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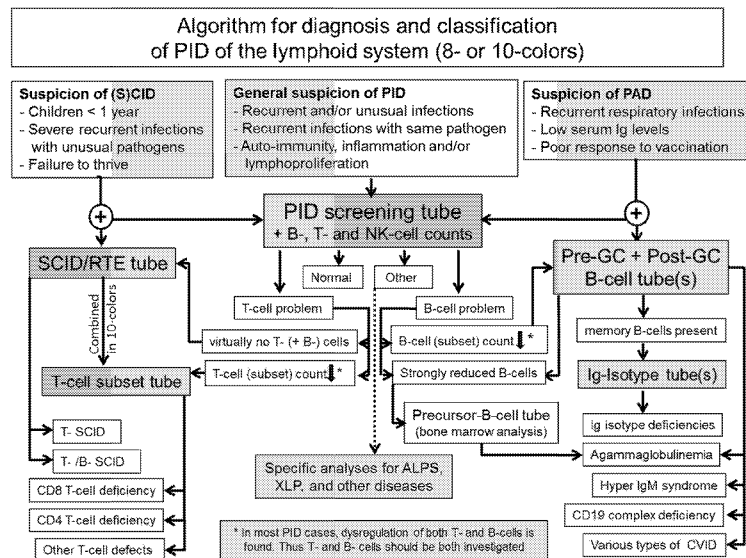
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[Continued on next page]

(54) Title: REAGENTS, METHODS AND KITS FOR DIAGNOSING PRIMARY IMMUNODEFICIENCIES

Figure 1A



(57) Abstract: This invention relates to the field of primary immunodeficiencies (PID), more specifically to means and method for the diagnosis of PID of the lymphoid system. Provided are unique reagent compositions for the flow cytometric immunophenotyping of leukocytes comprising fluorochrome-conjugated antibodies directed against various specific combinations of markers. Also provided are kits comprising the reagent compositions, and methods using the same.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER INV. G01N33/569 C07K16/00 G01N15/14 ADD.				
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 C07K				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched				
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, BIOSIS, EMBASE, WPI Data				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
A	EP 2 780 711 A1 (UNIV ERASMUS MEDICAL CT [NL]; UNIV SALAMANCA [ES]) 24 September 2014 (2014-09-24) the whole document, in particular page 8, line 31 to page 10, line 15; claims 1, 2, 7, 8, 11-13 <div style="text-align: center;">----- -/--</div>	1-3, 8-10, 13-17, 20-29		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.				
* Special categories of cited documents : <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: none; vertical-align: top;"> "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 </td> <td style="width: 50%; border: none; vertical-align: top;"> "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 </td> </tr> </table>			"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
"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			
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Weber, Peter			

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2015/050762

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>ANDREAS BOLDT ET AL: "Eight-color immunophenotyping of T-, B-, and NK-cell subpopulations for characterization of chronic immunodeficiencies", CYTOMETRY. PART B, CLINICAL CYTOMETRY, vol. 86, no. 3, 31 January 2014 (2014-01-31), pages 191-206, XP055254566, US ISSN: 1552-4949, DOI: 10.1002/cyto.b.21162 cited in the application the whole document, in particular title, abstract, table 1</p> <p style="text-align: center;">-----</p>	<p>1-3, 8-10, 13-17, 20-29</p>
A	<p>LOCKE BRADLEY A ET AL: "Laboratory Diagnosis of Primary Immunodeficiencies", CLINICAL REVIEWS IN ALLERGY AND IMMUNOLOGY, HUMANA PRESS, TOTOWA, NJ, US, vol. 46, no. 2, 26 February 2014 (2014-02-26), pages 154-168, XP035362242, ISSN: 1080-0549, DOI: 10.1007/S12016-014-8412-4 [retrieved on 2014-02-26] the whole document, in particular title; abstract; page 155, column 2, 5th line from bottom to page 156, column 1, line 27; page 160, column 1, lines 2-7</p> <p style="text-align: center;">-----</p>	<p>1-3, 8-10, 13-17, 20-29</p>
A	<p>HOLDEN T. MAECKER ET AL: "Standardizing immunophenotyping for the Human Immunology Project", NATURE REVIEWS IMMUNOLOGY, vol. 12, 17 February 2012 (2012-02-17), pages 191-200, XP055254686, cited in the application the whole document, in particular title; abstract; figure 2; table 2</p> <p style="text-align: center;">-----</p>	<p>1-3, 8-10, 13-17, 20-29</p>
A	<p>O'GORMAN MAURICE R G ET AL: "Flow cytometry assays in primary immunodeficiency diseases.", METHODS IN MOLECULAR BIOLOGY (CLIFTON, N.J.) 2011, vol. 699, 2011, pages 317-335, XP009188835, ISSN: 1940-6029 cited in the application the whole document, in particular title; abstract; page 320, paragraph 2.1</p> <p style="text-align: center;">-----</p> <p style="text-align: center;">-/--</p>	<p>1-3, 8-10, 13-17, 20-29</p>

