

### **Claims**

1. A library of prefabricated precursor-microparticles for making a library of prefabricated microparticles, said prefabricated microparticles being  
5 configured for performing specific detection of one or several analytes of interest in a sample, such specific detection occurring within such microparticles by a suitable chemical or biochemical reaction, each of said prefabricated precursor-microparticles in said library comprising:
  - 10 - a porous matrix, preferably a porous polymeric matrix, having a void volume for receiving an aqueous sample and for providing a reaction space for the specific detection of an analyte;
  - a reagent binding component allowing the attachment, preferably the reversible attachment, of an analyte-specific reagent to the precursor-microparticle; said reagent binding component being one of:
    - 15 (i) a polymer or polymer mixture that forms said porous matrix or is said porous matrix;
    - (ii) a reagent binding molecule attached to said porous matrix;
    - (iii) at least one ionisable group, or a plurality of ionisable groups,  
20 immobilized on said porous matrix, said ionisable group(s) being capable of changing its(their) charge(s) according to ambient conditions surrounding said precursor-microparticle;
    - (iv) at least one charged group, or a plurality of charged groups immobilized on said porous matrix;
    - 25 (v) a combination of any of (i) – (iv);
  - a label component attached to, contained within or otherwise associated with said precursor-microparticle for identifying said analyte-specific reagent, when attached to the precursor-microparticle.  
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2. The library according to claim 1, wherein, in said library, there are at least two separate subsets of prefabricated precursor-microparticles, preferably three or more separate subsets of prefabricated precursor-microparticles, with each subset having its distinct label component attached to, contained within or

otherwise associated with said precursor-microparticles within said subset, such that said at least two or more separate subsets of prefabricated precursor-microparticles differ by the respective label component attached to, contained within or otherwise associated with each subset.

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3. A library of prefabricated microparticles for performing specific detection of one or several analytes of interest in a sample, such specific detection occurring within such microparticles by a suitable chemical or biochemical reaction, each of said prefabricated microparticles comprising a prefabricated precursor-microparticle according to any of claims 1 – 2 and further comprising an analyte-specific reagent attached, preferably reversibly attached, to said precursor-microparticle.
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4. The library of prefabricated microparticles according to claim 3, wherein said analyte-specific reagent that is attached to each of said precursor-microparticles, is attached, preferably reversibly attached, through said reagent binding component by
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- a) direct binding of said analyte-specific reagent to said polymer or polymer mixture that forms said porous polymeric matrix or is part of said porous polymeric matrix (i);
  - b) said analyte-specific reagent being conjugated to a binding entity which, in turn, binds to said reagent binding molecule (ii);
  - c) direct binding of said analyte-specific reagent to said ionisable group(s) (iii) under conditions in which said ionisable group(s) has(have) a suitable net charge;
  - d) direct binding of said analyte-specific reagent to said charged group(s) (iv) on said polymer, wherein said analyte-specific reagent has at least one ionisable group, or a plurality of ionisable groups, said ionisable group(s) being capable of changing its(their) charge(s) according to ambient conditions surrounding said analyte-specific reagent; or
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e) a combination of any of (a) – (d).

5. The library of prefabricated precursor-microparticles according to any of claims 1 – 2 or the library of prefabricated microparticles according to any of claims 3 - 4, wherein

- said polymer (i) is a hydrogel-forming agent selected from the group comprising ia) synthetic polymers, such as poly(methyl)(meth)acrylate, polyamide; ib) silicone-based polymers, e.g. polydimethylsiloxanes; ic) naturally occurring polymers selected from polysaccharides, e.g. agarose, chitin, chitosan, dextran, alginate, carrageenan, cellulose, fucoidan, laminaran; gums selected from xanthan gum, arabic gum, ghatti gum, guar gum, locust bean gum, tragacanth gum, karaya gum, and inulin; polypeptides, e.g. collagens, gelatins; poly-amino acids, such as poly-lysine; polynucleotides; and said polymer mixture is a combination of any of the foregoing;
- said reagent binding molecule (ii) is selected from avidin, in particular tetrameric avidin; streptavidin; monomeric avidin; avidin having a nitrated tyrosine in its biotin binding site; other proteins, derived from or related to, avidin and retaining binding functionality of avidin; biotin; desthiobiotin; iminobiotin; biotin having a cleavable spacer arm; selenobiotin; oxybiotin; homobiotin; norbiotin; iminobiotin; diaminobiotin; biotin sulfoxide; biotin sulfone; epibiotin; 5-hydroxybiotin; 2-thiobiotin; azabiotin; carbobiotin; methylated derivatives of biotin; ketone biotin; other molecules, derived from or related to, biotin and retaining binding functionality of biotin;
- said at least one ionisable group (iii) or said ionisable group of claim 4 d) is selected from
  - N-2-acetamido-2-aminoethanesulfonic acid (ACES);
  - N-2-acetamido-2-iminodiacetic acid (ADA);
  - amino methyl propanediol (AMP);
  - 3-1,1-dimethyl-2-hydroxyethylamino-2-hydroxy propanesulfonic acid (AMPSO);
  - N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES);
  - N,N-bis(2-hydroxyethyl)glycine (BICINE);

