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(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.

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- with international search report (Art. 21(3))
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(54) Title: DETECTION OF ONE OR MORE PATHOGENS

(57) Abstract: Provided herein are media, methods, kits, primers and oligonucleotide probes for use in the molecular detection of pathogens. These may be used in combination for the rapid, high-throughput screening PCR-based techniques to simultaneously detect multiple pathogens. The multiplex-detection methods have improved sensitivity and specificity for the detection of multiple pathogens simultaneously. Real-time PCR assaying techniques using such primers include microarrays and multiplex arrays, the latter optionally simultaneously with oligonucleotide TaqMan probes.



WO 2016/164407 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/26140

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☐ forming part of the international application as filed:
☐ in the form of an Annex C/ST.25 text file.
☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☒ furnished subsequent to the international filing date for the purposes of international search only:
☒ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

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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.: 8, 11-25, 55, 143-145, 147-174, 183-186
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:

-Please See Supplemental Page-*

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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/26140

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68; C12N 1/06, 1/20, 15/10 (2016.01)

CPC - C12Q1/6806, 1/6813, 1/6816, 1/6818, 1/6844, 1/6869, 1/6876, 1/689

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): C12Q 1/68; C12N 1/06, 1/20, 15/10, 1/21, 15/85, 1/70; A61K 39/04, 45/00 (2016.01)

CPC: C12Q 1/6806, 1/6813, 1/6816, 1/6818, 1/6844, 1/6869, 1/6876, 1/689; A61K 38/00, 39/04, 45/06; C12N 1/20, 15/1093, 15/81, 15/85

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)

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Lens search database; Google Scholar; Pubmed; EBSO; NCBI database, ENA database; Keywords: nucleic acids, pathogen, PCR, hybridiz*, buffer, medium, extract*

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 2008/0182272 A1 (NAGAR, MS et al.) 31 July, 2008; abstract; paragraph [0045]; claim 1	1, 5/1, 6/1, 7/1, 49/1, 146/6/1 4, 5/4, 6/4, 7/4, 9/1, 9/4, 10/9/1, 10/9/4, 26/4, 27/26/4, 28/27/26/4, 29/28/27/26/4, 30/27/26/4, 31/30/27/26/4, 32/27/26/4, 33/32/27/26/4, 34/27/26/4, 35/34/27/26/4, 36/27/26/4, 37/36/27/26/4, 38/37/36/27/26/4, 39/27/26/4, 40/39/27/26/4, 41/4, 42/41/4, 43/42/41/4, 44/4, 45/44/4, 46/45/44/4, 47/4, 48/47/4, 49/2-4, 49/188, 50/4, 51/50/4, 52/4, 53/52/4, 54/52/4, 56/1, 56/4, 57/56/1, 57/56/4, 58/52/4, 59/58/52/4, 60/58/52/4, 61/1, 61/4, 146/6/4, 189-190



