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(54) **Title:** A TEST BAND FOR MINIMAL INHIBITION CONCENTRATION (MIC) DETECTION

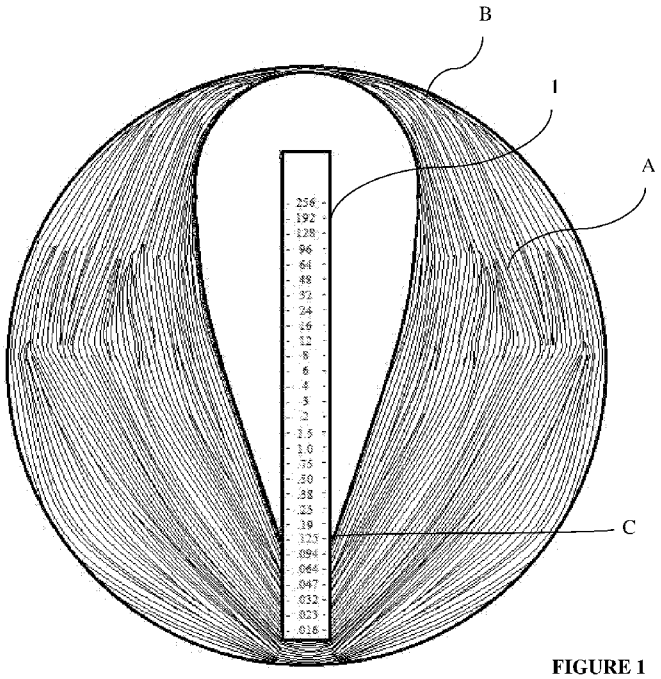


FIGURE 1

(57) **Abstract:** The present invention relates to a minimal inhibition concentration (MIC) detection test wherein bands (1), which are produced by applying antibiotics on cotton, linen and silk fabric, are used. The objective of the present invention is to use cotton fabric, linen fabric, silk fabric or fabrics of cotton-polymer mixture for the bands, which are used for carrying out measurements in the minimal inhibition concentration (MIC) detection test kit, and one surface of which has a ruler.

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A TEST BAND FOR MINIMAL INHIBITION CONCENTRATION (MIC) DETECTION

Field of the Invention

The present invention relates to a minimal inhibition concentration (MIC) detection test wherein bands, which are produced by applying antibiotics on cotton, linen and silk fabric, are used.

Background of the Invention

Antimicrobial susceptibility tests are tests which are applied in order to detect in vitro activity of an antimicrobial agent against a specific bacteria species and mainly two methods are used namely, “diffusion” “and “dilution”.

Dilution tests are applied to detect minimum concentration of an antimicrobial agent required to inhibit reproduction of a microorganism or to kill the same. Dilution tests are applied in two ways, namely “tube dilution” and “agar dilution”.

The publication of Irith Wiegand, Kai Hilpert and Robert E.W. Hancock in Centre for Microbial Diseases and Immunity Research (163-175) (2008) dated January 17, 2008 discloses that tube dilution method can be applied in two ways namely “macro” and “micro”. In principle, both methods are the same but test tubes are used in macrodilution whereas “U” or “V” based “microplates” are used in microdilution. In tube dilution method, cation (calcium and magnesium) added Mueller-Hinton broth is used as the medium. The antibiotics to be tested are first prepared in their special solvents and then are diluted in this broth to a two times lower concentration. A standard inoculum of the microorganism is prepared and it is added to each tube containing various dilutions of the antimicrobial agent at

equal amounts. Additionally, it is also added to the control tube which does not include antibiotic and which is the indicator of reproduction. A tube or a well, wherein bacteria are not inoculated but only the medium is disposed, is prepared as the control medium. The media are examined in terms of turbidity, which shows bacterial growth, after incubation of 16-20 hours at 37°C. The lowest drug concentration, which prevents bacterial growth and wherein there is no visible turbidity, is evaluated to be the minimum inhibitor concentration (MIC). The MIC values obtained as a result of the dilution tests provide information to the clinician about the antimicrobial drug concentration required to inhibit the microorganism causing the infection (Figure 6).

In a state-of-the-art application, the antibiotics were decreased from left to right and were respectively diluted in the broth, and an equal volume and equal concentration of microorganisms were added thereon. The tubes into which microorganisms were added were incubated at 37°C for 16-20 hours and the obtained results were evaluated. The antibiotic concentration in the tube, wherein microorganisms did not reproduce, was recorded as MIC value (6.25 µg/ml) [1, 2].

