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(57) Abstract

An improved test device for the detection of microorganisms and their susceptibilities to inhibitory agents, e.g., antibiotics, in sample specimens. The device is adapted to be inoculated by immersion in a specimen and to thereafter culture microorganisms therein by incubation in a sealable container. The device generally includes an absorbent matrix impregnated with a nutrient medium containing test reagents, an inhibitory agent or several different inhibitory agents localized in distinct regions in the matrix, and a culture-fixing agent for localizing colony location throughout the culturing period. This invention is particularly useful in cases involving urinary tract infection.

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TESTING DEVICE FOR DETERMINING THE SUSCEPTIBILITY OF MICROORGANISMS TO INHIBITORY AGENTS

Field of the Invention

The present invention is directed generally to an improved means for assessing the susceptibility of microorganisms to inhibitory agents in specimen samples, e.g., biological fluids. More specifically, the present invention combines semi-quantitative detection of microorganisms and their susceptibility to an inhibitory agent(s) into a single, simple reagent strip test.

Background of the Invention

Assays of liquid specimens for the presence of microorganisms are commonly performed in medicine, industry and by governmental agencies to determine the degree and type of microbiological contamination in various sample specimens. In many assays of this type, the presence of microorganisms is frequently of secondary importance or already known. Of paramount concern is the susceptibility of the microorganism or microorganisms in the specimen to agents intended for their control / eradication.

For example, early and accurate treatment with a suitable antibiotic in cases of infection, e.g., bacteriuria or mastitis, can prevent the development of more serious conditions, e.g., pyelonephritis or udder necrosis. Likewise, effective disinfection in the case of contaminated systems, e.g., water supply can reduce disease and economic losses. Hence, there is a need for a simple, reliable and inexpensive procedure for rapid determination of an agent's efficacy in combating microorganisms, which procedure avoids the problems presented with known procedures.

Presently, the most conventional test for the determination of the susceptibility of microorganisms to various antibiotics is a two-step test. First, a sample containing the pathogen is incubated in a conventional culture medium and allowed to grow. Next, the antibiotic susceptibility of the pathogen is measured by placing multiple small paper discs impregnated with various antibiotics, or alternatively various concentrations of antibiotic, on the culture medium containing the pathogen. After a second incubation period with the discs, antibiotics efficacy is indicated by the degree of growth inhibition found around the discs. Depending upon the absolute

magnitude of this so-called zone of inhibition, the microorganisms in the sample are classified as being either:

1) sensitive
2) intermediately sensitive or
3) resistant to the antibiotic in the disc, whereby the criteria are peculiar to each type of antibiotic disc, such that interpretation relies on accurate measurement of the diameter of the zone of inhibition for each disc.

The disadvantages inherent with the foregoing two-step test is the considerable assay time required of a dual incubation process, the need to prepare a culture medium, such medium's brief shelf life even under refrigerated storage, and the need for a considerable quantity of clean and sterilized laboratory equipment. In addition, considerable technical skill in conducting and interpreting the test is required. There is a need for a simple one-step test than can be performed quickly and without the need to independently prepare and/or store at < 4°C culture media.

Ericcson (U.S. Patent No. 5,028,529) teaches a variation on the two-step disc test. Ericcson's test uses a single rectangular carrier strip containing a gradient of a compound instead of multiple discs differing in concentration. The strip is placed on a culture medium inoculated with a cultured sample containing micro-organisms. Measurement of the growth inhibition zone's curve in the culture medium is used to determine the optimum antibiotic concentration necessary for treatment.

Though Ericcson's test uses a single strip, the test itself consists of multiple components and requires independent culturing of the microorganisms prior to testing for antibiotic efficacy.

Another method (Blume, U.S. Patent. No. 3,925,166), utilized to test antibiotic efficacy against microorganisms, involves the use of a sample plate with channels containing different concentrations and types of antibiotics. An agar solution containing a microbial sample is added into the channels and the plate is incubated. Bacterial colonies growing in the channels are counted and compared to determined which antibiotics best inhibit microbial growth.