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

21 October 2016 (21.10.2016)

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/26140

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	(TONGEREN, SPV et al.) Comparison of Three Rapid and Easy Bacterial DNA Extraction Methods for Use with Quantitative Real-Time PCR. European Journal of Clinical Microbiology and Infectious Diseases. 11 February, 2011; Vol. 30, No. 9; pages 1053-1061; abstract; page 1054, column 1, paragraph 4 - column 2, paragraphs 2-3; page 1055, column 1, paragraph 2 - column 2, paragraph 3; page 1056, column 1, paragraph 2; page 1056, column 2, paragraph 4; DOI: 10.1007/s10096-011-1191-4	2-3, 5/2-3, 5/187, 6/2-3, 6/188, 26/3, 26/187, 27/26/3, 27/26/187, 28/27/26/3, 28/27/26/187, 29/28/27/26/3, 29/28/27/26/187, 30/27/26/3, 30/27/26/187, 31/30/27/26/3, 31/30/27/26/187, 32/27/26/3, 32/27/26/187, 33/32/27/26/3, 33/32/27/26/187, 34/27/26/3, 34/27/26/187, 35/34/27/26/3, 35/34/27/26/187, 39/27/26/3, 39/27/26/187, 47/2-3, 47/194, 48/47/2-3, 48/47/194, 52/2-3, 52/193, 53/52/2-3, 53/52/193, 54/52/2-3, 54/52/193, 89-97, 101, 187-188, 191-194, 201 4, 5/4, 6/4, 7/4, 9/4, 10/9/4, 26/4, 27/26/4, 28/27/26/4, 29/28/27/26/4, 30/27/26/4, 31/30/27/26/4, 32/27/26/4, 33/32/27/26/4, 34/27/26/4, 35/34/27/26/4, 36/27/26/3-4, 36/27/26/187, 37/36/27/26/3-4, 37/36/27/26/187, 38/37/36/27/26/3-4, 38/37/36/27/26/187, 39/27/26/4, 40/39/27/26/3-4, 40/39/27/26/187, 41/3-4, 42/41/3-4, 43/42/41/3-4, 44/3-4, 45/44/3-4, 46/45/44/3-4, 47/4, 47/195, 48/47/4, 48/47/195, 49/2-4, 49/188, 50/2-4, 50/193, 51/50/2-4, 51/50/193, 52/4, 53/52/4, 54/52/4, 56/1-4, 56/201, 57/56/1-4, 57/56/201, 58/52/2-4, 58/52/193, 59/58/52/2-4, 59/58/52/193, 60/58/52/2-4, 60/58/52/193, 61/1-4, 61/201, 62-102, 103/78-91, 104/103/78-91, 105/104/103/78-91, 106/105/104/103/78-91, 107/106/105/104/103/78-91, 108/104/103/78-91, 109/108/104/103/78-91, 110/109/108/104/103/78-91, 111/104/103/78-91, 122-142, 146/6/4, 189-190, 195-200

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	US 2007/0141572 A1 (UBALIJORO, E et al.) 21 June, 2007; paragraphs [0026], [0031], [0074], [0077]	112-113 50/1-4, 50/193, 51/50/1-4, 51/50/193, 58/52/2-4, 58/52/193, 59/58/52/2-4, 59/58/52/193, 60/58/52/2-4, 81-84, 103/81-84, 104/103/81-84, 105/104/103/81-84, 106/105/104/103/81-84, 107/106/105/104/103/81-82, 108/104/103/81-84, 109/108/104/103/81-84, 110/109/108/104/103/81-84, 111/104/103/81-84, 114-119
X — Y	US 2011/0236891 A1 (LI, J et al.) 29 September, 2011; paragraphs [0064], [0098]-[0099]	120 50/1-4, 50/193, 51/50/1-4, 51/50/193, 60/58/52/2-4, 60/58/52/193, 81-84, 85, 103/81-82, 103/81-84, 104/103/81-84, 105/104/103/81-84, 106/105/104/103/81-84, 107/106/105/104/103/81-82, 108/104/103/81-84, 109/108/104/103/81-84, 110/109/108/104/103/81-84, 111/104/103/81-84, 121
Y	(POST, DE) Food Borne Pathogens Monograph 5 Escherichia Coli Shigella Species. Oxoid Setting Standards. March 1998; pages 33, 44, 45, 55	9/1, 9/4, 10/9/1, 10/9/4, 9/187, 10/9/187
Y	(YUAN, S et al.) Evaluation of Methods for the Extraction and Purification of DNA from the Human Microbiome. PLoS One. 23 March, 2012; Vol. 7, No. 3; pages 1-10; page 7, column 1, paragraph 4; DOI: 10.1371/journal.pone.0033865	36/27/26/3-4, 36/27/26/187, 37/36/27/26/3-4, 37/36/27/26/187, 38/37/36/27/26/3-4, 38/37/36/27/26/187, 40/39/27/26/3-4 and 40/39/27/26/187, 76-78, 80, 98-100, 102, 103/78, 103/80, 104/103/78, 104/103/80, 105/104/103/78, 105/104/103/80, 106/105/104/103/78, 106/105/104/103/80, 107/106/105/104/103/78, 107/106/105/104/103/80, 108/104/103/78, 108/104/103/80, 109/108/104/103/78, 109/108/104/103/80, 110/109/108/104/103/78, 110/109/108/104/103/80, 111/104/103/78, 111/104/103/80, 138-140, 142
Y	US 2013/0280787 A1 (MUELLER, M et al.) 24 October 2013; paragraph [0081]	41/3-4, 42/41/3-4, 43/42/41/3-4, 46/45/44/3-4, 47/195, 48/47/195, 103/78-84,