- bis-2-hydroxyethyliminotrishydroxymethylmethane (Bis-Tris);
- 1,3-bistrishydroxymethylmethylaminopropane (Bis-Tris Propane);
- 5 • 4-cyclohexylamino-1-butane sulfonic acid (CABS);
- 3-cyclohexylamino-1-propane sulfonic acid (CAPS);
- 3-cyclohexylamino-2-hydroxy-1-propane sulfonic acid (CAPSO);
- 2-N-cyclohexylaminoethanesulfonic acid (CHES);
- 10 • 3-N,N-bis-2-hydroxyethylamino-2-hydroxypropanesulfonic acid (DIPSO);
- N-2-hydroxyethylpiperazine-N-3-propanesulfonic acid (EPPS);
- N-2-hydroxyethylpiperazine-N-4-butanesulfonic acid (HEPBS);
- 15 • N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES);
- N-2-hydroxyethylpiperazine-N-2-propanesulfonic acid (HEPPSO);
- 20 • 2-N-morpholinoethanesulfonic acid (MES);
- 4-N-morpholinobutanesulfonic acid (MOBS);
- 3-N-morpholinopropanesulfonic acid (MOPS);
- 3-N-morpholino-2-hydroxypropanesulfonic acid (MOPSO);
- piperazine-N-N-bis-2-ethanesulfonic acid (PIPES);
- 25 • piperazine-N-N-bis-2-hydroxypropanesulfonic acid (POPSO);
- N-trishydroxymethyl-methyl-4-aminobutanesulfonic acid (TABS);
- N-trishydroxymethyl-methyl-3-aminopropanesulfonic acid (TAPS);
- 30 • 3-N-trishydroxymethyl-methylamino-2-hydroxypropanesulfonic acid (TAPSO);
- N-trishydroxymethyl-methyl-2-aminoethanesulfonic acid (TES);
- N-trishydroxymethylmethylglycine (TRICINE);
- 35 • trishydroxymethylaminomethane (Tris);

- polyhydroxylated amines;
  - imidazole, and derivatives thereof (i.e. imidazoles), especially derivatives containing hydroxyl groups;
  - triethanolamine dimers and polymers; and
- 5 di/tri/oligo/poly amino acids, such as for example Ala-Ala; Gly-Gly; Ser-Ser; Gly-Gly-Gly; or Ser-Gly. Oligo-His, Poly-His, Oligo-Lys, Poly-Lys.

10 6. The library of prefabricated precursor-microparticles according to any of claims 1 – 2, 5 or the library of prefabricated microparticles according to any of claims 3 - 5,

wherein said porous polymeric matrix, or said polymer or polymer mixture that forms or is part of said porous polymeric matrix, is composed of a polymer that is not crosslinked, wherein, preferably, said polymer or polymer mixture that forms or is part of said porous polymeric matrix, is composed of  
15 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).

20 7. The library of prefabricated microparticles according to any of claims 3 – 6, wherein 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  
25 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.

