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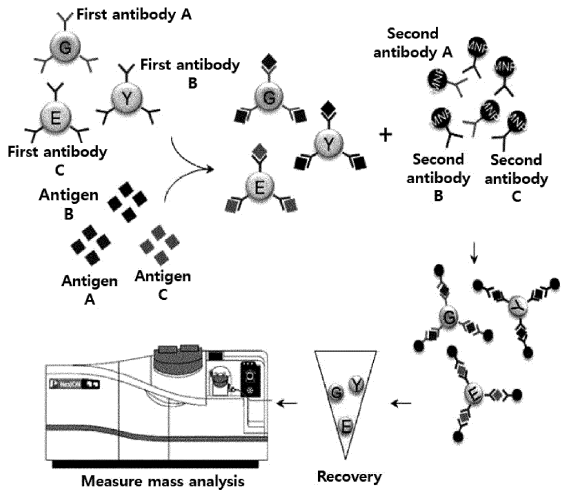
(54) SIMULTANEOUS ANALYSIS METHOD FOR MULTIPLE TARGETS USING MULTIPLE METAL NANO-TAGS

(57) The present invention relates to a method for simultaneous analysis of a target using a plurality of metal nano-tags and, more particularly, to a method for simultaneous analysis of a target using a plurality of metal nano-tags, wherein the method allows the convergence of a nano-particle technology based on an antigen-antibody reaction, which is a conventional biological immune response, and simultaneously diagnoses a plurality of target materials using a plurality of antigen-antibody reactions and a plurality of metal nano-tags, thereby enhancing diagnostic effect. The analysis method for a target material using metal nano-tags according to the present invention allows the convergence of a nanotechnology into a conventional biological immune response, and thereby enables accurate detection of even a trace amount of virus without the burden of inspection cost, in the business of verification/diagnosis of blood preparations, viruses, and other biomedicines as well as in the blood management business which deals with a large amount of blood samples.

Fig 1



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Description

BACKGROUND OF THE INVENTION

Field of the invention

[0001] The present invention relates to a method for simultaneous analysis of a target using a plurality of metal nano-tags and, more particularly, to a method for simultaneous analysis of a target using a plurality of metal nano-tags, in which the method allows the convergence of a nano-particle technology based on an antigen-antibody reaction, which is a conventional biological immune response, and simultaneously diagnoses a plurality of target materials using a plurality of antigen-antibody reactions and a plurality of metal nano-tags, thereby enhancing diagnostic effect.

Related Art

[0002] Major pathogenic viruses, for example, hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV), which are spread through blood or body fluids, are very important prognostic factors in blood management for transfusion.

[0003] Currently, the infection diagnosis of these pathogens is mainly performed by an enzyme-linked immunosorbent assay (ELISA), which can detect the presence of HBV surface antigen (HBsAg) or antibodies against HCV and HIV in the blood and thereby determine the presence of virus infection. However, ELISA has a problem in that the accuracy of the assay is low during the latent period until the antibody is formed after virus infection, while it is in a state of infection of viruses with different immune activities, or immune inactivity of the infected person.

[0004] In order to overcome the limitations of ELISA, a method that has been developed over the past several years is a nucleic acid test (NAT) which directly detects a genetic material of a virus consisting of DNA or RNA. The nucleic acid test is a diagnostic method for analyzing the presence/absence of a virus with enhanced sensitivity compared to enzyme immunoassay, using an oligo primer with nucleotide sequence specificity for viral nucleic acid. The nucleic acid test was expected to be appropriately utilized for screening pathogenic viruses that are transmitted through the blood in the fields of blood-associated business such as blood transfusion or biopharmaceutical business. However, it is difficult to utilize the nucleic acid test as a routine test method to handle a large amount of specimens due to the problem of cost incurring in the course of introducing and utilizing the test method.

[0005] In addition, there is a method called "multiplex NAT" to be used as a method for simultaneously detecting several kinds of viruses. The multiplex NAT has the effect of reducing the inspection time and effort to some extent if introduced. However, the method has difficulties in that the sensitivity may be degraded unless the optimization of the reaction conditions is warranted and that there is a risk of false positive or false negative, and also there is still difficulty in terms of inspection costs to utilize the method as a routine test method to handle a large amount of specimens.

[0006] Despite the advantages and disadvantages of various methods for the diagnosis of viruses as described above, the technology primarily used in clinical diagnostics at present to detect viruses is ELISA based on antigen-antibody reaction. Although ELISA method is commonly used because the operation of the measuring machine is simple and the sample can be processed rapidly, the assay has many problems in that the types of usable chromogens or phosphors are limited, there is a difficulty in tagging, reactivity of enzymes related to color development, and in the case of fluorescence, there are various constraints for measurements due to photo bleaching, quenching, etc. In particular, it is even more so in the field of applications where quantitative measurements are required.

[0007] Accordingly, there is a need for the development of a novel technology that enables accurate measurement and quantification as well as quantification of target materials such as various kinds of proteins in various matrices.

[0008] For this purpose, there is known a method which can detect even a trace amount of virus by using metal nano-tags instead of phosphors or other chromogenic compounds in the conventional ELISA method and measuring the mass of the metal.

[0009] However, conventionally, the method using a metal nano-tag has a problem in that it is difficult to simultaneously detect a plurality of targets because it includes only one kind of metal.

