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[Continued on next page]

(54) Title: METHODS AND DEVICES FOR IMMUNODIAGNOSTIC APPLICATIONS

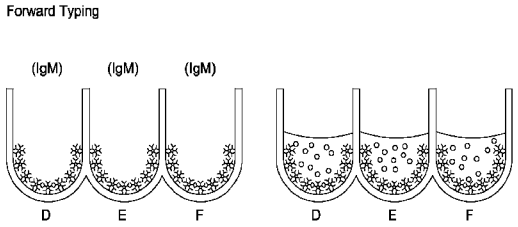

FIG. 1A FIG. 1B

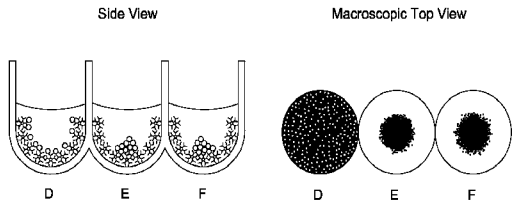

FIG. 1C FIG. 1D

(57) Abstract: Methods and devices for evaluating a sample, e.g., a plasma sample, from a subject for detecting a target red blood cell protein or antibody are disclosed. Antibody screening methods and devices significantly reduce the level of non-specific binding to a surface (e.g., a test surface bound with a red blood cell (rbcm) preparation), allowing for more efficient detection and reduced test time. The antibody screening method includes an immunoglobulin G (IgG) binding moiety that binds selectively and specifically to the plasma IgG present relative to the binding to the lysed rbcm preparation. An antibody screening method is disclosed whereby non-specific binding caused by lysed red blood cell membrane preparations can be reduced by an agent that specifically cleaves a human IgG in the hinge region. Additional methods and devices for target capturing include a substantially planar surface having an optimized angle for capture, or alternative solid phase geometries for capture.

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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:
18 October 2012

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/23553

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 5/07; G01N 33/555; C07K 16/18 (2012.01)
USPC - 435/326, 435/7.25, 530/387.1
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) - C12N 5/07; G01N 33/555; C07K 16/18 (2012.01)
USPC - 435/326, 435/7.25, 530/387.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - C12N 5/07; G01N 33/555; C07K 16/18 (2012.01), USPC - 435/326, 435/7.25, 530/387.1, 435/7.1, 435/7.4: keyword search, as below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
USPTO PubWest (databases: PGPB,USPT,USOC,EPAB,JPAB), Thompson Innovation (core patent databases), Google Scholar --- Search Terms: red blood cell, rbc, erythrocyte, membrane, antigen, type, antibody, sample, protease, proteinase, IgG, IgM, IgG-like, mimic, serum, centrifuge, migration, fractionation, magnetic, agglomeration, aggregation, clumping,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2010/0178656 A1 (BUFFIERE et al.) 15 July 2010 (15.07.2010) para [0012]; [0035]; [0040]-[0044]; [0080]; [0123]; [0057]; [0064]; [0101]; [0262]; [0124]; [0184]; [0001]; [0048]; [0049]; [0087]; [0068]; [0084]; [0085]; [0182]; [0206]; [0249]; [0406];	1-7, 11-26, 29-40, 43-48, 55, 94-96 and 108 --- 8-10, 41, 42, 49-54, 56-76, 194-235, 239, 243-277, 293-297, 299-301, 304-312, 359-361
Y	US 6,303,390 A (DEN BOER et al.) 16 October 2001 (16.10.2001) abstract; col 3, ln 56-59	8-10, 41, 42, 216, 217, 261, 262, 299, 311, 312, 359-361
Y	US 2010/0256005 A1 (PETRIK et al.) 7 October 2010 (07.10.2010) abstract; para [0015]	49-54, 267-272, 293-297, 299-301, 304-312, 359-361
Y	US 4,297,104 A (CLAUDE) 27 October 1981 (27.10.1981) abstract; Fig. 14b; Fig. 10; col 8, ln 8-9, col 9, ln 16-22; col 11, ln 12-16; col 9, ln 32-34	56-76, 194-235, 239, 243-277, 359-361

☐ 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	Date of mailing of the international search report
29 July 2012 (29.07.2012)	10 AUG 2012
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. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/23553

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.: 27, 28, 77-93, 97-107, 236-238, 240-242, 298, 302, 303, and 364-366
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:
This application contains the following inventions or groups of inventions which are not so linked 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 1-26, 29-76, 94-96, 108, 194-235, 239, 243-277, 293-297, 299-301, 304-312, and 359-361, directed to a method of evaluating a sample from a subject for an antibody of a G isotype (IgG antibody) against a red blood cell antigen (RBC).

Group II claims 109-143 and 372-382, directed to a method of evaluating a sample for a red blood cell antigen.

