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(54) Title: ADJUSTABLE TEST SYSTEM FOR THE DETERMINATION OF THE PRESENCE OF AN ANTIBIOTIC IN A FLUID

(57) Abstract: The present invention provides a test system, a test method and a test kit for the determination of the presence of an antibiotic in a fluid based on a test medium comprising a component that binds to an antibiotic. Preferably the component that binds to an antibiotic is an antibody or a penicillin binding protein.

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ADJUSTABLE TEST SYSTEM FOR THE DETERMINATION OF THE PRESENCE OF AN ANTIBIOTIC IN A FLUID

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Field of the invention

The present invention relates to an improved novel test system and method for the determination of the presence of antibacterial compounds in fluids such as milk, meat juice, serum and urine.

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Background of the invention

Microbiological test methods for the determination of antibacterial compounds, particularly residues of antibiotics such as cephalosporin, penicillin, tetracycline and derivatives thereof and chemotherapeutics such as sulfa's, in fluids such as milk, meat juice, serum and urine are known. Examples of such tests have been described in CA 2056581, DE 3613794, EP 0005891, EP 0285792, EP 0611001, GB A 1467439 and US 4,946,777. These descriptions all deal with ready to use tests that make use of a test organism and will give a result by the change indicated by an indicator molecule, for instance a change of color of a pH- and/or redox-indicator, added to the test system. A change in the indicator indicates the presence of a growing test organism. The principle is that when an antibacterial compound is present in a fluid in a concentration sufficient to inhibit growth of the test organism the color of the indicator will stay the same, while, when no inhibition occurs, growth of the test organism is accompanied by the formation of acid or reduced metabolites or other phenomena that will induce an indicator signal.

The known test systems mentioned above include a test medium, such as an agar medium, inoculated with a suitable test organism, preferably a strain of *Bacillus* or *Streptococcus*, and a pH indicator and/or a redox indicator. The suitable test organism and the indicator are introduced into an optionally buffered agar solution, optionally nutrients are added to the solution and optionally substances that change the sensitivity to certain antimicrobial compounds in a positive or a negative way are added to the solution. Finally the agar solution is allowed to solidify to form the test medium in such a way that the test organisms stay alive but cannot multiply because of lack of nutrients

and/or low temperature. Of course a suitable test should have the desired sensitivity with regard to the compounds to be tested for.

The problem with the test systems currently distributed on the market and/or described in the literature is that they do not provide a simple procedure by which the sensitivity towards specific analytes, such as β -lactams, can be adapted. From 5 DE 3613794, EP 0005891 and Galesloot *et al.* (Netherlands Milk and Dairy Journal 16, 1962, 89-95) it is known that certain folate inhibitors such as trimethoprim and tetroxoprim can be advantageously added to antibacterial test systems in order to enhance the sensitivity for the sulfa class of antibacterials, such as sulfadiazine. The 10 principle behind this mechanism is thought to be the synergistic effect between trimethoprim or tetroxoprim on the one hand and sulfadiazine on the other hand. There is however no test system available that allows for adjustment of the sensitivity towards β -lactam antibiotics. For example, a given test system may give an indicator change when the concentration of the β -lactam tested, *e.g.* penicillin G, exceeds a certain 15 detection threshold value, *e.g.* 1 ppb. However, when certain (local) requirements prescribe a higher threshold, or existing thresholds are changed for other reasons, said test systems cannot be easily adapted to a new threshold value. There is thus a need for an improved test method that may not have this problem.

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Summary of the invention

It is an object of the present invention to provide an adjustable test system and method for determining the presence or absence of antibiotics in fluids. We have found 25 that there is a remarkable effect attainable when adding certain proteins to the test medium.

The invention provides a test system for the determination of the presence or absence of an antibiotic in a fluid comprising a test medium comprising a test organism, at least one substance that provides a solid state, and an indicator, characterized in that 30 a component is present outside said test organism that binds to an antibiotic.