The invention disclosed in the United States patent document no. US47783758, known in the state of the art, relates to MIC tests used for in vitro diagnosis, and it is stated that the detection test band used is transparent, inert and has a nonporous surface. By means of this patent, instead of MIC detection carried out by tube dilution test, it was succeeded to inoculate on a band for the first time.

The MIC test bands of Oxoid Limited company mentioned in the publication titled "M.I.C.E valuators (M.I.C.E.) Simple, Convenient Method for Accurate MIC Values" in RAPIDMICROBIOLOGY dated 05/30/2008 are produced from polymer material. Furthermore, United States patent document no. US6010910A of the above mentioned company discloses that the bands in the test kits can be produced from plastic, cardboard or similar materials.

In the invention disclosed in the European patent document numbered EP2480682 of Liofilchem Srl company, it is stated that the detection test bands used for MIC tests are produced from paper material. It is stated that by using bands produced from paper material, formation of air bubbles in the band material adhered onto the medium, wherein bacteria are inoculated, can be prevented and air contact of the bands with the environment can be enabled. Additionally, in the method of the state of the art, it is pressed on the band material with a forceps in order to prevent formation of air bubbles after placing the band material on the medium. Therefore, formation of air bubbles can be prevented independent from the material used.

The invention disclosed in Chinese Utility Model document no. CN2564584, another state-of-the-art application, relates to a detection plate test band made of a membrane used for detecting minimum antibacterial concentration of bacteriophages. The filtration-membrane band is fixed on a substrate, thus leading to a detection plate of the bacterial drug sensitivity test. In the said invention, two detections can be carried out at once including drug sensitivity and minimum antibacterial concentration (MIC). It is stated that the said invention is advantageous due to its low cost, simple and easy application, and reliability.

When compared with the applications in the state of the art; it is seen that minimal inhibition concentration (MIC) detection test band production, wherein bands produced from cotton fabric, linen fabric, silk fabric or fabrics of cotton-polymer mixture, is advantageous in that the material used therein is easy to supply, has low cost, is produced from materials resistant against wear-out and tear, and allows easier and faster diffusion of the antibiotic material.

Summary of the Invention

The objective of the present invention is to use cotton fabric, linen fabric, silk fabric or fabrics of cotton-polymer mixture for the bands, which are used for

carrying out measurements in the minimal inhibition concentration (MIC) detection test kit, and one surface of which has a ruler.

Another objective of the present invention is to enable producing bands which are resistant to wear-out and tearing by using cotton fabric, linen fabric, silk fabric or fabrics of cotton-polymer mixture.

Another objective of the present invention is to enable producing bands which allow easier and faster diffusion of the antibiotic material by using cotton fabric, linen fabric, silk fabric or fabrics of cotton-polymer mixture.

A further objective of the present invention is to use bands produced from cotton fabric, linen fabric, silk fabric or fabrics of cotton-polymer mixture, which are more cost-effective and easily supplied, for the minimal inhibition concentration detection test kit.

Another objective of the present invention is to use two-layered and thin bands thereby eliminating the risk of easily bending.

Detailed Description of the Invention

The "Test Band For MIC (Minimal Inhibition Concentration) Detection" developed to fulfill the objective of the present invention is illustrated in the accompanying figures wherein,

Figure 1. is a view of the test band for MIC (Minimal Inhibition Concentration) Detection in a petri dish.

Figure 2. is a general view of the test band for MIC (Minimal Inhibition Concentration) Detection.

Figure 3. is a view of the layers of the test band for MIC (Minimal Inhibition Concentration) Detection.

Figure 4. is an exploded view of the layers of the test band for MIC (Minimal Inhibition Concentration) Detection.

Figure 5. is an exploded view of the test band for MIC (Minimal Inhibition Concentration) Detection.

Figure 6. is a view of the tube dilution method application.