SUMMARY OF THE INVENTION

[0010] In order to solve the problems in the conventional technologies, an object of the present invention is to provide a novel method of analysis which enables simultaneous analysis of a plurality of targets using a plurality of antibodies.

[0011] The present invention, in order to solve the above objects, provides a method for simultaneous analysis of a plurality of targets using a plurality of metal nano-tags, which includes:

(i) preparing an analysis platform to which a first antibody, that specifically binds to a target, is bound;
(ii) reacting the analysis platform including the first antibody with a sample containing a plurality of targets and thereby forming an analysis platform to which target materials are bound;
(iii) reacting a second antibody, that specifically binds to a target, with the analysis platform in which the first antibody and targets are bound; and
(iv) performing a quantitative analysis of the material

to which the second antibody is bound.

[0012] In the method for simultaneous analysis of targets using a plurality of metal nano-tags according to the present invention, the target molecule to be analyzed may be a biomolecule. The biomolecule is a material that is released or separated *in vivo* and it may include not only materials generated *in vivo,* but also materials put into a living body and remain therein for a predetermined time. For example, biomolecules can include antibiotics, nucleic acids, hormones, enzymes, cells, tumors, cancer cells, bacteria, viruses, secretions thereof, etc. Examples of the antibiotics may include salinomycin, enrofloxacin, ciprofloxacin, penicillin, cephalosporin, carbapenem, ampicillin, neomycin, gentamicin, isepamicin, sisomicin, erythromycin, clarithromycin, vancomycin, teicoplanin, lincomycin, sulfathiazole, tetracycline, oxytetracycline, sulfamerazine, etc., and examples of cell secretions may include prostate-specific antigens, which are proteins synthesized in prostate cells, but the antibiotics and cell secretions are not limited thereto.

[0013] In the method for simultaneous analysis of targets using a plurality of metal nano-tags by the present invention, the analysis platform to which the first antibody is bound is characterized by containing a plurality of types of antibodies.

[0014] In the method for simultaneous analysis of targets using a plurality of metal nano-tags by the present invention, the analysis platform to which the first antibody is bound may be silica nanoparticle which contains a metal-containing core and silica that coats the surface of the core, or a plate to which a plurality of types of first antibodies are bound. In the method for simultaneous analysis of targets using a plurality of metal nano-tags by the present invention, the case of silica nanoparticle where the analysis platform to which the first antibody is bound is silica nanoparticle which contains a metal-containing core and silica that coats the surface of the core is illustrated in FIG. 1, and the case of silica nanoparticle where the analysis platform to which the first antibody is bound is a plate to which a plurality of types of first antibodies are bound is illustrated in FIG. 2.

[0015] In the method for simultaneous analysis of targets using a plurality of metal nano-tags by the present invention, each of the silica nanoparticles is characterized by containing a single kind of metal, and the analysis platform to which the antibody is bound is characterized by containing at least two types of silica nanoparticles with different types of metals. The method for simultaneous analysis of targets using a plurality of metal nano-tags by the present invention is characterized in that the method can diversify the kinds of targets according to the type of the first antibody via analysis by attaching a different type of the first antibody to a plurality of the silica nanoparticles with different types of metals, and the method also enables a simultaneous analysis of two different kinds of metals when quantitative analysis is performed later using an inductively coupled plasma mass spectrometry (ICP-MS) by varying the kinds of the metals contained in the silica nanoparticles.

[0016] In the method for simultaneous analysis of targets using a plurality of metal nano-tags by the present invention, the metals contained in the silica nanoparticles may be selected from the group consisting of Au, Ag, Pt, Pd, Ir, Rh, Ru, Al, Cu, Te, Bi, Pb, Fe, Ce, Mo, Nb, W, Sb, Sn, V, Mn, Ni, Co, Zn, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Sc, Y, and Ti.

[0017] In the method for simultaneous analysis of targets using a plurality of metal nano-tags by the present invention, the analysis platform to which the first antibody is bound is characterized by having at least two types of a plurality of types of silica nanoparticles selected from the group consisting of silica nanoparticle including silica nanoparticle containing Au, silica nanoparticle containing Gd, silica nanoparticle containing Y, and silica nanoparticle containing Eu.

[0018] In the method for simultaneous analysis of targets using a plurality of metal nano-tags by the present invention, for the analysis platform to which the first antibody is bound, an appropriate metal may be selected depending on the target to be analyzed. Specifically, silica nanoparticle containing Au is desirable when the target to be analyzed is HBV in the blood; silica nanoparticle containing Gd is desirable when the target to be analyzed is HIV in the blood; and silica nanoparticle containing Eu is desirable when the target to be analyzed is HCV in the blood; and it is possible that at least two of these silica nanoparticles are used simultaneously.

[0019] In another exemplary embodiment of the present invention, it is possible to use the conventional ELISA method as it is except that the analysis platform to which the first antibody is bound is a plate to which a plurality of types of the first antibodies are bound and that a plurality of types of the first antibodies are bound.

[0020] In the method for simultaneous analysis of targets using a plurality of metal nano-tags by the present invention, when the analysis platform is a plate to which a plurality of types of the first antibodies are bound, the second antibodies are characterized by being bound to silica nanoparticles which contain a metal-containing core and/or silica that coats the surface of the core.

