



(43) International Publication Date
1 November 2012 (01.11.2012)

(10) International Publication Number
WO 2012/149188 A3

(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:
PCT/US2012/035253

(22) International Filing Date:
26 April 2012 (26.04.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
13/094,809 26 April 2011 (26.04.2011) US

(71) Applicant (for all designated States except US): **LONG-HORN VACCINES AND DIAGNOSTICS, LLC** [US/US]; 4520 East-west Highway, Suite 640, Bethesda, MD 20814 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **FISCHER, Gerald, W.** [US/US]; 6417 Lynbrook Drive, Bethesda, MD 20817 (US). **DAUM, Luke, T.** [US/US]; 318 Larkwood Drive, San Antonio, TX 78209 (US).

(74) Agents: **REMENICK, James** et al.; Remenick PLLC, 1025 Thomas Jefferson St., NW, Suite 175, Washington, DC 20007 (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, 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, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, 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, 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, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(88) Date of publication of the international search report:
27 February 2014

(54) Title: COMPOSITIONS AND METHODS FOR DETECTING AND IDENTIFYING NUCLEIC ACID SEQUENCES IN BIOLOGICAL SAMPLES

(57) Abstract: Compositions and methods for isolating, detecting, amplifying, and quantitating pathogen-specific nucleic acids in a biological sample are set forth. Diagnostic kits containing specific amplification primers, and labeled detection probes that specifically bind to the amplification products obtained therefrom are included. Compositions and methods for the isolation and characterization of nucleic acids that are specific to one or more pathogens, including for example Influenza virus and Mycobacterium tuberculosis, from a wide variety of samples including those of biological, environmental, clinical and/or veterinary origin are provided.



WO 2012/149188 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/35253

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68 (2012.01)

USPC - 435/6.1, 6.12, 91.2

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)

USPC: 435/6.1, 6.12, 91.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/6.1, 6.12, 91.2 (text search)

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

Electronic data bases: PubWEST (USPT, EPAB, JPAB, PGPB); Google Scholar

Search terms: PCR, PCR Master Mix, Ready Mix, PCR Ready, Taq polymerase, dNTPs, chelating, osmolarity, buffer, passive reference dye (e.g. ROX), nuclease-free H₂O

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 2010/0311739 A1 (GUNARATNAM et al.) 9 December 2010 (09.12.2010). Especially para [0106], [0108], [0109].	1, 2, 21, 22 3, 4
Y	US 2010/0151477 A1 (CAWTHON) 17 June 2010 (17.06.2010) abstract; para [0089]; [0091]	3, 4
A	US 7,695,904 B2 (CAWTHON) 13 April 2010 (13.04.2010)	1-4, 21, 22
A	US 7,361,489 B2 (ZHANG et al.) 22 April 2008 (22.04.2008)	1-4, 21, 22

☐ 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

"G" document member of the same patent family

Date of the actual completion of the international search

2 September 2012 (02.09.2012)

Date of mailing of the international search report

21 SEP 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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/35253

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.: 5-20, 23-25 and 29-34
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 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 1-4, 21-22, drawn to a PCR-ready composition for detection of a microorganism in a biological sample comprising:
a heat-stable polymerase present in an amount from about 0.05 U to about 1 U;
a mix of deoxynucleotide tri phosphates comprising about equivalent amounts of dATP, dCTP, dGTP and dTTP, collectively present in the composition at a concentration of about 0.1 mM to about 1 mM;
a chelating agent at a concentration of about 0.01 mM to about 1 mM;
- 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.:
1-4 and 21-22, limited to Taq polymerase

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 12/35253

Continuation of Box III: Lack of Unity of Invention

a PCR osmolarity agent at a concentration of about 1 mM to about 1 M;
albumin at a concentration of about 5 ng/ml to about 100 ng/ml;
at least two salts, the first being a potassium salt selected from the group consisting of potassium chloride and potassium glutamate and the second being a magnesium salt selected from the group consisting of magnesium chloride and magnesium sulfate, collectively present in the composition at a concentration of about 50 mM to about 1 M; and
a buffer a concentration of about 1 mM to about 1M and with a pH of about 6.5 to about 9.0, wherein the pKa of the buffer is within about one unit of the pH at a selected temperature,
wherein the components are combined with nuclease-free water. The first invention is restricted to a Taq polymerase. Should an additional fee(s) be paid, Applicant is invited to elect an additional heat-stable polymerase(s) to be searched.

