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William, G. [US/US]; 23 Cide Mill Place, Bahama, NC 27503 (US).

(74) Agents: BLACKSTONE, William, M. et al.; Akzo Nobel Patent Department, 1300 Piccard Drive, Suite 206, Rockville, MD 20850 (US).

(54) Title: DEVICE AND METHOD FOR MICROBIAL SUSCEPTIBILITY TESTING

(57) Abstract

A method and apparatus for performing microbial antibiotic susceptibility testing include disposable, multi–chambered susceptibility plates and an automated plate handler and image acquisition and processing instrument the susceptibility plates are inoculated with a microorganism (any suitable organism such as bacteria, fungi, protozoa, algae and viruses) and anti–microbial agent(s) are applied such that the microorganism is exposed to a variety of concentrations, or a gradient of each anti–microbial agent. The plates are then placed in the instrument, which monitors and measures the growth (or lack thereof) of the microorganisms. This data is used to determine the susceptibility of the microorganism to the antibiotics. Such a system automates antimicrobial susceptibility testing using solid media and Kirby–Bauer standardized result reporting. The system provides a level of automation previously associated only with broth microdilution testing, while retaining the advantages of the manual disk diffusion test. Figure (1) shows an illustration of agar plate for performing a disk diffusion antibiotic susceptibility test.

5   **DEVICE AND METHOD FOR MICROBIAL SUSCEPTIBILITY TESTING**

BACKGROUND OF THE INVENTION

Following detection of a microorganism in a patient sample, it is often

10   desirable to determine to which antibiotics the microorganism is susceptible.

There are now a number of bacterial species which increasingly exhibit

resistance to one or more classes of antimicrobial agents, making it that much

more important to perform susceptibility testing.   Failure of a particular

susceptibility test to accurately predict antimicrobial resistance in a patient's

15   isolate could significantly impact patient care if an antibiotic is used to which the

microorganism is not susceptible.

Different types of susceptibility tests can be used to test a

microorganism. The following brief descriptions give details of some known

20   susceptibility tests as well as some details that relate to the present invention.

One type of susceptibility test is the disk diffusion test, often referred to

as the Kirby-Bauer test. This is a standardized test that involves inoculating

(with 0.5 McFarland standardized suspension of a microbial isolate) a gel plate

25   (e.g. a 150-mm Mueller-Hinton agar plate) and placing thereon one or more

disks impregnated with fixed concentrations of antibiotics. After incubation (e.g.

18-24 hours at 35 degrees C), the diameter of zones of inhibition around the

disks (if present) determine the sensitivity of the inoculated microorganism to

the particular antimicrobial agent impregnated in each disk. Due to the

30   standardization of the Kirby-Bauer method, results of this method are analyzed

by comparing the diameter of the inhibition zone with information published by

NCCLS (National Committee on Clinical Laboratory Standards) in *Performance

Standards for Antimicrobial Disk Susceptibility Testing*, the subject matter of

which is incorporated herein by reference. The results of this test are semi-

35   quantitative in that there are three categories of susceptibility – namely

resistant, intermediate and susceptible. As can be seen in Fig. 1, an agar plate

10 with inoculum has a plurality of disks **12** placed thereon, which disks are

5   impregnated with antibiotics (of different types and/or concentrations). After

incubation, zones of microbial growth inhibition **14** are formed. These zones **14**

are interpreted to be resistant, intermediate or susceptible based on NCCLS

criteria.

10   Another method of antimicrobial susceptibility testing is the antibiotic

gradient method. This test utilizes an antibiotic gradient in a gel medium.

Paper or plastic strips are impregnated with an antibiotic concentration gradient.

A plurality of strips are placed on a Mueller-Hinton agar plate like spokes on a

wheel, with the plate having been inoculated with the microorganism to be

15   tested. After incubation, an antibiotic gradient is formed in the gel in an

elliptical shape around each test strip (if the microorganism is susceptible to the

antibiotic on the particular strip). The minimum concentration of the

antimicrobial agent that prevents visible microorganism growth is the endpoint

of the test (the minimum inhibitory concentration, or MIC). Put in other words,

20   in disk diffusion testing, the MIC is the concentration at the edge of the

inhibition zone (the growth/ no growth boundary). In this case, the MIC is the

point at which the elliptical growth inhibition area intersects the test strip. As

can be seen in Fig. 2, agar plate **1** has a plurality of test strips **3** that are

impregnated with an antibiotic gradient. Elliptical zones **5** are formed where

25   microorganism growth is inhibited by the antibiotic agent in/on the test strip.

Point **7** where the elliptical zone intersects the test strip is the MIC point.

A third type of susceptibility test is the broth microdilution test. In this

type of test, dilutions of antibiotics (e.g. consecutive two-fold dilutions) are

30   prepared. Often, at least ten concentrations of a drug are prepared in tubes or

microwells. Each tube or well having the various concentrations of antibiotics is

inoculated with a particular microorganism (a standardized suspension of test

bacteria is added to each dilution to obtain a final concentration of 5×10^5

CFU/ml). A growth control well and an uninoculated control well are included on

35   each plate. After incubation (e.g. for 16-24 hours at 35 degrees C), the wells or

tubes are examined manually or by machine for turbidity, haze and/or pellet.

Indicators can be placed in the wells to facilitate the visualization of microbial

5   growth. As with other tests, the minimum concentration of antimicrobial agent
     that prevents visible microbial growth is the MIC.

          Commercial microdilution tests are typically performed on standard 96
     well plates, each well holding approximately 100 to 200 microliters with
10   commercially prepared antibiotic test panels. With 96 wells and 2 to 10 different
     dilutions for each antibiotic, numerous antibiotics can be tested on a single
     plate. A significant problem with such commercial microdilution systems is the
     inflexibility of the standard antibiotic test panels. The commercial plates are
     manufactured with various amounts of frozen, dried or lyophilized antimicrobial
15   agents in the wells. This avoids the time consuming task of preparing the
     plates. However, due to the availability of many antibiotics (more than fifty in
     the United States), it is often problematic for a laboratory to find a standard
     commercial test panel which is ideal for that laboratory's needs. Fig. 3 is an
     illustration of a 96-well plate used in such a microdilution system.

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          A variation of the broth microdilution method is set forth in U.S. patent
     5,501,959. This system uses microtiter plates with 168 wells, each containing a
     paper disk attached to the bottom of the well. The disks contain serial two-fold
     dilution concentrations of various antimicrobial agents, as well as a redox
25   indicator. Up to 20 different antimicrobial agents can be tested on a plate. This
     use of paper disks simplifies the manufacture of the custom panels. However,
     higher costs are involved when a susceptibility test is custom made for a
     customer.

30   Current instruments that offer the highest degree of automation in
     susceptibility testing are typically based on automating the tasks performed in
     the manual broth microdilution method mentioned above. One such example is
     the instrument described in U.S. patent 4,448,534. This instrument uses multi-
     well plates that are pre-loaded with serial two-fold dilution concentrations of
35   antimicrobial agents. Plates are inoculated manually and placed in the
     instrument, where they are incubated. At the appropriate times, the wells on
     the plate are read by a photometer/fluorometer to determine the results of the

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5   test. Another automated system is described in U.S. patent 3,957,583. This
     instrument uses small multi-chamber cards that are pre-loaded with serial two-
     fold dilution concentrations of antimicrobial agents. Cards are inoculated
     automatically, incubated, and monitored within the instrument. This instrument
     reads the chambers in the card periodically using a photometer. These kinetic

10   measurements yield growth curves that allow the instrument to determine the
     results of the test. Though the aforementioned instruments perform testing in 4
     to 8 hours, they may fail to detect induced resistance of the microorganism,
     which could result in an incorrect susceptibility report. Unfortunately, the
     degree of automation that is provided by instruments based on broth

15   microdilution is not available for methods such as disk diffusion.

     SUMMARY OF THE INVENTION
             The present invention relates to a system for performing microbial
     antibiotic susceptibility testing. The system is comprised of disposable, multi-

20   chambered susceptibility plates and an automated plate handler and image
     acquisition and processing instrument. The susceptibility plates are inoculated
     with a microorganism (any suitable organism such as bacteria, fungi, protozoa,
     algae or viruses) and anti-microbial agent(s) are applied such that the
     microorganism is exposed to a variety of concentrations, or a gradient of each

25   anti-microbial agent. The plates are then placed in the instrument, which
     monitors and measures the growth (or lack thereof) of the microorganisms.
     This data is used to determine the susceptibility of the microorganism to the
     antibiotics. Such a system automates antimicrobial susceptibility testing using
     solid media and Kirby-Bauer standardized result reporting. Thus, the present

30   invention provides a level of automation previously associated only with broth
     microdilution testing, while retaining the advantages of the manual disk diffusion
     test.
             The present invention also relates to a kit for determining the existence
     and/or degree of resistance of a microorganism to one or more antimicrobial

35   agents, each at one or more concentrations, which comprises one or more
     antimicrobial agents provided on substrates, a container having a plurality of
     separate compartments, at least when assembled, growth medium within the

5   container or for adding to the container, the growth medium when in the
       container forming a solid or semi-solid growth medium and the growth medium
       being sufficiently solid so that the antimicrobial agent when applied to the
       growth medium will diffuse over time and form a concentration gradient.

10   BRIEF DESCRIPTION OF THE DRAWINGS
            Fig. 1 is an illustration of an agar plate for performing a disk diffusion
       antibiotic susceptibility test;
            Fig. 2 is an illustration of an antibiotic gradient method for determining
       susceptibility of a microorganism to particular antimicrobial agents;
15   Fig. 3 is an illustration of a device for performing a broth microdilution
       antibiotic susceptibility test;
            Fig. 4 is an illustration of one embodiment of the present invention
       having a bottom portion with a bottom gel plate having internal partitions, and a
       top cover;
20   Figs. 5a and 5b are top views of two embodiments of the invention
       where Fig. 5a illustrates elongated channels with antibiotic disks, and where Fig.
       5b illustrates shorter channels, some with antibiotic disks therein;
            Fig. 6a illustrates an embodiment with elongated channels each having
       an antibiotic strip therein, whereas Fig. 6b illustrates an embodiment having thin
25   shorter channels with antibiotic disks at one end thereof;
            Figs. 7a and 7b are views of a susceptibility plate with *E. coli*, where Fig.
       7a shows a raw image and Fig. 7b shows a processed image;
            Figs. 8a and 8b are views of a susceptibility plate with *S. aureus*, where
       Fig. 8a shows a raw image and Fig. 8b shows a processed image;
30   Figs 9a to 9b are additional views of a susceptibility plate for *Kleb.*
       pneumoniae, where Fig. 9a is an unprocessed image after 4 hours, Fig. 9b is a
       processed image after 4 hours, Fig. 9c is an unprocessed image after 18 hours,
       and Fig. 9d is a processed image after 18 hours; and
            Fig. 10 is an illustration of one system for moving a plurality of
35   susceptibility plates past imaging devices for detecting and processing images of
       the plates; and

5   DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

One embodiment of the susceptibility plate of the present invention is
illustrated in Fig. 4. Such a plate is provided to be disposable and have a low
cost for manufacture, and is preferably made of plastic. A top **40** is provided
which fits onto bottom **42**. Top **40** is preferably transparent or otherwise

10   having properties that can allow viewing (manually or with machine) of microbial
growth in the channels in bottom **42**. Bottom **42** is provided with a plurality of
channels 44 or otherwise mutually isolated chambers. Such channels can be
formed within an insert 46 that fits into bottom **42**, or bottom **42** and insert **46**
can be integrally formed as a single piece (and either or both of the bottom and

15   insert can be opaque). Preferably, marks such as those illustrated along each
channel in Figs. 5 and 6 (or other markings such as numbers) are provided for
aiding in manual measurement of the length of inhibition in the channel, if such
is desired. This is a definite advantage compared to the standard manual disk
diffusion system, where calipers are used to measure a diameter of the

20   inhibition zone, a system that is more labor intensive and less accurate. Each
channel **44** contains a growth medium that is solid (or semi-solid). Such growth
medium may optionally contain an indicator additive for improving the
readability of growth patterns in the various channels. Also, an indicator may be
provided in a separate layer (a "sensor layer"), a conditioning layer may be

25   provided, and various components can be provided in the gel layer, such as
described in U.S. patent application 08/989,560, filed December 12, 1997, the
subject matter of which is incorporated herein by reference.

