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*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: COMPOSITIONS, METHODS AND KITS FOR DETERMINING THE PRESENCE OF CRYPTOSPORIDIUM OR-
GANISMS IN A TEST SAMPLE

(57) Abstract: The present invention describes novel oligonucleotides targeted to nucleic acid sequences derived from *Cryptosporid-
ium* organisms, and *Cryptosporidium parvum* organisms in particular, which are useful for determining the presence of *Cryptosporid-
ium* organisms in a test sample. The oligonucleotides of the present invention include hybridization assay probes, helper probes and
amplification primers. The present invention further describes a novel method for obtaining purified ribonucleic acid from viable
oocysts.



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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/68

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 7 C12Q

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 practical, search terms used)

BIOSIS, EPO-Internal, WPI Data, PAJ, MEDLINE, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE NCBI [Online] National Institute of Health; Cryptosporidium parvum 18S rRNA, 30 June 1993 (1993-06-30) CAI, J.: "PCR cloning and nucleotide sequence determination of the 18S rRNA genes and internal transcribed spacer 1 of the protozoan parasites Cryptosporidium parvum and Cryptosporidium muris" retrieved from NCBI, accession no. gi:7001 Database accession no. X64340 XP002228938 the whole document & CAI, J. ET AL.: "PCR cloning and nucleotide sequence..." BIOCHIM. BIOPHYS. ACTA, vol. 1131, no. 3, 16 July 1992 (1992-07-16), pages 317-320, the whole document -/--	1-22, 47-63, 70,76,78

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in 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 document 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

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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	--- DATABASE NCBI [Online] NIH; 8 March 1996 (1996-03-08) PIENIAZEK, N.J. ET AL.: "Cryptosporidium parvum 18S ribosomal RNA gene, complete" retrieved from NATIONAL INSTITUTE OF HEALTH, accession no. gi:290015 Database accession no. L16996 XP002228939 the whole document	1-22, 47-63, 70,76,78
Y	--- HALLIER-SOULIER SYLVIE ET AL: "An immunomagnetic separation polymerase chain reaction assay for rapid and ultra-sensitive detection of Cryptosporidium parvum in drinking water." FEMS MICROBIOLOGY LETTERS, vol. 176, no. 2, 15 July 1999 (1999-07-15), pages 285-289, XP002228935 ISSN: 0378-1097 the whole document	1-22, 47-63, 70,76,78
Y	--- DEERE D ET AL: "Evaluation of fluorochromes for flow cytometric detection of Cryptosporidium parvum oocysts labelled by fluorescent in situ hybridization." LETTERS IN APPLIED MICROBIOLOGY, vol. 27, no. 6, December 1998 (1998-12), pages 352-356, XP008013001 ISSN: 0266-8254 the whole document	1-22, 47-63, 70,76,78
Y	--- WANG JOSEPH ET AL: "Electrochemical biosensor for detecting DNA sequences from the pathogenic protozoan Cryptosporidium parvum." TALANTA, vol. 44, no. 11, 1997, pages 2003-2010, XP002228936 ISSN: 0039-9140 the whole document	1-22, 47-63, 70,76,78
Y	--- DENG MING QI ET AL: "Immunomagnetic capture PCR to detect viable Cryptosporidium parvum oocysts from environmental samples." APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 63, no. 8, 1997, pages 3134-3138, XP002923030 ISSN: 0099-2240 the whole document --- -/--	1-22, 47-63, 70,76,78

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/42192

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LABERGE I ET AL: "Detection of Cryptosporidium parvum in raw milk by PCR and oligonucleotide probe hybridization." APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 62, no. 9, 1996, pages 3259-3264, XP002228937 ISSN: 0099-2240 the whole document ---	1-22, 47-63, 70,76,78
Y	LINDQUIST H D A: "Rapid communication. Probes for the specific detection of cryptosporidium parvum" WATER RESEARCH, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 31, no. 10, 1 October 1997 (1997-10-01), pages 2668-2671, XP004090502 ISSN: 0043-1354 the whole document -----	1-22, 47-63, 70,76,78

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/42192

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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 II Observations where unity of invention is lacking (Continuation of item 2 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 fee, this Authority did not invite payment of any additional fee.
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.:

1-22, 47-63, 70, 76, 78 (partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ 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: 1-22, 47-63, 70, 76, 78; (partially)

Invention Number 1:

A Cryptosporidium-specific oligonucleotide probe, namely Sequence Identity Number 1, being a hybridization assay probe specific for said organism, being comprised in a probe mix, for use in determining the presence of a Cryptosporidium organism in a test sample.

2. Claims: 1-22, 47-63, 70, 76, 78; (partially)

Invention Number 2 - 4:

A Cryptosporidium-specific oligonucleotide probe, namely Sequence Identity Numbers 2 - 4, being a hybridization assay probe specific for said organism, being comprised in a probe mix, for use in determining the presence of a Cryptosporidium organism in a test sample.

3. Claims: 19-22, 76, 78, 79 (partially);

Invention Number 5:

A Cryptosporidium-specific oligonucleotide probe, namely Sequence Identity Number 21, being comprised in a probe mix, being comprised in a kit.

4. Claims: 19-22, 76, 78, 79 (partially);)

Invention Number 6 - 12:

A Cryptosporidium-specific oligonucleotide probe, namely Sequence Identity Number 22 - 28, being comprised in a probe mix, being comprised in a kit.

5. Claims: 23-49, 51-60, 62-75, 77, 113-162 (partially)

Invention 13:

A Cryptosporidium-specific oligonucleotide probe, namely Sequence Identity Number 45, being an amplification primer, said amplification primer being contained in a set, said oligonucleotide probe being used in determining the presence of a Cryptosporidium organism, said oligonucleotide probe being used within a method for amplifying the nucleic acid of said organism, being comprised in kits.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

6. Claims: 23-49, 51-60, 62-75, 77, 113-162 (partially)

Inventions 14 - 37:

A Cryptosporidium-specific oligonucleotide probe, namely Sequence Identity Numbers 46 - 69, being an amplification primer, said amplification primer being contained in a set, said oligonucleotide probe being used in determining the presence of a Cryptosporidium organism, said oligonucleotide probe being used within a method for amplifying the nucleic acid of said organism, being comprised in kits.

7. Claims: 80-116, 129-159 (partially);

Invention Number 38:

A Cryptosporidium-specific oligonucleotide probe, namely Sequence Identity Number 5, it being a hybridization assay probe, it being comprised in a probe mix, for use in determining the presence of a Cryptosporidium parvum organism in a test sample, it being comprised in a kit.

8. Claims: 80-116, 129-159 (partially);

Invention Number 39 - 53:

A Cryptosporidium-specific oligonucleotide probe, namely Sequence Identity Numbers 6 - 20, it being a hybridization assay probe, it being comprised in a probe mix, for use in determining the presence of a Cryptosporidium parvum organism in a test sample, it being comprised in a kit;

9. Claims: 102-112, 160-162 (partially);

Invention 54:

A Cryptosporidium-specific oligonucleotide probe, namely Sequence Identity Number 29, it being comprised in a probe mix, it being comprised in kits.

10. Claims: 102-112, 160-162 (partially);

Invention 55 - 69:

A Cryptosporidium-specific oligonucleotide probe, namely Sequence Identity Number 30 - 44, it being comprised in a probe mix, it being comprised in kits.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

11. Claims: 163-165 (completely);

Invention Number 70:

A method of obtaining purified RNA from a viable oocyst.