While the latter method may be considered an improvement over the first described method because only one incubation period is required, the testing process still requires wet media, sterilized equipment, and greater technical skill than a one-step dry test would require. Also, the equipment must be cleaned and resterilized before each use.

Determination of potential resistance of microorganisms to disinfectants, e.g., on inanimate surfaces, has also conventionally relied on inoculation of wet media, where efficacy is demonstrated by comparing samples collected before and after application of the agent. In addition to the aforementioned disadvantages, improper physical application of an otherwise effective agent could lead to incorrect conclusions on its inherent suitability in any given case.

Given the limitations of the prior known art, there is clearly a need for a simplified, dry media, single step test which will identify both the presence of microorganisms and their susceptibility to inhibitory agent(s).

Summary of the Invention

The testing device of this invention provides a bibulous matrix impregnated with a nutrient medium containing test reagents, an inhibitory agent or several inhibitory agents immobilized in distinct areas within the matrix, and a culture-fixing agent. A substrate and container may be provided to form an integral test device. In use, the impregnated matrix is dipped momentarily into, or otherwise saturated with, liquid to inoculate the matrix which absorbs a known volume of sample. The inoculated device is then incubated in a sealable container for a period of up to forty-eight hours. Microorganisms present on the matrix will be inhibited from propagating and forming distinct colonies if the test agent in the matrix is effective against those microorganisms. Efficacy is measured by comparing colony formation within impregnated regions against a control matrix or region of matrix containing no inhibitory agent.

This unitized system consisting of both growth medium and inhibitory agent co-existing in a single phase allows testing fluids not only for the presence of microorganisms, but also to detect efficacy of an inhibitory agent against said microorganisms for the purposes of their control / eradication. Furthermore, the

present invention provides a simple and inexpensive test that is less generative of waste as compared to currently utilized procedures.

Brief Description of the Drawings

Fig. 1 is an elevational view of a test device of this invention enclosed within a container.

Fig. 2 is an enlarged fragmentary sectional view of the device of Fig. 1 removed from the container, said view being taken along the line 2 - 2 of Fig. 1, and Figs. 3, 4, 5, and 6 are plan views of device matrices showing colony locations in conjuntions therewith. Fig. 7 is a plan view of a matrix in which no colonies have grown.

Description of the Preferred Embodiment

Referring to the drawings, and particularly to Fig. 1, the test device of this invention is indicated generally by the numeral 10. The test device 10 generally includes absorbent control and test matrices 11c & 11t, both attached to a substrate 12 enclosable within a sealable container 13.

The present invention provides for a test device for analyzing sample specimens, such as liquids, for microorganisms and their susceptibility to inhibitory agents. Although any specimen sample suspected of having microorganisms therein (e.g., food, tissue, ground water, cooling water, pharmaceutical products, sewage, etc.) can be assayed, the invention is particularly useful for bacterial detection in aqueous liquids, such as human and animal biological fluids (e.g., urine, spinal fluid, blood, milk and the like, as well as stool secretions) and suspensions of animal or human tissues. The preferred biological fluid used in practicing this invention is urine (diluted or undiluted).

A similar device is described in U.S. Patent No. 3,881,993 and herein incorporated by reference. However, the test device described in Patent No. 3,881,993 does not allow for the determination of susceptibility to inhibitory agents. The present invention incorporates one or more inhibitory agent(s) into the matrix allowing for

detection of its (their) efficacy, so crucial for treatment of disease or for effective disinfection.

The matrices 11c & 11t are formed from a flat bibulous material, such as absorbent filter paper or the like, impregnated with a suitable nutrient medium, a reagent, and a culture-fixing agent. The impregnation mixture for matrix 11t includes an inhibitory agent(s) as well. The matrices are adapted to be inoculated by dipping them into the sample specimen to be assayed and absorbing a portion thereof, thereby obviating the necessity to measure and/or dilute the sample. It is necessary for the matrix to have a known constant absorption capacity for purposes of semi-quantitative determinations. Thus, if each matrix is known to absorb a known volume of the sample, i.e., 0.1 milliliter, the concentration of microorganisms in the sample and their susceptibility to the inhibitory agent(s) at a particular concentration can be easily determined, as hereinafter described.