[0021] In the method for simultaneous analysis of targets using a plurality of metal nano-tags by the present invention, the core of the silica nanoparticles to which the second antibodies are bound is characterized by containing at least two metals selected from the group consisting of Au, Ag, Pt, Pd, Ir, Rh, Ru, Al, Cu, Te, Bi, Pb, Fe, Ce, Mo, Nb, W, Sb, Sn, V, Mn, Ni, Co, Zn, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Sc, Y, and Ti.

[0022] In the method for simultaneous analysis of targets using a plurality of metal nano-tags by the present invention, the core of the silica nanoparticles to which the second antibodies are bound is characterized by containing Au.

[0023] In the method for simultaneous analysis of tar-

gets using a plurality of metal nano-tags by the present invention, the first antibody is characterized to be a monoclonal antibody.

[0024] In the method for simultaneous analysis of targets using a plurality of metal nano-tags by the present invention, it is possible that the first antibody and the second antibody to be used are the same.

[0025] In the method for simultaneous analysis of targets using a plurality of metal nano-tags by the present invention, it is possible that the first antibody is a monoclonal antibody and the second antibody is a polyclonal antibody.

[0026] In the method for simultaneous analysis of targets using a plurality of metal nano-tags by the present invention, step (iv) of performing a quantitative analysis of the material to which the second antibody is bound includes:

(iv-1) capturing the target material to which the second antibody is bound by applying an external magnetic force; and

(iv-2) analyzing the captured target material to which the second antibody is bound using a spectrophotometer. That is, in the case of using silica nanoparticles as the analysis platform to which the first antibody is bound, the target to which the second antibody is bound is separated by the magnetism of the magnetic nanoparticles which are connected to the second antibody, and the tagged metal of the separated target to which even the second antibody is bound is subjected to quantitative analysis for the analysis of the target.

[0027] In another exemplary embodiment of the present invention, in the case of using the first antibody bound to a plate as the analysis platform to which the first antibody is bound, step (iv) of performing a quantitative analysis of the material to which the second antibody is bound is characterized in that it includes:

(iv-1) separating the material, which is bound to the first antibody of the plate, to which the second antibody is bound; and

(iv-2) analyzing only the material, which is bound to the first antibody of the plate, to which the second antibody is bound using a spectrophotometer. That is, since the target to which even the second antibody is bound is fixed onto a plate, it is possible to perform a quantitative analysis for a plurality of targets by subjecting the tagged metal bound to the second antibody after the simple separation.

[0028] In the method for simultaneous analysis of targets using a plurality of metal nano-tags by the present invention, step (iv-2) of analyzing the captured target material to which the second antibody is bound using a spectrophotometer is characterized by performing the analysis using an inductively coupled plasma mass spectrometry (ICP-MS) or graphite furnace atomic absorption spectrophotometer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029]

FIGS. 1 and 2 show schematic diagrams illustrating a method for simultaneous analysis of targets using a plurality of metal nano-tags by the present invention.

FIGS. 3 and 4 show the analysis results by ICP-MS in the blood according to an exemplary embodiment of the present invention.

BEST MODE FOR CARRYING OUT THE INVENTION

[0030] The method for simultaneous analysis of a plurality of targets using a plurality of metal nano-tags according to the present invention may include:

(i) preparing an analysis platform to which a first antibody, that specifically binds to a target, is bound;

(ii) reacting the analysis platform including the first antibody with a sample containing a plurality of targets and thereby forming an analysis platform to which target materials are bound;

(iii) reacting a second antibody, that specifically binds to a target, with the analysis platform in which the first antibody and targets are bound; and

(iv) performing a quantitative analysis of the material to which the second antibody is bound.

[0031] In the method for simultaneous analysis of a plurality of targets using a plurality of metal nano-tags according to the present invention, the analysis platform to which the first antibody is bound may include a plurality of types of antibodies.

[0032] In the method for simultaneous analysis of a plurality of targets using a plurality of metal nano-tags according to the present invention, the analysis platform to which the first antibody is bound may be a silica nanoparticle which contains a magnetic metal-containing core and a silica that coats the surface of the core. The silica nanoparticle contains a single type of metal; and the analysis platform to which the antibody is bound contains at least two types of silica nanoparticles containing different types of metals. The metals contained in the silica nanoparticle may be selected from the group consisting of Au, Ag, Pt, Pd, Ir, Rh, Ru, Al, Cu, Te, Bi, Pb, Fe, Ce, Mo, Nb, W, Sb, Sn, V, Mn, Ni, Co, Zn, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Sc, Y, and Ti. The analysis platform to which the first antibody is bound may include at least two types of a plurality of types of silica nanoparticles selected from the group consisting of a silica nanoparticle containing a silica nanoparticle containing Au, a silica nanoparticle containing Gd, a silica nanoparticle containing Y, and a silica nan-

oparticle containing Eu. The second antibody may be bound to a silica nanoparticle, which contains a magnetic metal-containing core and a silica that coats the surface of the core.

[0033] In the method for simultaneous analysis of a plurality of targets using a plurality of metal nano-tags according to the present invention, the analysis platform to which the first antibody is bound may be a plate to which a plurality of types of the first antibody is bound.

[0034] In the method for simultaneous analysis of a plurality of targets using a plurality of metal nano-tags according to the present invention, the second antibody may be bound to a silica nanoparticle which contains a magnetic metal-containing core and a silica that coats the surface of the core.