30 8. The library of prefabricated microparticles according to any of claims 3 – 7, wherein, for each of said microparticles, said analyte-specific reagent is reversibly attached to said microparticle through said reagent binding component by b) said analyte-specific reagent being conjugated to a binding entity which, in turn, binds to said reagent binding molecule (ii), wherein

- said binding entity is independently selected from biotin, desthiobiotin, iminobiotin, biotin having a cleavable spacer arm, selenobiotin, oxybiotin, homobiotin, norbiotin, iminobiotin, diaminobiotin, biotin sulfoxide, biotin sulfone, epibiotin, 5-hydroxybiotin, 2-thiobiotin, azabiotin, carbobiotin, methylated derivatives of biotin, ketone biotin, other molecules, derived from or related to, biotin and retaining binding functionality of biotin; and said reagent binding molecule is independently selected from avidin and streptavidin; or vice versa; or
  - said binding entity is biotin, and said reagent binding molecule is selected from monomeric avidin, avidin having a nitrated tyrosine in its biotin binding site, and other proteins, derived from or related to, avidin and retaining binding functionality of avidin; or vice versa.
9. The library of prefabricated microparticles according to any of claims 3 – 8, wherein said library is for performing a specific detection of a single analyte of interest in several samples, wherein, in said library, there are at least two separate subsets of prefabricated microparticles, preferably three or more separate subsets of prefabricated microparticles,
- with each subset
- having its distinct label component attached to, contained within or otherwise associated with said microparticles of said subset; and
- all of said at least two, three or more separate subsets having
- the same analyte-specific reagent attached to the porous matrix of said microparticles of said subsets, said analyte-specific reagent being specific for one analyte of interest;
- such that said at least two or more separate subsets of prefabricated microparticles are identical in terms of the analyte-specific reagent attached, but differ by
- the respective label component attached to, contained within or otherwise associated with said microparticles of each subset;

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

5           10.     The library of prefabricated microparticles according to any of claims 3 – 8, wherein said library is for performing a specific detection of multiple analytes of interest in a single sample, wherein, in said library, there are at least two separate subsets of prefabricated microparticles, preferably three or more separate subsets of prefabricated microparticles,

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with each subset

- having its distinct label component attached to, contained within or otherwise associated with said microparticles of said subset; and
- 15       - having a different analyte-specific reagent attached to the porous matrix of said microparticles of said subset; each analyte-specific reagent being specific for one analyte of interest;

such that said at least two or more separate subsets of prefabricated microparticles differ by

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- the respective label component attached to, contained within or otherwise associated with said microparticles of each subset; and
- the respective analyte-specific reagent attached to each subset;

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with each subset being unambiguously defined and identifiable by said respective label component and said respective analyte-specific reagent.

30           11.     The library of prefabricated microparticles according to any of claims 3 – 8, wherein said library is for performing a specific detection of multiple analytes of interest in several samples, wherein, in said library, there are a plurality of different separate subsets of prefabricated microparticles,

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with each subset

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

5 wherein, in said plurality of separate subsets of prefabricated microparticles, there are different classes of separate subsets of prefabricated microparticles, with each of said classes comprising several subsets of microparticles and each class having a different analyte-specific reagent attached to the porous matrix of said microparticles, and all subsets of microparticles within one class having  
10 the same analyte-specific reagent attached; each analyte-specific reagent being specific for one analyte of interest;

such that, in said library, said plurality of different separate subsets of prefabricated microparticles differ by

- 15
- the respective label component attached to, contained within or otherwise associated with said microparticles of each subset; and

each separate subset of microparticles forms part of one class of subsets of  
20 microparticles;

with each subset being unambiguously defined and identifiable by said respective label component and said respective analyte-specific reagent; and such that said different classes of subsets of microparticles differ by the respective analyte-specific reagent attached to the porous matrix of said  
25 microparticles; and each of said different classes comprises several subsets of microparticles, all of which subsets within one class having the same analyte-specific reagent attached.