Physically, the external geometry of the susceptibility plate could be
30   made similar to that of a standard microwell plate (128 mm x 86 mm).
However, other shapes and sizes are envisioned. The plate could be made to be
almost any geometric shape, including square or even round like a standard
agar plate. Whatever the external geometry, internally the plate is partitioned
into separate chambers or channels in which the solid (or semi-solid) medium is

35   held. The wells or chambers within the plate are preferably elongated channels,
though triangular, pie-shaped, circular or square wells, or other geometrically
shaped wells, are also envisioned. As an example, Fig. 5a illustrates one

5   embodiment where mutually isolated channels **50** extend almost fully across the width of the plate. Antibiotic disks **52** are placed in each channel at the end or middle of the channel. Shorter channels **54** could also be formed in the plate, such as those illustrated in Fig. 5b.   Fig. 6a illustrates a further example where antibiotic gradient strips **61** are disposed in elongated channels **63**. In this

10   embodiment, an MIC (minimum inhibitory concentration) can be determined (the MIC is the concentration at the edge of the inhibition zone – the growth/no growth boundary). The separated channels could also be made much narrower, such as illustrated in Fig. 6b. However, if it is desired to use standard antibiotic disks **65** such as those commercially provided for standard Kirby-Bauer antibiotic

15   testing (which disks are approximately 6mm in diameter), then larger antibiotic disk receiving areas should be provided, such as at the end of each narrow channel. Generally, the channel length is greater than 8 mm (preferably from 20 mm to 45 mm in length), and the channel width is greater than 6 mm (preferably from 8 mm to 16 mm in width). A channel width of approximately 8

20   mm is most preferred if standard antibiotic disks are used (which are approximately 6 mm in diameter). Of course, if antibiotic disks of different size are used, the channel dimensions could be made larger or smaller. A length of from about 30 to 35 mm is most preferred as this allows sufficient length to detect and measure inhibition zones resulting from nearly all

25   antibiotic/microorganism combinations used in susceptibility testing. The depth of the solid or semi-solid growth medium in the channels should be more than 1 mm, preferably from about 2 mm to about 20 mm, and more preferably from about 5 mm to about 15 mm.

One of the purposes of the isolated chambers is to increase the ease and

30   reproducibility of susceptibility testing, as well as to maximize the number of tests that can be performed on one susceptibility plate. Whereas standard disk diffusion (Kirby-Bauer) tests are physically restricted to a density of 12 tests or less per 150-mm Mueller-Hinton plate (one test per14.73-cm^2), the present invention easily allows for 24 tests or more on a 128 mm x 85 mm plate (at least

35   one test per 4.53-cm^2), a density of more than 3 times that of the standard disk diffusion plate. Initially it was thought that the length of inhibition area in a channel in the present invention would not correlate with the radius of inhibition

on a standard disk diffusion plate (when using the same microorganism and antibiotic). Instead, it was found that the measured length of inhibition was substantially the same in the present invention as the radius measured in the standard plate. By "substantially the same" it is meant that the measured lengths in the present invention and in a standard Kirby-Bauer test after the same period of time were either exactly the same, or close enough that the ultimate outcome of the test (susceptible, intermediate or resistant as defined by the National Committee for Clinical Laboratory Standards, or NCCLS) correlated over 80% of the time, and were within the control ranges outlined by the NCCLS. In most cases the results correlated over 90% of the time. And, in only in a small percentage (<1.1%) of cases did the results of the present invention indicate susceptible when the standard Kirby-Bauer test (with same microorganism and antibiotic) indicated resistant, or vice-versa (<0.9%).

The susceptibility plates of the present invention are used as follows:

1) Disks containing single concentrations, or strips containing several concentrations, of each antibiotic being tested are placed (manually or automatically) onto the inoculated surface of the growth medium in each chamber of the susceptibility plate. Once the disks or strips are placed on the plate, the antibiotics start to diffuse into the growth medium forming an antibiotic gradient within the growth medium. Antibiotic panels are flexible and can be user-configurable and/or pre-configured.

2) The susceptibility plate is placed in the instrument (manually or automatically) where it is incubated, promoting the growth of microorganisms within the chambers, except where inhibited by antibiotics diffused into the growth medium.

3) The susceptibility plate is inspected manually or automatically to determine the presence and length of inhibition zones in the chambers. Ruler markings or numberings along each channel facilitates manual zone measurements. Automatic zone

measurements are performed by the instrument via image capture and image processing.

As mentioned above, the length of the inhibition in the channel of the susceptibility plate can be measured manually, or automatically. If automatically, an instrument is provided which is responsible for performing three main functions: susceptibility plate incubation, image acquisition/capture, and image processing. The instrument provides a controlled environment to incubate the plates. Susceptibility plates are inoculated and placed in the incubator where they are subsequently scanned by an image acquisition device during the incubation period. The instrument provides for image acquisition using one or more color and/or gray scale imaging devices: CCD linear array scanner, a CCD line-scan camera, a CCD 2D array camera (still or motion video), a laser scanning camera, or other device that would provide a sufficiently clear image of the susceptibility plate that can be used alone or after further processing. By "image" it is meant any information, such as optical information, from the susceptibility plate that is $\geq$ a 1 x 1 pixel. The image acquisition is performed at regular pre-programmed intervals, with the captured image obtained from one or more views and angles of the susceptibility plate.

One example of how susceptibility plates could be moved relative to one or more imaging devices is illustrated in Fig. 10. As can be seen in this figure, imaging devices **101** and **102** are provided for capturing the image of the top and bottom, respectively, of each susceptibility plate. Top and bottom imaging transport systems **105** and **106** are provided for moving the respective imaging devices past a plurality of plates when the plates are disposed at the top or bottom imaging station. Each susceptibility plate **107** at each imaging station has its image captured by an imaging device. Susceptibility plates are moved to the imaging stations by a plate transport system **108** that, as in Fig. 10, moves plates both upwardly and downwardly from the imaging stations. Of course other plate and/or imaging device transport systems could be

utilized. A cartridge system could be used, where one plate at a time is removed from a stack for imaging (and either the stack or imaging device is moved to position the imaging device and selected plate in proximity to each other). A turntable or other rotary system could be used to position each plate proximate to an imaging device, or a conveyor belt or wheel system could be used, where plates (with solid media) are affixed to a belt or wheel in rows and are turned upside down depending upon which side of the belt or wheel the plate is on.

Images acquired during the incubation period are analyzed using one or more image processing techniques. In the example of Fig. 10, images are acquired from both the top and bottom of the plate at regular intervals (though imaging only one side of the plate is also envisioned). Typically the interval is from 5 minutes to 4 hours, though preferably the interval is between one half-hour and 2 hours, and most preferably every hour. Scanning at regular intervals provides kinetic growth data, which may be used to help characterize the microorganisms. The image-processing algorithm implemented to determine susceptibility is comprised of one or more of the following steps:

a) Image Masking – to isolate the area of interest from extraneous image data;

b) Antibiotic Disk or Strip Detection – to determine identity and concentration of antibiotic under test;

c) Image Subtraction – to isolate the areas of change between two images taken at different time intervals;

d) Image Equalization – to amplify the magnitude of the changes appearing in the subtracted image;

e) Image Blurring – to reduce the effects of single pixel noise in the equalized image (low pass filter);

f) Image Contrast and Brightness Enhancement – to further amplify localized differences in the filtered image; and/or

g) Inhibition Zone Detection, Measurement, and Inspection – to determine the susceptibility of microbial organisms to a particular antibiotic.

5   Results of susceptibility testing with system of the present

invention are shown in Figs. 7-9.   Fig. 7a shows a grayscale image of *E.*

coli on a susceptibility plate (using several different antibiotic disks)

taken 18 hours after inoculation and antibiotic disk placement. Fig. 7b is

10   the same plate image after image processing. The image taken at

inoculation was subtracted from the image taken after 18 hours of

incubation, the difference image was histogram equalized and blurred,

and the zone measurement algorithm was applied to the resulting image.

Inhibition zones and equivalent diameter measurements are shown in

15   Fig. 7b.   Similarly, Figs. 8a and 8b show a grayscale image and

processed image for *S. aureus* using the same timing and processing

techniques as for Fig. 7.

Analyses of images acquired each hour from 1 to 17 hours after

inoculation demonstrate the presence of additional information regarding

20   the interaction between the microorganisms and the antibiotics.

Properties such as the growth rates of the microorganisms, the diffusion

rate of the antibiotics, and the characteristics of the antimicrobial effect

on the microorganism are evident. In fact, in a number of instances, the

inhibition zones are defined as early as 4 to 6 hours after plate

25   inoculation and using image processing. As an example, Figs. 9a and 9b

show a grayscale image and processed image, respectively, of *Kleb.*

pneumoniae only four hours after inoculation (Figs. 9c and 9d are

grayscale and processed images, respectively, of the same plate after 18

hours).

30   The invention is also envisioned as comprising a top plate and a

bottom plate where the bottom plate is a single chamber filled with the

solid or semi-solid nutrient medium for the microorganisms and the top

plate is provided with a plurality of ribs or dividers. When the top and

bottom plates are fitted together (after placement of antibiotic discs

35   within the "chambers" of the top plate) the nutrient medium is separated

into isolated chambers as in the embodiment of the invention illustrated

in Fig. 4. Of course, the ribs or dividers could be provided as a separate element from the top plate.

Another aspect of the invention is, rather than determining susceptible, intermediate or resistant, the determination of the MIC. In the present invention, MICs can be determined using a regression analysis of the zone measurement (the length of inhibition along the channel), as the diffusion of antibiotic out of the disk forms a highly predictable logarithmic gradient. Determining the MIC from the length of the disk diffusion can be performed by any known method, such as that of BIOMIC™ System by Giles Scientific (NY).