[0035] In the method for simultaneous analysis of a plurality of targets using a plurality of metal nano-tags according to the present invention, the core of the silica nanoparticle bound to the second antibody may contain at least two metals selected from the group consisting of Au, Ag, Pt, Pd, Ir, Rh, Ru, Al, Cu, Te, Bi, Pb, Fe, Ce, Mo, Nb, W, Sb, Sn, V, Mn, Ni, Co, Zn, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Sc, Y, and Ti.

[0036] In the method for simultaneous analysis of a plurality of targets using a plurality of metal nano-tags according to the present invention, the core of the silica nanoparticle bound to the second antibody may contain Au.

[0037] In the method for simultaneous analysis of a plurality of targets using a plurality of metal nano-tags according to the present invention, the first antibody and the second antibody may be the same.

[0038] In the method for simultaneous analysis of a plurality of targets using a plurality of metal nano-tags according to the present invention, the first antibody may be a monoclonal antibody and the second antibody may be a polyclonal antibody.

[0039] In the method for simultaneous analysis of a plurality of targets using a plurality of metal nano-tags according to the present invention,

step (iv) of performing a quantitative analysis of the material to which the second antibody is bound may include:

(iv-1) capturing the target material to which the second antibody is bound by applying an external magnetic force; and

(iv-2) analyzing the captured target material to which the second antibody is bound using a spectrophotometer.

[0040] In the method for simultaneous analysis of a plurality of targets using a plurality of metal nano-tags according to the present invention,

step (iv) of performing a quantitative analysis of the material to which the second antibody is bound may include:

(iv-1) separating the material, which is bound to the first antibody of the plate, to which the second antibody is bound; and

(iv-2) analyzing only the material, which is bound to the first antibody of the plate, to which the second antibody is bound using a spectrophotometer.

[0041] In the method for simultaneous analysis of a plurality of targets using a plurality of metal nano-tags according to the present invention,

step (iv-2) of analyzing the captured target material to which the second antibody is bound using a spectrophotometer may be to analyze using an inductively coupled plasma mass spectrometry (ICP-MS) or graphite furnace atomic absorption spectrophotometer.

DESCRIPTION OF EXEMPLARY EMBODIMENTS

[0042] Hereinafter, the present invention will be described in more detail with reference to examples. However, the present invention is not further limited by the following examples.

< Example 1> Case using plate as analysis platform containing first antibody

[0043] After attaching a Human anti-p24 monoclonal antibody as a first antibody to a plate, HBsAg was attached as a second antibody to the plate, and silica nanoparticles containing an Au particle were prepared using the Gold Nanoparticle Conjugation kit

[0044] A blood sample was allowed to flow through the plate to induce a reaction between the first antibody and a target in the blood sample and unreacted impurities were removed by washing. The HBsAg was attached the resultant and allowed to react with a second antibody containing an Au particle.

[0045] Then, the conjugate bound to the second antibody was separated and recovered by a reaction with nitric acid and the weight of the conjugate was measured using an ICP-MS. The results are shown in FIG. 3.

<Example 2> Case using silica nanoparticle as analysis platform containing first antibody

[0046] Gadolinium-doped silica nanoparticles, yttrium-doped silica nanoparticles, and europium-doped silica nanoparticles were synthesized as an analysis platform containing the first antibody, respectively.

[0047] Human anti-p24 monoclonal antibody was attached to each of the synthesized silica nanoparticles as a first antibody and mixed, and thereby an analysis platform containing silica nanoparticles was prepared.

[0048] Iron nanoparticles were prepared as magnetic nanoparticles and by attaching human anti-p24 monoclonal antibody thereto as a second antibody.

[0049] Silica nanoparticles, in which Gadolinium-doped silica nanoparticles, yttrium-doped silica nanoparticles, and europium-doped silica nanoparticles were mixed, were reacted with a sample containing target materials. After removing the unreacted materials, the conjugate bound to the second antibody was separated and recovered by a reaction with nitric acid, and the weight of the resultant was measured by ICP-MS. The results are shown in FIG. 4.

[0050] It was confirmed that a plurality of targets can be quantitatively analyzed when a method for simultaneous analysis of a plurality of targets using a plurality of metal nano-tags according to the present invention.

ADVANTAGEOUS EFFECTS OF INVENTION

[0051] The analysis method for a target material using metal nano-tags according to the present invention fuses a nanotechnology to a conventional biological immune response, and the method thereby makes it possible to accurately detect even a trace amount of virus without the burden of inspection cost, in the business of verification/diagnosis of blood preparations, viruses, and other biomedicines as well as in the blood management business which deals with a large amount of blood samples.

Claims

1. A method for simultaneous analysis of a plurality of targets using a plurality of metal nano-tags, comprising:

   (i) preparing an analysis platform to which a first antibody, that specifically binds to a target, is bound;
   (ii) reacting the analysis platform comprising the first antibody with a sample comprising a plurality of targets and thereby forming an analysis platform to which target materials are bound;
   (iii) reacting a second antibody, that specifically binds to a target, with the analysis platform in which the first antibody and targets are bound; and
   (iv) performing a quantitative analysis of the material to which the second antibody is bound.

2. The method of claim 1, wherein the analysis platform to which the first antibody is bound comprises a plurality of types of antibodies.

3. The method of claim 1, wherein the analysis platform to which the first antibody is bound is silica nanoparticle, comprising a metal-comprising core and silica that coats the surface of the core.

4. The method of claim 3, wherein the silica nanoparticle comprises a single type of metal; and the analysis platform to which the antibody is bound comprises at least two types of silica nanoparticles comprising different types of metals.

