



(51) International Patent Classification:

G01N 30/72 (2006.01) G01N 33/543 (2006.01)
G01N 33/53 (2006.01)

(21) International Application Number:

PCT/US2015/033278

(22) International Filing Date:

29 May 2015 (29.05.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/004,640 29 May 2014 (29.05.2014) US

(71) Applicant: SIEMENS HEALTHCARE DIAGNOSTICS INC. [US/US]; 511 Benedict Ave., Tarrytown, New York 10591 (US).

(72) Inventors: PUGIA, Michael; 14342 Taddington Dr., Granger, Indiana 46540 (US). PHILIP, Julia; 831 S. 28th St., South Bend, Indiana 46615 (US). MARFURT, Karen; 27153 Redfield St., Edwardsburg, Michigan 49112 (US).

(74) Agents: STEIN, Kevin et al.; Siemens Corporation, IPD, 3501 Quadrangle Blvd Ste 230, Orlando, Florida 32817 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

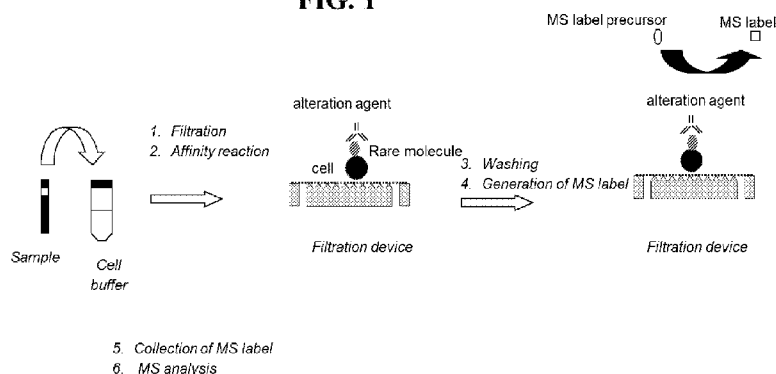
- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(88) Date of publication of the international search report:

21 January 2016

(54) Title: RARE MOLECULE DETECTION

FIG. 1



(57) Abstract: A concentrated sample having enhanced concentration of the one or more different populations of target rare molecules is incubated with, for each different population of target rare molecules, a particulate or non-particulate affinity agent that comprises a specific binding partner that is specific for and binds to a target rare molecule. The affinity agent comprises a mass spectrometry (MS) label precursor or a first alteration agent, which either facilitates the formation of an MS label from the MS label precursor or releases an entity that comprises the MS label precursor from the affinity agent. The MS label corresponds to one of the populations of target rare molecules. A second alteration agent is employed if the first alteration agent does not facilitate the formation of an MS label from the MS label precursor. MS analysis is used to determine each different MS label.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/33278

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 30/72; G01N 33/53; G01N 33/543 (2015.01)

CPC - G01N 33/53, 33/543, 33/6848, 33/54366, 33/54313

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)

IPC(8)- G01N 30/72; G01N 33/53; G01N 33/543 (2015.01)

CPC: G01N 33/53, 33/543, 33/6848, 33/54366, 33/54313

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/7.2; 436/173, 518, 523 (text search)

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

Electronic data bases: PatBase; Google Patents; Google Scholar

Search terms: Rare cell and molecule capture and detection, particle beads capture or affinity agent (i.e. antibody), MS label, MS label precursor, precursor alteration agent, filtration, enzyme linked immuno mass spectrometry

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2009/0188864 A1 (ZHENG et al.) 30 July 2009 (30.07.2009). Especially para [0010], [0059], [0063], [0079], [0081].	1-12, 16-20
Y	FLORENTINUS-MEFAILOSKI et al. Enzyme linked immune mass spectrometric assay (ELIMSA). J Proteomics January 2014 Vol 16 No 96 Pages 343-352. Especially abstract, pg 345 col 1 para 1 and col 2 para 2-3, pg 346 fig 1, pg 350 col 2 para 1.	1-12, 16-20
Y	US 2009/0137060 A1 (LUND-JOHANSEN) 28 May 2009 (28.05.2009). Especially para [0018], [0031], [0060], pg 7-9 Table 1.	5-11

☐ Further documents are listed in the continuation of Box C.

* 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

26 September 2015 (26.09.2015)

Date of mailing of the international search report

10 NOV 2015

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/33278

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.:
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:
-----Go to Extra Sheet for continuation-----

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.:
Claims 1-12, 16-20

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.

-----continuation of Box III (Lack of Unity of Invention)-----

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-12 and 16-20, drawn to a method of detecting one or more different populations of target rare MOLECULES in a sample suspected of containing the one or more different populations of rare molecules and non-rare molecules.

Group II: Claims 13-15, drawn to a method detecting one or more different populations of target rare CELLS in a sample suspected of containing the one or more different populations of rare cells and non-rare cells.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I has the special technical feature of concentration enhancement and detection of rare molecules isolated from a fluid, not required by Group II.

Group II has the special technical feature of concentration enhancement and detection of rare cells isolated from a biological fluid, not required by Group I.

