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(54) Title: MULTI-PRIMER ASSAY FOR MYCOPLASMA DETECTION

(57) Abstract: Disclosed is a multi-primer amplification assay, method and kits for detecting Mycoplasma species and closely related species utilizing a plurality of oligonucleotide primers in contact with a sample in a single vessel and detecting the amplification product, wherein the presence of an amplification product indicates Mycoplasma in the sample.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68; C12P 19/34; G01N 33/48 (2011.01)
USPC - 435/6; 435/91.2, 436/94
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; C12P 19/34; G01N 33/48 (2011.01)
USPC - 435/6; 435/91.2, 436/94

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)
USPTO PubWest (PGPB,USPT,USOC,EPAB,JPAB); Thompson Innovation (core patent databases); Google Scholar: mycoplasma, multiplex, multiple, plurality, primer, pcr, amplification, single, same, reaction, vessel, tube, well, discriminatory, positive control, extract; Petrauskene, Brzoska, Tebbs, detect, assay, determine, evaluation

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 2008/0187916 A1 (Ikonomi et al.) 7 August 2008 (07.08.2008) abstract; Table 1 and 3; para [0009]; [0017]; [0023]-[0024]; [0027]; [0050]; [0066]; [0069]; [0079]; [0098]; [0106]; [0110]-[0113]; [0115]; [0146]; [0149]-[0150]; [0170]	28 --- 1-3, 9, 12-27, 29-30 and 33-36
X	US 2004/0077015 A1 (VOJDANI et al.) 22 April 2004 (22.04.2004) abstract, para [0012]; [0015]; [0032]	28
Y	US 2009/0181368 A1 (IWAKIRI) 16 July 2009 (16.07.2009) abstract; SEQ ID NO: 1	1-3, 9, 12-27, 29-30 and 33-36
Y	US 2009/0075274 A1 (SLEPNEV et al.) 19 March 2009 (19.03.2009) abstract; para [0017]; [0079]	18-23, 26 and 30
A	US 2008/0233570 A1 (Hall et al.) 25 September 2008 (25.09.2008)	1-3, 9, 12-30 and 33-36
A	US 2009/0053703 A1 (BERGERON et al.) 26 February 2009 (26.02.2009)	1-3, 9, 12-30 and 33-36
A	US 5,491,062 A (MCKENZIE et al.) 13 February 1996 (13.02.1996)	1-3, 9, 12-30 and 33-36
A	GRUTEKE et al. Practical Implementation of a Multiplex PCR for Acute Respiratory Tract Infections in Children. J. Clin. Microbiol. 2004, 42(12):5596-5603	1-3, 9, 12-30 and 33-36

☐ 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
23 May, 2011 (23.05.2011)	02 JUN 2011
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.
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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.:
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.

Group I+: claims 1-30, 33-36, drawn to a multi-primer assay for detecting Mycoplasma comprising:
a) contacting a sample with the plurality of oligonucleotide primers in a single vessel;
b) performing a multi-primer amplification reaction in the vessel, wherein each of the plurality of oligonucleotide primers is present for participation in amplifying a target nucleic acid in the sample to produce an amplification product; and
c) detecting the amplification product, wherein the presence of an amplification product indicates Mycoplasma in the sample. The first invention is restricted to primers of SEQ ID NO: 1, 63, 135 (identical); SEQ ID NO: 2-3 (identical); SEQ ID NO: 4, 64, 136 (identical).
NOTE: Claims 4-8 and 10-11 were ecluded from the first invention, because they are drawn to a non-elected subject matter.]

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.:
1-3, 9, 12-30 and 33-36, limited to SEQ ID NOs: 1-4, 63, 64, 135 and 136.

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.

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********** Supplemental Box **********

Continuation of Box III: Lack of Unity of Invention

Should an additional fee(s) be paid, Applicant is invited to elect an additional SEQ ID NO(s) to be searched. The exact claims searched will depend on the specifically elected SEQ ID NO(s). Due to the number of sequences in this application, an additional invention(s) of Group I+ will be defined as necessary depending on Applicant's ultimate payment of additional fees. The additional sequences will be searched if applicant pays for each additional sequence or shows that the sequences share a special technical feature, i.e. a common structure or feature that defines a contribution over the prior art. Note that each additional sequence to be searched must be specified by the Applicant in the response to this invitation and must either (1) have an additional invention fee paid or (2) have a showing that the sequences share a common structure or feature that defines a contribution over the prior art.
NOTE: Claims 4-8 and 10-11 were ecluded from the first invention, because they are drawn to a non-elected subject matter.]