In accordance with the invention, the antimicrobial agent can be applied to a plurality of compartments in differing concentrations. The solid or semi-solid growth medium should be sufficiently solid so that the antimicrobial agent when applied to the growth medium, will diffuse over time and form a concentration gradient (the concentration gradient can be formed in a horizontal direction and can continue to diffuse over a period of up to 10 to 18 hours or more). The solid or semi-solid growth medium in each chamber of the susceptibility plate is inoculated (e.g., swabbed) with a McFarland 0.5 standardized suspension of the microorganism being tested. If the microorganism is a bacterium, it can be an aerobic gram-positive organism, aerobic gram negative organism, anaerobic gram positive organism, anaerobic gram negative organism or a cell wall deficient organism.

The solid or semi-solid growth medium may comprise one or more of routine media, selective media, differential media, selective-differential media, enriched media, susceptibility media, anaerobic media and fungal media. If the media is routine media, it can comprise one or more of trypticase soy blood agar, trypticase soy agar, tryptic soy, BHI blood agar, BHI agar, Casman blood, HBT bi-layer media, and standard methods agar. If the media is selective media, it can comprise one or more of, columbia CNA blood, azide blood agar, chocolate selective, Brucella blood, blood SxT, Strep selective I & II, PEA, Bile Esculin agar, *clostridium difficile* agar, skirrow, CCFA, CLED, *Pseudomonas cepacia*

agar, SxT blood agar, TCBS agar, CIN, *moraxella catarrhalis* media, and charcoal selective. If the media is differential media, it can comprise one or more of brilliant green, CYE – Legionella, centrimide, DNA-se, hektoen enteric agar, Jordans tartrate, mannitol salt, LIA, TSI, FLO – Pseudomonas F, TECH – Pseudomonas P, Sellers, starch agar, thermonuclease,Tinsdale agar, McCarthy, LSM, sorbitol-McConkey, MUG-McConkey.

If the media is selective and differential media, it can comprise one or more of MacConkey, EMB, Baird Parker, BHI blood with antibiotics, BiGGY – mycologic, CIN, *clostridium difficile* agar, McBride, Pseudomonas isolation agar, S-S agar, turgitol 7, and XLD agar. If the media is enriched media, it can comprise one or more of chocolate, GC chocolate, BHI chocolate, Borget Gengou, heart infusion agar, McCarthy, Regan-Lowe, Thayer-Martin, transgrow medium, cystein tellurite blood, cystein tellurite heart, BHT, heart infusion, Loefflers, and serum tellurite. If the media is anaerobic media, it can comprise one or more of columbia base, PEA, CAN, LKV, BBE, Brucella, BHI blood base, KBE, McClung-Toabe, oxgall, Schaedlers, and Wilkens-Chalgren. And, if the media is a fungal media, it can comprise one or more of BHIbase, BiGGY, birdseed, corn meal, coton seed, DTM, sabourauds dextrose, Fuji medium, inhibition mold, Littman oxgall, mycologic, mycophil, Nickersons, SABHI, and trichophytin.

The antimicrobial agents can be one or more of a beta-lactam antibiotic, a cepheme antibiotic, a glycopeptide antibiotic, an aminoglycoside antibiotic, a macrolide antibiotic, a tetracycline antibiotic, and a quinalone antibiotic. If the antimicrobial agent is a beta-lactam antibiotic, it can comprise one or more of penicillins, uredopenicillins, synthetics, carbapenems and beta-lactam/inhibitors. If it is a cepheme antibiotic, it can comprise one or more of cephalosporins generations I to IV, and carbacephems. Also, the one or more antimicrobial agent can comprise one or more of sulfa agents and derivatives, chloramphenicol, clindamycin, nitrofurantoins, polymyxins and chemical agents.

5   The foregoing description is sufficient to enable one skilled in the
art to practice the invention. The examples herein should not be
construed as limiting the scope of the claims in any way. Indeed, various
modifications of the invention in addition to those shown and described
herein will become apparent to those skilled in the art from the foregoing
10   description and fall within the scope of the appended claims.

5   We claim:

1) A method for determining the existence and/or degree of resistance
of a microorganism to one or more antimicrobial agents, each at one
or more concentrations, comprising:

10   providing a container having a plurality of separate
compartments, each compartment having therein a solid or semi-
solid microbial growth medium;

adding a microorganism to one or more of said separate
compartments;

15   placing an antimicrobial agent onto a surface of, or into, the
microbial growth medium in one or more of said plurality of
separate compartments;

incubating before and/or after said placing step, said container
having said microorganism and antimicrobial agent in at least one

20   compartment thereof; and

determining the existence and/or extent of resistance of said
microorganism to said antimicrobial agent based on the existence
and/or size of the inhibition zone in said compartment.

25   2) The method according to claim 1, wherein the determination of the
existence and/or extent of resistance of said microorganism is a
determination of susceptible, intermediate or resistant.

3) The method according to claim 2, wherein the determination of

30   susceptible, intermediate or resistant corresponds at least 80% of the
time with the determination made with the same microorganism and
antimicrobial agent in a standard Kirby-Bauer disk diffusion method
performed in accordance with NCCLS standards.

35   4) The method according to claim 3, wherein the correspondence is
more than 90% of the time.

5   5) The method according to claim 1, wherein said separate
         compartments are elongated compartments.

     6) The method according to claim 5, wherein adjacent one or more of
         said elongated compartment are provided markings for aiding

10   measurement of microbial growth in the adjacent compartment.

     7) The method according to claim 1, wherein said solid or semi-solid
         growth medium comprises one or more of routine media, selective
         media, differential media, selective-differential media, enriched

15   media, susceptibility media, anaerobic media and fungal media.

     8) The method according to claim 1, wherein the microorganism is a
         bacterium and is selected from the group consisting of aerobic gram
         positive organisms, aerobic gram negative organisms, anaerobic

20   gram positive organisms, anaerobic gram negative organisms, and
         cell wall deficient organisms.

     9) The method according to claim 1, wherein one or more antimicrobial
         agents are applied in one or more concentrations to the solid or

25   semi-solid growth medium in the plurality of compartments of said
         container.

     10) The method according to claim 1, wherein a minimum inhibitory
          concentration (MIC) of the antimicrobial agent for the microorganism

30   is determined.

     11) The method according to claim 10, wherein the MIC is determined
          by regression analysis.

35   12) The method according to claim 1, wherein for the same, the
          antimicrobial agent is applied to a plurality of compartments in
          differing concentrations.

13) The method according to claim 9, wherein the one or more antimicrobial agents comprise one or more of a betalactam antibiotic, a cepheme antibiotic, a glycopeptide antibiotic, an aminoglycoside antibiotic, a macrolide antibiotic, a tetracycline antibiotic, and a quinalone antibiotic.

14) The method according to claim 1, wherein said solid or semi-solid growth medium is sufficiently solid so that the antimicrobial agent when applied to the growth medium, will diffuse over time and form a concentration gradient.

15) The method according to claim 1, said step of determining the existence and/or degree of susceptibility of said microorganism to said antimicrobial agent, comprises capturing an image of said compartments of said container at one or more intervals with at least one image capture device, and determining the length of the inhibition zone within each compartment.

16) The method according to claim 15, wherein said image capture occurs at intervals of between 5 minutes to 4 hours.

17) The method according to claim 15, wherein said image capture device is comprised of one or more color and/or gray scale imaging devices.

18) The method according to claim 17, wherein said one or more color and/or gray scale imaging devices comprises a CCD linear array scanner, a CCD line-scan camera, a still CCD 2D array camera, a motion video CCD 2D array camera or a laser scanning camera.

19) The method according to claim 17, wherein a plurality of containers having said plurality of compartments and solid or semi-solid growth

media are provided, and wherein said plurality of plates are manually or automatically moved relative to the image capture device and sufficiently close to the image capture device so that an image of each of the containers can be captured.

20) The method according to claim 19, wherein images of both the top and bottom of the containers are captured by one or more image capture devices.

21) The method according to claim 19, wherein said image capture device is moved to be adjacent each container.

22) The method according to claim 19, wherein each container is moved to be adjacent to the image capture device.

23) The method according to claim 15, further comprising one or more of:

   a) image masking for isolating area to be measured from extraneous image data;

   b) antimicrobial agent detection for determining the identity and/or concentration of the antimicrobial agent being tested;

   c) image subtraction for isolating areas of change between two images taken at different time intervals;

   d) image equalization for amplifying a magnitude of changes appearing in a subtracted image;

   e) image blurring for reducing effects of single pixel noise in an equalized image;

   f) image contrast and brightness enhancement for amplification of localized differences; and

   g) inhibition zone detection, measurement and inspection, for determining the susceptibility of the microorganism to the antimicrobial agent.

24) The method according to claim 1, wherein the separate compartments are elongated compartments having a length greater than about 8mm.

25) The method according to claim 24, wherein the length is between about 20 to about 45 mm.

26) The method according to claim 24, wherein the separate compartments have a width greater than about 6 mm.

27) The method according to claim 26, wherein the width is from about 8 to about 16 mm.

28) The method according to claim 19, wherein both the containers and the image capture device are moved so that each container can be positioned adjacent the image capture device.

29) The method according to claim 16, wherein said interval is between one-half and two hours.

30) The method according to claim 5, wherein the elongated compartments are provided parallel to each other.

31) The method according to claim 10, wherein the minimum inhibitory concentration is determined by a plurality of concentrations of an antimicrobial agent on a substrate.

32) The method according to claim 1, wherein the determination of the existence and/or degree of resistance of said microorganism to said antimicrobial agent includes analysis of kinetic growth data of the microorganism provided by multiple images taken by said one or more image capture devices over time.

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33) The method according to claim 7, wherein said routine media comprises one or more of trypticase soy blood agar, trypticase soy agar, tryptic soy, BHI blood agar, BHI agar, Casman blood, HBT bi-layer media, and standard methods agar.

34) The method according to claim 7, wherein said selective media comprises one or more of, columbia CNA blood, azide blood agar, chocolate selective, Brucella blood, blood SxT, Strep selective I & II, PEA, Bile Esculin agar, *clostridium difficile* agar, skirrow, CCFA, CLED, *Pseudomonas cepacia* agar, strep selective, SxT blood agar, TCBS agar, CIN, *Moraxella catarrhalis* media, and charcoal selective.

35) The method according to claim 7, wherein said differential media comprises one or more of brilliant green, CYE – Legionella, centrimide, DNA-se, hektoen enteric agar, Jordans tartrate, mannitol salt, LIA, TSI, FLO – Pseudomonas F, TECH – Pseudomonas P, Sellers, starch agar, thermonuclease, Tinsdale agar, McCarthy, LSM, sorbitol-McConkey, MUG-McConkey.

36) The method according to claim 7, wherein said selective and differential media comprises one or more of MacConkey, EMB, Baird Parker, BHI blood with antibiotics, BiGGY – mycologic, CIN, *clostridium difficile* agar, McBride, Pseudomonas isolation agar, S-S agar, turgitol 7, and XLD agar.

37) The method according to claim 7, wherein said enriched media comprises one or more of chocolate, GC chocolate, BHI chocolate, Borget Gengou, heart infusion agar, McCarthy, Regan-Lowe, Thayer-Martin, transgrow medium, cystein tellurite blood, cystein tellurite heart, HBT, heart infusion, Loefflers, and serum tellurite.