12. A kit for making a library of prefabricated microparticles according to  
30 any of claims 3 - 11, said kit comprising:

- at least two containers, namely a first and a second container, and, optionally, further containers, each containing:
  - a subset of prefabricated precursor-microparticles as defined in any  
35 of claims 1 - 2, 5 and 6, with each subset having its distinct label

component attached to, contained within or otherwise associated with said precursor-microparticles within said subset, such that said two subsets of prefabricated precursor-microparticles differ by the respective label component attached to, contained within or otherwise associated with each subset;

- a further container containing:

- a conditioning solution to enable the attachment, preferably the reversible attachment, of an analyte-specific reagent to said subset of said precursor-microparticles through said reagent binding component by

a) direct binding of said analyte-specific reagent to said polymer or polymer mixture that forms said porous polymeric matrix or is part of said porous polymeric matrix (i);

b) said analyte-specific reagent being conjugated to a binding entity which, in turn, binds to said reagent binding molecule (ii);

c) direct binding of said analyte-specific reagent to said ionisable group(s) (iii) under conditions in which said ionisable group(s) has(have) a suitable net charge;

d) direct binding of said analyte-specific reagent to said charged group(s) (iv) on said polymer, wherein said analyte-specific reagent has at least one ionisable group, or a plurality of ionisable groups, said ionisable group(s) being capable of changing its(their) charge(s) according to ambient conditions surrounding said analyte-specific reagent; or

e) a combination of any of (a) – (d);

said reagent binding component being as defined in claim 1, said reagent binding molecule being as defined in any of claims 1, 4 and 5, and said binding entity being as defined in any of claims 4 – 5; said polymer or polymer mixture being as defined in any of claims 1, 4 - 6; and said ionisable groups being as defined in any

of claims 1, 4, and 5, and said analyte-specific reagent being as defined in any of claims 3 - 4, 7, and 8.

5           13.    A method of making a library of prefabricated microparticles according to any of claims 3 - 11, said method comprising:

-   Providing, in any order:

- 10                     •   a library of prefabricated precursor-microparticles as defined in any of claims 1 - 2, 5 and 6;
- at least one analyte-specific reagent, as defined in any of claims 3 - 4, 7, and 8;

15           -   mixing said library of prefabricated precursor-microparticles, or selected subsets thereof, and the at least one analyte-specific reagent under conditions allowing the attachment, preferably the reversible attachment, of said at least one analyte-specific reagent to some or all of said prefabricated precursor-microparticles, thus generating a library of prefabricated microparticles according to any of claims 5 - 13;

20           -   optionally, washing said prefabricated microparticles to remove any unattached analyte-specific reagent therefrom.

14.    A kit for detecting an analyte in a sample, said kit comprising:

a)

25           -   a container containing a generic detection composition comprising reagents for performing a chemical or biochemical detection reaction of an analyte, wherein said chemical or biochemical detection reaction of an analyte is a nucleic acid amplification, said generic detection composition comprises a buffer, mono-nucleoside-triphosphates, an amplification

30                     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, OR

35           -   a container containing a first detection composition comprising reagents for performing a chemical or biochemical detection reaction of an analyte, and yet a further container containing a second detection composition

comprising a detection reagent, wherein said chemical or biochemical detection reaction of an analyte is an immunochemistry detection reaction, said first detection composition comprises 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 said second detection composition comprises, as a detection reagent, a suitable substrate for said reporter enzyme which substrate upon having been reacted by said reporter enzyme, becomes detectable, preferably optically detectable, more preferably detectable by fluorescence, OR

- a container containing a first detection composition comprising reagents for performing a chemical or biochemical detection reaction of an analyte, and yet a further container containing a second detection composition comprising a detection reagent, wherein the chemical or biochemical reaction is an immunochemistry detection reaction, said first detection composition comprises reagents for performing an immunochemistry detection reaction, such as a buffer, and a secondary antibody or secondary antibody fragment coupled to a suitable oligonucleotide tag and being specific for the same analyte as a primary antibody, used as analyte-specific reagent (ASR) in said immunochemistry detection reaction; and said second detection composition comprises, as detection reagent(s), 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 detection of an amplification product, such as an amplified nucleic acid, and primers suitable for amplifying the oligonucleotide tag attached to the secondary antibody;

and

b)

- a container containing a non-aqueous phase, such as an oil, e.g. fluorocarbon oil, optionally supplemented with an emulsifier;

and

c)

- a mixing container for mixing components, wherein, preferably, said kit further comprises

d)

- a container for performing a detection reaction; and wherein, more preferably, said kit further comprises

e)

- the kit according to claim 12, or  
- a library of prefabricated microparticles according to any of claims 3 - 11.