Furthermore, the invention provides a method for determining the presence or absence of an antibiotic in a fluid comprising:

(a) contacting a fluid sample with a test medium comprising a test organism and

at least one indicator;

- (b) incubating the test organism with the fluid under conditions whereby growth of the test organism occurs if no antibiotic is present in the fluid sample; and
(c) detecting any growth or inhibition of growth of the test organism as appropriate
5 by means of an indicator,

characterized in that a component that binds to said antibiotic is added to the fluid sample and/or the test medium in step (a) and/or to the fluid in step (b).

Furthermore, the invention provides a kit suitable for the determination of the presence or absence of an antibiotic in a fluid comprising:

- 10 (a) at least one container partially filled with a test medium comprising a thermophilic test organism, at least one substance that provides a solid state, nutrients and an indicator, and;
(b) a device for adding fluid to the test medium,

characterized in that in said test medium a component is present outside said test
15 organism that binds to an antibiotic.

Additionally, the invention provides the use of a component that binds an antibiotic in an assay for the detection of an antibiotic.

20 **Detailed description of the invention**

The terms and abbreviations given below are used throughout this disclosure and are defined as follows.

25 The term 'CFU' is an abbreviation of Colony Forming Units and refers to the number of organisms, spores of organisms, partially germinated spores of organisms or vegetative cells capable of producing colonies of organisms.

The term 'component' refers to a compound, or to a compound as part of a mixture, that binds to a certain species such as an analyte. Preferably said binding is specific, meaning that binding occurs only with one species or with one genus. Binding
30 can take place through covalent attachment, formation of hydrogen bridges, electronic or ionic interactions, encapsulation and similar phenomena. Examples of components within the scope of the present invention are antibodies, antigens, catalysts, clathrates,

complexing agents, enzymes, polymers, polynucleotides, polysaccharides, proteins, zeolites and the like, or multi-particle mixtures comprising said components.

The term 'fluid' refers to a substance (as a liquid, not a gas) tending to flow or conform to the outline of its container.

5 The term 'gelling agent' refers to a compound that assists in changing a mixture into or taking on the form of a gel.

The term 'indicator' refers to a substance used to show (for example by change of color or fluorescence) the condition of a mixture such as a solution or a gel with respect to the presence of a particular material (for example an acid, a base, oxidizing or
10 reducing agents). For instance, the term 'indicator' may refer to one or more compounds that are known as pH-indicators, but also to one or more compounds that are known as redox-indicators. Also, the term 'indicator' may refer to mixtures of two or more different types of indicators, such as a combination of a pH- and a redox-indicator.

The term 'lateral flow binding assay' refers to an assays based on the binding
15 reaction between an antigen and its complementary antibody; such assays are also known as immunochromatographic assays. However, the term lateral flow binding assay also encompasses assays that are based on the recognition and/or binding of an analyte to any suitable binding particle which can be either natural or non-natural, for example a protein suitable for binding; not necessarily an antibody. Lateral flow binding assay
20 devices generally comprise a fluid sample receiving section, an analyte detection section and an absorption section that are all attached to one side of an essentially flat surface, such as a strip, usually made of inert material such as glass, metal or preferably a plastic. The principle of this assay is visualization of the binding between analyte and a suitable binding particle in the analyte detection section. To this end, a label can be
25 attached to the suitable binding particle. Examples of compounds that are used as label are compounds that produce a visual signal in the analyte detection section, such as dyes (*i.e.* chromogenic or fluorescent dyes), certain metal particles (*i.e.* gold sols), other colored particles (*i.e.* latex particles), or particles based on other means of detection, such as radioactive compounds. In order to achieve visualization, the analyte detection
30 section contains a capture site where the complex between analyte and the suitable binding particle is retained, for instance by means of an immobilized antigen. Also, there are many examples wherein more than one caption sites with different functionalities are present in the analyte detection section. Alternatively, the assay may be designed in such

a way that visualization only occurs when there is no binding between analyte and the suitable binding particle, *i.e.* in the case when there is no analyte present in the sample. As outlined above, an essential step in lateral flow binding assay technology is contacting the sample to be analyzed with a suitable binding particle to which in most cases a label is attached.

The term 'nutrient' refers to one or more nutritive substances or ingredients that promote and/or are required for the growth of test organisms as used in the method of the present invention.

The term 'spore' refers to a primitive usually unicellular often environmentally resistant dormant or reproductive body produced by plants or organisms and which is capable of development into a new individual.