5. The method of claim 4, wherein the metals comprised in the silica nanoparticle are selected from the group consisting of Au, Ag, Pt, Pd, Ir, Rh, Ru, Al, Cu, Te, Bi, Pb, Fe, Ce, Mo, Nb, W, Sb, Sn, V, Mn, Ni, Co, Zn, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Sc, Y, and Ti.

6. The method of claim 4, wherein the analysis platform to which the first antibody is bound comprises at least two types of a plurality of types of silica nanoparticles selected from the group consisting of silica nanoparticle comprising silica nanoparticle comprising Au, silica nanoparticle comprising Gd, silica nanoparticle comprising Y, and silica nanoparticle comprising Eu.

7. The method of claim 4, wherein the second antibody is bound to a silica nanoparticle, which comprises a magnetic metal-comprising core and silica that coats the surface of the core.

8. The method of claim 1, wherein the analysis platform to which the first antibody is bound is a plate to which a plurality of types of the first antibody is bound.

9. The method of claim 8, wherein the second antibody is bound to a silica nanoparticle which comprises a metal-comprising core and silica that coats the surface of the core.

10. The method of claim 9, wherein the core of the silica nanoparticle bound to the second antibody comprises at least two metals selected from the group consisting of Au, Ag, Pt, Pd, Ir, Rh, Ru, Al, Cu, Te, Bi, Pb, Fe, Ce, Mo, Nb, W, Sb, Sn, V, Mn, Ni, Co, Zn, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Sc, Y, and Ti.

11. The method of claim 9, wherein the core of the silica nanoparticle bound to the second antibody comprises Au.

12. The method of claim 1, wherein the first antibody and the second antibody are the same.

13. The method of claim 1, wherein the first antibody is a monoclonal antibody and the second antibody is a polyclonal antibody.

14. The method of claim 1, wherein step (iv) of performing a quantitative analysis of the material to which the second antibody is bound comprises:

    (iv-1) capturing the target material to which the second antibody is bound by applying an exter-

nal magnetic force; and
(iv-2) analyzing the captured target material to which the second antibody is bound using a spectrophotometer.

15. The method of claim 1, wherein step (iv) of performing a quantitative analysis of the material to which the second antibody is bound comprises:

(iv-1) separating the material, which is bound to the first antibody of the plate, to which the second antibody is bound; and
(iv-2) analyzing only the material, which is bound to the first antibody of the plate, to which the second antibody is bound using a spectrophotometer.

16. The method of claim 14 or claim 15, wherein step (iv-2) of analyzing the captured target material to which the second antibody is bound using a spectrophotometer is to analyze using an inductively coupled plasma mass spectrometry (ICP-MS) or graphite furnace atomic absorption spectrophotometer.

