyte-specific reagent (ASR), such analyte-specific reagent
35 allowing an enrichment of an analyte of interest and/or allowing a specific

signal or amplification reaction involving said analyte; wherein said analyte-specific reagent is capable of specifically binding to an analyte of interest, wherein, preferably, said analyte-specific reagent is selected from nucleic acids, including aptamers, Spiegelmers; antibodies or antibody fragments; non-antibody proteins capable of specifically binding an analyte or analyte complex, such as receptors, receptor fragments, and affinity proteins; wherein, preferably, said analyte-specific reagent is selected from nucleic acids, in particular nucleic acid oligomers and nucleic acid primers.

20. The kit according to any of claims 18 – 19, wherein said analyte of interest is a nucleic acid, said detection reaction is a nucleic acid amplification, and said detection composition is a composition for performing a nucleic acid amplification which comprises a buffer, mono-nucleoside-triphosphates, an amplification enzyme, such as a suitable nucleic acid polymerase, e.g. Taq polymerase, and a nucleic acid dye for the detection of an amplification product, such as an amplified nucleic acid, and, optionally, a pair of primers, if such pair of primers are not already provided as analyte-specific reagent(s) (ASR) being attached to or contained in said microparticles; OR

wherein said analyte of interest is a protein or other non-nucleic acid molecule, said detection reaction is an immunochemistry detection reaction, and said detection composition is a composition for performing such immunochemistry detection reaction and is provided in said kit in two separate compartments or containers; wherein said detection composition comprises, in a first compartment or container, necessary reagents for performing an immunochemistry detection reaction, such as a buffer, and a secondary antibody or secondary antibody fragment coupled to a suitable reporter enzyme and being specific for the same analyte as a primary antibody, antibody fragment, or non-antibody protein, used as analyte-specific reagent (ASR) in said immunochemistry reaction; and, optionally, a primary antibody, antibody fragment, or a non-antibody protein capable of specifically binding said protein analyte or other non-nucleic acid analyte, if such a primary antibody, antibody fragment, or non-antibody protein is not

already provided as analyte-specific reagent(s) (ASR) being attached to or contained in said microparticles; and wherein said detection composition comprises, in a second compartment or container, as a detection reagent, a suitable substrate for said suitable reporter enzyme which substrate upon having been reacted by said reporter enzyme, becomes detectable, preferably optically detectable, more preferably detectable by fluorescence.

21. The kit according to any of claims 18 – 20, wherein each of said porous microparticles has the same analyte-specific reagent attached to its porous matrix or contains the same analyte-specific reagent.

22. The kit according to claim 21, wherein, in said plurality of porous microparticles, there are different subsets of microparticles,

with each subset

- having its distinct label component attached to, contained in or otherwise associated with said microparticles of said subset; and

all of said different subsets having

- the same analyte-specific reagent attached to or contained in said microparticles of said subsets, said analyte-specific reagent being specific for one analyte of interest;

such that said different subsets of microparticles are identical in terms of the analyte-specific reagent attached or contained, but differ by

- the respective label component attached to, contained in or otherwise associated with said microparticles of each subset;

with each subset being unambiguously defined and identifiable by said respective label component and being provided in a separate container.

23. The kit according to any of claims 21 – 22, wherein said kit is a kit for detecting one analyte of interest in a plurality of biological liquid samples, wherein the number of different subsets of microparticles provided in said kit equals the number of separate biological liquid samples provided.

24. The kit according to any of claims 18 - 20, wherein, in said plurality of porous microparticles, there are several different analyte-specific reagents attached to or contained in said microparticles.

5 25. The kit according to claim 24, wherein there are different subsets of microparticles,

with each subset

10 - having its distinct label component attached to, contained in or otherwise associated with said microparticles of said subset; and

wherein furthermore, in said plurality of porous microparticles, there are different classes of subsets of microparticles with each class of subsets

15 - having a different analyte-specific reagent attached to the porous matrix of said microparticles or contained in said microparticles; wherein, preferably, there are at least two different classes of subsets of microparticles, more preferably at least three or more different classes of subsets of microparticles;

20 such that said different subsets of microparticles differ by the respective label component attached to, contained in or otherwise associated with said microparticles of each subset; and each subset of microparticles forms part of one class of subsets of microparticles; with each subset being unambiguously defined and identifiable by the respective label component and the respective
25 analyte-specific reagent and being provided in a separate container; and

such that said different classes of subsets of microparticles differ by the respective analyte-specific reagent attached to the porous matrix of said microparticles or contained in said microparticles; and each of said different
30 classes comprises several subsets of microparticles, all of which subsets have the same analyte-specific reagent attached or contained.

26. The kit according to any of claims 24 - 25, wherein said kit is a kit for
35 detecting more than one analyte of interest in a plurality of biological liquid samples, wherein, the number of different subsets of microparticles

provided in said kit equals the number of separate biological liquid samples provided, multiplied by the number of analytes of interest to be detected, and wherein, in said kit, there are as many classes of subsets of microparticles provided as the number of analytes of interest to be detected.

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27. A cartridge for performing a method of detecting and/or quantitating an analyte of interest in a plurality of biological liquid samples according to any of claims 1 – 17, wherein, preferably, said cartridge comprises a plurality of sample-specific modules, a plurality of storage chambers, at least one non-aqueous phase chamber for storing a non-aqueous phase, and either a single combined mixing and detection chamber, or a combination of a separate mixing chamber and a separate detection chamber;

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wherein each sample-specific module comprises a sample compartment having its own separate sample inlet, each sample-specific module being configured to separately receive exactly one biological sample only, in the respective sample compartment; each sample-specific module being furthermore configured to receive microparticles in said sample compartment, said microparticles being as defined in any of claims 1 - 17; each sample-specific module being further configured to facilitate a phase-transfer of said microparticles from an aqueous environment to a non-aqueous environment.

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