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2015/050762

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LIEKE S. KAMPHUIS ET AL: "Perigranuloma Localization and Abnormal Maturation of B Cells", AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE., vol. 187, no. 4, 15 February 2013 (2013-02-15), pages 406-416, XP055254490, US ISSN: 1073-449X, DOI: 10.1164/rccm.201206-10240C	1,20-24
A	the whole document, in particular page 407, column 2, lines 7-20; figure 2	2,3, 8-10, 13-17, 25-29
X	----- K Warnatz ET AL: "Immune phenotyping in primary immunodeficiency", Ipidnet, 10 March 2010 (2010-03-10), pages 1-5, XP055254334, Retrieved from the Internet: URL:http://www.ipidnet.org/protocol-data-1 [retrieved on 2016-03-01]	1,20-24
A	the whole document, in particular title; antibodies listed on page 2	2,3, 8-10, 13-17, 25-29
X	----- MIRZOKHID RAKHMANOV ET AL: "Circulating CD21low B cells in common variable immunodeficiency resemble tissue homing, innate-like B cells", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 106, no. 32, 11 August 2009 (2009-08-11), pages 13451-13456, XP055254282, US ISSN: 0027-8424	1,20-24
A	the whole document, in particular paragraph spanning pages 13451 and 13452, more particularly page 13452, column 1, lines 13-14	2,3, 8-10, 13-17, 25-29
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL2015/050762

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:

see additional sheet

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 an 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.:

2, 3(completely); 1, 8-10, 13-17, 20-29(partially)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 2, 3(completely); 1, 8-10, 13-17, 20-29(partially)

A reagent composition for the flow cytometric immunophenotyping of leukocytes comprising fluorochrome-conjugated antibodies directed against marker combination (a) of claim 1. Corresponding set of at least two reagent compositions, comprising said marker combination of claim 1 and at least one other marker combination of claim 1. Corresponding diagnostic kit. Corresponding multi-color flow cytometric method for immunophenotyping of leukocytes.

2. claims: 1, 8-10, 13-15, 20-29(all partially)

A reagent composition for the flow cytometric immunophenotyping of leukocytes comprising fluorochrome-conjugated antibodies directed against marker combination (b) of claim 1. Corresponding set of at least two reagent compositions, comprising said marker combination of claim 1 and at least one other marker combination of claim 1. Corresponding diagnostic kit. Corresponding multi-color flow cytometric method for immunophenotyping of leukocytes.

3. claims: 4(completely); 1, 8, 13-17, 20-29(partially)

A reagent composition for the flow cytometric immunophenotyping of leukocytes comprising fluorochrome-conjugated antibodies directed against marker combination (c) of claim 1. Corresponding set of at least two reagent compositions, comprising said marker combination of claim 1 and at least one other marker combination of claim 1. Corresponding diagnostic kit. Corresponding multi-color flow cytometric method for immunophenotyping of leukocytes.

4. claims: 5, 6(completely); 1, 8, 10, 13-15, 18-29(partially)

A reagent composition for the flow cytometric immunophenotyping of leukocytes comprising fluorochrome-conjugated antibodies directed against marker combination (d) of claim 1. Corresponding set of at least two reagent compositions, comprising said marker combination of claim 1 and at least one other marker combination of claim 1. Corresponding diagnostic kit. Corresponding multi-color flow cytometric method for immunophenotyping of leukocytes.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

5. claims: 1, 8, 10, 13-15, 20-29(all partially)

A reagent composition for the flow cytometric immunophenotyping of leukocytes comprising fluorochrome-conjugated antibodies directed against marker combination (e) of claim 1. Corresponding set of at least two reagent compositions, comprising said marker combination of claim 1 and at least one other marker combination of claim 1. Corresponding diagnostic kit. Corresponding multi-color flow cytometric method for immunophenotyping of leukocytes.

6. claims: 7, 11(completely); 1, 8, 12-15, 17, 20-29(partially)

A reagent composition for the flow cytometric immunophenotyping of leukocytes comprising fluorochrome-conjugated antibodies directed against marker combination (f) of claim 1. Corresponding set of at least two reagent compositions, comprising said marker combination of claim 1 and at least one other marker combination of claim 1. Corresponding diagnostic kit. Corresponding multi-color flow cytometric method for immunophenotyping of leukocytes.

7. claims: 1, 8, 12-15, 17, 18, 20-29(all partially)

A reagent composition for the flow cytometric immunophenotyping of leukocytes comprising fluorochrome-conjugated antibodies directed against marker combination (g) of claim 1. Corresponding set of at least two reagent compositions, comprising said marker combination of claim 1 and at least one other marker combination of claim 1. Corresponding diagnostic kit. Corresponding multi-color flow cytometric method for immunophenotyping of leukocytes.

8. claims: 1, 8, 19-28(all partially)

A reagent composition for the flow cytometric immunophenotyping of leukocytes comprising fluorochrome-conjugated antibodies directed against marker combination (h) of claim 1. Corresponding set of at least two reagent compositions, comprising said marker combination of claim 1 and at least one other marker combination of claim 1. Corresponding diagnostic kit. Corresponding multi-color flow cytometric method for immunophenotyping of leukocytes.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/NL2015/050762

Patent document cited in search report	Publication date	Patent family member(s)	Publication date	
EP 2780711	A1	24-09-2014	EP 2780711 A1	24-09-2014
			US 2014315749 A1	23-10-2014
			WO 2013073932 A1	23-05-2013