15. A method of detecting and/or quantitating an analyte of interest in an aqueous sample, said method comprising the steps:

- providing, in any order:

- an aqueous sample known or suspected to contain an analyte of interest;
- a generic detection composition comprising reagents for performing a chemical or biochemical detection reaction of the analyte;
- a library of prefabricated microparticles according to any of claims 3 - 11, wherein said analyte-specific reagent(s) attached to said microparticles, is(are) chosen such that it is (they are) capable of specifically binding to or reacting with said analyte of interest;

- optionally, mixing said aqueous sample and said generic detection composition;
- incubating said aqueous sample with said library of prefabricated microparticles, thereby allowing said library of microparticles to absorb aqueous sample in the void volumes of said microparticles and, optionally, to bind said analyte of interest, if present in said sample;
- optionally, washing said microparticles;
- adding said generic detection composition to the microparticles, if said aqueous sample has not been previously mixed with said generic detection composition;

- transferring said library of prefabricated microparticles into a non-aqueous phase and removing any aqueous phase surrounding the individual prefabricated microparticle(s), thereby creating a plurality of insulated reaction spaces for detecting said analyte, which reaction spaces comprise an aqueous phase and are confined to said void volume(s) of said microparticles;
  - optionally, releasing the analyte-specific reagent(s) attached to said microparticles by applying an external trigger;
  - performing a detection reaction of said analyte of interest;
  - detecting and/or quantitating said analyte of interest.
16. The method according to claim 15, wherein said analyte of interest is a nucleic acid, and
- said analyte-specific reagent is a nucleic acid or a pair of nucleic acids, sufficiently complementary to said analyte of interest to be able under hybridizing conditions to hybridize to said analyte of interest, wherein, preferably, said analyte-specific reagent is a suitable primer or primer pair for amplification of said analyte of interest;
  - said generic detection composition comprises reagents for performing an amplification reaction of said nucleic acid analyte of interest, except for primers, wherein, in particular, said generic detection composition 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;
  - said step of transferring is a step wherein said prefabricated microparticles are transferred into a non-aqueous phase and suspended and, optionally, repeatedly washed with said non-aqueous phase, said step more optionally involving also a filtration or mechanical agitation to ensure the formation of a monodisperse suspension, of microparticles with no aqueous phase or no substantial aqueous phase remaining outside of any of the microparticles;
  - said optional step of releasing the analyte-specific reagent(s) attached to said microparticles by applying an external trigger is a step of temporarily increasing the temperature, changing the pH, or changing the salt

conditions, preferably of increasing the temperature, more preferably from ambient temperature to a temperature  $>90^{\circ}\text{C}$ .

- said step of performing a detection reaction is a step of performing an amplification reaction of said analyte if present in said aqueous sample; and
- said step of detecting and/or quantitating said analyte of interest is a step of detecting and/or quantitating said amplified analyte.

17. A method of detecting and/or quantitating an analyte of interest in an aqueous sample, said method comprising the steps:

- providing, in any order:
  - an aqueous sample known or suspected to contain an analyte of interest;
  - a first detection composition comprising necessary reagents for performing a chemical or biochemical detection reaction of the analyte;
  - a second detection composition comprising a detection reagent;
  - a library of prefabricated microparticles according to any of claims 3 - 11, wherein said analyte-specific reagent(s) attached to said microparticles, is(are) chosen such that it is (they are) capable of specifically binding to or reacting with said analyte of interest;
- optionally, mixing said aqueous sample and said first detection composition;
- incubating said aqueous sample with said library of prefabricated microparticles and, if said aqueous sample has not already been mixed with said first detection composition, also with said first detection composition, thereby allowing said library of microparticles to absorb aqueous sample and said first detection composition in the void volumes of said microparticles and, optionally, to bind said analyte of interest, if present in said sample;
- optionally, washing said library of prefabricated microparticles to remove any non-absorbed or non-reacted first detection composition;

- incubating said library of prefabricated microparticles including absorbed aqueous sample with said second detection composition, thereby allowing said library of microparticles to absorb said second detection composition;
- optionally, further washing said library of prefabricated microparticles to remove any non-absorbed or non-reacted second detection composition;
- transferring said library of prefabricated microparticles into a non-aqueous phase and removing any aqueous phase surrounding the individual prefabricated microparticle(s), thereby creating a plurality of insulated reaction spaces for detecting said analyte which reaction spaces comprise an aqueous phase and are confined to said void volume(s) of said microparticles; wherein, preferably, said step of transferring into a non-aqueous phase is performed immediately after said library of prefabricated microparticles have been incubated with said second detection composition;
- optionally, releasing the analyte-specific reagent(s) attached to said microparticles by applying an external trigger;
- detecting and/or quantitating said analyte of interest by detecting and/or quantitating said detection reagent.

