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(54) Title: METHOD FOR DETERMINING THE PRESENCE OF BACTERIA RESISTANT TO CELL LYSING ANTIBIOTICS

(57) Abstract: A method is provided for determining the presence of a target bacteria based on its resistance to a cell lysing antibiotic. Said antibiotic is used to lyse cells of non-target bacteria in a sample and hence facilitate isolation of the target prior to detection by known means.

METHOD FOR DETERMINING THE PRESENCE OF BACTERIA RESISTANT TO CELL LYSING ANTIBIOTICS

The present invention is directed to a method for determining the presence of one or more target bacteria in a sample, which target bacteria are resistant to a cell-lysing antibiotic or antibiotics. The invention is particularly, although not exclusively, directed to a method for determining the presence of one or more methicillin-resistant bacteria in a clinical sample.

The emergence of bacteria having resistance to commonly used antibiotics is an increasingly prevalent problem with serious implications for the treatment of infected individuals. Indeed, these so-called superbugs, such as *Staphylococcus aureus*, are often resistant to all but the most powerful antibiotics. The problem has become particularly acute in the hospital environment where nosocomial infection of individuals with weakened immune systems by bacteria resistant to antibiotics can lead to severe complications and even death. Consequently, there is a need to minimise the risk of infection in such an environment. One way of minimising the risk relies on the early determination of the presence of such bacteria so as to enable individuals and an environment to be carefully monitored and, if necessary, treated at an early stage.

Present methods for the determination of the presence of bacteria resistant to antibiotics are, however, time consuming and require that the bacteria first be cultured in pure form and then exposed to a set of antibiotics to see whether their growth is inhibited. The whole process can take at least two days and can result in an often, critical delay before an optimum treatment regimen can be administered to an infected individual. Of course, such delays are not only deleterious to the health of individuals but also result in longer stays in the hospital environment.

Consequently, there is a need for a method for the rapid determination of the presence of bacteria which are resistant to certain antibiotics in a patient or environment.

WO 99/37799, Applicant's co-pending application, teaches a rapid method for determining whether a particular bacterium, known to be present in a culture, is susceptible or resistant to a particular antibiotic. The method is, however, limited in that it preferably requires that the bacterium is present as a pure culture or is a pure isolate. The method, however, can be adapted for determining the antibiotic resistance or susceptibility of a particular bacterium in a mixed culture. In that case the method generally relies on a preliminary separation step prior to treatment with the particular antibiotic or other action specific for the particular bacterium.

Alternatively, and provided the mode of action of the antibiotic is known, the method can reveal the antibiotic resistance or susceptibility of a particular bacterium known to be present in a mixed culture through a comparative analysis of the intracellular adenylate kinase content of four aliquots derived from the mixed culture and cultured in the presence of i) no antibiotic ii) antibiotic, iii) a bacteriophage specific to the bacterium and iv) a mixture of both the antibiotic and the bacteriophage. A bacterium susceptible to a cell-lysing antibiotic, for example, may be deduced on the basis that intracellular adenylate kinase is reduced in the sample cultured with the antibiotic and bacteriophage when compared with the sample cultured with the bacteriophage alone.

Although it might be supposed that the methods disclosed in WO 99/37799 might simply indicate the presence of bacterium in a mixed culture having resistance to a particular

antibiotic, in practice such a result cannot be unambiguously and reliably obtained especially where concentrations of resistant bacteria are low.

Even, if it could, it will be apparent that the determination of that presence still requires the performance of a number of repetitive steps involving the culture, treatment and assay of at least four different aliquots from the mixed culture. Such repetitions are inelegant and increase the possibility of error. Further, the method comprises an inherent delay in that the treatment of an aliquot of the mixed culture with a bacteriophage requires time to ensure that the bacteria are established in the log phase of their growth.

Consequently, there remains a need for a simple, rapid method for determining the presence of bacteria having antibiotic resistance in a sample. In particular, there remains a need for a simple, and rapid method for determining the presence of bacteria having antibiotic resistance in a clinical sample.

The present invention therefore seeks to provide a simple and rapid method, involving a minimum of necessary steps, for determining whether antibiotic resistant bacteria are present in a sample, especially a clinical sample, taken, for example, by swabbing infected individuals.

The present invention starts from the realisation that a wide range of bacteria are generally susceptible to cell lysing antibiotics and that, in consequence, the intracellular material of susceptible bacteria in a mixed culture can be advantageously discarded in the first instance.

