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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**:
6 March 2014

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(54) **Title**: DETECTION OF MYCOPLASMA IN CELL CULTURES AND CELL CULTURE DERIVED BIOLOGICALS

(57) **Abstract**: Sequences having specificity for.Mycoplasma and related Mollicutes genus strains and uses thereof. Methods of use include detection of samples contaminated with Mycoplasma. Kits are provided and comprise one or more oligonucleotides for the detection of Mycoplasma and related Mollicutes genus strains.

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A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2013.01)
USPC - 435/6.1, 6.11, 6.12
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, 6.11, 6.12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/6.1, 6.11, 6.12 (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic data bases: PatBase; Google Patents; Google Scholar; GenCore sequence search (NT)
Search terms: Mycoplasma, Mollicutes, 16S-23S rRNA intergenic region (ITS), M. genitalium ATCC 33530, A. laidlawii ATCC 23206, nucleic acid analog, locked nucleic acid (LNA), peptide nucleic acid (PNA), detection, probe, PCR

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	WANG et al. Simultaneous detection and identification of common cell culture contaminant and pathogenic mollicutes strains by reverse line blot hybridization. Appl Environ Microbiol. March 2004 Vol 70 No 3 Pages 1483-1486. Especially pg 1484 table 1, ; pg 1485 Fig 1.	1, 2, 5-8, 13 ---------- 3, 4
X ---- Y ----- A	US 20090018031 A1 (TRINKLEIN et al.) 15 January 2009 (15.01.2009). Especially SEQ ID NO: 8254, para [0037]	9, 10 -------- 11,12 ---------- 14, 28-31, 34 and 35
Y	US 2007/0065828 A1 (KIM et al.) 22 March 2007 (22.03.2007). Especially abstract, para [0024]	3, 4, 11, 12
A	WO 2011/153277 A1 (GREEN et al.) 8 December 2011 (08.12.2001). Especially SEQ ID NO: 136	15-20, 28-31, 34 and 35

☐ 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
26 December 2013 (26.12.2013)	15 JAN 2014
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

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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:
please go to extra sheet for continution

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.:
Claims 1-20 and 28-35,limited to SEQ ID NO: 1 (claims 1-20, 28-31 and 34-35)

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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Continuation of Box III (Lack of Unity of Invention)

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 1-20 and 28-35, drawn to a nucleic acid sequence, wherein the nucleic acid sequence is complimentary to at least five consecutive nucleic acids in a 5' to 3' direction or 3' to 5' direction, of any one or more sequences set forth as SEQ ID NOs: 1-15. The first invention is restricted to SEQ ID NO: 1. Group I+ will be searched to the extent that it reads on SEQ ID NO: 1, without fee. It is believed that claims 1-20, 28-31 and 34-35 of Group I+ read on the first named invention. Applicants must indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be SEQ ID NO: 10 (claims 1-20, 28, 29, 32-35).

Group II+; Claims 21-27 and 36-39, drawn to a method/composition of detecting whether a sample is contaminated with Mycoplasma and/or related Mollicutes genus strains comprising: incubating a sample with one or more primers comprising nucleic acid sequences set forth as SEQ ID NOs: 1-15; assaying the sample thereby, detecting whether the sample is contaminated with Mycoplasma and/or related Mollicutes genus strains. The first invention is restricted to SEQ ID NO: 1. Group II+ will be searched to the extent that it reads on SEQ ID NO: 1. It is believed that claims 21-31 and 34-39 of Group II+ read on the first named invention. Applicants must indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first claimed invention to be searched/examined.

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:

Special Technical Features
Groups I+ includes the special technical feature of an isolated nucleic acid hybridizes to at least 5 consecutive nucleotide sequences in the 5'-3' direction or 3'-5' direction of a region of a 16S-23S ribosomal intergenic region nucleic acid of Mycoplasma and/or related Mollicutes genus, not required by Group II+.

Group II+ includes the special technical feature of a method of detecting whether a sample is contaminated with Mycoplasma and/or related Mollicutes genus, not required by Group I+.

Another special technical feature of the inventions listed as Groups I+ and II+ is the specific sequences recited therein. The inventions do not share a technical feature because no significant structural similarities can readily be ascertained among recited sequences.

Common Technical Features
Groups I+ and II+ share the technical feature of a nucleic acid (oligomer and/or primer) hybridizes to a nucleic acid region of interest of Mycoplasma and/or related Mollicutes genus.

The inventions of Groups I+ share the technical feature of the nucleic acid sequence complimentary to at least five consecutive nucleic acids in a 5' to 3' direction or 3' to 5' direction.

The inventions of Group II+ share the technical feature of a method of detecting whether a sample is contaminated with Mycoplasma and/or related Mollicutes genus strains comprising: incubating a sample with one or more primers or oligomers, assaying the sample, and detecting whether the sample is contaminated with Mycoplasma and/or related Mollicutes genus strains.

However, these shared technical features do not represent a contribution over prior art as being anticipated by the publication entitled "Simultaneous detection and identification of common cell culture contaminant and pathogenic mollicutes strains by reverse line blot hybridization" by WANG et al (hereinafter "Wang") [published March 2004 in: Appl Environ Microbiol. Vol 70 No 3 Pages 1483-1486]. Wang discloses claim 1 of Group I+, an isolated or synthetic nucleic acid, wherein the isolated or synthetic nucleic acid hybridizes to at least 5 consecutive nucleic acids in a 5' to 3' or 3' to 5' direction of a 16S-23S ribosomal intergenic region nucleic acid of Mycoplasma and/or related Mollicutes genus strains (pg 1484 table 1; sequences of 20 probes greater than 5 nucleotides in length [sequences derived from GenBank accession numbers, as indicated in table] used to detect specific Mycoplasma and related Mollicutes genus strains; pg 1485 Fig 1- detection of various Mycoplasma and related Mollicutes: "Ten mollicute species reference strains identified by the RLB hybridization method. The positions of 20 probes specific for mollicute species are shown on the left-hand side. The 16S-23S rRNA intergenic spacer region PCR amplicons were from the following species (top to bottom): A. laidlawii ATCC 23206; M. fermentans ATCC 19989 ...").

As to claim 21 of Group II+, Wang discloses a method of detecting whether a sample is contaminated with Mycoplasma and/or related Mollicutes genus strains (pg 1484 col 1 para 4; "We tested 92 contaminated cell cultures, 100 clinical isolates or specimens, and 21 reference strains by mollicute species-specific PCR assay, based on the 16S-23S rRNA intergenic spacer region"), comprising: incubating a sample with one or more primers or oligomers (pg 1484 table 1; primers and probes indicated); assaying the samples thereby, detecting whether the sample is contaminated with Mycoplasma and/or related Mollicutes genus strains (pg 1484 col 1 para 4; pg 1485 Fig 1-e.g., M. fermentans: positive indicator).

As the common technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups. The inventions lack unity with one another.

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

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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 filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search

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 in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

GenCore ver 6.4.1 SEQ ID NO: 1