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(54) Title: METHODS AND COMPOSITIONS TO DETECT BACTERIA USING MULTIPLEX PCR

(57) Abstract: A novel multiplex polymerase chain reaction assay for detecting a bacteria (e.g. *Helicobacter pylori*) in a specimen uses multiple oligonucleotide primer pairs based on the sequences of multiple loci of the bacteria. In one application, up to five loci in the genomic DNA sequences of *Helicobacter pylori* were amplified. Two fragments of *H. pylo* were amplified from each locus, wherein a second fragment was an internal fragment of the first fragment.



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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US05/14389

## A. CLASSIFICATION OF SUBJECT MATTER

IPC: C12Q 1/68( 2006.01);C12P 19/34( 2006.01)

USPC: 435/6,91.2

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)

U.S. : 435/6,91.2

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)  
WEST (patent database), pubmed, STN; search terms: helicobacter or H pylori, multiplex, nested, amplification

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                   | Relevant to claim No.  |
|------------|--------------------------------------------------------------------------------------------------------------------------------------|------------------------|
| X          | Database BIOSIS on STN, MENG, X et al. "Digestive Disease Week Abstracts and Itinerary Planner". 2003, Vol. 2003, abstract No S1217. | 1-3, 6-8, 11-12, 17-18 |
| X          | MONSTEIN, H. et al. Differential Virulence-gene mRNA expression in coccoid forms of                                                  | 1-3, 5-6, 11           |
| ---        | Helicobacter pylori. Biochemical and Biophysical Research Communication. 2001, Vol.                                                  |                        |
| Y          | 285, pages 530-536, entire document.                                                                                                 |                        |
| X          | MONSTEIN, H. et al. Molecular typing of Helicobacter pylori by virulene-gene based                                                   | 1-3, 5-6, 9-11, 19-20  |
| ---        | multiplex PCR and RT-PCR analysis. Helicobacter. 2002, Vol. 7, No. 5, pages 287-296,                                                 | -----                  |
| Y          | entire document.                                                                                                                     | 7-8, 17-18             |

☒ 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 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 September 2006 (21.09.2006)

Date of mailing of the international search report

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US05/14389

## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                        | Relevant to claim No.         |
|------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|
| Y          | KAWAMATA, O. et al. Nested-polymerase chain reaction for the detection of Helicobacter pylori infection with novel primers designed for sequence analysis of Urease A gene in clinically isolated bacterial strains. Biochemical and Biophysical Research Communications. 1996, Vol. 219, pages 266-272, entire document. | 1-3, 6-8, 11-12, 14-15, 17-18 |
| Y          | MULLER, U et al. Cloning and comparison of ten gene sequences of a Chilean H. pylori strain with other H. pylori strains revealed higher variability for VacA and CagA virulence factors. Biological Research. 2002, Vol. 35, No. 1. pages 67-84, entire document.                                                        | 4 and 16                      |
| Y          | COUSINS, D.V. et al. Use of Polymerase Chain Reaction for rapid detection of tuberculosis. J. Clinical Microbiology. 1992, Vol. 30, No. 1, pages 255-258, entire document.                                                                                                                                                | 13                            |

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US05/14389

## 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 See Continuation 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 additional fees, this Authority did not invite payment of any 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.: 1-20

- Remark on Protest
- |                          |                                                                                                                                                                       |
|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <input type="checkbox"/> | The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.                                           |
| <input type="checkbox"/> | 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. |
| <input type="checkbox"/> | No protest accompanied the payment of additional search fees.                                                                                                         |

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US05/14389

### BOX 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, claim(s) 1-20, drawn to a method of detecting bacteria.

Group II, claim(s) 21-26, drawn to a diagnostic kit comprising primers and amplification reagents.

The inventions listed as Groups I-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 Groups I-II are not linked by a special technical feature which distinguishes over the disclosure of the prior art. The first broadest product, the diagnostic kit of claim 21, within Group II, is disclosed in the prior art. Monstein et al. (Biochemical and Biophysical Research Communications, 2001, vol. 283, p. 530-536) teaches the amplification of multiple *H. pylori* loci using a plurality of primers (see Table 1, p. 532; where sequences of the plurality of primer sets are disclosed). The amplification reagents were taught by Monstein and were included in reverse-transcription PCR amplification reactions (p. 531, col. 1, 'Multiplex reverse-transcription PCR amplification' heading).