38) The method according to claim 7, wherein said anaerobic media comprises one or more of columbia base, PEA, CAN, LKV, BBE,

Brucella, BHI blood base, KBE, McClung-Toabe, oxgall, Schaedlers, and Wilkens-Chalgren.

39) The method according to claim 7, wherein said fungal medium comprises one or more of BHI base, BiGGY, birdseed, corn meal, cotton seed, DTM, sabourauds dextrose, Fuji medium, inhibition mold, Littman oxgall, mycologic, mycophil, Nickersons, SABHI, and trichophytin.

40) The method according to claim 13, wherein the beta-lactam antibiotic comprises one or more of penicillins, uredopenicillins, synthetics, carbapenems and beta-lactam/inhibitors.

41) The method according to claim 13, wherein the cepheme antibiotic comprises one or more of cephalosporins generations I to IV, and carbacephems.

42) The method according to claim 9, wherein the one or more antimicrobial agents comprises one or more of sulfa agents and derivatives, chloramphenicol, clindamycin, nitrofurantoins, polymyxins and chemical agents.

43) The method according to claim 1, wherein the solid or semi-solid growth medium is provided in each compartment at a depth of greater than 1 mm.

44) The method according to claim 43, wherein the depth of the growth medium is from about 2 mm to about 15 mm.

45) The method according to claim 44, wherein the depth of the growth medium is from about 5 mm to about 15 mm.

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46) The method according to claim 1, wherein the microorganism is a
     bacterium, fungus, protozoa, algae or virus.

47) The method according to claim 1, wherein said separate
     compartments are formed upon assembly of the container and after
     addition of said microorganism and growth medium.

48) A kit for determining the existence and/or degree of resistance of a
     microorganism to one or more antimicrobial agents, each at one or
     more concentrations, comprising:
         one or more antimicrobial agents provided on substrates;
         a container having a plurality of separate compartments, at least
         when assembled;
         growth medium within said container or for adding to said
         container, said growth medium when in said container forming a
         solid or semi-solid growth medium and said growth medium being
         sufficiently solid so that the antimicrobial agent when applied to
         the growth medium will diffuse over time and form a
         concentration gradient

49) A method for determining the existence and/or degree of
     resistance of a microorganism to one or more antimicrobial agents
     each at one or more concentrations, comprising:
         providing a container having a plurality of separate
         compartments, each compartment having therein a solid or semi-
         solid microbial growth medium for growth of a microorganism to
         be tested for antimicrobial susceptibility, and for a corresponding
         antimicrobial agent;
         adding a sample containing microorganisms to a respective
         compartment containing solid or semi-solid microbial growth
         medium;
         placing an antimicrobial agent onto said solid or semi-solid
         microbial growth medium;

5   incubating said container having said microorganism and

antimicrobial substance in at least one compartment thereof; and

determining the existence and/or the degree of resistance of said

microorganism to said antimicrobial agent based on the existence

and/or size of the inhibition zone in said compartment.

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5   incubating said container having said microorganism and antimicrobial substance in at least one compartment thereof; and determining the existence and/or the degree of resistance of said microorganism to said antimicrobial agent based on the existence and/or size of the inhibition zone in said compartment.

10
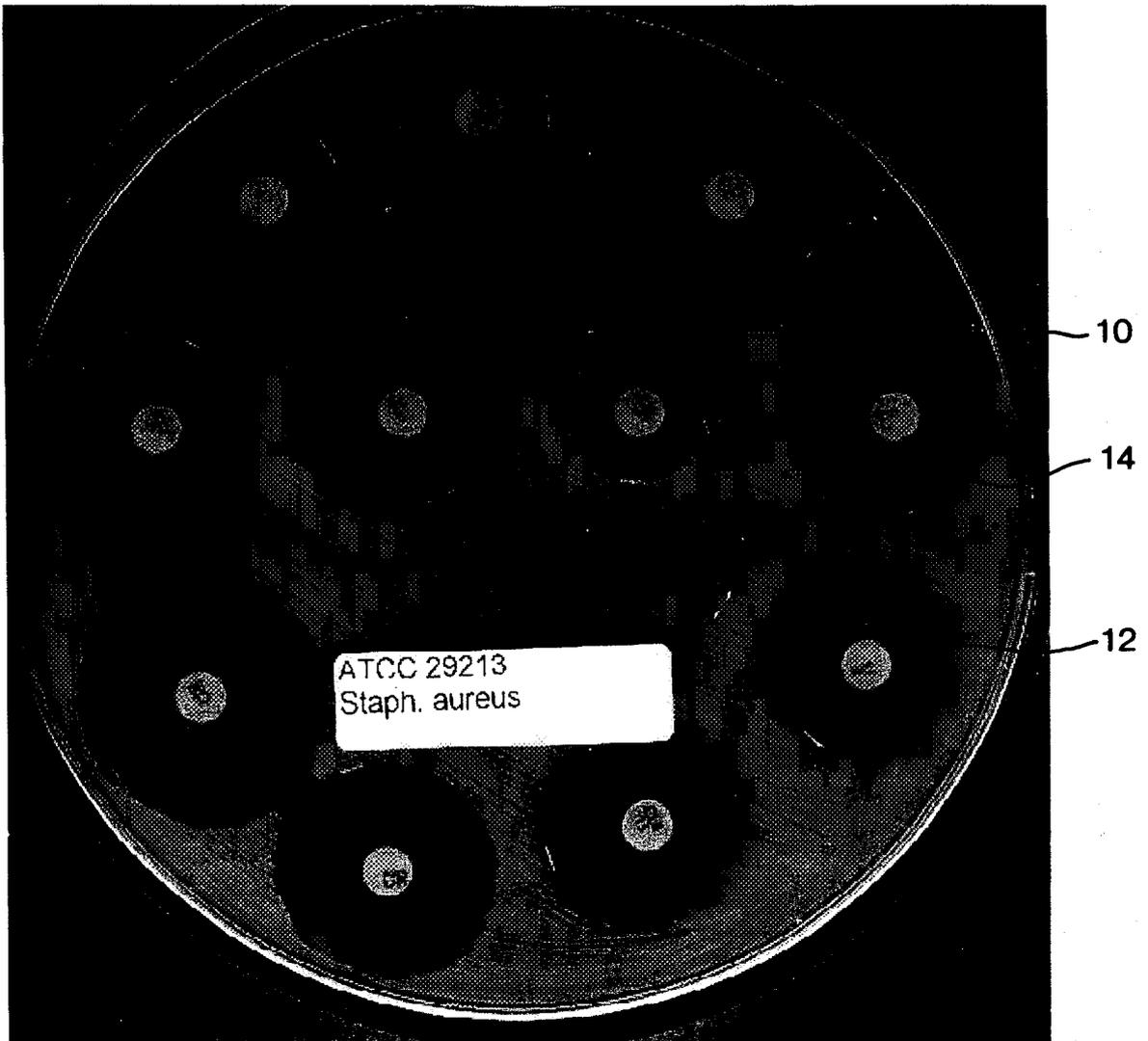
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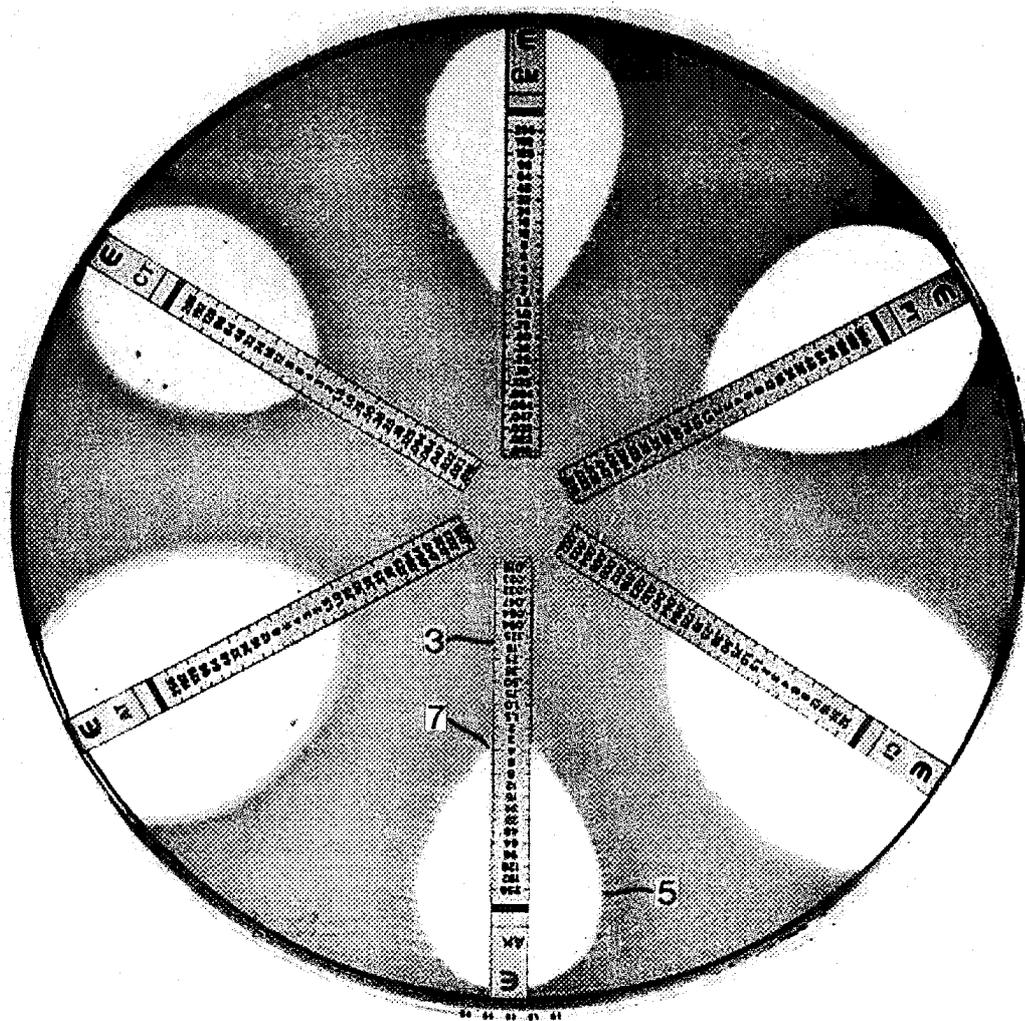
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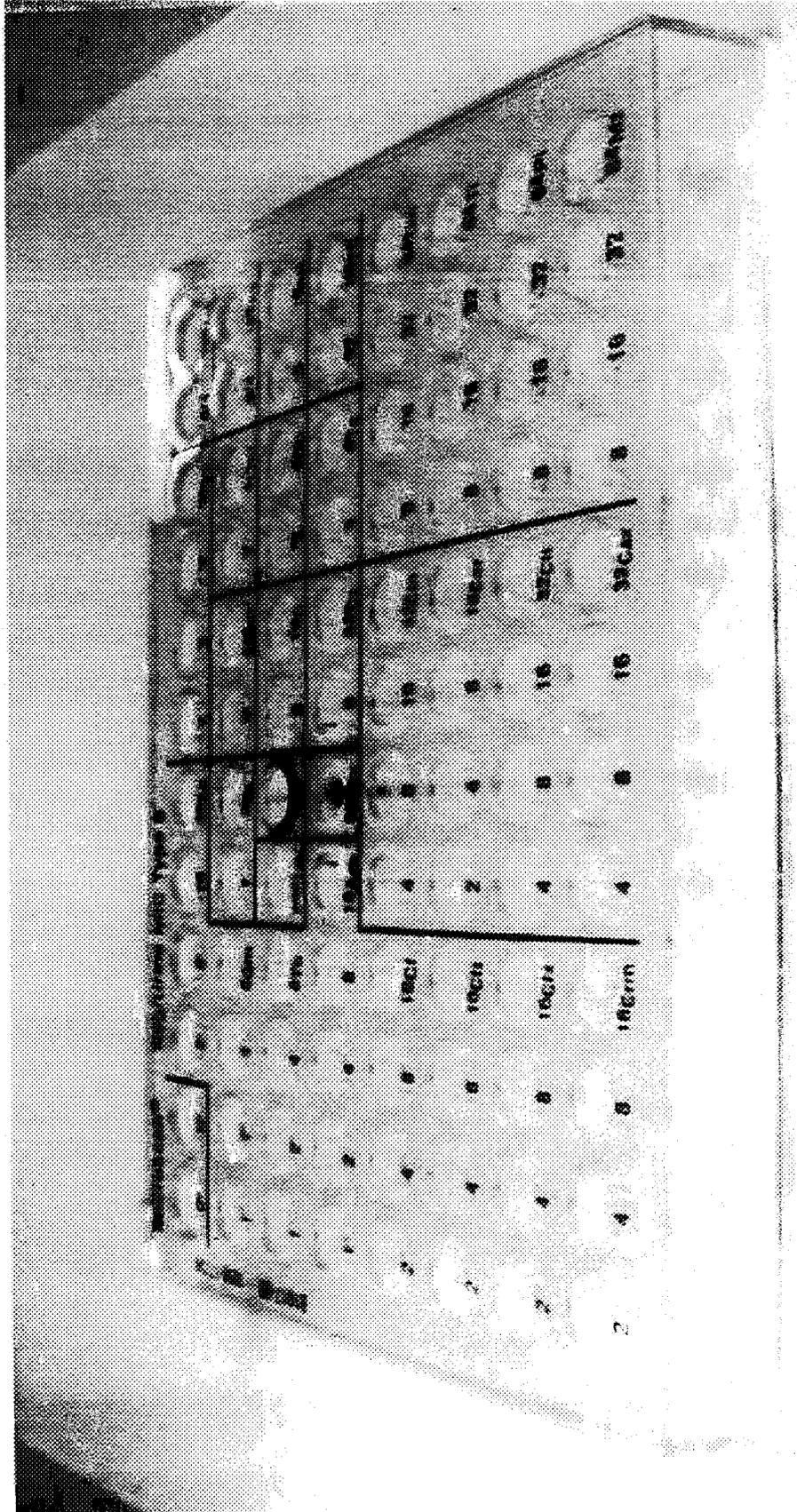
FIG. 3
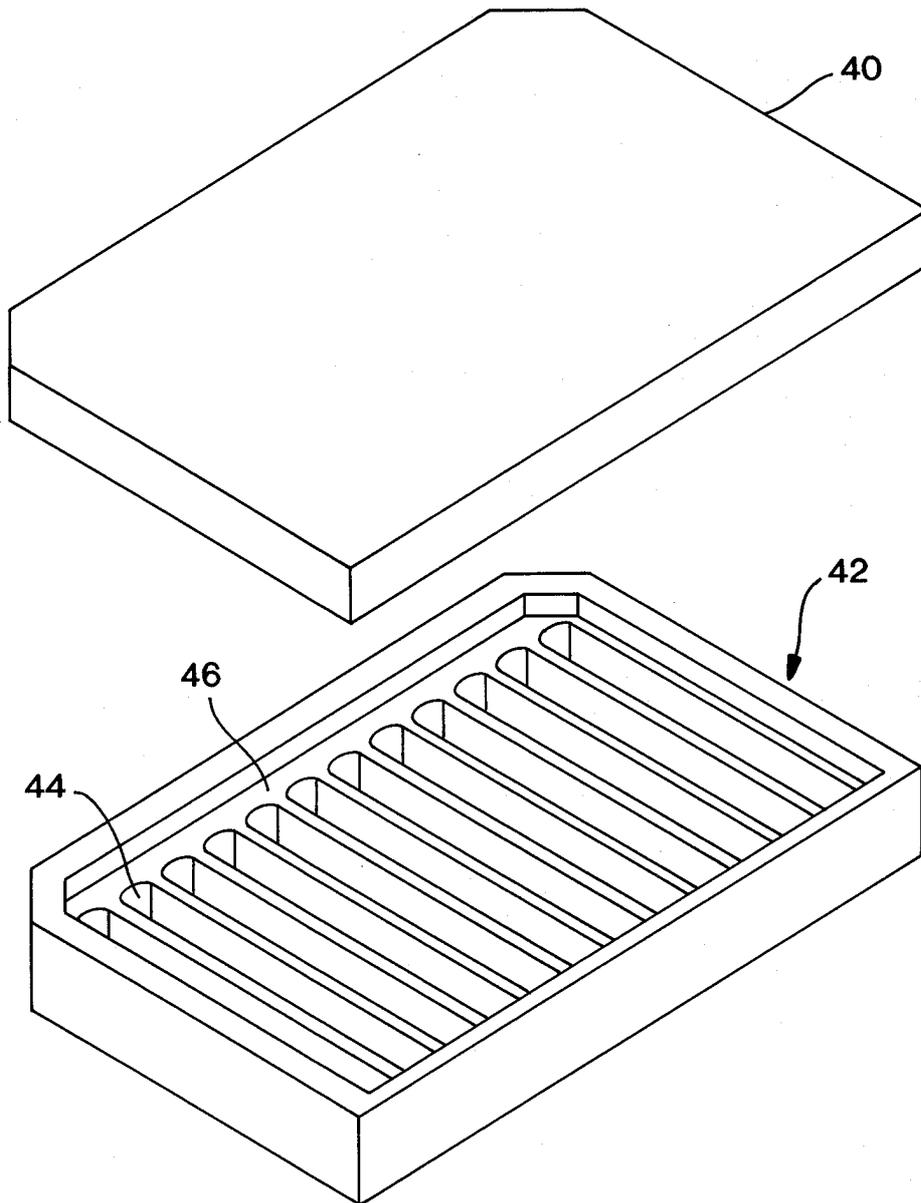
FIG. 4