Group I has the special technical feature of an affinity reagent specific for a rare molecule, not required by Group II.

Group II has the special technical feature of an affinity reagent specific for a rare cell, not required by Group I.

Common Technical Features:

Groups I and II share the following common technical features:

1. Enhancing the concentration of a rare component of a population (i.e. of molecules or cells).
2. Incubating the component with an affinity agent specific for the component, where the affinity agent comprises a mass spectrometry label precursor and/or alteration agent
3. Forming a retentate and a filtrate by contacting the incubated sample with a porous matrix.
4. Subjecting one or both of the retentate and the filtrate to mass spectrometry analysis to determine the presence and/or amount of each different mass spectrometry label
5. Relating the presence and/or amount of each different mass spectrometry label to the present and/or amount of each different population of target rare components in the sample.

However, said common technical features do not represent a contribution over the prior art, and is obvious over US 2009/0188864 A1 to ZHENG et al. (hereinafter "Zheng"), in view of the publication titled "Enzyme linked immune mass spectrometric assay (ELIMSA)" by Florentinus-Mefailoski (hereinafter "Florentinus") [J Proteomics January 2014 Vol 16 No 96 Pages 343-352].

Concerning common technical features #1 and #3, Zheng teaches enhancing the concentration of a rare component of a population (i.e. of molecules or cells) and forming a retentate and a filtrate by contacting the incubated sample with a porous matrix (claims 2-4; The microfiltration patch according to claim 1, wherein diameters of several top membrane pores are sized to capture particles having selected particle diameters and the gap distance positions the bottom porous membrane to provide back pressure to particles captured within at least one of the top membrane pores; 3. The microfiltration patch according to claim 2, wherein the top membrane pores and bottom membrane pores have diameters greater than diameters of blood cells and smaller than diameters of circulating tumor cells and wherein the gap distance is smaller than the diameters of the circulating tumor cells.; 4. The microfiltration patch according to claim 1, wherein diameters of several top membrane pores are sized larger than particle diameters of particles to be captured and diameters of the bottom membrane pores are sized smaller than particle diameters of particles to be captured and the gap distance is smaller than particle diameters of particles to be captured"). Furthermore, Zheng makes reference to capture of target molecules or cells using target specific antibodies conjugated to microparticles (para [0010]), such that it would have been obvious that target molecules captured and complexed to the microparticles could have been isolated and concentrated using the membrane microfiltration patch taught by Zheng.

-----continued on next sheet-----

-----continued from previous sheet-----

Concerning common technical feature #2, Florentinus teaches incubating the component with an affinity agent specific for the component, where the affinity agent comprises a mass spectrometry label precursor and/or alteration agent (abstract; "The central concept of ELIMSA is that the reporter enzymes HRP and AP [i.e. alteration agents conjugated to a target specific antibody] yield product [e.g. AP substrate [and mass spectroscopy precursor] ASMX naphthol phosphate converted to ASMX naphthol, the mass spectroscopy label; see Florentinus pg. 345 col 2 para 2-3] that ionize efficiently with a high signal to noise ratio that can be measured by mass spectrometry. The reporter enzymes HRP or AP may be covalently attached to a specific detection probe such as a protein or an antibody to bind their target analyte and then catalyze the rapid production of ionizable, small-molecules. The use of mass spectrometry to measure small molecule products may commonly reach femto to picomol amounts on the column with high signal to noise ratio"; pg 346 fig 1). It would have been obvious that the enzyme reactions to produce mass spectroscopic ionizable products taught by Florentinus could have been performed directly on the microfilters taught by Zhang, or in the filtrate, thereof, before performing the mass spectroscopic measurement.

Concerning common technical feature #4, Florentinus teaches subjecting one or both of the retentate and the filtrate to mass spectrometry analysis to determine the presence and/or amount of each different mass spectrometry label (abstract; HRP and AP yield product that ionize efficiently with a high signal to noise ratio that can be measured by mass spectrometry [....] catalyze the rapid production of ionizable, small-molecules. The use of mass spectrometry to measure small molecule products may commonly reach femto to picomol amounts).

Concerning common technical feature #5, Florentinus teaches relating the presence and/or amount of each different mass spectrometry label to the present and/or amount of each different population of target rare components in the sample (abstract; "There is an urgent need to detect and quantify low abundance proteins such as hormones, chemokines, cytokines, and others that exist at attomolar concentrations under physiological conditions by ELIMSA. A sensitive method for the quantification of immunological assays is obtained by applying mass spectrometry to detect the products of the alkaline phosphatase (AP) and horseradish peroxidase (HRP) enzyme reactions. There are many molecules from human subjects or micro-organisms that are of great importance to medicine"; pg 350 col 2 para 1- A quantitative analytical method that can confidently measure analytes to attomol levels is required for biomedical and environmental testing and research. Here the equivalent of about 6 amol of PSA on the column was detectable by ELIMSA with a capture and detection antibody in a 96 well plate").

As the common technical features were known in the art at the time of the invention, they cannot be considered common special technical features that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I and II lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.