The term 'test medium' refers to a solid composition, preferably in the form of a sol or a gel, which may comprise a gelling agent. Suitable examples of gelling agents are agar, alginic acid and salts thereof, carrageenan, gelatin, hydroxypropylguar and derivatives thereof, locust bean gum (Carob gum), processed eucheuma seaweed and the like. However, the person skilled in the art will understand that other types of solid test media may be based on carrier materials such as ceramics, cotton, glass, metal particles, paper, polymers (in any shape or form), silicates, sponges, wool and the like.

Usually, a test medium contains one or more indicators, however, these compounds may also be added during the test method. The test medium comprises one or more types of test organisms as detecting agents. Optionally, the test medium may also contain nutrients, stabilizers, and/or viscosity-increasing agents. Examples of viscosity-increasing agents are ascorbyl methylsilanol pectinate, carbomer, carboxymethyl cellulose, cetaryl alcohol, cetyl alcohol, cetyl esters, cocamide DEA, emulsifying wax, glucose, hydroxyethyl cellulose, hydroxypropylmethyl cellulose, lauramide DEA, linoleamide DEA, magnesium aluminum silicate, maltodextrins, PEG-8 distearate, polyacrylamide, polyvinyl alcohol, PVP/hexadecene copolymer, sodium chloride, sodium sulfate, soyamidopropyl betaine, xanthan gum and the like. Alternatively, the optional ingredients of the test medium mentioned above may be added during the test method.

The term 'threshold' refers to the concentration value above which a given analyte is to be regarded as present and below which said analyte is to be regarded as absent. Generally, a threshold value is given for particular analytes in particular samples by local,

regional or interregional authorities but it can also be pre-set for certain research purposes.

In a first aspect of the invention there is provided a test system that comprises a test medium. The test medium comprises a test organism, nutrients, a substance that provides a solid state, at least one indicator and a component, present outside said test organism, which binds to an antibiotic.

Suitable test organisms are thermophilic test organisms, examples of which are *Bacillus* species, preferably *Bacillus stearothermophilus*, *Escherichia coli* species, or *Streptococcus* species, preferably *Streptococcus thermophilus*. These species may be introduced in the test as units capable of producing colonies, or Colony Forming Units (CFU's). Said CFU's may be living test organisms, spores, vegetative cells or any mixture of two or more of these units. The concentration of said CFU's is expressed as Colony Forming Units per ml of test medium (CFU.ml⁻¹) and is usually in the range of 1 x 10⁵ to 1 x 10¹² CFU.ml⁻¹, preferably 1 x 10⁶ to 1 x 10¹⁰ CFU.ml⁻¹, more preferably 2 x 10⁶ to 1 x 10⁹ CFU.ml⁻¹, most preferably 5 x 10⁶ to 1 x 10⁸ CFU.ml⁻¹, or still more preferably 5 x 10⁶ to 2 x 10⁷ CFU.ml⁻¹.

Suitable nutrients are carbon-sources and nitrogen-sources of which many commercially available variants exist. Typical constituents are amino acids, mono-saccharides, oligosaccharides vitamins and the like.

The skilled artisan will appreciate that many indicators are suitable for the purpose of the present invention. Particularly useful are indicators that, upon changing from one state to the other, provide a visually detectable signal such as a change in color or fluorescence. The amount of indicator in the test medium is between 0.01 and 50 g.l⁻¹ test medium, preferably between 0.1 and 10 g.l⁻¹, more preferably between 0.5 and 5 g.l⁻¹, most preferably between 1 and 3 g.l⁻¹. Such indicators may be easily selected from handbooks such as 'H.J. Conn's Biological Stains', R.D. Lillie ed., Baltimore, 1969. Preferred indicators are pH-indicators and/or redox indicators. Examples of suitable indicators are Acid Blue 120, Acid Orange 51, Acid Yellow 38, Alizarin acid, Alizarin Blue, Azure A, Azure B, Basic Blue 3, Brilliant Black, Brilliant Cresyl Blue, Brilliant Crocein MOO, Brilliant Yellow, Bromocresol Green, Bromocresol Purple, Bromophenol Blue, Bromophenol Red, Bromothymol Blue, Chlorocresol Green, Congo Red, m-Cresol Purple, Gallocyanine, Indigo Carmine, Janus Green B, Litmus,

Methylene Blue, Nile Blue A, Nitrazol Yellow (also referred to as Nitrazine Yellow), *o*-Nitrophenol, *p*-Nitrophenol, 1-10 Phenanthroline, Phenolphthalein, Safranin O, Thionin, Thymol Blue, Toluidine Blue and Xylenol Blue.