50   52
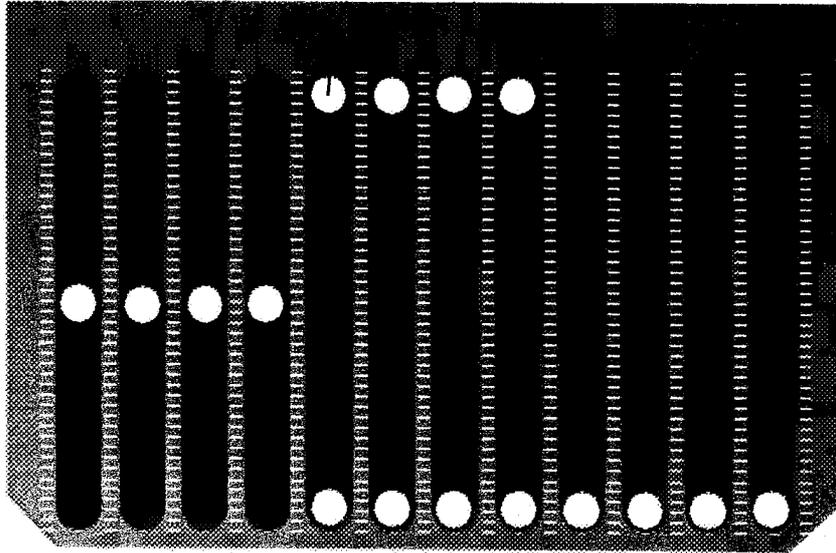
FIG. 5A

54
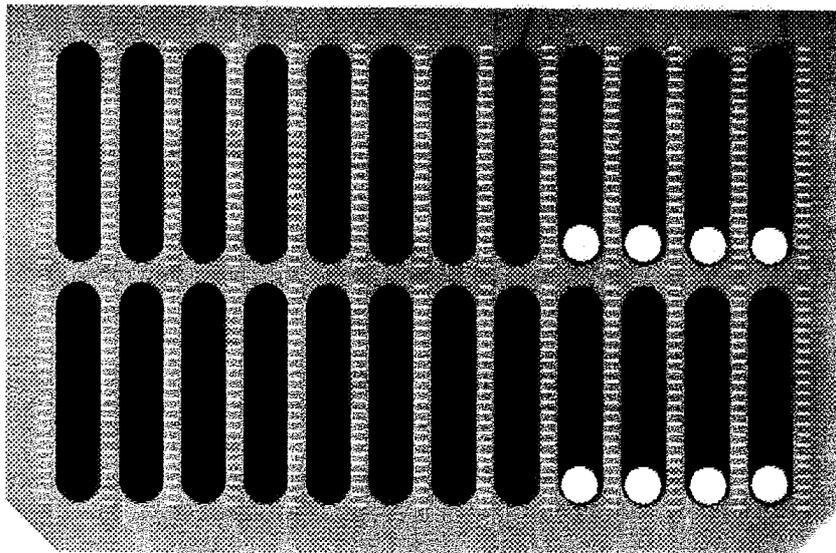
FIG. 5B
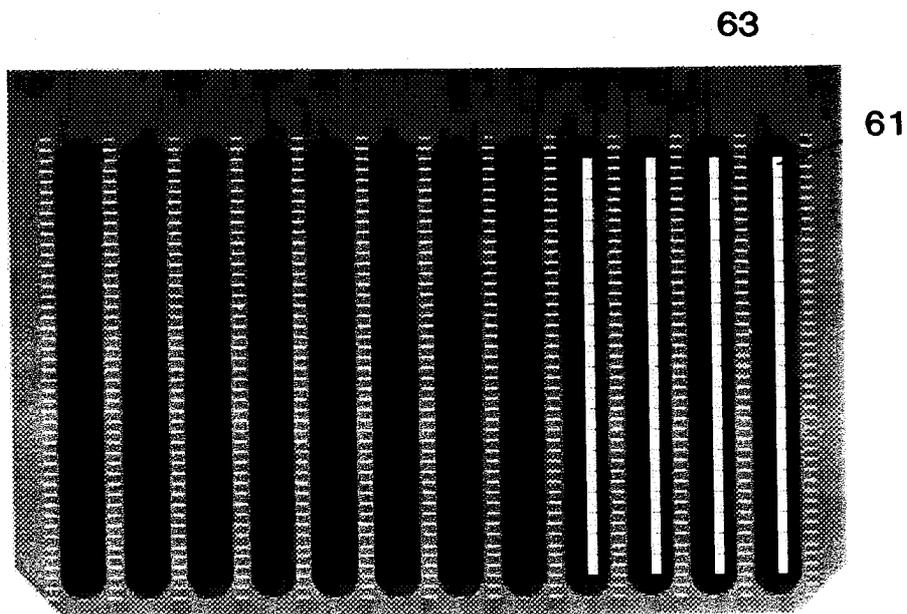
FIG. 6A
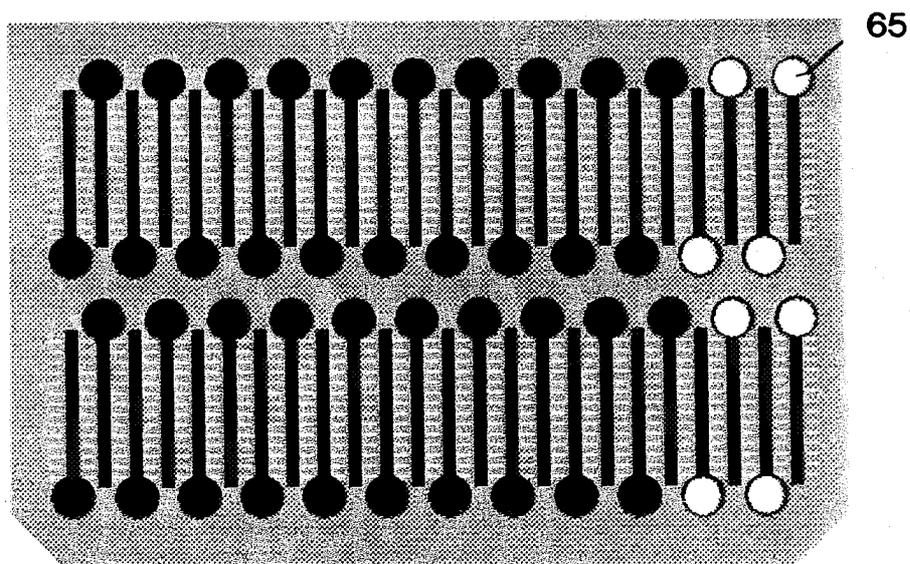
FIG. 6B