Accordingly, the present invention provides a method for determining the presence of target bacteria in a sample, which target bacteria are resistant to a cell lysing antibiotic, comprising the sequential steps of:

i) incubating the sample in an incubating medium including the cell lysing antibiotic,

ii) capturing unlysed cells of the target bacteria on a solid support comprising a capture agent,

iii) exposing the unlysed cells of the target bacteria to an agent capable of causing cell lysis thereof,

iv) determining the presence of intracellular material from the lysed cells of the target bacteria.

In one embodiment of the present invention the method further comprises a washing step and/or a filtration step preceding step iii), so as to remove intracellular and material derived from cells susceptible to the cell lysing antibiotic.

It will be understood that throughout this application the phrase "cell lysing antibiotic" describes an agent which disrupts bacterial cell wall synthesis during cell replication. Thus, any of the β-lactam antibiotics are suitable for the performance of the method of the present invention. In a preferred embodiment of the present invention the cell lysing antibiotic is a penicillin such as ampicillin or methicillin.

In one embodiment of the present invention the capture agent may be specific only to a particular species of bacteria. In other embodiments the capture agent may be specific to a particular strain of bacteria. In a preferred embodiment the capture agent is fibrinogen

which binds to *Staphylococcus aureus*. Alternatively the capture agent is an antibody specific to *Staphylococcus aureus*.

It will of course be realised that the method of the present invention may include an additional intervening step or steps so as to enable the presence of more than one target bacteria to be determined. Typically, these steps may comprise capturing unlysed cells of the target bacteria on a solid support comprising one or more capture agents.

Advantageously, the culture medium for performing the present invention comprises a liquid broth. In this embodiment the solid support may comprise magnetic beads coated with a suitable antibody or other capture agent. Alternatively, the solid support may comprise a spatula, paddle or filter at least in part coated with a suitable antibody or other capture agent.

In a further embodiment of the present invention the agent capable of causing cell lysis is selective towards the target bacteria. Preferably the agent is selective to *Staphylococcus aureus*. Still more preferably the agent capable of causing cell lysis is lysostaphin.

In another embodiment of the present invention, the agent capable of causing cell lysis is a bacteriophage selective to the target bacteria or a lysin derived therefrom. In a further embodiment the agent capable of causing cell lysis is a colicin.

Of course it will also be realised that the method of the present invention may include a number of such steps so that the presence of other target bacteria may be also determined.

The determination of intracellular material derived from lysed cells of the target bacteria may comprise any appropriate assay as is known to the art. Preferably, the assay comprises a bioluminesence assay. Still more preferably, the bioluminescence assay is based on adenylate kinase (AK) as is described, for example, in Applicant's co-pending applications WO 94/17202 and WO 96/02665. However, the assay may alternatively comprise a colourimetric or fluorimetric assay based on intracellular enzyme markers – for example, phosphatase or peroxidase.

One aspect of the present invention provides for test kits for performing the method of the invention. Preferably, the test kit is a disposable test kit. The test kit may comprise suitable components so as to allow the chosen assay to be carried out. Preferably, the test kit comprises a suitable culture medium containing a chosen cell lysing antibiotic. Still more preferably, the test kit comprises a solid support including a capture agent specific to the target bacteria, a wash solution and an agent capable of causing cell lysis of the target bacteria.

In one embodiment of the present invention, the test kit comprises a liquid broth comprising methicillin. Preferably, the liquid broth containing methicillin is supplied in a freeze-dried form.

Advantageously, the test kit may be supplied in the form of a multi-well container such as that described in Applicant's co-pending patent application GB 0110476.9.

One advantage of the method of the present invention provides a rapid (about 3 hours) determination for the presence of bacteria resistant to a cell lysing antibiotic involving a

single assay for intracellular material. In addition, the method avoids the need for multiple, comparative assays and further confers the advantage that the results may be interpreted in an unambiguous, independently qualitative way, rather than in a comparative or quantitative way.

The present invention will now be described by reference to non-limitative embodiments and the following drawings in which

Figure 1 is a schematic illustration of the essential steps of the method of the present invention;

Figure 2 is a graph highlighting the difference between negative and positive tests;

Figure 3 is a graph showing test results on methicillin-resistant and methicillin–sensitive strains of *Staphylococcus aureus* after an 3.5 h incubation with 50 μg/ml of ampicillin or 4 μg/ml methicillin; and

Figure 4 is a section view of a test kit according to the present invention.