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
		103/86-88, 103/89-91, 104/103/78-84, 104/103/86-88, 104/103/89-91, 105/104/103/78-84, 105/104/103/86-88, 105/104/103/89-91, 106/105/104/103/78-84, 106/105/104/103/86-88, 106/105/104/103/89-91, 107/106/105/104/103/78-84, 107/106/105/104/103/86-88, 107/106/105/104/103/89-91, 108/104/103/78, 108/104/103/80-85, 108/104/103/89-91, 109/108/104/103/78, 109/108/104/103/80-85, 109/108/104/103/89-91, 110/109/108/104/103/78, 110/109/108/104/103/80-85, 110/109/108/104/103/89-91, 111/104/103/78-84, 111/104/103/86-88 111/104/103/89-91, 122-142, 195-200
Y	(REISCHL, U et al.) Rapid Identification of Methicillin-Resistant Staphylococcus aureus and Simultaneous Species Confirmation Using Real-Time Fluorescence PCR. Journal of Clinical Microbiology. June, 2000; Vol. 38, No. 6; pages 2429-2433; page 2430, column 1, paragraph 2	44/3-4, 45/44/3-4, 46/45/44/3-4, 108/104/103/78-91, 109/108/104/103/78-91, 110/109/108/104/103/78-91, 127, 200
Y	US 2003/0219770 A1 (ESHLEMAN, JR et al.) 27 November, 2003; abstract; paragraph [0013]	56/1-4, 56/201, 57/56/1-4, 57/56/201
Y	US 2014/0273185 A1 (SRC, INC.) 18 September, 2014; paragraph [0009]	61/1-3, 61/201
Y	WO 2010/046930 A1 (GENESIS LABS LIMITED.) 29 April, 2010; abstract; page 4, paragraphs 1, 5	62-88, 103/78-88, 104/103/78-88, 105/104/103/78-88, 106/105/104/103/78-88, 107/106/105/104/103/78-88, 108/104/103/78-88, 109/108/104/103/78-88, 110/109/108/104/103/78-88, 111/104/103/78-88
Y	(OOKA, T et al.) Clinical Significance of Escherichia albertii. Emerging Infectious Diseases. March, 2012; Vol. 18, No. 3; pages 488-492; page 490, column 1, paragraph 2; DOI: 10.3201/eid1803.111401; Genbank supplement pages 1-2	50/1-4, 50/193, 51/50/1-4, 51/50/193, 81-82, 103/81-82, 104/103/81-82, 105/104/103/81-82, 106/105/104/103/81-82, 107/106/105/104/103/81-82, 108/104/103/81-82, 109/108/104/103/81-82, 110/109/108/104/103/81-82, 111/104/103/81-82, 114-115

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	(OGURA, Y et al.) Comparative Genomics Reveal the Mechanism of the Parallel Evolution of O157 and non-O157 Enterohemorrhagic Escherichia coli. Proceedings of the National Academy of Sciences of United States of America. 06 October, 2009; Vol. 106, No. 42; pages 17939-17944; abstract; page 17939, column 2, paragraph 3; DOI: 10.1073/pnas.0903585106; Genbank supplement pages 1-6	50/1-4, 50/193, 51/50/1-4, 51/50/193, 81-82, 103/81-82, 104/103/81-82, 105/104/103/81-82, 106/105/104/103/81-82, 107/106/105/104/103/81-82, 108/104/103/81-82, 109/108/104/103/81-82, 110/109/108/104/103/81-82, 111/104/103/81-82, 116-117
Y	(YAN, X et al.) Genome Sequencing and Comparative Genomics Provides Insights on the Evolutionary Dynamics and Pathogenic Potential of Different H-Serotypes of Shiga Toxin-Producing Escherichia coli O104. BMC Microbiology. 03 April 2015; Vol. 15, No. 83; pages 1-20; abstract; page 10, column 1, paragraph 2; DOI: 10.1186/s12866-015-0413-9; Genbank supplement pages 1-6	50/1-4, 50/193, 51/50/1-4, 51/50/193, 81-82, 103/81-82, 104/103/81-82, 105/104/103/81-82, 106/105/104/103/81-82, 107/106/105/104/103/81-82, 108/104/103/81-82, 109/108/104/103/81-82, 110/109/108/104/103/81-82, 111/104/103/81-82, 118-119
Y	US 2006/0240442 A1 (VEVEA, DN) 26 October, 2006; paragraph [0017].	58/52/2-4, 58/52/193, 59/58/52/2-4, 59/58/52/193, 60/58/52/2-4, 83-84, 103/83-84, 104/103/83-84, 105/104/103/83-84, 106/105/104/103/83-84, 107/106/105/104/103/83-84, 108/104/103/83-84, 109/108/104/103/83-84, 110/109/108/104/103/83-84, 111/104/103/83-84, 115
Y	(HASSAN, J et al.) Escherichia coli strain BAUMH1 shiga toxin 1 subunit (stx1) gene, partial cds. NCBI PDB Accession No. KM596779, Escherichia coli strain BAU-MH1 shiga toxin 1 subunit (stx1) gene, partial cds. Submitted 22 September, 2014; downloaded from the internet < https://www.ncbi.nlm.nih.gov/nuccore/KM596779 > on 19 October 2016, page 1	58/52/2-4, 58/52/193, 59/58/52/2-4, 59/58/52/193, 60/58/52/2-4, 83-84, 103/83-84, 104/103/83-84, 105/104/103/83-84, 106/105/104/103/83-84, 107/106/105/104/103/83-84, 108/104/103/83-84, 109/108/104/103/83-84, 110/109/108/104/103/83-84, 111/104/103/83-84, 117