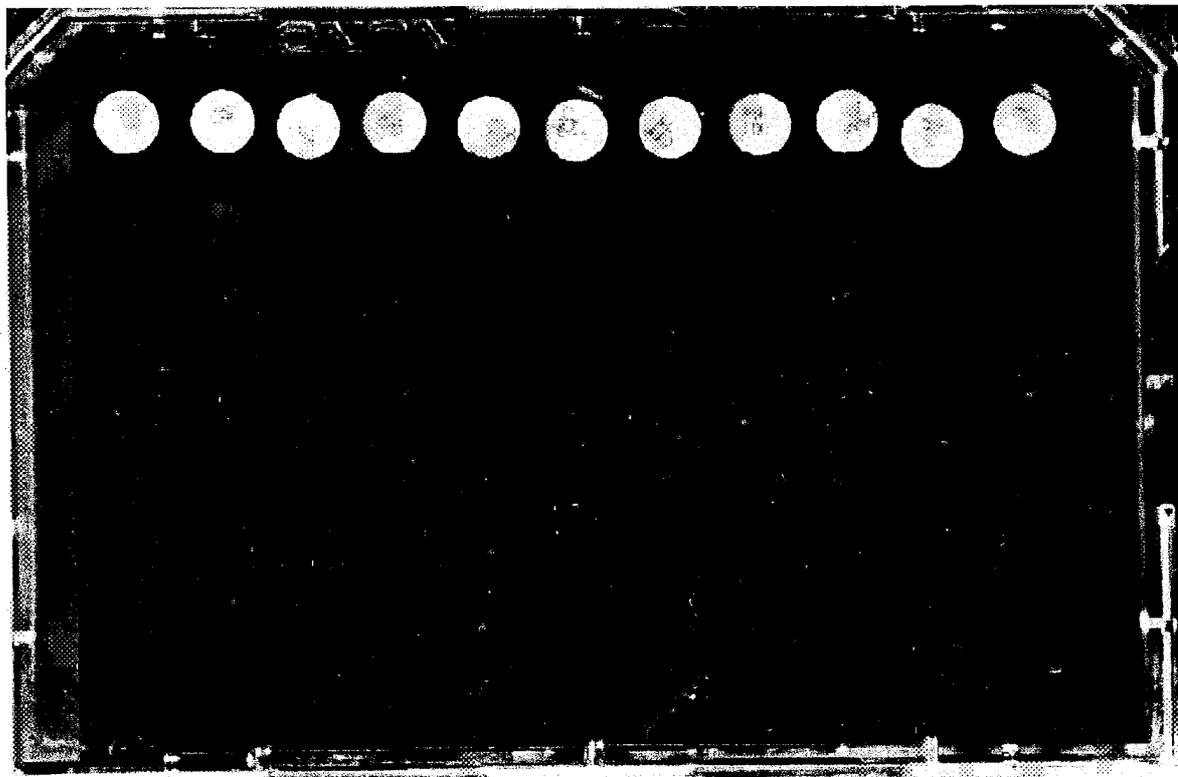
FIG. 7A
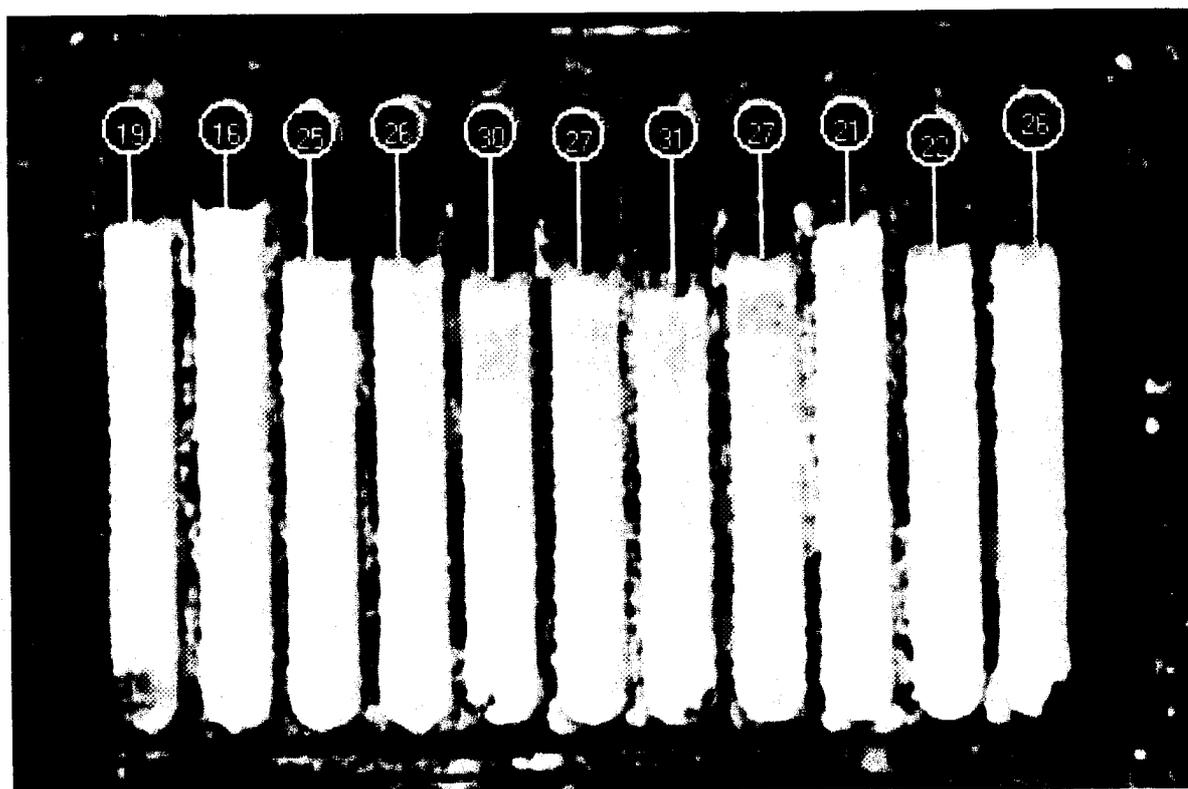
FIG. 7B
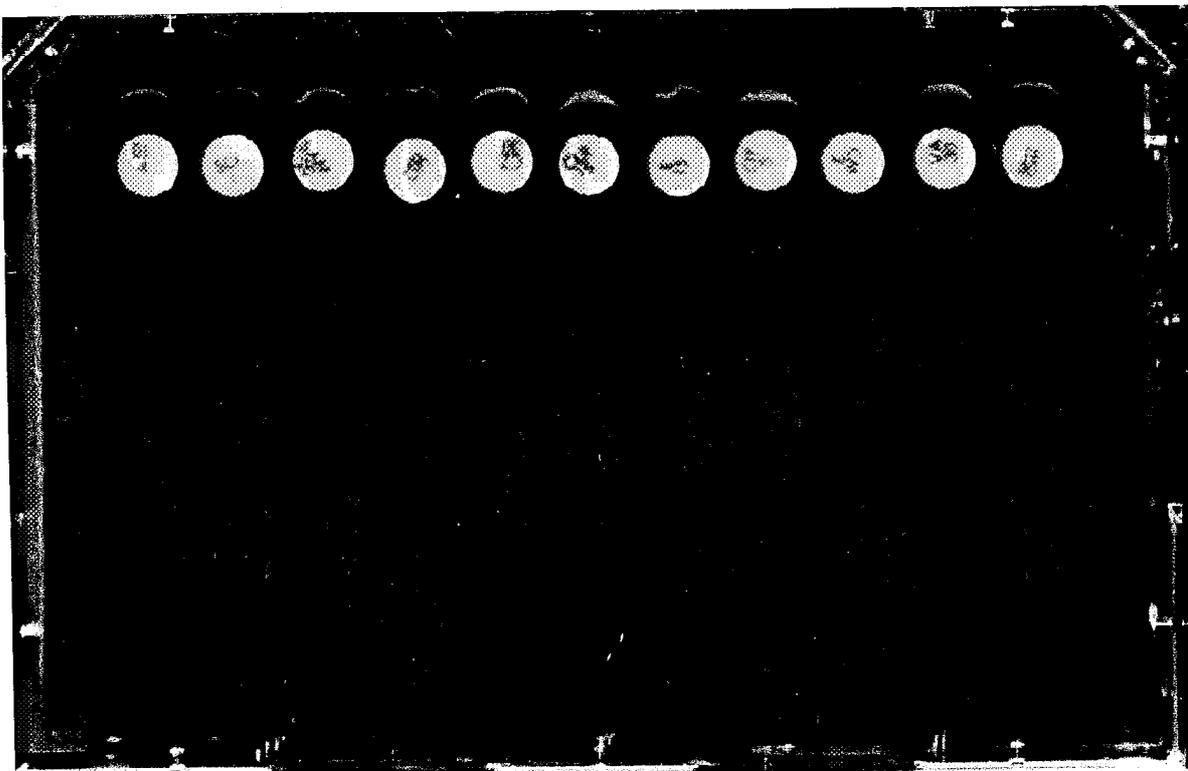
FIG. 8A
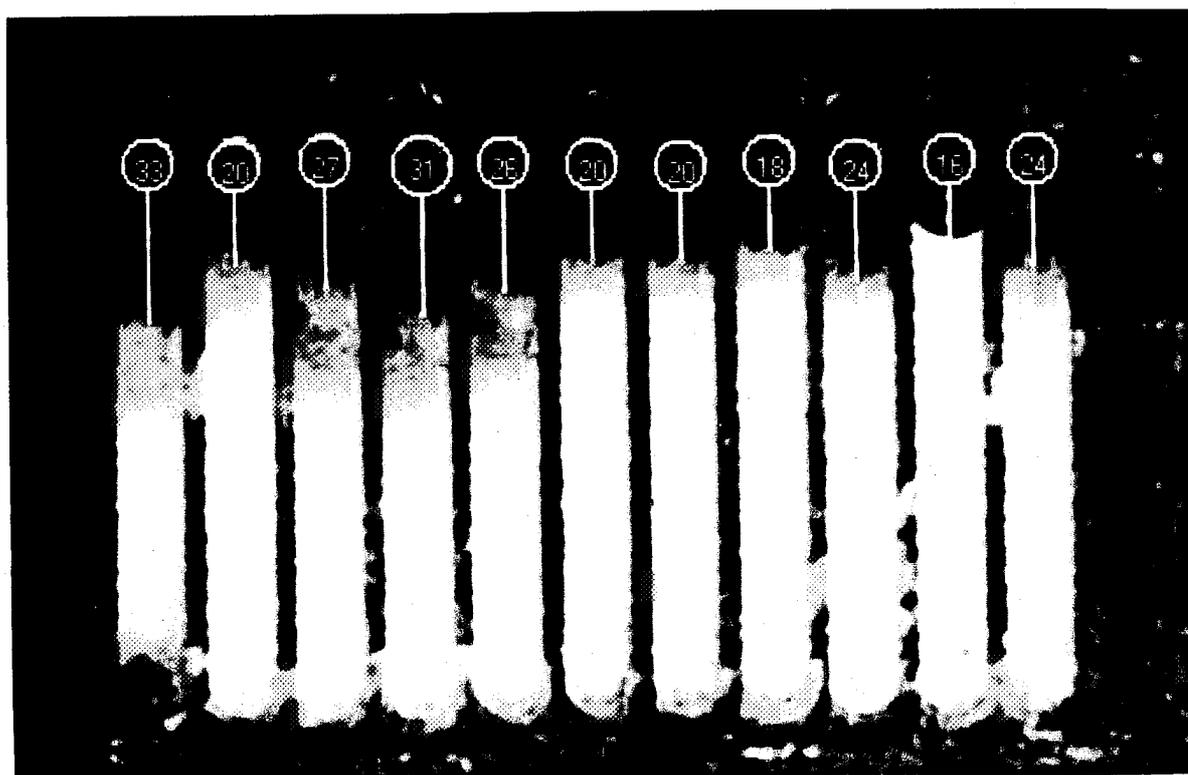
FIG. 8B
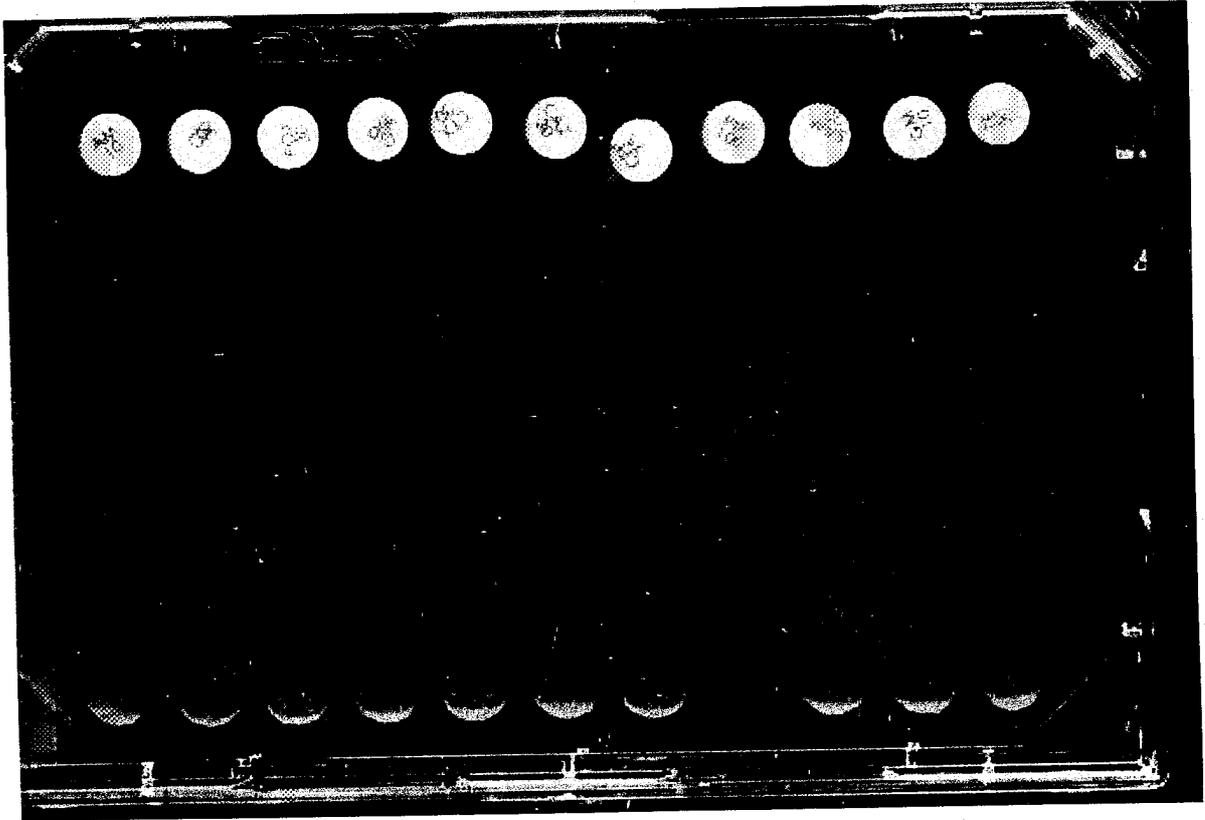