18. The method according to claim 17, wherein said analyte of interest is a protein or other non-nucleic acid molecule, and
- said analyte-specific reagent is a primary antibody, antibody fragment, or a non-antibody protein capable of specifically binding said protein analyte or other non-nucleic acid analyte;
  - said first 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 said analyte;
  - said second 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;
  - said step of incubating said aqueous sample with said library of prefabricated microparticles and with said first detection composition is a step of performing an immunochemistry reaction involving the binding of said analyte, if present, in said aqueous sample, to said primary antibody,

primary antibody fragment, or non-antibody protein, thus forming a complex of analyte and said primary antibody, primary antibody fragment, or non-antibody protein; said immunochemistry reaction further involving a binding of said secondary antibody to said complex, thus forming a sandwich between primary antibody, antibody fragment or non-antibody protein, analyte, and secondary antibody;

- said first optional step of washing said library is a step of removing any non-bound secondary antibody from said library;
- said step of incubating said library of prefabricated microparticles including absorbed aqueous sample with said second detection composition is a step of allowing said substrate to be reacted by said reporter enzyme;
- said further optional step of washing said library is a step of removing any non-reacted substrate from said library;
- said step of transferring is a step wherein said prefabricated microparticles are transferred into a non-aqueous phase and suspended and, optionally, repeatedly washed with said non-aqueous phase, more optionally involving also a filtration or mechanical agitation to ensure the formation of a monodisperse suspension of microparticles with no aqueous phase or no substantial aqueous phase remaining outside of any of the microparticles;
- said optional step of releasing the analyte-specific reagent(s) attached to said microparticles by applying an external trigger is a step of temporarily increasing the temperature, changing the pH, or changing the salt conditions, preferably a step of increasing the temperature, more preferably from ambient temperature to a temperature  $>90^{\circ}\text{C}$ ;
- said step of detecting and/or quantitating said analyte of interest is a step of detecting and/or quantitating said reacted substrate.

19. A method of detecting and/or quantitating an analyte of interest in an aqueous sample, said method comprising the steps:

- providing, in any order:

- an aqueous sample known or suspected to contain an analyte of interest;
  - a first detection composition comprising necessary reagents for performing a chemical or biochemical detection reaction of the analyte;
  - a second detection composition comprising a detection reagent;
  - a library of prefabricated microparticles according to any of claims 3 - 11, wherein said analyte-specific reagent(s) attached to said microparticles, is(are) chosen such that it is (they are) capable of specifically binding to or reacting with said analyte of interest;
- optionally, mixing said aqueous sample and said first detection composition;
- incubating said aqueous sample with said library of prefabricated microparticles and, if said aqueous sample has not already been mixed with said first detection composition, also with said first detection composition, thereby allowing said library of microparticles to absorb aqueous sample and said first detection composition in the void volumes of said microparticles and, optionally, to bind said analyte of interest, if present in said sample;
- optionally, washing said library of prefabricated microparticles to remove any non-absorbed or non-reacted first detection composition;
- incubating said library of prefabricated microparticles including absorbed aqueous sample with said second detection composition, thereby allowing said library of microparticles to absorb said second detection composition;
- optionally, further washing said library of prefabricated microparticles to remove any non-absorbed or non-reacted second detection composition;
- transferring said library of prefabricated microparticles into a non-aqueous phase and removing any aqueous phase surrounding the individual prefabricated microparticle(s), thereby creating a plurality of insulated reaction spaces for detecting said analyte which reaction spaces comprise an aqueous phase and are confined to said void volume(s) of said microparticles;
- optionally, releasing the analyte-specific reagent(s) attached to said microparticles by applying an external trigger;

- performing a detection reaction of said analyte of interest; and
- detecting and/or quantitating said analyte of interest.

