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GQ, GW, ML, MR, NE, SN, TD, TG).

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(54) Title: METHOD FOR DETECTING LOW CONCENTRATIONS OF A TARGET BACTERIUM THAT USES PHAGES TO INFECT TARGET BACTERIAL CELLS

(57) Abstract: The invention is directed to a method for detecting low concentrations of bacteria in liquid solution that may or may not be complex liquid solutions. In one embodiment, immunomagnetic separation (IMS) is used to separate target bacterium that may be in a liquid mixture from other constituents in the mixture. A low concentration of a bacteriophage for the target bacteria is subsequently used to infect target bacterial cells that have been captured using the IMS technique. If at least a certain concentration of target bacterium are present, the bacteriophage will multiply to a point that is detectable. Matrix assisted laser desorption ionization/time-of-flight-mass spectrometry (MALDI/TOF-MS) is then used to produce a mass spectrum that is analyzed to determine if one or more proteins associated with the bacteriophage are present, thereby indirectly indicating that target bacterium were present in the liquid mixture.



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**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US03/11253

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : C12Q 1/70, 1/02, 1/06, 1/18  
 US CL : 435/5, 29, 32, 39

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. : 435/5, 29, 32, 39

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, PubMed

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHATTERJEE et al., "A high yielding mutant of mycobacteriophage L1 and its application as a diagnostic tool," FEMS Microbiology Letters, July 2000, Vol 188 No. 1, pages 47-53, esp. 52-53.	1-6, 15-45
Y	TAN et al., "Rapid Simultaneous detection of two orchid viruses using LC- and/or MALDI-mass spectrometry," Journal of Virological Methods,	4, 6, 29, 30, 44
Y	US 6,037,118 A (THOMAS et al.) 14 March 2000 (14.03.2000), while document, esp. columns 1 and 3.	4, 6, 29, 30, 44
Y	SIUZDAK et al., "Probing Viruses with Mass Spectrometry," Journal of Mass Spectrometry, 1998, Vol 33, pages 203-11.	4, 6, 29, 30, 44
Y	OLSVIK et al., "Magnetic Separation Techniques in Diagnostic Microbiology," Clinical Microbiology Reviews, January 1994, Vol 7 No. 1, pages 43-54.	15-34, 36, 38-41
Y	SUN et al., "Use of bioluminescent Salmonella for assessing the efficiency of constructed phage-based biosorbent," Journal of Industrial Microbiology & Biotechnology, 2000, Vol 25, pages 273-75.	15-34, 36, 38-41



Further documents are listed in the continuation of Box C.



See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance	"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
"E"	earlier application or patent published on or after the international filing date	"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
"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)	"&"	document member of the same patent family
"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		

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## INTERNATIONAL SEARCH REPORT

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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.
Y	NAKAMURA et al., "A Visualization Method of Filamentous Phage Infection and Phage-Derived Proteins in Escherichia coli Using Biotinylated Phages," Biochemical and Biophysical Research Communications, November 2001, Vol. 289 No. 1, pages 252-56.	1-6, 15-45
Y	FAVRIN et al., "Development and optimization of a novel immunomagnetic separation-bacteriophage assay for detection of Salmonella enterica serovar enteritidis in broth," Applied and Environmental Microbiology, January 2001, Vol 67 No. 1, pages 217-24.	1-6, 15-45
Y	STEWART et al., "The specific and sensitive detection of bacterial pathogens within 4 h using bacteriophage amplification," Journal of Applied Microbiology, May 1998, Vol 84 No. 5, pages 777-83.	1-6, 15-45.
Y	US 5,888,725 A (SANDERS) 30 March 1999 (30.03.1999), whole document.	1-6, 15-45
Y	US 5,498,525 A (REES et al.) 12 March 1996 (12.03.1996), whole document.	1-6, 15-45
A	WO 95/05483 (THE MINISTER OF AGRICULTURE, FISHERIES AND FOOD IN HER BRITANNIC MAJESTY'S GOVERNMENT OF THE UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND) 23 February 1995 (23.02.1995), whole document.	1-5, 15-45

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/11253

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claim 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.  Claim 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:  
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 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.: 1-6 and 15-45
- Remark on Protest**
- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING**

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid.

Applicant is required to elect both one of species (A)-(H) below. The species are as follows:

Species (A)-(H) represent the claimed invention wherein the step of analyzing comprises, in the step of analysis, use of

- (A) Mass spectrum;
- (B) MALDI;
- (C) MALDI-TOF;
- (D) Electro-spray ionization mass spectrometry;
- (E) Ion mobility spectrometry
- (F) Immuno-analysis;
- (G) Chromatographic analysis; or
- (H) Aptamer analysis.

The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: the common technical feature among these inventions is the use of a bacteriophage to detect low levels of a bacterium in a sample by the detection of a biomarker to the phage after infection of the bacteria. Such a method is taught in the art. See e.g., Stewart et al., *Journal of Applied Microbiology*, Volume 84, pages 777-83, esp. pages 777, right column (teaching the use of phage to detect bacteria by detecting replication of phage in a sample suspected of containing bacteria); and Tan et al., *Journal of Virological Methods*, Volume 85, pages 93-99 (teaching the use of MALDI-mass spectrometry for the detection of viruses). The art therefore renders obvious the common technical features of the claims. Because the art suggests the claimed method, the inventions share no common special technical feature. Unity is therefore lacking.