The components in the figures are given reference numbers as follows:

1. Test band for MIC (Minimal Inhibition Concentration) Detection

2. Interaction layer

3. Ruler layer

A. Microorganisms

B. Petri dish

C. MIC value

The inventive test band (1) for MIC (Minimal Inhibition Concentration) detection is placed into a petri dish (B) containing suitable agar medium such that its layer, on which a drug is inoculated, is contacted with the microorganisms (A) inoculated into the petri dish (B) with the purpose of detecting the measure of interaction (MIC value (C)) of the microorganisms (A) with the antibacterials (in antibiotic, antimycostatics or other biological fluids), by using an in vitro diagnosis device (IVD); and it is preferably in the form of a rectangular plate and is placed into the petri dish (B) such that its layer, on which antibiotic is inoculated, contacts the microorganisms (A) in the petri dish (B) and all parts thereof are ensured to be adhered by lightly pressing thereon via a penset; and **comprises**

- at least one interaction layer (2) which is in the form of a rectangular plate and is positioned so as to contact the microorganisms (A) in the petri dish (B),

- at least one ruler layer (3) which is in the same form and size with the interaction layer (2) and is connected to the interaction layer (2) from the face thereof that does not contact the microorganisms (A);

and is **characterized by**

- the interaction layer (2); on which an antibiotic, whose interaction with microorganisms (A) will be examined, is applied on the surface thereof that contacts the microorganisms (A) by gradually decreasing amounts between the short sides of its rectangular form; and which is produced from fabrics comprising cotton fabric, linen fabric, silk fabric or cotton – polymer mixture,
- the ruler layer (3), which, in order to figure out the antibiotic concentration applied onto the interaction layer (2), includes numbers denoting the concentration value and lines denoting the positions corresponding to these concentrations.

In one embodiment of the invention, the ruler layer (3) is produced by using cotton fabric similar to the interaction layer (2). Accordingly, in this embodiment of the invention, the ruler layer (3) and the interaction layer (2) are produced together as a single piece and are not connected. The bands produced in the scope of the present invention by using preferred cotton fabric, linen fabric, silk fabric or a fabric comprised of cotton-polymer mixture do not have air permeability.

In one embodiment of the invention, the polymer used in the structures comprised of cotton fabric and polymer combination is preferred to be polypropylene and ethylene butylacrylate copolymer.

In one embodiment of the invention, the interaction layer (2) includes chambers, which enable the inoculated antibiotic to easily diffuse to the medium, on the surface thereof contacting the microorganisms (A).

In a different embodiment of the invention, a polymer-cotton fabric mixture, whose absorption capacity is higher than that of the paper applications in the state of the art, is used as the interaction layer (2). In this sense, synthetic fabrics having 50% or more cotton content are also used as cotton fabric. As an alternative to cotton fabric; cotton cloth, cotton and polyester mixture cloth, linen cloth or silk cloth are also used in the scope of the invention.

In a different embodiment of the invention, the ruler layer (3) is produced from aluminum, plastic or paper sticker.

In one embodiment of the invention, the ruler layer (3) is in the form of a sticker and is adhered onto the surface of the interaction layer (2) that does not contact the microorganisms (A). After the ruler layer (3) is produced, the numerical values and lines, which enable to read measurements, are provided on the surface thereof by printing in the printing press.

The MIC test band (1) of the present invention enables both inoculation of the drug and the inoculated drug to adhere to the surface and remain there evenly. Drug inoculation is performed after the ruler layer (3) and the interaction layer (2) are connected and thus the MIC test band (1) structure of the present invention is formed. The drug inoculation on the interaction layer (2) is performed between the two short sides of the interaction layer (2) in a decreasing manner, i.e. gradient manner, in accordance with the concentration values in the ruler layer (3). The MIC test bands according to the present invention are preferably produced in the form of a rectangular band. This way, it can be easily placed on a medium such as in a petri dish (B) in which the microorganisms are inoculated, and the intersection point of the interaction between the drug and the bacteria can be detected more specifically by means of the numbers on the ruler. This way, being able to express the minimal antibiotic concentration, at which microorganism-antibiotic interaction takes place, denoted as MIC value, as a numeric value facilitates the procedures for the user.

The MIC test bands of the present invention can be stored within the range of -20 to 8°C. On the other hand, MIC test bands comply with two standard values accepted worldwide, namely CLSI (Clinic and Laboratory Standards Institute) in the United States and EUCAST (European Committee on Antimicrobial Susceptibility Testing) in European Union.

Gradually decreasing amounts of antibiotic are added to the test bands produced by using cotton fabric, linen fabric, silk fabric or fabrics of cotton-polymer mixture; and a ruler is printed on the layer of the test band, on which a drug (antibiotic) is not inoculated, and which is produced from aluminum material, in order to be able to evaluate the experiment result. The minimum antibiotic concentration (mg/l), at which bacterial growth is inhibited, of the ruler arranged according to the MIC value ranges known in the literature is defined as MIC value.