FIG. 9A
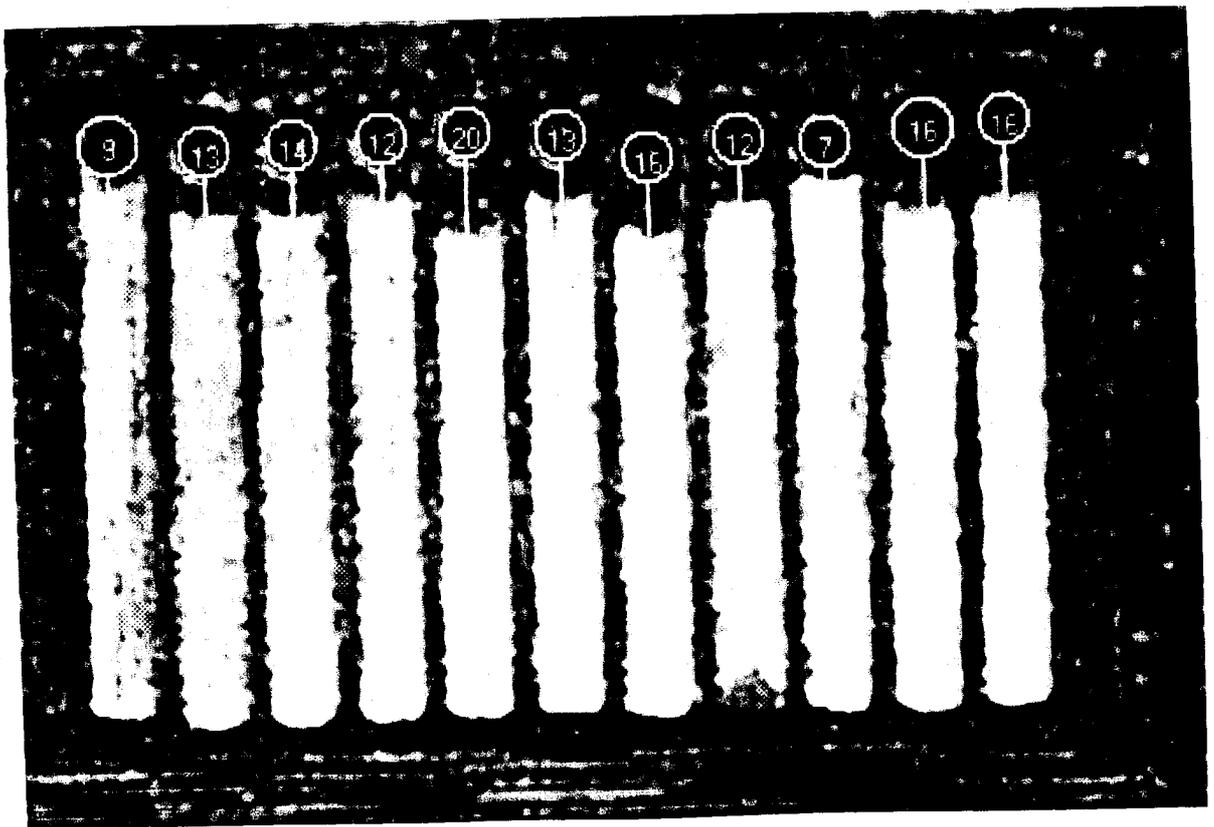
FIG. 9B
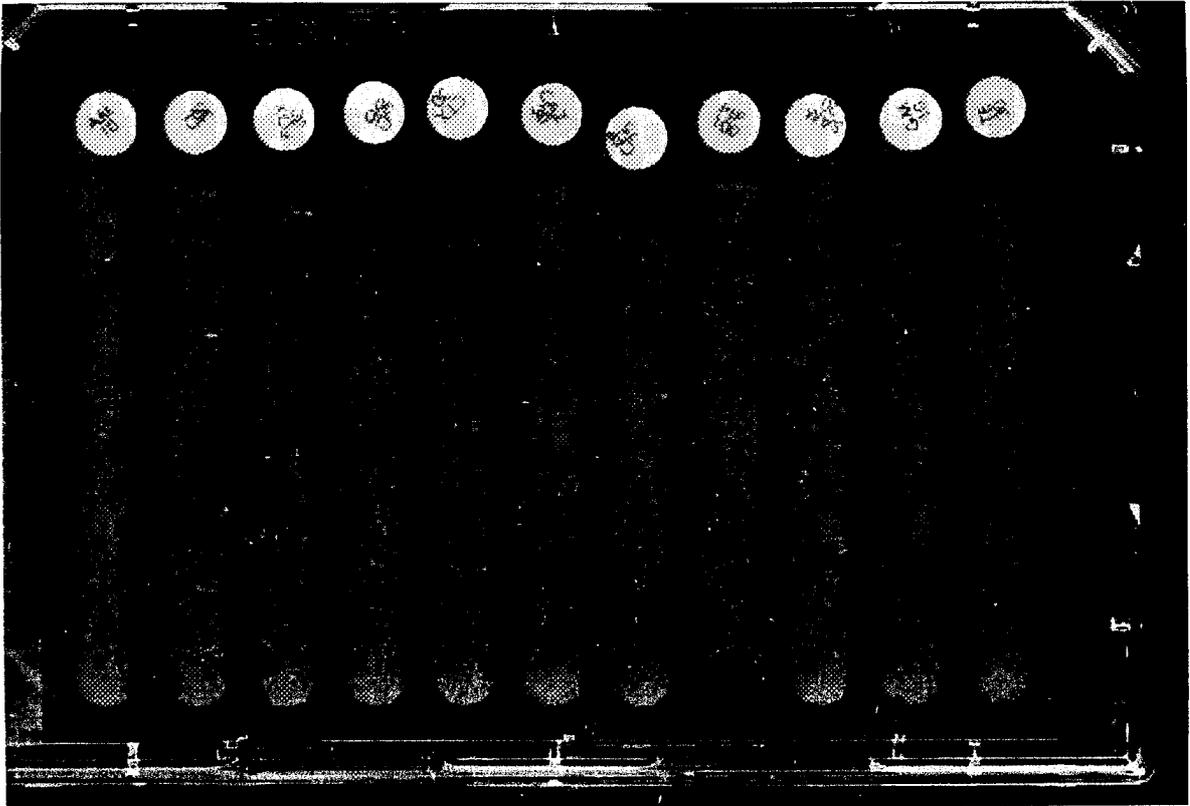
FIG. 9C
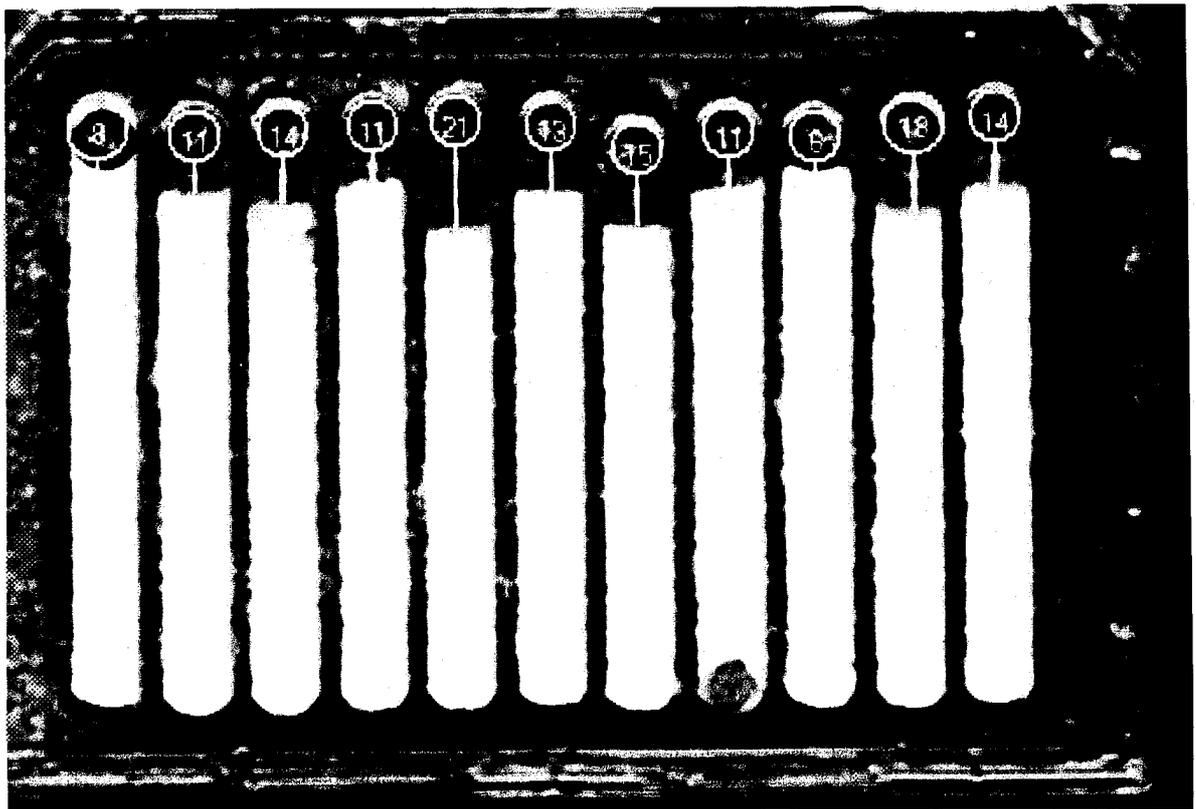
FIG. 9D
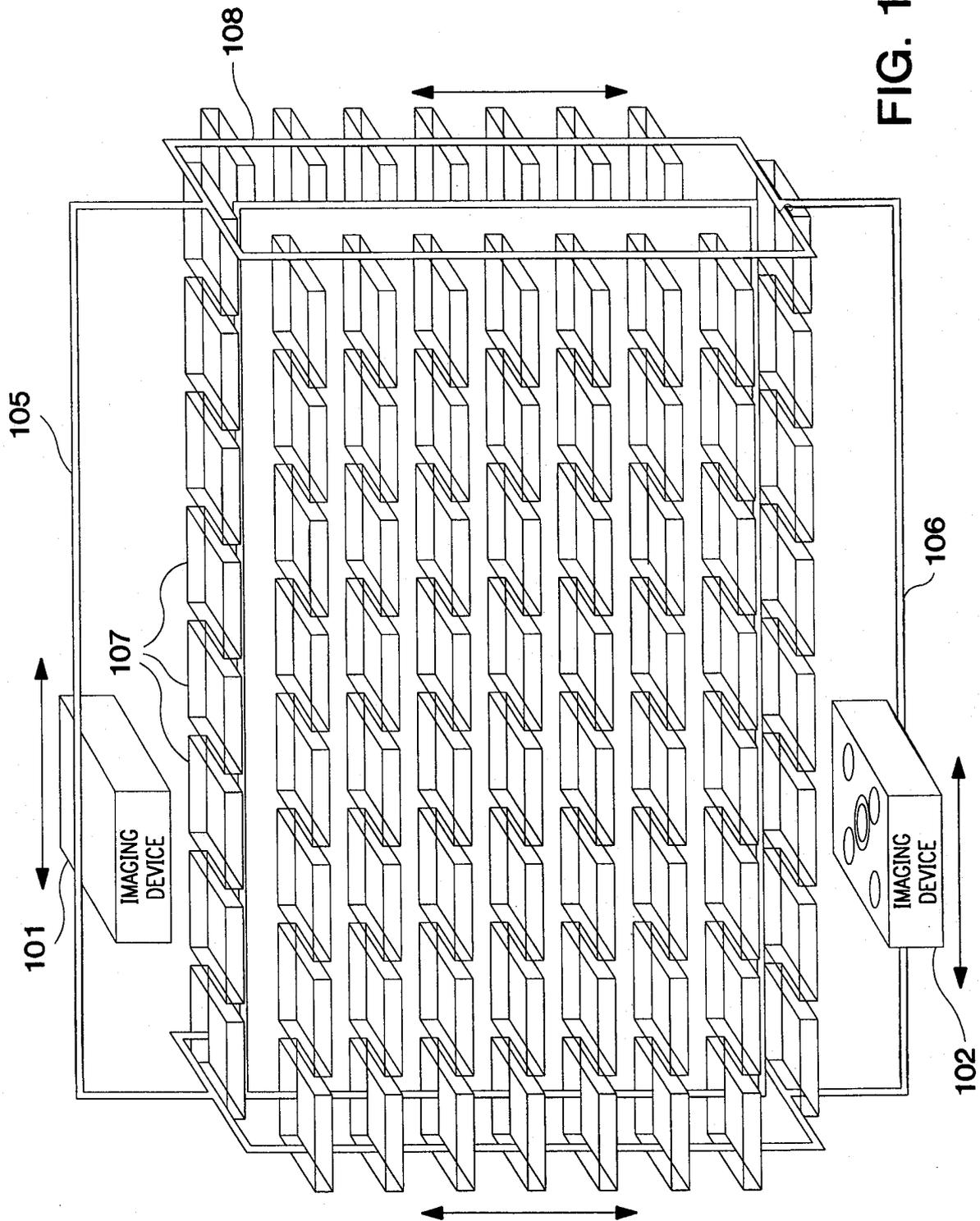
FIG. 10