Referring now to Figure 1, a sample taken in the form of a swab, for example from a patient, and comprising a mixture of bacteria containing, generally methicillin resistant, bacteria 11 and, generally susceptible bacteria 12, is cultured at 37°C in a liquid broth of about 1 ml volume containing about 4 μg of the antibiotic.

Bacteria resistant to methicillin 11 survive and may multiply. Bacteria susceptible to methicillin 12, however, after an initial growth phase, develop weakened cell walls 13 and lyse, releasing their intracellular material 14 to the liquid broth.

After a period of 2 to 4 hours the liquid broth therefore comprises a mixture of methicillin-resistant, non-lysed bacteria and methicillin-susceptible lysed bacteria.

The presence of the particular bacteria, *Staphylococcus aureus* for example, is then determined by the addition to the liquid broth of paramagnetic beads 15 coated with fibrinogen. Fibrinogen is a specific binding agent for *Staphylococcus aureus*. After a period of 2 to 10 minutes the beads are magnetically removed from the broth and washed prior to an analysis step.

The beads 15 and the attached target bacteria 11 are then treated in a separate vessel with a detergent capable of lysing the cells. The intracellular material thus obtained is analysed for adenylate kinase using a luciferin-luciferase bioluminescence protocol. The presence of methicillin resistant *Staphylococcus aureus* is revealed by a signal 16. The signal 16 may, for the purposes of certainty, be compared to a suitable control signal so as to exclude anomalies arising from background concentrations of adenylate kinase.

It will be apparent that only those cells which remain impervious to methicillin and are immobilised on the magnetic beads provide a significant signal in response to the adenylate kinase assay.

Of course, the presence of other methicillin resistant target species may be investigated by an alternative or additional step or steps comprising the addition of beads comprising other coatings or beads including one or more capture agents in their coatings.

Alternatively or additionally specificity can be introduced into the method by targeting a particular variety of the bacterial species in the final cell lysis step. For example, a bacteriophage or a detergent combination which is specific to *Staphylococcus aureus* may be used.

Figure 2 shows the bioluminescence results obtained for the determination of the presence of *Staphylococcus aureus* in a series of clinical samples. As may be seen a positive test result (sample numbers M421000M and M421148L) is readily distinguished from a negative test result (sample numbers M421117S and M421131P).

Figure 3 shows the bioluminescence results obtained for different strains of *Staphylococcus aureus* cultured in the presence of ampicillin as well as methicillin. In each case the method distinguishes the presence of antibiotic resistant strains from antibiotic susceptible strains.

Referring now to Figure 4, a test kit for use in the method of the present invention comprises a solid, injection moulded polypropylene housing, generally designated 17, comprising a number of wells 18, 19, 20, 21, 22 which act as storage chambers and/or reaction chambers for various suitable tools or reagents (as are described below).

Chamber 18 stores a liquid broth 22 containing a cell lysing antiobiotic which is suitable for incubating a swab sample obtained from an infected individual. The chamber 18 is provided with a solid rod 23 carrying a swab 24 for obtaining this sample. Rod 23 carries a transverse portion 25 which acts as a handle for rod 18 and conveniently provides a lid 26 sealing the liquid broth 22 containing the cell lysing antibiotic in chamber 18.

Adjacent chambers 19, 20 respectively store an aqueous solution containing a suspension of magnetic beads 27 and an aqueous, phosphate buffered, saline, wash solution 28. Suitable magnetic beads are those obtained from Dynal under the name Dynabeads ™. As mentioned previously the beads are coated with an agent capable of capturing the target bacteria.

Chamber 21, which acts as the reaction chamber of the kit, is provided with an aqueous, buffer solution 29 suitable for performance of a bioluminescence assay based on adenylate kinase. The chamber is also provided with foil sealed capsules 30, 31 above the buffer solution 29 containing respectively a luciferin/luciferase based bioluminescent reagent and an aqueous solution of adenosine diphosphate containing a detergent or agent capable of lysing cells of the target bacteria. A source of magnesium, such as magnesium acetate, which acts as a promoter for bioluminescence assay, is provided in the space 32 dividing the foil sealed capsules

Chamber 22 is provided with a hollow wand 33 which carries a sharp conical tip 34 suitable for piercing the foil sealed capsules 30, 31. Wand 33 is provided with a transverse portion 35 carrying engagement means (eg. slots – not shown) for engaging a mechanism suitable for moving the wand 33 in vertical and horizontal directions under the control of control hardware or software (not shown). Transverse portion 35 is also provided with an aperture (not shown) allowing the wand 33 to receive a retractable cylindrical magnet (not shown).