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2008/0107653 A1 (VERMEIJ, P) 08 May, 2008; paragraph [0006]	58/52/2-4, 58/52/193, 59/58/52/2-4, 59/58/52/193, 60/58/52/2-4, 83-84, 103/83-84, 104/103/83-84, 105/104/103/83-84, 106/105/104/103/83-84, 107/106/105/104/103/83-84, 108/104/103/83-84, 109/108/104/103/83-84, 110/109/108/104/103/83-84, 111/104/103/83-84, 119
Y	US 2006/0177824 A1 (PROCOP, GW) 10 August, 2006; paragraph [0029]	58/52/2-4, 58/52/193, 59/58/52/2-4, 59/58/52/193, 60/58/52/2-4, 60/58/52/193, 83-84, 103/83-84, 104/103/83-84, 105/104/103/83-84, 106/105/104/103/83-84, 107/106/105/104/103/83-84, 108/104/103/83-84, 109/108/104/103/83-84, 110/109/108/104/103/83-84, 111/104/103/83-84, 121
A	(POORAFSHAR, M et al.) Ornithorhynchus anatinus coagulation factor X (F10), mRNA. NCBI PDB Accession No. NM_001127614 XM_001515062, Ornithorhynchus anatinus coagulation factor X (F10), mRNA. Submitted November, 2000; downloaded from the internet < https://www.ncbi.nlm.nih.gov/nuccore/189027043/ > on 19 October 2016, pages 1-2	175-182

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
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-Continued from Box No. III: Observations where unity of invention is lacking-

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 1-7, 9, 10, 26-54, 56-142, 146, 175-178 and 187-201 are directed toward method comprising: a. conducting a first sample lysis and a second sample lysis on a sample or a portion thereof, wherein the second sample lysis is performed at a temperature higher than the temperature of the first sample lysis; a lysis buffer and amplification primers used therewith; and kits therefor.

Group II, Claims 179-182 are directed toward a pair of single-stranded DNA primers for determination of a nucleotide sequence of an internal control by a polymerase chain reaction.

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: the special technical features of Group I include second lysis of a sample, not present in Group II; the special technical features of Group II include an internal control, not present in Group I.

Groups I and II share the technical features including: a pair of single-stranded DNA primers for determination of a nucleotide sequence by a polymerase chain reaction, wherein the use of the primers in a polymerase chain reaction results in the synthesis of DNA.

However, these shared technical features are previously disclosed by US 2011/0312705 A1 to Azimi et al. (hereinafter 'Azimi').

Azimi discloses a pair of single-stranded DNA primers (a pair of single-stranded DNA primers; paragraph [0024]) for determination of a nucleotide sequence (for identification (determination) of a nucleotide sequence; paragraph [0035]) by a polymerase chain reaction (by RT-PCR (by a polymerase chain reaction); paragraph [0035]), wherein the use of the primers in a polymerase chain reaction results in the synthesis of DNA (wherein the use of the primers in a polymerase chain reaction results in the synthesis of DNA; paragraph [0035]).

Since none of the special technical features of the Groups I and II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Azimi reference, unity of invention is lacking.