Fig 1

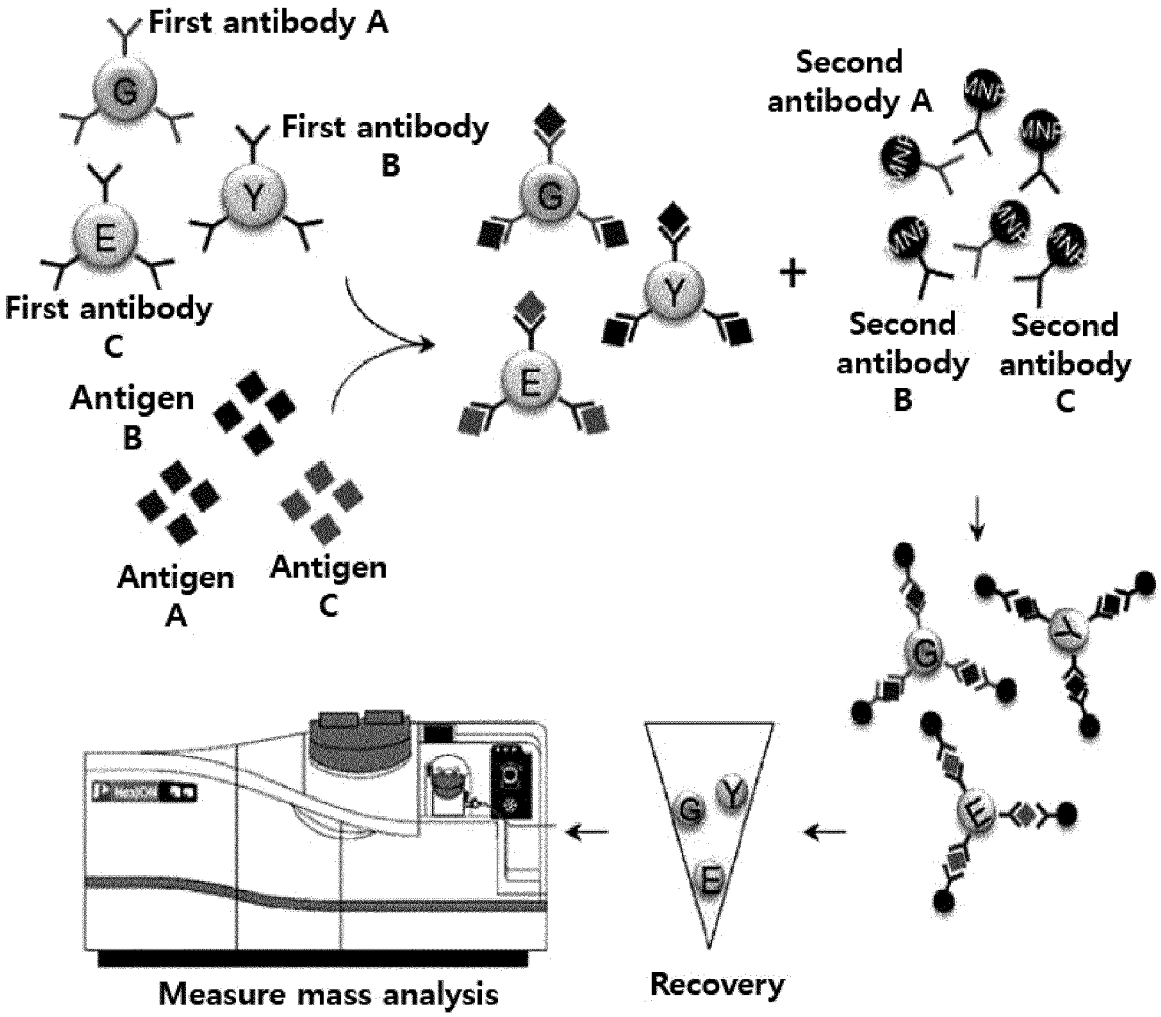

First antibody A
First antibody B
First antibody C
Antigen B
Antigen A
Antigen C
Second antibody A
Second antibody B
Second antibody C
Recovery
Measure mass analysis

Fig 2

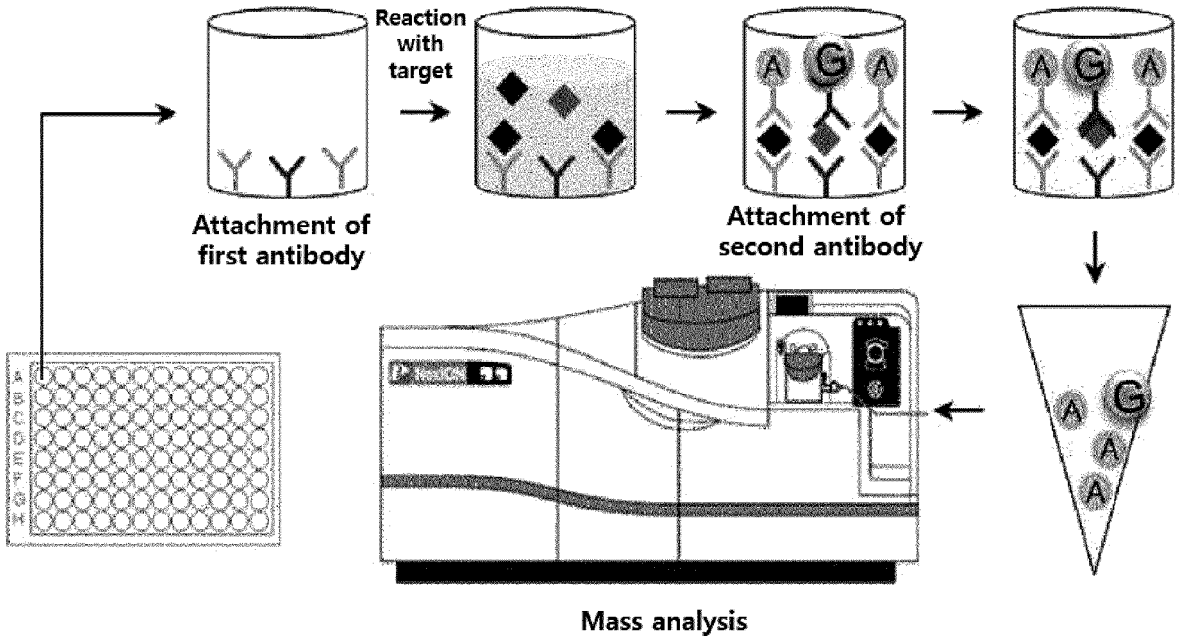

Reaction with target
Attachment of first antibody
Attachment of second antibody
A
G
A
Mass analysis