Preferably, the substance providing for a solid state is a gelling agent and/or a carrier material. The amount of gelling agent in the test medium is between 1 and 200 g.l⁻¹ test medium, preferably between 2 and 50 g.l⁻¹, more preferably between 5 and 20 g.l⁻¹, most preferably between 7 and 15 g.l⁻¹. Preferred gelling agents are agar and gelatin.

The test system of the present invention comprises a test medium in which a component is present that binds to an antibiotic. Such components are usually present within organisms, also within test organisms that are normally used in test media suitable for the determination of the presence of an antibiotic in a fluid. In contrast however, in the present invention said component is also present exogenously, *i.e.* not as part of the contents of an intact test organism. Said component advantageously binds to an antibiotic for which the test system is designed, thus enabling adjustment of the test system by tuning the amount of said component present in the test medium. Different test media can thus be made depending on the required application and/or desired threshold values. The preferred antibiotic is a β -lactam antibiotic and thus the preferred component is a component that binds to β -lactam antibiotics. Suitable β -lactam antibiotics are cephalosporin and penicillin derivatives. Examples of such derivatives are amoxicillin, ampicillin, cefadroxil, cefradine, ceftiofur, cephalexin, penicillin G, penicillin V and ticarcillin. Suitable components that bind to said β -lactam antibiotics are proteins, particularly antibodies and the so-called cephalosporin binding proteins and penicillin binding proteins. The latter are, amongst others, obtainable from test organisms according to well-known procedures. The form in which said component is present in the test medium may vary widely. The component may be a crystalline or amorphous product obtained from purification procedures. Generally the purity of such components is anywhere between 10% and 100%. Alternatively, the component may be present in the form of lysed cells or other mixtures obtainable from disruption of cell walls of test organisms. If the component that binds to an antibiotic is a protein, such as an antibody or a penicillin binding protein, possible concentrations are between 0.1 and 100 mg.l⁻¹ (expressed as weight of active protein per volume of test medium), suitable concentrations are between 0.2 and 50 mg.l⁻¹, preferred concentrations are between

0.3 and 25 mg.l⁻¹, more preferred concentrations are between 0.5 and 10 mg.l⁻¹, and the most preferred concentrations are between 1.5 and 5 mg.l⁻¹.

In a second aspect of the invention, there is provided a method for determining the presence or absence of an antibiotic in a fluid comprising the steps of contacting a fluid
5 sample with a test medium comprising CFU's of a test organism and at least one indicator. The method of the present invention comprises the addition of a component that binds to an antibiotic, as described above in the first aspect of the invention. Such a component may be present in the test medium, but may also be added to the sample
10 fluid prior to or during testing.

The system may also comprise nutrients. Preferably, the test medium is a sol or gel comprising a gelling agent and/or a carrier material. Advantageously, the method also provides for conditions whereby there is minimal growth of a test organism prior to the addition of fluid sample. Such conditions comprise an unfavorable temperature and/or an
15 unfavorable pH-value and/or the absence of nutrients essential for growth, provided these conditions do not cause irreversible damage to all CFU's present. After addition of the fluid sample, growth of the test organism is allowed to take place during a period sufficiently long for the test organisms to grow in case no antibiotic is present. Growth is encouraged by adding nutrients, optionally before the contacting of said fluid sample,
20 and/or raising the temperature, and/or providing for a pH-value at which the test organism is able to grow. Alternatively, these conditions may be established prior to contact of the fluid sample with the test medium. Growth of the test organism is detected by observing the presence or absence of a change of the indicator.