Chambers 19, 20, 21, 22 are provided with laminate sealing means in the form a metal foil 36. In contrast to the sealing means provided to chamber 18, the laminate sealing means is not intended to be re-usable after it has been broken.

In use, the solid rod 23 carrying swab 24 is manually removed from chamber 18 by the handle 25 and used to obtain a sample from an infected individual. The rod 23 is immediately returned to the chamber so that the swab 24 is immersed in the liquid broth 22 and the seal between the lid 26 and the chamber 18 is reformed.

The test kit is held under suitable conditions for a period of about 3h so as to incubate the patient sample. At the end of this period rod 23 is removed from chamber 18 and the metal foil sealing chambers 19, 20, 21, 22 removed.

The hollow wand 33 (containing the retractable magnet) is engaged with the moving mechanism. Under the control of the hardware or software control wand 33 is moved to chamber 19 where it collects the magnetic beads from suspension 27 and thence to chamber 18 where retraction of the magnet by, for example worm drive means, causes the beads to be deposited in the broth.

After a suitable period, and if appropriate stirring, the magnet is returned to the wand 33 so that it collects the beads. Wand 33 is then removed to chamber 20 where the beads are deposited in the wash solution 28 and after a further suitable period retrieved as previously described.

Wand 33 carrying the washed beads is subsequently removed to the reaction chamber 21 so that the sharp, conical tip 34 is caused to release the contents of foil sealed capsules 30, 31 to the buffer solution 29. The beads are then released to the solution 29 by retraction of the magnet from wand 33 and the wand returned to chamber 22.

A photomultiplier (not shown) placed beneath the chamber 21 of polypropylene housing 17 detects the emitted light from the bioluminescence reaction that follows. (A detailed description of the reaction is given in Applicant's co-pending applications WO 94/17202 and WO 96/02665).

CLAIMS

1. A method for determining the presence of target bacteria in a sample, which target bacteria are resistant to a cell lysing antibiotic, comprising the sequential steps of:

i) incubating the sample in an incubating medium including the cell lysing antibiotic,

ii) capturing unlysed cells of the target bacteria on a solid support comprising a capture agent,

iii) exposing the unlysed cells of the target bacteria to an agent capable of causing cell lysis thereof,

iv) determining the presence of intracellular material from the lysed cells of the target bacteria.

2. A method according to Claim 1, in which step iii) is preceded by a washing and/or a filtration step.

3. A method according to Claim 1 or Claim 2, in which the capture agent is an antibody specific to *Staphylococcus aureus*.

4. A method according to Claim 1 or Claim 2, in which the capture agent is fibrinogen.

5. A method according to Claim 4, in which the agent capable of causing cell lysis is selective towards *Staphylococcus aureus*.

6. A method according to Claim 5, in which the agent capable of causing cell lysis is lysostaphin.

7. A method according to any of Claims 1 to 4, in which the agent capable of causing cell lysis is a bacteriophage.

8. A method according to any preceding Claim, in which the culture medium is a liquid broth.

9. A method according to any preceding Claim, in which the solid support comprises magnetic beads.

10. A method according to any of Claims 2 to 9, in which step iv) comprises a bioluminescence assay.

11. A method according to Claim 10, in which the bioluminescence assay is based on adenylate kinase.

12. A method according to any of Claims 2 to 9, in which step iv) comprises a colourimetric or fluorimetric assay based on an intracellular enzyme marker.

13. A method according to any preceding Claim, in which the cell lysing antibiotic is methicillin.

14. A test kit, for determining the presence of a target bacteria, comprising a culture medium containing a cell lysing antibiotic, one or more solid supports comprising a capture agent specific for the target bacteria, one or more agents capable of causing cell lysis of the target bacteria and reagents for determining the presence of intracellular material from lysed cells of the target bacteria.

15. A test kit according to Claim 14, in which the cell lysing antibiotic is methicillin.

16. A test kit according to Claim 14 or Claim 15, in which the one or more capture agents include a capture agent specific to *Staphylococcus aureus*.

17. A test kit according to any of Claims 14 to 16, in which the reagents for determining the presence of intracellular material from the lysed cells of the target bacteria are suitable for effecting a bioluminescence, colourimetric or fluorimetric assay for intracellular enzyme marker.

Fig.1.