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12Q 1/18, 1/02, 1/22, 1/20; C12N 1/00; G01N 33/53

US CL : 435/32, 29, 30, 31, 32, 33, 975, 253.3, 842

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/32, 29, 30, 31, 32, 33, 975, 253.3, 842

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS: microbial resistance, antimicrobial, inhibition zone, Kirby-Bauer, bacterium, fungus, protozoa, algae, virus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,252,904 A (NELSON et al) 24 February 1981, see entire document.	1-49
A	US 4,090,920 A (STUDER, JR.) 23 May 1978, see entire document.	1-49

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"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
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 MAY 2000	19 JUL 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer LOUISE LEARY
Facsimile No. (703) 305-3230	Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)*

专利名称(译)	用于微生物敏感性测试的装置和方法
公开(公告)号	EP1163362A4	公开(公告)日	2004-12-29
申请号	EP2000917807	申请日	2000-03-09
[标]申请(专利权)人(译)	阿克佐诺贝尔公司
申请(专利权)人(译)	AKZO NOBEL N.V.
当前申请(专利权)人(译)	AKZO NOBEL N.V.
[标]发明人	MATSUMURA PAUL M HYMAN JONES M JEFFREY SCOTT R MARESCH MARTIN J THORPE THURMAN C BARRON WILLIAM G
发明人	MATSUMURA, PAUL, M. HYMAN, JONES, M. JEFFREY, SCOTT, R. MARESCH, MARTIN, J. THORPE, THURMAN, C. BARRON, WILLIAM, G.
IPC分类号	C12M1/34 C12Q1/18 C12Q1/20 C12R1/01 G01N21/25 C12N1/00 C12Q1/02 C12Q1/22 G01N33/53
CPC分类号	G01N21/253 C12Q1/18
代理机构(译)	HARRISON GODDARD FOOTE
优先权	09/267863 1999-03-12 US
其他公开文献	EP1163362B1 EP1163362A1
外部链接	Espacenet

摘要(译)

用于进行微生物抗生素敏感性测试的方法和设备包括一次性多室易感性板和自动化板处理器以及图像采集和处理仪器，易感板用微生物接种（任何合适的生物，例如细菌，真菌，原生动物，藻类）。应用抗病毒剂和抗微生物剂使得微生物暴露于各种浓度或每种抗微生物剂的梯度。然后将板置于仪器中，仪器监测并测量微生物的生长（或缺乏）。该数据用于确定微生物对抗生素的敏感性。这样的系统使用固体培养基和Kirby-Bauer标准化结果报告自动化抗菌药敏试验。该系统提供了以前仅与肉汤微量稀释测试相关的自动化水平，同时保留了手动纸盘扩散测试的优点。图（1）显示了用于进行盘扩散抗生素敏感性试验的琼脂平板的图示。