It should be understood that upon rehydrating the bibulous matrices in a liquid sample, specific and equal volumes of the sample are absorbed by each matrix and any microorganisms present in the absorbed sample are likewise absorbed by the matrices. In this manner the matrices are inoculated. When the inoculated matrices, impregnated with suitable nutrient media, are incubated, the microorganisms are cultured and form colonies, unless inhibited by agent(s) present in the matrix, if any.

For the purpose of this disclosure, the term "microorganisms" refers to the general class of microorganism comprising yeast, other fungi, bateria, protozoa, etc. present in the sample to be analysed. The term "colony" refers to the "microorganisms" subsequent to culturing. The expression "colony location" indicates the site of a colony as observed on the matrices and is synonymous, for the purpose of this disclosure, with the term colony.

The nutrient medium can be any conventional medium, or modification thereof, known to provide a suitable environmental for the selected test. For semi-quantitative testing, a general nutrient medium such as Bacto Brain Heart Infusion, commercially available from Difco Laboratories, Detroits, Michigan, USA is preferred. This type of medium is considered a general medium and is capable of culturing most microorganisms.

The reagent preferably includes an indicator capable of providing a color change in the matrix in response to a positive culturing of microorganisms. The reagent may be included in the nutrient medium and may comprise a simple pH indicator such as phenol red or the reagent may include a reduction-oxidation type indicator such as one of the various tetrazolium compounds, i.e., 2,3,5-triphenyltetrazolium chloride (colorless), which compound is reduced to a formazan, i.e., triphenyl-tetraformazan (intense red), by most microorganisms during their growth. In preparing the matrices for a semi-quantitative test, triphenyltetrazolium as an indicator is preferred, since the reduced product, triphenyltetraformazan, not only provides a bright visible color, it also has the characteristics of being insoluble. The insoluble property, combined with the hereinafter described culture-fixing agent, facilitates the formation of separate and distinct colony locations.

The addition of a culture-fixing agent to the impregnation formulations is necessary to localize motile microorganisms in the matrices subsequent to inoculation. The ability of the culture-fixing agent to localize the microorganisms, and thereby enable the formation separate and distinct colonies in and on the matrix, is particularly important to enable the cultured colonies to be counted for purposes of performing meaningful semi-quantitative tests. The fixing agent is used in the impregnated formulation in concentrations ranging from about 0.1 to about 5.0 percent by weight, with a range of about 0.5 to about 3.0 percent by weight preferred.

The fixing agent is characterized as being soluble in an aqueous solution to form a viscous colloidal suspension. Fixing agents found to be suitable, either alone or in combination for this purpose, are inert gums, such as carrageens and various linear polysaccharides or alginates.

As discussed, this invention is distinguishable from the prior art by virtue of one or more inhibitory component(s) added to the bibulous matrix. The addition of the inhibitory component(s) allows its (their) suitability for the task at hand, e.g., antibiotic susceptibility, to be determined in one simple test. Presently known antibiotic efficacy tests require long incubation periods, multiple phases and highly trained personnel. The present invention incorporates such efficacy testing into a single, simple diagnostic test.

For the purpose of this disclosure, the term "inhibitory agent" refers to any compound capable of killing or inhibiting the growth of a microorganism. Preferred inhibitory agents include classical groups of antibiotics such as the aminoglycosides, beta-lactams, cephalosporins, chloramphenicols, macrolides, nitrofuranes, sulfonamides, tetracyclines, quinolones and any derivatives thereof, e.g., salts, adducts, etc. In addition, "inhibitory agents" refers to any chemical substance which is active against microorganisms, such as disinfectants, anti-mycotics, antiviral agents, and anti-tuberculosis agents. However, it should be understood that not all inhibitory agents are operable for this purpose. By way of example, the determination of whether a given agent is operable and within the scope of the present invention is dependent upon the agent's inherent stability characteristics and its compatibility with the growth medium selected and/or the indicator system employed. Because of their favorable characteristics, quinolone antibiotics, such as ciprofloxacin and enrofloxacin, are especially preferred for this application. When other types of antibiotics are employed, particular attention must be given to the aforementioned factors, among others.