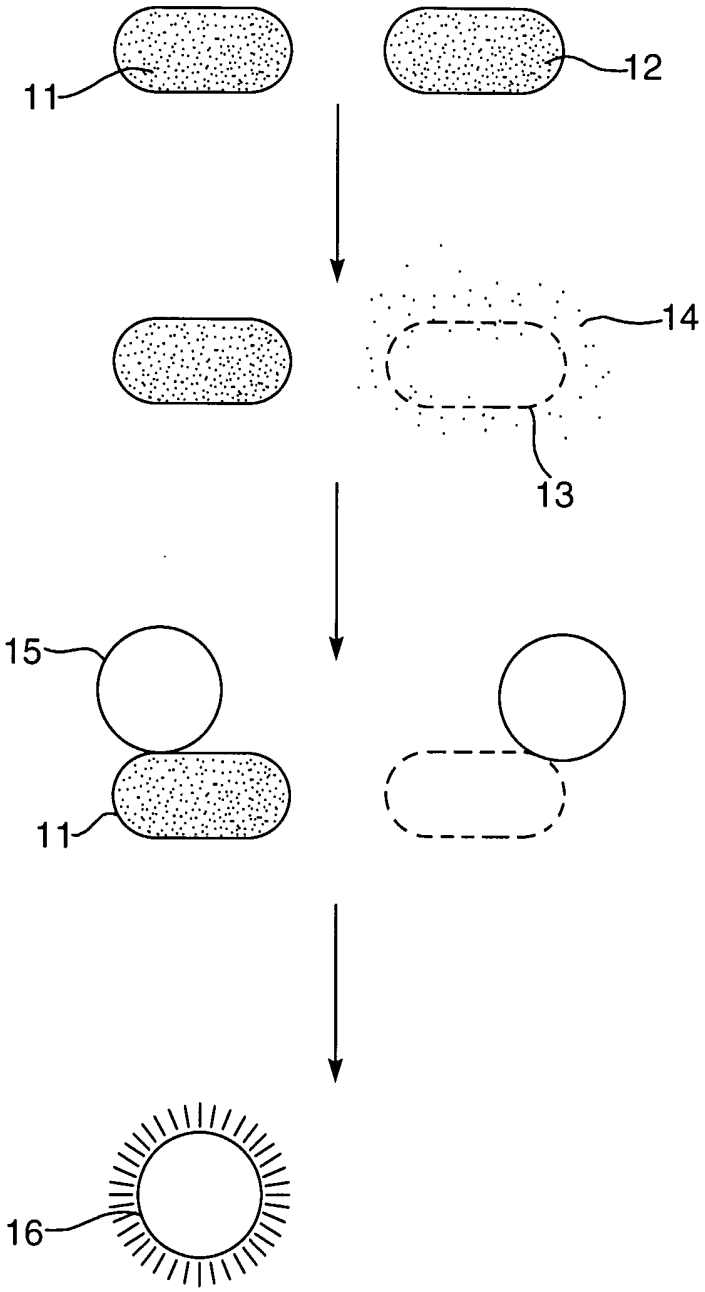
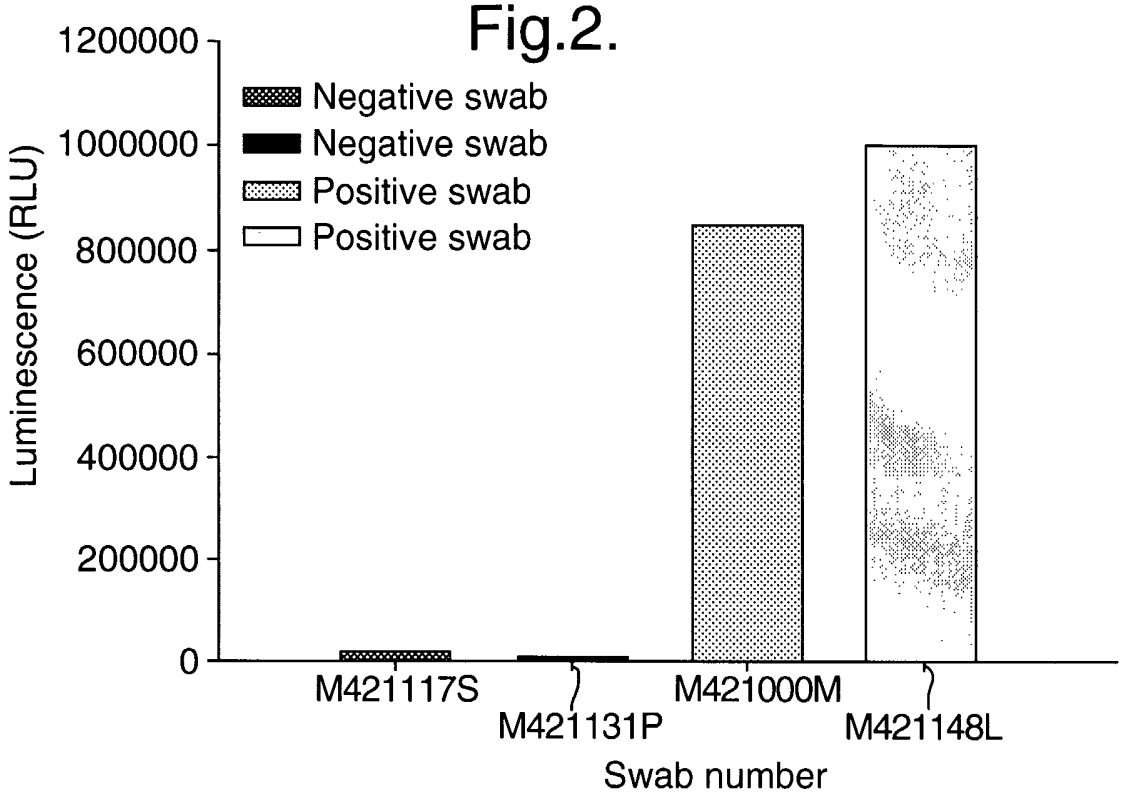