The method of the present invention also includes mixing samples (*e.g.* with other
25 samples, but also with salts, buffering compounds, nutrients, stabilizers, isotope-labeled compounds, fluorescence-labeled compounds and the like), concentrating and/or diluting (*e.g.* with diluting liquids such as water, milk or liquids derived from milk, blood or liquids derived from blood, urine and/or solvents) samples prior to addition to the test medium.

In one embodiment of the present invention, the antibiotic is a β -lactam antibiotic
30 such as a cephalosporin or a penicillin derivative. Examples of such derivatives are amoxicillin, ampicillin, cefadroxil, cefradine, ceftiofur, cephalixin, penicillin G, penicillin V and ticarcillin, but of course many other similar β -lactam derivatives are known and applicable in the method of the present invention. Advantageously, it was established that

the method of the present invention displays adjustability of the sensitivity for β -lactam antibiotics. The sensitivity for these compounds could be adjusted whilst simultaneously the sensitivity for other types of antibiotics and/or chemotherapeutics such as sulfa's remained virtually unchanged. This phenomenon is of utmost importance in test systems
5 where a change of sensitivity for one analyte is called for, whereas the sensitivity for another analyte is already satisfactory, a situation that occurs quite frequently in practice.

In another embodiment of the method of the invention, the test organism is incubated for a predetermined period, preferably within a time span of 0.5 to 4 hours, more preferably between 0.75 to 3 hours, most preferably between 1.0 to 2.75 hours.
10 Preferably the test organism is incubated at a predetermined temperature, preferably the optimal growth temperature of the test organism. When, for example, thermophilic test organisms are used, said temperature is preferably between 40 and 70°C, more preferably between 50 and 65°C, most preferably between 60 and 64°C. Optionally said reaction can be carried out with the aid of a thermostatic device. Alternatively, the time
15 required for growth of the test organism is equal to the time that is required for a calibration sample with a known amount of analyte(s) to induce a change in the indicator.

The presence or absence of an antibiotic is determined by the presence or absence of a change of the indicator or indicators used. When, for example such a
18 change is a color change, said color change may be observed visually. However in one
20 embodiment of the invention said color change is determined using an arrangement that generates digital image data or an arrangement that generates analog image data and converts said analog image data into digital image data followed by interpretation of said digital image data by a computer processor. Such an arrangement, which may for instance be a sample-reading device such as a scanner coupled to a personal computer,
25 is described in International Patent Application WO 03/033728, incorporated by reference, and briefly summarized below.

The arrangement can be used for detecting residues of antibiotics in milk. The commercially available Delvotest[®] and BR[®]-test are commonly used. Delvotest[®] comprises an agar matrix, CFU's of an acid forming test organism, as well as a color
30 indicator. With the arrangement mentioned above it is possible to automatically scan the bottom side of each of the samples in a test plate. The color and the brightness of the reflected light are registered in three variables, each describing one color component, for instance the so-called L*a*b* model. In the L*a*b* model, the color spectrum is divided in

a two-dimensional matrix. The position of a color in this matrix is registered by means of the two variables "a" and "b". The variable L indicates the intensity (for instance, from light blue to dark-blue). It is possible to make a criterion comprising the a-value, b-value and L-value to make a composite function as follows:

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$$Z = w_L \cdot L + w_a \cdot a + w_b \cdot b$$

where w_L , w_a and w_b are weighting factors for the L-value, a-value and b-value, respectively. The values of these weighting factors can be calculated by means of "discriminant analysis", such that the group mean shows a maximum distance in relation to the spreading. By combining two or more of the color components in the L*a*b* model in a predetermined manner that depends on the type of residue and the sample, an accurate detection is possible. In practice, a certain value of Z at which a test should switch between positive and negative result (the threshold value) can be experimentally predetermined.

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In a third aspect of the invention there is provided a kit for carrying out the method of the second aspect of the present invention. Such a kit comprises one or more containers filled with test medium as described in the first aspect of the invention and optionally a sampling device. The containers may be test tubes of any shape and size and from any material available, provided that observation of indicator changes is possible. Also, the containers may be wells such as those incorporated in micro-titer plates. Said sampling device is a device with the aid of which fluid can be added to said test medium. Preferably, such a device is a container, optionally with volume markings. More preferably, such a device is a syringe, a pipette or an automated pipetting system. Such a syringe or pipette may be designed in such a fashion that with only one mode of operation a predetermined volume can be withdrawn from the fluid to be analyzed. Optionally, systems known in the art with which more than one syringe or pipette can be operated with one single handling may be applied. It is the object of the third aspect of the present invention to provide a kit that allows for simple addition of the amounts of fluid to be added according the second aspect of the invention. Optionally, said kit comprises means for sealing of said containers filled with test medium during incubation and/or an insert with instructions for use and/or a means for setting the time needed for incubation.