Fig 3

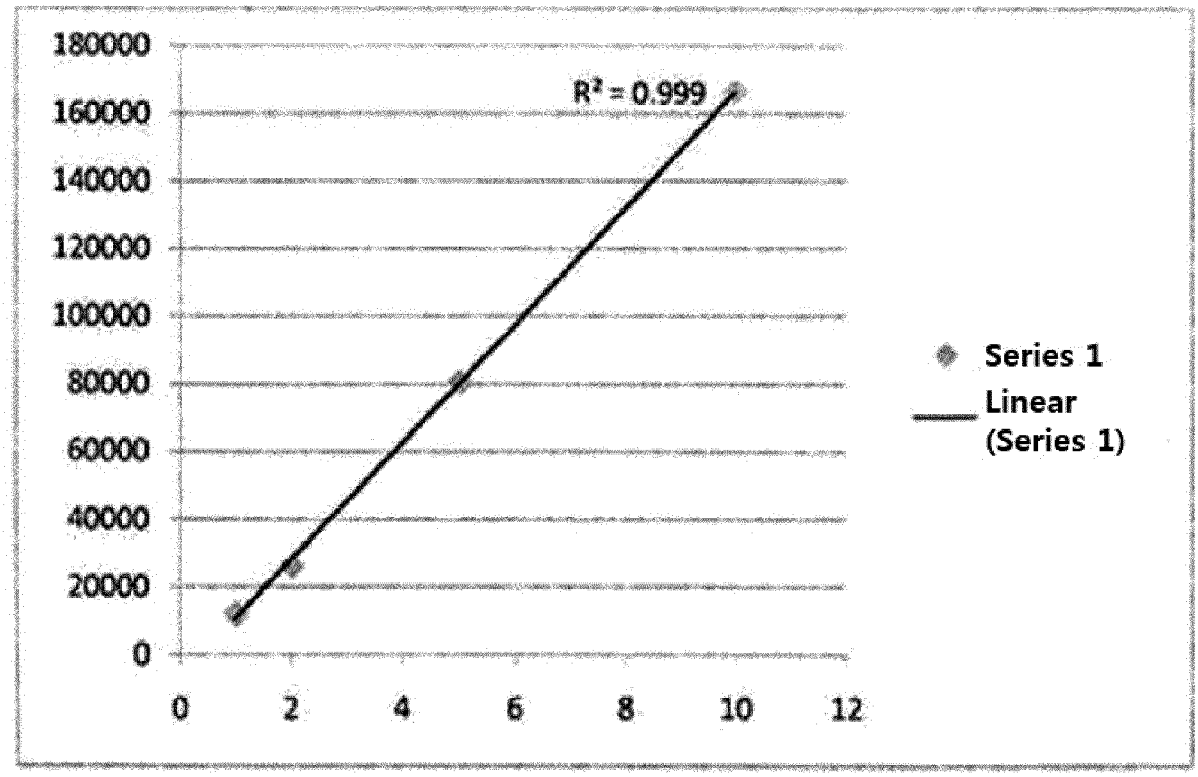
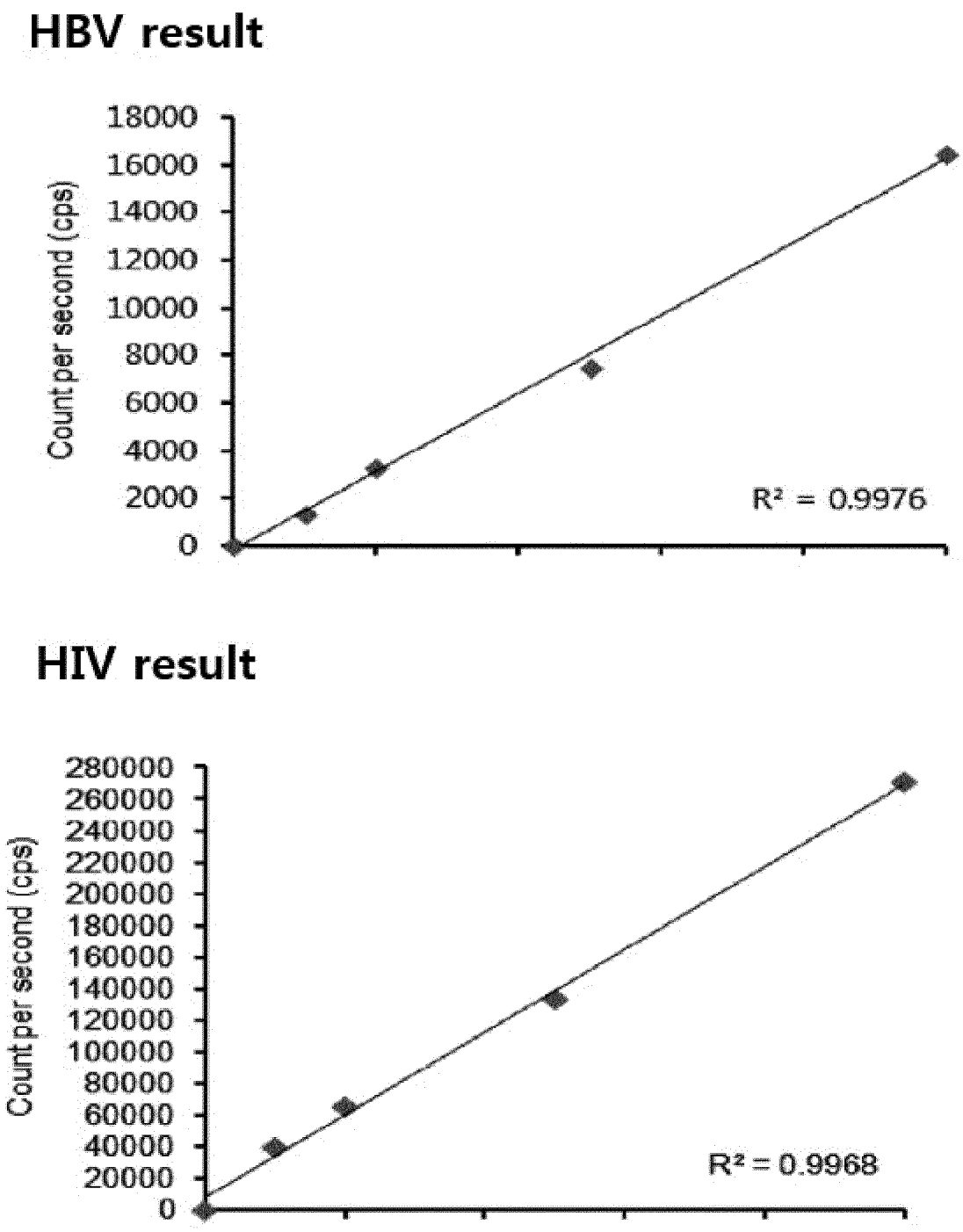