Fig.2.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Negative swab
Positive swab
Positive swab
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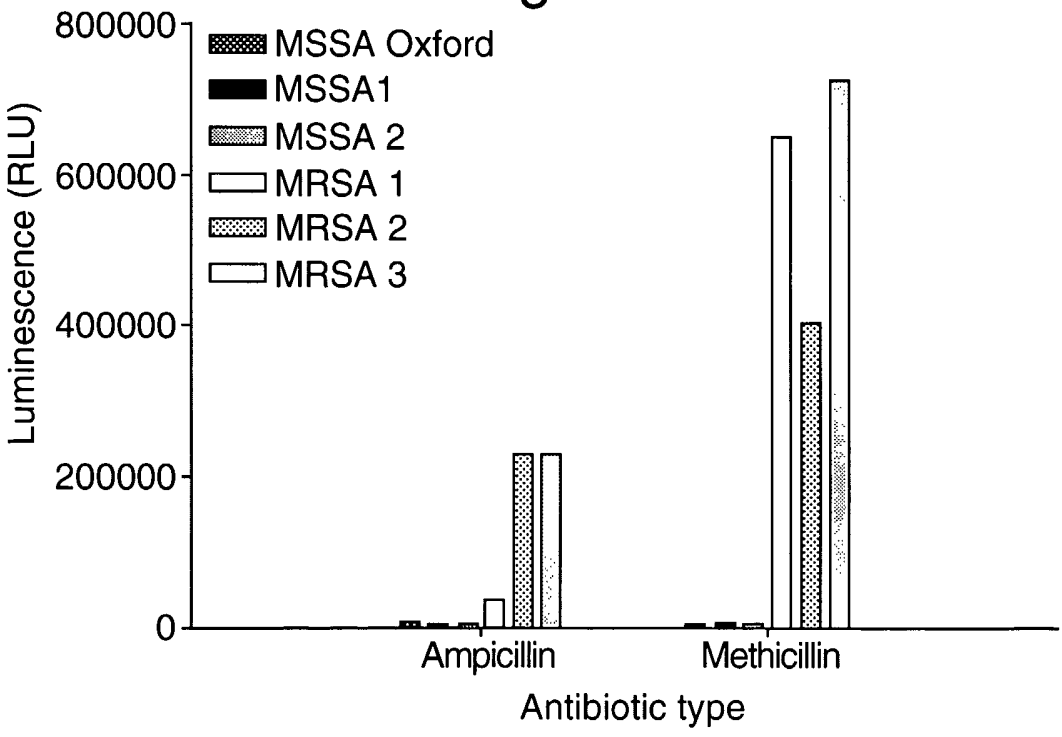
M421148L
Swab number