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In another embodiment of the third aspect of the present invention, said kit comprises a thermostatic device, with the aid of which test samples can be kept at a pre-set temperature, such as the temperature at which the test organism shows sufficient growth. Preferably, said thermostatic device is designed in such a fashion that it
5 can hold said containers filled with test medium. Optionally the thermostatic device is coupled to a means for setting the time needed for incubation such that heating and/or cooling is stopped after lapse of a pre-set period.

In yet another embodiment of the third aspect of the invention, said kit comprises a data carrier loaded with a computer program suitable for instructing a computer to
10 analyze digital data obtained from a sample-reading device. Said data carrier may be any carrier suitable for storing digital information such as a CD-ROM, a diskette, a DVD, a memory stick, a magnetic tape or the like. Advantageously, said data carrier loaded with a computer program provides for easy access to the latest available computer programs suitable for use in the method of the present invention.

15 In a fourth aspect of the present invention there is provided the use of a component that binds an antibiotic in an assay for the detection of an antibiotic. Suitable components are those that are mentioned in the first aspect of the invention. The component may be used in a microbiological test system as described above. However,
20 the component may also be used in a lateral flow-binding assay. Lateral flow binding assays are used for the detection of analytes in fluids. The analytes to be tested can be of various origins, for example antibiotics, bacteria, carbohydrates and hormones. A well-known example of such an assay is the pregnancy test. Other examples can be found in the area of antibiotic assays, such as meat, milk or urine tests as described in
25 US 5,434,053.

In one embodiment of the fourth aspect of the invention, the component is preferably a Penicillin Binding Protein (PBP). Said PBP is added to the sample to be analyzed and/or is incorporated in the lateral flow-binding assay with the aim of binding
30 certain amounts of β -lactam antibiotics. It is known that different PBP's exist that are structurally closely related and all have different affinities towards β -lactam antibiotics. Preferably different PBP's are added to the sample to be analyzed and/or are incorporated in the lateral flow-binding assay. By using the PBP's in this fashion, the presence of certain antibiotics can be wholly or partly nullified with respect to other

antibiotics. Hence, tests that have different sensitivities towards different analytes can be easily made available.

EXAMPLES

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Example 1

Sensitivity of a microbiological test for different antibiotics as a function of the amount of Penicillin Binding Protein present in the test

Commercially available Delvotest[®] MCS, prepared according to the methods described in EP 0005891, was adapted by the addition of various concentrations of Penicillin Binding Protein (PBP, obtained by centrifuging lysed cells of *Bacillus stearothermophilus*, applying the obtained supernatant to a 7-ACA Affi-gel 10 matrix, followed by washing of the matrix and eluting with a 0.8 M solution of hydroxylamine in water, followed by dialysis and concentration). PBP concentrations of 0, 0.6, 1.6, and 3.2 mg.l⁻¹ test medium were investigated (see Table below).

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Antibiotic	Penicillin Binding Protein (in mg.l ⁻¹ test medium)			
	0	0.6	1.6	3.2
<u>β-Lactams:</u>				
Amoxicillin	4	4	4-6	6-8
Ampicillin	4	4-5	5-6	6-8
Penicillin G	2	2-3	3	4-5
<u>Non-β-lactams:</u>				
Neomycin	400	400	400	400
Spiramycin	400	400	400	400
Sulfadiazine	100	100-200	100	100
Tetracycline	200	200	200	200

Table Effect of different concentrations PBP on the sensitivity of microbial inhibition tests towards different antibiotics. Experiments were read after 2.25 h; observed sensitivities are the threshold concentrations of antibiotic in ppb at which the test medium no longer changes color (*i.e.* tests that are positive for that antibiotic).