20. The method according to claim 19, wherein said analyte of interest is a protein or other non-nucleic acid molecule, and

- said analyte-specific reagent is a primary antibody, antibody fragment, or a non-antibody protein capable of specifically binding said protein analyte or other non-nucleic acid analyte;
- said first 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 oligonucleotide tag and being specific for the same analyte as said primary antibody;
- said second detection composition comprises, as detection reagent(s), 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 detection of an amplification product, such as an amplified nucleic acid, and primers suitable for amplifying the oligonucleotide tag attached to the secondary antibody;
- said step of incubating said aqueous sample with said library of prefabricated microparticles and with said first detection composition is a step of performing an immunochemistry reaction involving the binding of said analyte, if present, in said aqueous sample, to said primary antibody, primary antibody fragment, or non-antibody protein, thus forming a complex of analyte and said primary antibody, primary antibody fragment, or non-antibody protein; said immunochemistry reaction further involving a binding of said secondary antibody to said complex, thus forming a sandwich between primary antibody, antibody fragment or non-antibody protein, analyte, and secondary antibody;
- said first optional step of washing said library is a step of removing any non-bound secondary antibody from said library;
- said step of incubating said library of prefabricated microparticles including absorbed aqueous sample with said second detection composition is a step of allowing said primers in said second detection

composition hybridize to the oligonucleotide tag attached to the secondary antibody;

- said further optional step of washing said library is a step of removing any non-hybridized primers from said library;
  - 5 - said step of transferring is a step wherein said prefabricated microparticles are transferred into a non-aqueous phase and suspended and, optionally, repeatedly washed with said non-aqueous phase, more optionally involving also a filtration or mechanical agitation to ensure the formation of a monodisperse suspension, of microparticles with no aqueous phase or
  - 10 no substantial aqueous phase remaining outside of any of the microparticles;
  - said optional step of releasing the analyte-specific reagent(s) attached to said microparticles by applying an external trigger is a step of temporarily increasing the temperature, changing the pH, or changing the salt
  - 15 conditions, preferably a step of increasing the temperature, more preferably from ambient temperature to a temperature  $>90^{\circ}\text{C}$ ;
  - said step of performing a detection reaction is a step of performing an amplification reaction of said oligonucleotide tag; and
- said step of detecting and/or quantitating said analyte(s) of interest is a step of
- 20 detecting and/or quantitating said amplified oligonucleotide tag(s).

21. The method according to any of claims 15 – 20, wherein said step of releasing the analyte-specific reagent(s) attached to said microparticles by applying an external trigger is performed by raising the temperature

25 to which said microparticles are exposed.

22. The method according to any of claims 15 – 21, wherein,

- the method is a method of detecting and/or quantitating a single analyte in
- 30 a plurality of aqueous samples, e.g. from different patients,
- in said method, there are provided a plurality of different aqueous samples known or suspected to contain an analyte of interest, e.g. samples from different patients,
- the library of prefabricated microparticles that is provided in said method,
- 35 is a library according to claim 9, wherein, in such library, there are as many

- or at least as many separate subsets of microparticles provided, as there are different aqueous samples provided and to be tested, wherein all of said separate substeps have the same analyte-specific reagent attached to the porous matrix of said microparticles of said subsets, said analyte-specific reagent being specific for one analyte of interest;
- 5
- in said step of incubating said aqueous samples with said library of prefabricated microparticles, each of said different aqueous samples is incubated separately with a separate subset of microparticles of said library;
- 10
- in said step of incubating said library of prefabricated microparticles including absorbed aqueous sample with a second detection composition, each of said separate subsets of microparticles of said library is incubated separately with said second detection composition;
- 15
- said step of transferring said library into a non-aqueous phase is performed for each subset of microparticles separately, i.e. each separate subset of microparticles is separately transferred into a non-aqueous phase, and the aqueous phase surrounding the individual microparticles is removed for each subset separately, thereby creating, for each subset separately, a plurality of insulated reaction spaces for detecting said analyte which
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- reaction spaces comprise an aqueous phase and are confined to said void volume(s) of said microparticles,

wherein said method further comprises, after said step of transferring said library into a non-aqueous phase, a step of mixing said plurality of insulated reaction spaces

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of all the separate subsets in said non-aqueous phase together, before subsequently a detection reaction of said analyte of interest is performed, and before detecting and/or quantitating said analyte of interest.