Fig.3.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200000
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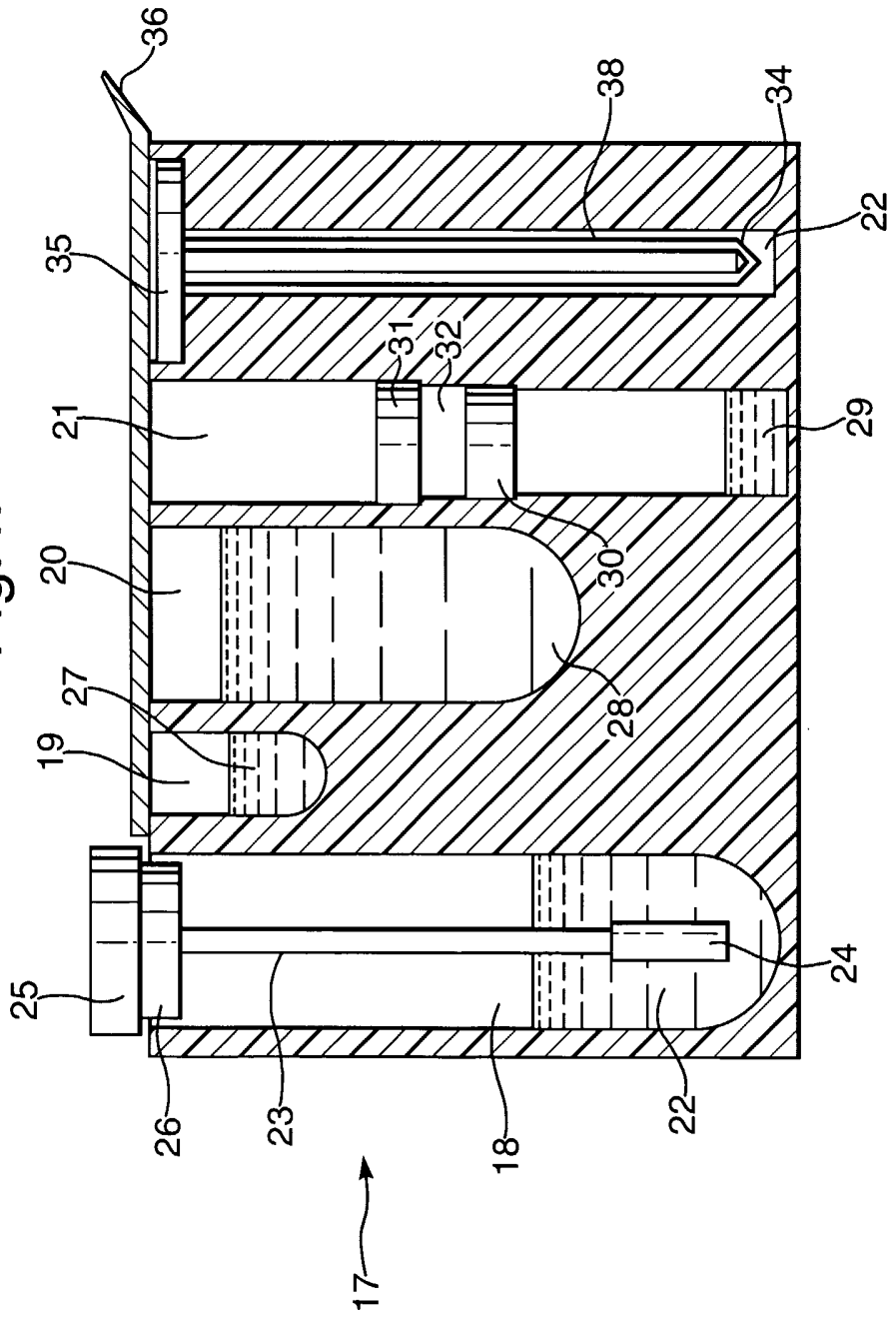
MSSA 2
MRSA 1
MRSA 2
MRSA 3
Ampicillin
Methicillin
Antibiotic type

Fig.4.



INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/GB 02/03990

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/04 C12Q1/00

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)
WPI Data, PAJ, EMBASE, MEDLINE, BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 37799 A (MURPHY MELANIE JANE; PRICE RACHEL LOUISE (GB); SQUIRRELL DAVID JAM) 29 July 1999 (1999-07-29) cited in the application page 6, line 17 -page 7, line 28 page 3, line 20 -page 4, line 25; claims 1-24; figure 4; examples 1-6	14,15,17
Y		16
A		1-13

Y	US 5 695 946 A (BENJAMIN THOMAS L ET AL) 9 December 1997 (1997-12-09) column 4, line 1 -column 8, line 60; claims 1-8; example 1	16
A		1-15,17

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[X] Further documents are listed in the continuation of box C.