In this regard, one or more of the zones of the elements of this invention can contain a variety of other desirable, but optional, components, including buffers, surfactants, binders and/or (micro-)encapsulating agents, as is know in the art.

If, after saturation and incubation with a sample, colonies of microorganisms are inhibited from growing on portions of the bibulous matrix contaning impregnated inhibitory agent(s), but do grow on a control matrix with no inhibitory agents, the impregnated agent(s) is (are) effective in inhibiting growth and would therefore be effective in combating the microorganism.

The term "bodily fluids" includes such fluids as blood, liquor, pus, sputum, bladder content or urine, and any other secretion which may be isolated from an animal source in order to diagnose microbial infection.

As used herein, the term "animal" refers to human beings as well as the wide arrays of types of animals normally treated by veterinarians including, but not limited to dogs, cats, rabbits, horses, cows and pigs.

One the matrices 11c &11t are impregnated, they are then dried. Since a high temperature may affect some of the nutrient and test materials impregnated into the matrices, it has been found that drying the impregnated matrices in a vacuum or forced air oven at about 40°-60°C for 1 to 3 hours is preferred, although temperatures as high as 100°C for times as brief as 15 minutes have been successfully employed.

To provide a convenient integral test device 10, the dried impregnated matrices 11c & 11t are attached to a substrate or handle 12. The substrate 12 is an elongated member preferably formed of a semi-stiff, insoluble material, such as a strip of polyethylene terephthalate, polystyrene, or the like. Since the matrices 11c & 11t are to be adapted for macrobiological purposes, special care must be taken to secure them to the substrate 12 with a microbiologically inert adhesive (not shown). Certain double-faced tapes, such as No. Y9494, commercially available from 3M Company, Minneapolis, Minnesota, USA, have been found suitable for this purpose. The matrices 11c & 11t can also be readily attached to the substrate 12 by bonding without affecting them, the impregnated medium, the reagent or the inhibitory agent(s). To facilitate this, a thin polyethylene sheet 14 (Fig. 2) is disposed between the substrate 12 and each matrix 11c & 11t, and sufficient heat is applied to the side 16 of the substrate 12 distal to each matrix 11c & 11t to cause the sheet 14 to melt and bond the matrices 11c & 11t to the substrate 12.

The matrices 11c & 11t (Fig.1) and attached substrate 12 are then placed in the sealable container 13, and the container 13 is sealed. Generally, any conventional container 13 capable of being resealed has been found suitable for this purpose. It has been found that an envelope of polyethylene, or the like, which is sealed along it sides 17 & 18 and bottom 19 is acceptable for this purpose. The top 21 of the container envelope 13 is preferably provides with a conventional interlocking seal lip 22 which frictionally seals the container 13 upon the application of pressure thereon and thus obviates the necessity for special sealing instruments. Preferably, the container 13 is formed of a clear transparent material which the container 13 without exposing either the operator or laboratory to the cultured microorganisms or to the inhibitory agent(s).

In use, the contaner 13 is unsealed and the substrate 12 and attached matrices 11c & 11t are removed therefrom. The matrices are dipped into the liquid sample to be

tested, such as urine, milk, water, or the like, to rehydrate and inoculate the matrices 11c & 11t. The inoculated matrices 11c & 11t and attached substrate 12 are aseptically replaced in the container 13, and the container 13 is resealed and incubated at the optimum temperature, depending upon the suspected micro-organisms. Most microorganisms are optimumly incubated at temperatures ranging from about 4° to about 40°C, and preferably from 35° to about 39°C. Following the incubation period, the matrices 11c & 11t are visually observed and the results of the particular test are read. The number of colored spots, indicating colony locations, are counted and recorded, as hereinafter described. Once the results of the test are known, the device 10 may be sterilized and discarded. It has been found, however, that sterilization does not affect most color changes formed in the matrices 11c & 11t as a result of the culturing of the microorganisms, and the device 10, or merely the matrices 11c & 11t, may be retained as a permanent record if desired.