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The sensitivities for various antibiotics were determined by investigating samples containing different concentrations of antibiotic in different test systems. After the sample was added, the tests were incubated for 2.25 hours at 64°C and the concentration ranges of antibiotics were visually inspected with regard to color changes. If there are no or little antibiotics that inhibit the growth of the test organism, acid is formed by the growing test organisms. Then, the color of the indicator changes from blue/purple to yellow. However, if there are sufficient antibiotics to inhibit that growth, the color of the indicator does not change and remains purple. The concentration of antibiotic at which there no longer occurred a change of color was determined as threshold value. The results clearly show that sensitivity towards β -lactam antibiotics can be adjusted by the addition of PBP whereas the sensitivity towards other classes of antibiotics remains unchanged.

Example 2

Sensitivity of a microbiological test for different β -lactam antibiotics as a function of the amount of Penicillin Binding Protein present in the test

Example 1 was repeated with the exception that the incubation time was 3.17 h. Furthermore, only β -lactam antibiotics and only PBP concentrations of 0 and 3.2 mg.l⁻¹ test medium were investigated. Again, the results as outlined in the Table below clearly show that sensitivity towards β -lactam antibiotics can be adjusted by the addition of PBP.

Antibiotic	Penicillin Binding Protein (in mg.l ⁻¹ test medium)	
	0	3.2
<u>β-Lactams:</u>		
Amoxicillin	3	4
Ampicillin	< 3	5
Penicillin G	2	> 5

Table Effect of different concentrations PBP on the sensitivity of microbial inhibition tests towards different β -lactam antibiotics. Experiments were read after 3.17 h; observed sensitivities are the threshold concentrations in ppb at which the test medium no longer changes color (*i.e.* tests that are positive for that antibiotic).

CLAIMS

1. Test system for the determination of the presence or absence of an antibiotic in a fluid comprising a test medium comprising a test organism, at least one substance that provides a solid state, and an indicator, characterized in that a component is present outside said test organism that binds to an antibiotic.
2. Test system according to claim 1, wherein said test organism is a living test organism.
3. Test system according to any one of claims 1 to 2, wherein said component is present in a concentration higher than or equal to 0.5 mg per liter of said test medium.
4. Test system according to any one of claims 1 to 3, wherein said component is a protein.
5. Test system according to claim 4, wherein said protein is an antibody and/or a penicillin binding protein.
6. Test system according to any one of claims 1 to 5 wherein said test organism is selected from the group consisting of *Bacillus* species, *Escherichia* species and *Streptococcus* species.
7. A method for determining the presence or absence of an antibiotic in a fluid comprising:
- (a) contacting a fluid sample with a test medium comprising a test organism and at least one indicator;
 - (b) incubating the test organism with the fluid under conditions whereby growth of the test organism occurs if no antibiotic is present in the fluid sample; and
 - (c) detecting any growth or inhibition of growth of the test organism as appropriate by means of an indicator,
- characterized in that a component that binds to said antibiotic is added to the fluid sample and/or the test medium in step (a) and/or to the fluid in step (b).

8. A method according to claim 7, wherein said component is added in the form of a suspension of dead and/or lysed test organisms comprising penicillin binding protein.

5 9. A method according to any one of claims 7 to 8, wherein said component is a protein.

10 10. A method according to claim 9, wherein said protein is an antibody and/or a penicillin binding protein.

11. A method according to any one of claims 7 to 10, wherein the fluid sample comprises a fluid obtainable from an animal or human body.

12. A method according to claim 11, wherein the fluid is milk.

15 13. A method according to any one of claims 7 to 12, wherein the conditions for growth of the test micro-organism comprise:

- (a) adding nutrients; and/or
- (b) incubating at an appropriate temperature; and/or
- 20 (c) incubating for a sufficient period of time.

14. Kit suitable for the determination of the presence or absence of an antibiotic in a fluid comprising:

- 25 (a) at least one container partially filled with a test medium comprising a thermophilic test organism, at least one substance that provides a solid state, nutrients and an indicator, and;
- (b) a device for adding fluid to the test medium,

characterized in that in said test medium a component is present outside said test organism that binds to an antibiotic.