Using cotton fabric, linen fabric, silk fabric or fabrics of cotton-polymer mixture for their production enabled to obtain bands, which are more resistant against tearing and breaking, more advantageous in terms of cost and easy to supply, and which allow the antibiotic material to diffuse more easily and rapidly. Furthermore, it is observed that the interaction between the antibiotic and bacteria can be prevented from getting affected by the air in the outer environment by using bands not having air permeability.

In another embodiment of the invention, the layer of the test bands, on which drug is not inoculated and a ruler is printed, is produced from a plastic or paper material.

In another embodiment of the invention, both the interaction layer and the ruler layer are produced by using fabric material.

REFERENCES

[1]. BauerJD, Ackerman PG, Toro G. Clinical laboratory methods 8th ed. The C.V.mosby company. Sant Louise, USA 1974; 661-662

[2]. Bauer J.D. Clinical l9th ed. The c.v.mosby company .St.Louise 1982 ; 883-884

CLAIMS

1. A test band (1) for MIC (Minimal Inhibition Concentration) detection which is placed into a petri dish (B) containing suitable agar medium such that its layer, on which a drug is inoculated, is contacted with the microorganisms (A) inoculated into the petri dish (B) with the purpose of detecting the measure of interaction (MIC value (C)) of the microorganisms (A) with the antibacterials (in antibiotic, antimycostatics or other biological fluids), by using an in vitro diagnosis device (IVD); and which is preferably in the form of a rectangular plate and is placed into the petri dish (B) such that its layer, on which antibiotic is inoculated, contacts the microorganisms (A) in the petri dish (B) and all parts thereof are ensured to be adhered by lightly pressing thereon via a penset; and **comprising**

- at least one interaction layer (2) which is in the form of a rectangular plate and is positioned so as to contact the microorganisms (A) in the petri dish (B),
- at least one ruler layer (3) which is in the same form and size with the interaction layer (2) and is connected to the interaction layer (2) from the face thereof that does not contact the microorganisms (A); and **characterized by**
- the interaction layer (2); on which an antibiotic, whose interaction with microorganisms (A) will be examined, is applied on the surface thereof that contacts the microorganisms (A) by gradually decreasing amounts between the short sides of its rectangular form; and which is produced from fabric material,
- the ruler layer (3), which, in order to figure out the antibiotic concentration applied onto the interaction layer (2), includes numbers denoting the concentration value and lines denoting the positions corresponding to these concentrations.

2. A test band (1) for MIC (Minimal Inhibition Concentration) detection according to Claim 1, **characterized** by a ruler layer (3) which is produced by using cotton fabric similar to the interaction layer (2), and which is formed as a single piece with the interaction layer (2).

3. A test band (1) for MIC (Minimal Inhibition Concentration) detection according to Claim 1, **characterized** by the interaction layer (2), which does not have air permeability, and which is comprised of cotton fabric, linen fabric, silk fabric or fabrics of cotton-polymer mixture that are more resistant against tearing and breaking, more advantageous in terms of cost and easy to supply, and which allow the antibiotic material to diffuse more easily and rapidly.

4. A test band (1) for MIC (Minimal Inhibition Concentration) detection according to Claim 1, **characterized** by the interaction layer (2), wherein the polymer used in the fabric structures comprised of cotton-polymer mixture is polypropylene and ethylene butylacrylate copolymer.

5. A test band (4) for MIC (Minimal Inhibition Concentration) detection according to Claim 4, **characterized** by the interaction layer (2), wherein the cotton-polymer mixture fabric is a synthetic fabric having 50% or more cotton content.

6. A test band (1) for MIC (Minimal Inhibition Concentration) detection according to Claim 1, **characterized** by the ruler layer (3), which is produced from aluminum, plastic or paper material.

7. A test band (1) for MIC (Minimal Inhibition Concentration) detection according to Claim 1, **characterized** by the ruler layer (3), on a surface of which numerical values and lines that enable to read measurements are printed in a printing press during production thereof.

8. A test band (1) for MIC (Minimal Inhibition Concentration) detection according to Claim 1, **characterized** by the ruler layer (3), which is in the form of a sticker, and which is adhered and fixed on the interaction layer (2).

9. A test band (1) for MIC (Minimal Inhibition Concentration) detection according to Claim 1, **characterized in that** the interaction layer (2) and the ruler layer (3) are produced using fabric material.