[X] 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

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"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

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Date of the actual completion of the international search	Date of mailing of the international search report
4 December 2002	18/12/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stachowiak, O

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Ir ional Application No
PCT/GB 02/03990

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 441 469 A (PROMEGA CORP) 14 August 1991 (1991-08-14) abstract; claims 1-26; figures 1-4; examples 1,2	1-17
A	US 5 798 214 A (SQUIRRELL DAVID JAMES) 25 August 1998 (1998-08-25) page 9, column 2	1-17
A	US 4 647 536 A (MOSBACH KLAUS ET AL) 3 March 1987 (1987-03-03) column 1-2; claims 1-6; example 7	4-6
A	STRANDEN ANNE M ET AL: "Cell wall monoglycine cross-bridges and methicillin hypersusceptibility in the femAB null mutant of methicillin-resistant Staphylococcus aureus." JOURNAL OF BACTERIOLOGY, vol. 179, no. 1, 1997, pages 9-16, XP002222628 ISSN: 0021-9193 page 9, column 2	6
A	WO 99 50441 A (UREN JACK R; HAYDOCK PAUL V (US); SAIGENE CORP (US)) 7 October 1999 (1999-10-07) abstract; claims 1-14	1-17
A	WO 95 16039 A (LENCASTRE HERMINIA DE; TOMASZ ALEXANDER (US); UNIV ROCKEFELLER (US) 15 June 1995 (1995-06-15) abstract; claims 13,14	1-17
A	WO 95 14105 A (ROBERTS MICHAEL STEPHEN; AQUACULTURE DIAGNOSTICS LIMITE (GB)) 26 May 1995 (1995-05-26) claims 1-11	1-17

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 02/03990

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9937799 A	29-07-1999	AU 751388 B2	15-08-2002
		AU 2065399 A	09-08-1999
		BR 9907161 A	24-10-2000
		CA 2318367 A1	29-07-1999
		CN 1294634 T	09-05-2001
		EP 1049798 A1	08-11-2000
		GB 2348702 A	11-10-2000
		WO 9937799 A1	29-07-1999
		HU 0101276 A2	28-08-2001
		JP 2002500892 T	15-01-2002
		NO 20003708 A	21-09-2000
		NZ 505847 A	27-09-2002
US 5695946 A	09-12-1997	US 5491068 A	13-02-1996
		CA 2112603 A1	01-07-1994
		EP 0605003 A2	06-07-1994
		JP 7067693 A	14-03-1995
		AT 189310 T	15-02-2000
		CA 2042726 A1	15-08-1992
		DE 69131939 D1	02-03-2000
		EP 0498920 A2	19-08-1992
		JP 2986961 B2	06-12-1999
		JP 6324039 A	25-11-1994
EP 0441469 A	14-08-1991	AU 6847890 A	25-07-1991
		EP 0441469 A1	14-08-1991
US 5798214 A	25-08-1998	AT 184054 T	15-09-1999
		AU 691940 B2	28-05-1998
		AU 2931995 A	16-02-1996
		BR 9508392 A	26-05-1998
		CA 2194458 A1	01-02-1996
		CN 1157638 A ,B	20-08-1997
		DE 69511876 D1	07-10-1999
		DE 69511876 T2	31-05-2000
		DK 774011 T3	20-03-2000
		EP 0774011 A1	21-05-1997
		ES 2136866 T3	01-12-1999
		WO 9602666 A1	01-02-1996
		GB 2304892 A ,B	26-03-1997
		GR 3031877 T3	29-02-2000
		HU 76447 A2	29-09-1997
		JP 10502815 T	17-03-1998
		NO 970106 A	13-03-1997
		NZ 289464 A	28-07-1998
		ZA 9505846 A	19-02-1996
US 4647536 A	03-03-1987	SE 441009 B	02-09-1985
		AT 32348 T	15-02-1988
		DE 3375581 D1	10-03-1988
		DK 509483 A	07-11-1983
		EP 0102985 A1	03-02-1988
		JP 59500350 T	08-03-1984
		SE 8201401 A	09-09-1983
		WO 8303102 A1	15-09-1983
WO 9950441 A	07-10-1999	AU 3206199 A	18-10-1999
		CA 2325851 A1	07-10-1999

INTERNATIONAL SEARCH REPORT

In ional Application No
PCT/GB 02/03990

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9950441 A		EP 1064396 A1	03-01-2001
		JP 2002509731 T	02-04-2002
		WO 9950441 A1	07-10-1999
WO 9516039 A	15-06-1995	AU 1398295 A	27-06-1995
		WO 9516039 A1	15-06-1995
		US 6063613 A	16-05-2000
		US 6251647 B1	26-06-2001
WO 9514105 A	26-05-1995	WO 9514105 A2	26-05-1995