23. The method according to any of claims 15 – 21, wherein,

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- the method is a method of detecting and/or quantitating multiple analytes in a single aqueous sample,
  - in said method, there is provided a single aqueous sample known or suspected to contain multiple analytes of interest,

- the library of prefabricated microparticles that is provided in said method, is a library according to claim 10, wherein, in such library, there are as many or at least as many separate subsets of microparticles provided, as there are different analytes of interest to be detected, with each of said separate subsets having a different analyte-specific reagent attached to the porous matrix of said microparticles of said subset; each analyte-specific reagent being specific for one analyte of interest;
- in said step of incubating said aqueous sample with said library of prefabricated microparticles, said aqueous sample is incubated with all of said separate subsets of microparticles of said library together;
- in said step of incubating said library of prefabricated microparticles including absorbed aqueous sample with a second detection composition, all of said separate subsets of microparticles of said library are incubated together with said second detection composition;
- said step of transferring said library into a non-aqueous phase is performed for all subsets of microparticles together, i.e. all subsets of microparticles are transferred together into a non-aqueous phase, and the aqueous phase surrounding the individual microparticles is removed, thereby creating a plurality of insulated reaction spaces for detecting and/or quantitating said multiple analytes which reaction spaces comprise an aqueous phase and are confined to said void volume(s) of said microparticles.

24. The method according to any of claims 15 – 21, wherein,

- the method is a method of detecting and/or quantitating multiple analytes in a plurality of aqueous samples,
- in said method, there are provided a plurality of different aqueous samples known or suspected to contain multiple analytes of interest, e.g. samples from different patients,
- the library of prefabricated microparticles that is provided in said method, is a library according to claim 11, wherein, in such library, there are different classes of separate subsets of prefabricated microparticles, with each of said classes comprising several subsets of microparticles and each of said classes having a different analyte-specific reagent attached to the porous matrix of said microparticles, and all subsets of microparticles

within one class having the same analyte-specific reagent attached; each analyte-specific reagent being specific for one analyte of interest; wherein, in said method, in said step of providing said library, there are

- 5                   • as many or at least as many different classes of separate subsets of microparticles provided, as there are different analytes to be detected,
- as many or at least as many different subsets of microparticles provided within each class, as there are aqueous samples provided and to be tested,
- 10               • as many or at least as many different subsets of microparticles provided within the library as there are different aqueous samples to be tested multiplied by the number of different analytes of interest to be detected,
- 15               - in said step of incubating said aqueous sample with said library of prefabricated microparticles, , exactly one aqueous sample is incubated with exactly one subset of microparticles from each class, with each aqueous sample being incubated with as many different subsets of microparticles from different classes together as there are classes of microparticles in said library,
- 20               - in said step of incubating said library of prefabricated microparticles including absorbed aqueous sample with a second detection composition, the combined subsets from different classes of microparticles with which subsets one respective aqueous sample had been previously incubated, are incubated together with said second detection composition,
- 25               - said step of transferring said library into a non-aqueous phase is performed for each aqueous sample separately, namely the combined subsets of microparticles with which one respective aqueous sample had been previously incubated, are transferred together into a non-aqueous phase, and the aqueous phase surrounding the individual microparticles is
- 30               removed, thereby creating, for each aqueous sample separately, a plurality of insulated reaction spaces for detecting said multiple analytes which reaction spaces comprise an aqueous phase and are confined to said void volume(s) of said microparticles,

wherein said method further preferably comprises, after said step of transferring said library into a non-aqueous phase, a step of mixing said pluralities of insulated reaction spaces of all the separate samples in said non-aqueous phase together, before subsequently a detection reaction of said analytes of interest is performed, and  
5 before detecting and/or quantitating said analytes of interest.

25. The method according to any of claims 15 – 24, wherein, in said step of detecting and quantitating said analyte of interest, quantitation of said analyte is performed by a method selected from:

- 10 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  
15 immunochemistry detection methods, such as digital immunoassays, e.g. digital enzyme-linked immunosorbent assays (ELISAs);
- d) immunochemistry detection methods combined with nucleic acid amplification, such as immuno-polymerase chain reaction;  
20 in particular digital immune-PCR;

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.

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