In microbiological populations, cell numbers are often of such magnitude that it is difficult to express them easily or exactly. To simplify the handling of these large numbers, the microbiologist uses exponents of 10. For example, a concentration of microorganisms per milliliter of specimen expressed as 10^2 is equivalent to 100 microorganisms per milliliter, 10^3 to 1,000, 10^4 to 10,000, and so on. Thus, the exponent of 10 represents the number of zeros to the right of the digit 1. Since only the relative concentration of microorganisms in a sample is sufficient for microbiological purposes, this manner of expressing the number of micro-organisms per milliliter is not only adequate, but expedient.

The ability to semi-quantitatively assess the number of microorganisms in the sample being tested is an essential aspect of the device, since the concept of inhibitory agent(s) efficacy is only meaningful in relation to real and significant microbiological load. As such, the intention of the device is two-fold: First, it must demonstrate that the number of microorganisms present is significant enough to warrant further action. Provided that this condition is met, the second task of the device is to permit an assessment of the efficacy of a particular inhibitory agent or agents in combating the microorganisms present.

For example, although urine is sterile under normal conditions, contamination from the external genitalia may contribute up to 1,000 (10^3) organisms per milliliter in

properly collected and transported specimens. In such cases, treatment is generally unnecessary and/or inappropriate. Urinary tract infection (UTI), however, is usually associated with baterial counts of 100,000 (10^5) organisms per milliliter, a condition referred to as significant bacteriuria, and here it is crucial to be certain that a given antibiotic is effective against the pathogens at hand before selecting it for treatment.

By the way of further example, similar considerations apply to decisions regarding the disinfection of an inanimate surface, water supply, etc.

Relating the above explanation to the present invention, the significance of the culture-fixing agent can be best illustrated in Figs. 3-6. With reference to Fig. 3, the appearance of 4 locations 23a can be observed. This number would be indicative of 20 microorganisms per milliliter or a concentration expressed as 10^1. The locations 23b appearing on Fig. 4 would indicate a concentration of 10^2. The locations 23c and 23d on Figs 5 and 6, respectively, while clear and distinct, become too numerous to easily count and can best be interpreted by comparing same to a standard or chart showing the approximate number of locations that would appear on a corresponding matrix, and assigning concentrations of 10^3 and 10^4 to the Figs. 5 and 6, respectively. Concentrations of samples exceeding 10^6 microorganisms per milliliter create such a multiplicity of colony locations thereon, the matrices take on a substantially solid color (e.g., pink) thereby portraying a confluent appearance. Finally, matrices in which no bacterial growth has occured will retain the native, homogenous color (e.g., beige) of their original state, as in Fig. 7.

By comparing the appearance of the control and test matrices 11c & 11t following incubation, it will be evident whether or not and, if so, to what extent the inhibitory agent(s) has (have) effectively suppressed the growth of and/or killed the microorganisms contained in the sample. Hence, the relative appearance of the test matrix 11t will allow the microorganisms to be classified as either:

1) sensitive
2) intermediately sensitive or
3) resistant, as is customary in conventional antibiotic susceptibility testing, as explained above. In other words, if the microorganisms are resistant to the

inhibitory agent(s), the general appearance and number of colony locations on matrices 11c & 11t will be strictly comparable. In cases of intermediate sensitivity, some colony locations will appear on the test matrix 11t, however their number will be less than on the control matrix 11c. Finally, microorganisms that are sensitive to the inhibitory agent(s) will form no colony locations on the rest matrix 11t, regardless of the number formed on the control matrix 11c by the uninhibited microorganisms.

The following examples will illustrate the improved test device of this invention without restricting it to these examples.

EXAMPLE I

A Test Device

12.5 grams of xanthan gum, a fixing agent (Keltrol ®), Kelco Division of Merck & Co., San Diego, California, USA), were added to 1 liter of distilled water and agitated until dissolved.