Group II: claims 26-28, drawn to a method of providing for detection of a microorganism in a biological sample comprising providing a PCR-ready composition containing as components:
a heat-stable polymerase present in an amount from about 0.05U to about 1 U;
a mix of deoxynucleotide tri phosphates comprising about equivalent amounts of dATP, dCTP, dGTP and dTTP, collectively present in the composition at a concentration of about 0.1mM to about 1 mM;
one or more chelating agents present in the composition at a concentration of about 0.01 mM to about 1 mM;
one or more PCR osmolarity agents present in the composition at a concentration of about 1 mM to about 1 M;
one or more albumin proteins present in the composition at a concentration of about 5 ng/ml to about 100 ng/ml;
one or more salts present in the composition at a concentration of about 50 mM to about 1M; and
one or more buffers present in the composition at a concentration of about 1 mM to about 1 M and with a pH of about 6.5 to about 9.0, wherein the pKa of the buffer is within about one unit of the pH at a selected temperature,
wherein the components are combined with nuclease-free water.

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 inventions of Group I+ do not include the inventive concept of a method of providing for detection of a microorganism in a biological sample comprising providing a PCR-ready composition, as required by Group II.

The inventions of Group I+ share the technical feature of a PCR-ready composition of claim 1. However, this shared technical feature does not represent a contribution over prior art as being obvious over US 2010/0311739 A1 to Gunaratnam, et al. (hereinafter "Gunaratnam") that discloses a PCR-ready composition (para [0109], "a second PCR master mix was prepared...") comprising:
a heat-stable polymerase present in an amount from about 0.05U to about 1 U (para [0109], 2 U of taq polymerase);
a mix of deoxynucleotide tri phosphates comprising about equivalent amounts of dATP, dCTP, dGTP and dTTP, collectively present in the composition at a concentration of about 0.1 mM to about 1 mM (para [0109], 0.5 mM dNTPs);
a chelating agent at a concentration of about 0.01 mM to about 1 mM (para [0106] and [0109], TRAP buffer contains 1 mM EGTA);
a PCR osmolarity agent at a concentration of about 1 mM to about 1 M (para [0106] and [0109], nonionic detergent, TRAP buffer contains 0.05% v/v Tween-20);
albumin at a concentration of about 5 ng/ml to about 100 ng/ml (para [0109], BSA (5 mkg));
at least two salts, the first being a potassium chloride and the second being a magnesium chloride, collectively present in the composition at a concentration of about 50 mM to about 1 M (para [0106] and [0109], TRAP buffer contains 68 mM KCl, 1.5 mM MgCl₂.sub.2); and
a buffer a concentration of about 1 mM to about 1M and with a pH of about 6.5 to about 9.0, wherein the pKa of the buffer is within about one unit of the pH at a selected temperature, (para [0106] and [0109], TRAP buffer contains 20 mM Tris-HCl (pH 8.3)), wherein the components are combined with nuclease-free water (para [0108], PCR-grade water).
The master mix of Gunaratnam differs from the claimed composition by having twice as claimed amount of Tag polymerase and double collective amount of dNTPs. However, it would have been obvious to one of ordinary skill in the art how to, in the course of routine experimentation and with a reasonable expectation of success, determine an optimal amounts of Tag polymerase and dNTPs in the composition.
Gunaratnam does not disclose that said PCR master mix is for detection of a microorganism in a biological sample. However, it would have been obvious for one of ordinary skill in the art how to use the PCR master mix of Gunaratnam such as to detect, with a reasonable expectation of success, a microorganism in a biological sample. As said PCR ready composition would have been obvious to one of ordinary skill in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Another special technical feature of the inventions listed as Group I+ is the specific heat-stable polymerase recited therein. As the claimed polymerases were known in the art at the time of the invention (Gunaratnam, para [0109], Taq polymerase), the inventions do not share a special technical feature. Without a shared special technical feature, the inventions lack unity with one another.

Groups I+ and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.