180000
160000
140000
120000
100000
80000
60000
40000
20000
0
0
2
4
6
8
10
12
R² = 0.999
Series 1
Linear (Series 1)

Fig 4


HBV result
Count per second (cps)
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16000
14000
12000
10000
8000
6000
4000
2000
0
R² = 0.9976
HIV result
Count per second (cps)
280000
260000
240000
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
R² = 0.9968

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2016/005116

A. CLASSIFICATION OF SUBJECT MATTER

G01N 33/58(2006.01)i, G01N 33/532(2006.01)i, G01N 33/569(2006.01)i, G01N 27/62(2006.01)i, C12Q 1/70(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N 33/58; B05D 7/00; G01J 3/44; B32B 5/16; G01N 33/543; G01N 21/65; G01N 33/532; G01N 33/569; G01N 27/62; C12Q 1/70

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Utility models and applications for Utility models: IPC as above
Japanese Utility models and applications for Utility models: IPC as above

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

eKOMPASS (KIPO internal) & Keywords: metal nano tag, silica, coating, antibody, magnetic metal, simultaneous analysis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GONG, Ji - Lai et al., Ag/SiO2 Core-shell Nanoparticle-based Surface-enhanced Raman Probes for Immunoassay of Cancer Marker using Silica-coated Magnetic Nanoparticles as Separation Tools, Biosensors and Bioelectronics, 2007, vol. 22, no. 7, pages 1501-1507 See abstract; and pages 1501-1506.	1-15
Y	WU, Shijia et al., Magnetic Nanobead-based Immunoassay for the Simultaneous Detection of Aflatoxin B1 and Ochratoxin A using Upconversion Nanoparticles as Multicolor Labels, Biosensors and Bioelectronis, 2011, vol. 30, no. 1, pages 35-42 See abstract; and pages 35-41.	1-15
A	US 2015-0038347 A1 (JOHNSON, Patrick A. et al.) 05 February 2015 See paragraphs [0010]-[0124]; claims 1-55; and figures 1-10.	1-15
A	US 2014-0308756 A1 (METALOR THEUNOLOGIES INTERNATIONAL SA.) 16 October 2014 See the entire document.	1-15
A	US 8481115 B2 (TOKORO, Hisato et al.) 09 July 2013 See the entire document.	1-15

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 AUGUST 2016 (09.08.2016)	**09 AUGUST 2016 (09.08.2016)**
Name and mailing address of the ISA/KR Korean Intellectual Property Office Government Complex-Daejeon, 189 Seonsa-ro, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2016/005116

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: **16**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (January 2015)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR2016/005116

Patent document cited in search report	Publication date	Patent family member	Publication date
US 2015-0038347 A1	05/02/2015	WO 2011-116402 A2	22/09/2011
		WO 2011-116402 A3	22/12/2011
US 2014-0308756 A1	16/10/2014	CH 705758 A1	15/05/2013
		CH 705758 B1	31/03/2016
		CN 103988081 A	13/08/2014
		EP 2780710 A2	24/09/2014
		JP 2015-507078 A	05/03/2015
		KR 10-2014-0092390 A	23/07/2014
		WO 2013-072213 A2	23/05/2013
		WO 2013-072213 A3	01/08/2013
US 8481115 B2	09/07/2013	CN 101977711 A	16/02/2011
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		EP 2272608 A1	12/01/2011
		EP 2272608 A4	27/11/2013
		EP 2272608 B1	17/09/2014
		US 2011-0293940 A1	01/12/2011
		WO 2009-119757 A1	01/10/2009

Form PCT/ISA/210 (patent family annex) (January 2015)

专利名称(译)	利用多种金属纳米标记同时分析多个目标的方法		
公开(公告)号	EP3296745A4	公开(公告)日	2019-01-02
申请号	EP2016793048	申请日	2016-05-13
[标]发明人	MUN HAE RAN KIM JONG SU KIM INAE		
发明人	MUN, HAE RAN KIM, JONG SU KIM, INAE		
IPC分类号	G01N33/58 G01N33/532 G01N33/569 G01N27/62 C12Q1/70		
CPC分类号	C12Q1/70 G01N33/54333 G01N33/552 G01N33/5761 G01N33/587 G01N33/6848 G01N2458/15 G01N27/62 C01G7/00 C01P2004/64 G01N33/532 G01N33/54346 G01N33/553 G01N33/569		
代理机构(译)	J A KEMP		
优先权	1020150066820 2015-05-13 KR		
其他公开文献	EP3296745A1		
外部链接	Espacenet		

摘要(译)

本发明涉及使用多个金属纳米标签同时分析靶的方法，更具体地，涉及使用多个金属纳米标签同时分析靶的方法，其中该方法允许收敛基于抗原 - 抗体反应的纳米粒子技术，其是常规的生物免疫应答，并且使用多个抗原 - 抗体反应和多个金属纳米标签同时诊断多种靶材料，从而增强诊断影响。根据本发明的使用金属纳米标签的靶材料的分析方法允许将纳米技术收敛到常规的生物免疫应答中，从而能够在没有检查成本负担的情况下准确检测甚至痕量的病毒，血液制剂，病毒和其他生物药物的验证/诊断业务以及处理大量血液样本的血液管理业务。