To this solution, the following formulation was added:

Bovine Brain Heart Infusion (Difco Labs, Detroit, Michigan, USA) ..... 200 grams
Triphenyltetrazolium Chloride (Aldrich, Milwaukee, Wisconsin, USA)...0.15 grams

The ingredients were mixed thoroughly until dissolved in the xanthan gum solution to provide a final pH of 7.0-7.5 (= impregnation solution for control matrix 11c).

This procedure was repeated as above, with the exception that 4.4 milligrams of Enrofloxacin HCl (90.8%; Bayer AG, Leverkusen, Germany) were included in the formulation as well, to obtain an approximate activity of 4.0 micrograms/milliliter (= impregnation solution for test matrix 11t).

One sheet of ED 1236 filter paper (Ahlstrom, Mt. Holly Springs, Pennsylvania, USA) was immersed in the control solution until thoroughly saturated and drawn between two closely fitted glass to remove excess fluid therefrom and to insure

uniformity of impregnation. The procedure was repeated for a second sheet with the test solution. Both sheets were then dried in a forced air oven at about 100°C for 15 minutes. The dehydrated sheets were laminated onto double-backed adhesive No. 9494 (3M, Minneapolis, Minnesota, USA) and cut ribbons measuring approximately 1 x 20 centimeters. One each of control and test ribbons were then laminated onto 8.5 x 20 centimeter sheets of polystyrene (American National Can, Cleveland, Ohio, USA) in parallel rows spaced approximately 0.5 centimeters apart. By slitting the laminated composite along its width, individual strips were obtained, measuring 1 x 8.5 centimeters overall and containing the two matrices 11c and 11t, each measuring 1 x 1 centimeter, which had been found to absorb ≈ 100 microliters of liquid specimen apiece. The individual strips were then stored in desiccated plastic bottles prior to use.

EXAMPLE II

Semi-Quantitative Tests

a. Preparing Test Samples

Test samples were prepared by propagating pure cultures of Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Proteus vulgaris, Serratia marcesens, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus faecalis in brain heart infusion broth to a maximum turbidity of ≈ 10^9 cells per milliliter. Tenfold serial dilution of each culture were prepared using sterile saline as a diluent to provide serial bacterial suspensions of each culture at 10^3, 10^5 and 10^7 colony forming units (CFU) per milliliter.

b. Testing the Samples

To assay each of the above test sample dilutions, a device as described in Example 1 was employed. The bottle was opened and the strip removed therefrom. The dehydrated matrices were dipped into the sample dilutions for five seconds to inoculate and rehydrate the pads. The inoculated strips were then placed in a sealable, transparent, plastic container. The container was sealed to prevent dehydration and placed in an incubation chamber maintained at 36° ± 1°C for 18 - 24 hours.

Agar surface streak plates were made to confirm the quantitative estimate of viable bacterial cells in the test suspensions. Moreover, the susceptibility of each culture to enrofloxacin was determined in additional agar surface streak plates according to standardized disc diffusion techniques, in which enrofloxacin 5 µg discs were used (Difco, Product Code 6028).

Subsequent to the incubation period, the devices were withdrawn from the incubator and the matrices were observed through the transparent containers. The reduction of the colorless triphenyltetrazolium to the magenta colored triphenyltetraformazan was visible in all of the control pads. The physical distribution of this color formation stood in definite and distinct correlation to the concentration of microorganisms in the test suspensions.

The pads containing enrofloxacin remained colorless in all cases where the disc diffusion technique had shown the test cultures to be sensitive to the antibiotic.

Consistent with the intermediate sensitivity of Streptococcus faecalis seen in the disc diffusion test, a slight pink color development was observed on the enrofloxacin pads sa 10^7 CFU/ml. Where the disc diffusion method indicated resistance to the antibiotic, i.e., Pseudomonas aeruginosa, growth on the enrofloxacin pad was essentially identical to that seen in the control pad.

While the above disclosure and examples have been directed primarily to a test device having a matrix attached to a substrate and enclosed within a container, it should be recognized that the invention also contemplates the production of only the impregnated matrices. For example, conventional laboratory equipment, such as forceps and slide mailers, can be used to replace the substrate 12 and container 13, respectively. The dehydrated matrix could be held by the forceps and dipped into the samples to be analyzed and subsequently placed in a slide mailer and incubated. Also, the impregnated matrices 11c & 11t may be rehydrated and inoculated, if desired, by adding a measured volume of the sample directly to each matrix, rather than by the immersion procedure hereinbefore described.