Group II+, claims 31-32, drawn to a method of identifying a sample that contains any of the microorganisms listed in Table 6 by a) PCR amplifying at least one target nucleic acid from the sample that contains any of the microorganisms listed in Table 6 to form at least one target amplicon, wherein the PCR contains a plurality of primers capable of amplifying any of the microorganisms listed in Table 6; and b) detecting the at least one target amplicon to identify the sample that contains any of the microorganisms listed in Table 6. The first invention of Group II+ is restricted to identifying Acholeplasma granularum. Should an additional fee(s) be paid, Applicant is invited to elect an additional specific microorganism(s) to be searched.

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 Group I+ do not include the inventive concept of identifying a sample that contains any of the specific Mycoplasma microorganisms listed in Table 6 an agent, as required by Group II+.

The inventions Group II+ do not include the inventive concept of a multi-primer assay for detecting Mycoplasma, said method comprising using specific primers of specific SEQ ID NOs, as required by Group I+.

The inventions of Group I+ share the technical feature of a multi-primer assay for detecting Mycoplasma comprising: a) contacting a sample with the plurality of oligonucleotide primers in a single vessel; b) performing a multi-primer amplification reaction in the vessel, wherein each of the plurality of oligonucleotide primers is present for participation in amplifying a target nucleic acid in the sample to produce an amplification product; and c) detecting the amplification product, wherein the presence of an amplification product indicates Mycoplasma in the sample. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2004/0077015 A1 to Vojdani et al. (hereinafter "Vojdani", which discloses a method for determining the presence of one or more Mycoplasma species (abstract, claim 1, "A method for determining an increased likelihood of the presence of chronic fatigue syndrome (CFS), fibromyalgia (FMS), or rheumatoid arthritis (RA) in an individual, comprising the steps of: isolating peripheral blood mononuclear cells (PBMC) from said individual; and detecting the presence of at least one mycoplasma species in said PBMC, wherein the presence of at least one of said species indicates an increased likelihood of the presence of CFS, FMS or RA", para [0027]-[0033]), wherein the detection step comprises multiplex PCR (para [0012]), wherein multiple primers are used in a single reaction simultaneously (para [0015]) and in the same vessel (claim 4, "said detecting step comprises multiplex PCR", wherein "multiplex PCR" inherently discloses "the same vessel" limitation), and detecting the amplification products (para [0032]). As said method was known at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

The inventions of Group II+ lack unity for the reasons stated for Group I+, and further because Vojdani discloses the use of multi-plex PCR for identification of specific species of Mycoplasma (para.[0033], TABLE 3, "Percentages of positive results from each sample group tested", discloses identification of Mycoplasma genus, M. fermentans; M. hominis; and M. penetrans).

Another special technical feature of the inventions listed as Group I+ is the specific nucleic acid sequence recited therein. The inventions do not share a special technical feature, because US 20090181368 A1 (Iwakiri) (16 July 2009) discloses the claimed SEQ ID NO:1, 63, 135 (Acholeplasma laidlawii, nucleotides 709-732 of SEQ ID NO 1, 100% identity); SEQ ID NO: 2-3 (Acholeplasma laidlawii, nucleotides 705-726 of SEQ ID NO 1, 100% identity), and Mycoplasma synoviae 16s rRNA gene SEQ ID:1260, and further because JP2009072168-A to Fukuda, et al. (hereinafter "Fukuda") (09-APR-2009) discloses the claimed SEQ ID NO:4, 64, 136 (16s ribosomal RNA of Mycoplasma synovia, nucleotides 391-414 of SEQ ID NO 1260, 100% identity). Without a shared special technical feature, the inventions lack unity with one another.

Another special technical feature of the inventions listed as Group II+ is the specific species of Mycoplasma recited therein. The inventions do not share a special technical feature, because said species were known in the art, as evidenced by both, Iwakiri and Fukuda, as set forth in the immediately preceding paragraph. 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.