- Please see 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.:
1-26, 29-76, 94-96, 108, 194-235, 239, 243-277, 293-301, 304-312, and 359-361.

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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/23553

Continuation of Box III: Lack of Unity of Invention

Group III claims 144-160 and 383-385 directed to a method of evaluating a sample for a red blood cell (RBC) antigen-specific antibody for reverse grouping or typing.

Group IV claims 161-193 and 367-371 directed to a method of evaluating a sample for an analyte.

Group V claims 278-292, 313-316 directed to a method of providing a substrate having red blood cells, or a red blood cell membrane preparation bound thereto.

Group VI claims 317-358 directed to a device for evaluating a sample from a subject for an anti-RBC antigen antibody.

Group VII claims 362 and 363 directed to a kit.

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

The special technical features of the Group I is contacting a first red blood cell membrane preparation (a rbcm preparation) comprising a first RBC antigen, with the sample from said subject, under conditions sufficient for the formation of an immune complex between said first RBC antigen and an anti-first-RBC antigen IgG antibody in said sample; and providing a detection reagent under conditions sufficient for the formation of the immune complex between said detection reagent and the first-RBC antigen IgG antibody in said sample, said detection reagent comprising an IgG-specific binding moiety, wherein the behavior, or the positional distribution, of said detection reagent is correlated with the presence or absence of said anti-RBC antigen antibody in said sample, thereby evaluating a sample for an anti-RBC antigen IgG antibody. However, this is not an improvement over the prior art of US 5,302,512 A to Pernelle. Pernelle specifically teaches a method of evaluating a sample from a subject for an antibody of a G isotype (IgG antibody) against a red blood cell antigen (RBC) comprising contacting a first red blood cell preparation (a rbcm preparation) comprising a first RBC antigen, with the sample from said subject, under conditions sufficient for the formation of an immune complex between said first RBC antigen and an anti-first-RBC antigen IgG antibody in said sample (col 5 ln 16-22 'Reaction on opaline plates'); and providing a detection reagent under conditions sufficient for the formation of the immune complex between said detection reagent and the first-RBC antigen IgG antibody in said sample, said detection reagent comprising an IgG-specific binding moiety (col 6 ln 64-68 'anti-IgG anti-immunoglobulin'), wherein the behavior, of said detection reagent is correlated with the presence or absence of said anti-RBC antigen antibody in said sample, thereby evaluating a sample for an anti-RBC antigen IgG antibody (col 7 ln 1-3; col 5 ln 23-40). Pernelle does not specifically teach a red blood cell membrane preparation, however, such a preparation would have been obvious to one of ordinary skill in the art as an alternative to whole RBCs in order further elucidate appropriate binding complexes. Groups I-III and V-VII have the shared technical feature of a red blood cell or red blood cell membrane preparation and a red blood cell antigen. Group IV does not require a red blood cell or red blood cell membrane preparation. As above, the shared technical feature of a red blood cell or red blood cell membrane preparation and and a red blood cell antigen are taught by Pernelle (col 5 ln 23-40). All Groups I-VII have the further shared technical feature of a detection reagent. Likewise, this is not an improvement over the prior art of Pernelle that specifically teaches detection reagent (col 6 ln 64-68 "anti-IgG anti-immunoglobulin").Group II has the special technical feature of 'complexed cells' and 'uncomplexed cells' and subsequent separation of the two which is unique to Group II and not shared with any of the other groups. Group III has the special technical features of evaluating a sample for a red blood cell (RBC) antigen-specific antibody for reverse grouping or typing comprising, inter alia, one or more indicator cells, a multi-valent binding agent, and applying an acceleration to the sample which is not shared by any of the other groups which are not shared with any of the other groups. Group IV has the special technical features of a capture agent, wherein, said capture agent is disposed on a substrate or a surface, and the angle between said substrate or a surface and the direction of an applied force chosen from a centrifugal, a gravitational, a fluid magnetic, an electric or a fluid, force, that causes migration of detection reagent, is non-orthogonal or other than 90 degrees which are not shared with any of the other Groups. Group V has the special technical features of providing a substrate capable of binding red blood cells; contacting said substrate with a solution of red blood cells to form a solution-contactedsubstrate; centrifuging said solution-contacted-substrate for a time sufficient to cause red blood cells in said solution to settle onto said substrate which are not shared by the other Groups. Group VI has the special technical feature of a device comprising a channel which is not required by any of the other Groups. The special technical feature of Group VII is a kit which is not shared by any of the other Groups. Therefore the inventions listed as Groups I-VII do not relate to a single general inventive concept under PCT Rule 13.1 because they do not share a same or corresponding special technical feature. In order for all inventions to be examined, the appropriate examination fees must be paid.