In addition, it will be apparent to those skilled in the art that the device is adaptable for detecting the presence of microorganisms in samples other than

liquids. For use in this manner, the matrix 11 can be rehydrated with sterile water, or the like, and inoculated by contact with the "solid" sample to be analyzed.

It is also understood that the present invention is not limited to the specific bibulous materials, nutrient media, reagents, inhibitory agents, or fixing agents described in the above examples, as one skilled in the art will recognize that a wide variety or combination of bibulous material, nutrient media, reagents, inhibitory agents and fixing agents can be employed to carry out the purpose of the novel test device.

What is claimed is:

1. A test device for analyzing a sample for microorganisms and for detecting the efficacy of inhibitory agent(s) against such microorganisms, said device being adapted to be inoculated with the sample and incubated in a sealable container, the device comprising: at least two matrices of equivalent absorptive capacity: one of which (control matrix) is impregnated with a nutrient medium, a reagent, and a water soluble culture-fixing agent capable of localizing microorganisms on said matrix; the others of which (test matrix/matrices) is (are) additionally impregnated with at least one inhibitory agent each.

2. The test device as defined in claim 1 wherein said device is used to
   a) diagnose the presence of infection in an animal or plant and
   b) determine the susceptibility of such infection to an antibiotic(s).

3. The test device as defined in claim 1 wherein said device is used to determine
   a) the bacterial load in substances intended for administration to or intake by animals or plants, and
   b) the efficacy of a disinfectant(s) in reducing such bacterial load.

4. The test device as defined in claim 1 wherein said device is used to determine
   a) the bacterial load on any inanimate surface, and
   b) the efficacy of a disinfectant(s) in reducing such bacterial load.

5. The test device as defined in claim 1 wherein said sample comprises bodily fluids from an animal.

6. The test device as defined in claim 5 wherein said animal is a veterinary animal.

7. The test device as defined in claim 5 wherein said animal is a human.

8. The test device as defined in claim 1 wherein said matrix is a flat bibulous material.

9. The test device as defined in claim 8 wherein said bibulous material has a known constant absorption capacity.

10. The test device as defined in claim 1 wherein said nutrient medium is bovine brain heart infusion.

11. The test device as defined in claim 1 wherein said reagent includes an indicator.

12. The test device as defined in claim 1 and claim 11 wherein said reagent includes a color indicator.

13. The test device as defined in claim 1 and claim 11 wherein said indicator is triphenyltetrazolium chloride.

14. The test device as defined in claim 1 wherein said inhibitory agent is an antibiotic.

15. The test device as defined in claim 1 and claim 14 wherein said inhibitory agent is an antibiotic belonging to the group of aminoglycosides, beta-lactams, cephalosporins, chloramphenicols, macrolides, nitrofuranes, sulfo-namides, tetracyclines, quinolones, or a combanation thereof.

16. The test device as defined in claim 1 and claim 14 wherein said inhibitory agent is an antibiotic belonging to the group of quinolones.

17. The test device as defined in claim 16 wherein said quinolone is enro-floxacin.

18. The test device as defined in claim 16 wherein said quinolone is cipro-floxacin.

19. The test device as defined in claim 1 wherein said inhibitory agent is an anti-mycotic, anti-viral, anti-tuberculosis agent, or a combination thereof.

20. The test device as defined in claim 1 wherein said inhibitory agent is a disinfectant.

21. The test device as defined in claim 1 and claim 20 wherein said inhibitory agent is a disinfectant belonging to the group of quaternary ammonium compounds, surfactants, or a combination thereof.

22. The test device as defined in claim 1 wherein said culture-fixing agent is an inert gum.

23. The test device as defined in claim 1, wherein said culture-fixing agent is a xanthan gum.

24. The test device as defined in claim 1 wherein said culture-fixing agent is a linear polysaccharide.

25. The test device as defined in claim 1 wherein said culture-fixing agent is sodium alginate.

26. A test device for analyzing a sample for microorganisms and for detecting the efficacy of inhibitory agent(s) against such microorganisms, said device being adapted to be inoculated with the sample and incubated in a sealable container, the device comprising: at least two matrices of equivalent absorptive capacity; one of which (control matrix) is impregnated with a nutrient medium, a reagent, and a water soluble culture-fixing agent capable of localizing microorganisms on said matrix; the others of which (test matrix/matrices) is (are) additionally impregnated with at least one inhibitory agent each; a substrate attached to said matrices; and a sealable container enclosing said matrices and substrate.

27. The test device as defined in claim 26 wherein said substrate is a stiff elongated member.

28. The test device as defined in claim 26 wherein said container is transparent.

29. The test device as defined in claim 26 wherein said container includes an interlocking seal lip.

Fig.1

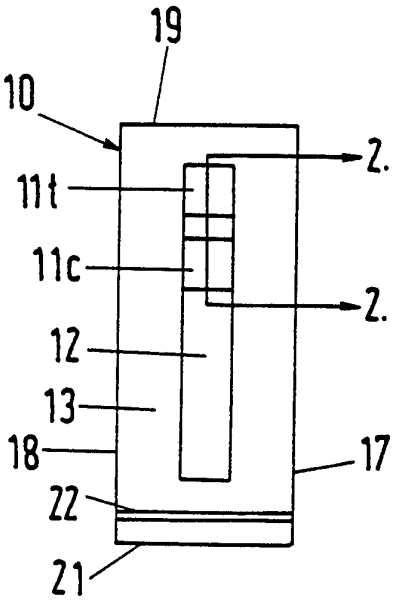


Fig.2

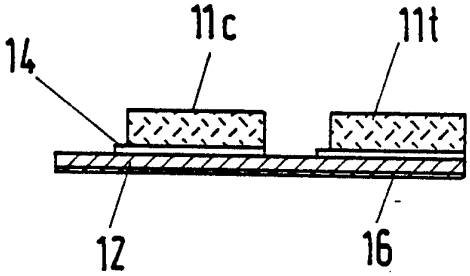


Fig.3

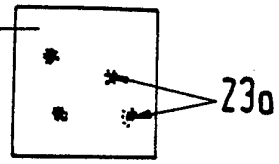


Fig.4

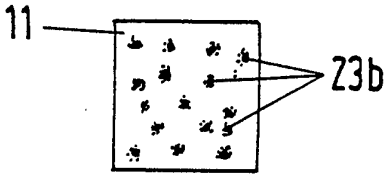


Fig.5

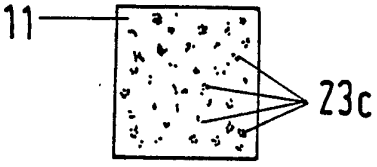


Fig.6

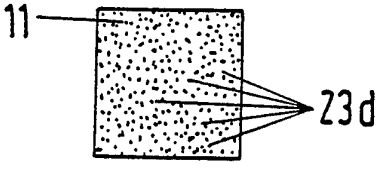
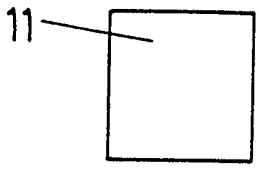


Fig.7



INTERNATIONAL SEARCH REPORT

International Application No
PC./EP 96/02400

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/18 C12M1/18 C12M1/26

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 6 C12Q C12M

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A,3 881 993 (FREAKE RONALD ET AL) 6 May 1975 cited in the application see claims; figures	1-29
Y	AU,A,1 157 876 (MCDONNEL DOUGLAS CORPORATION) 8 September 1977 see claims	1-29
Y	EP,A,0 322 591 (ABBOTT LAB) 5 July 1989 see example 6	1-29
Y	EP,A,0 550 903 (BAYER AG) 14 July 1993 see claims 9-13	14-19

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 October 1996	04. 11. 96
Name and mailing address of the ISA 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	Authorized officer Hoekstra, S

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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PCT/EP 96/02400

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