FIGURE 1

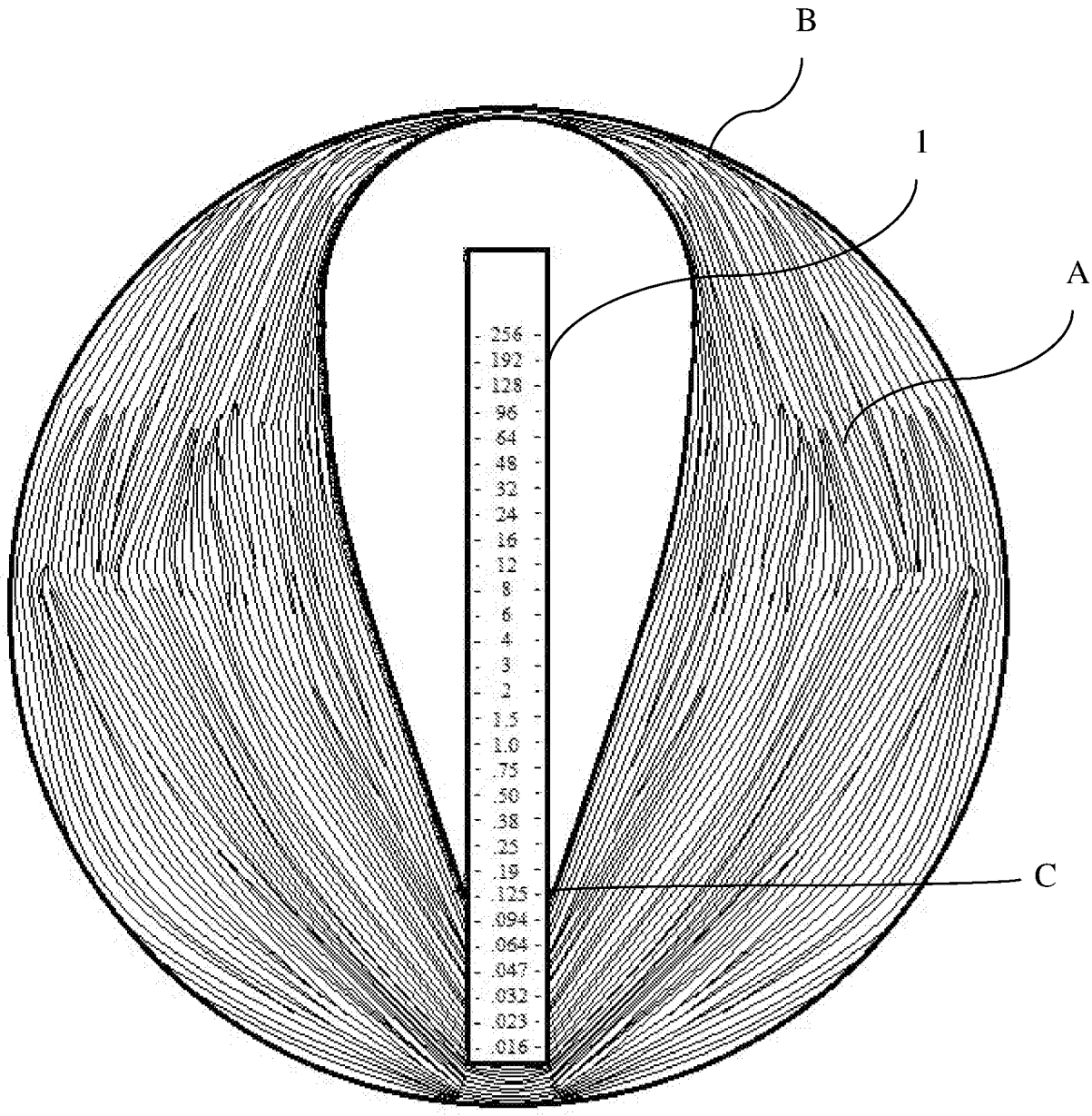

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FIGURE 2

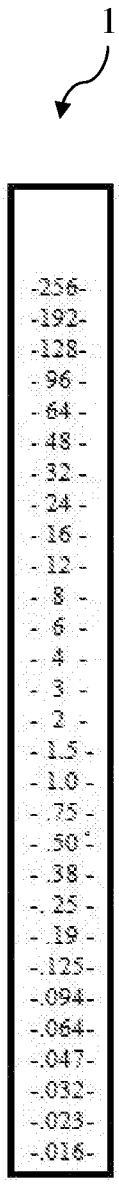


FIGURE 3

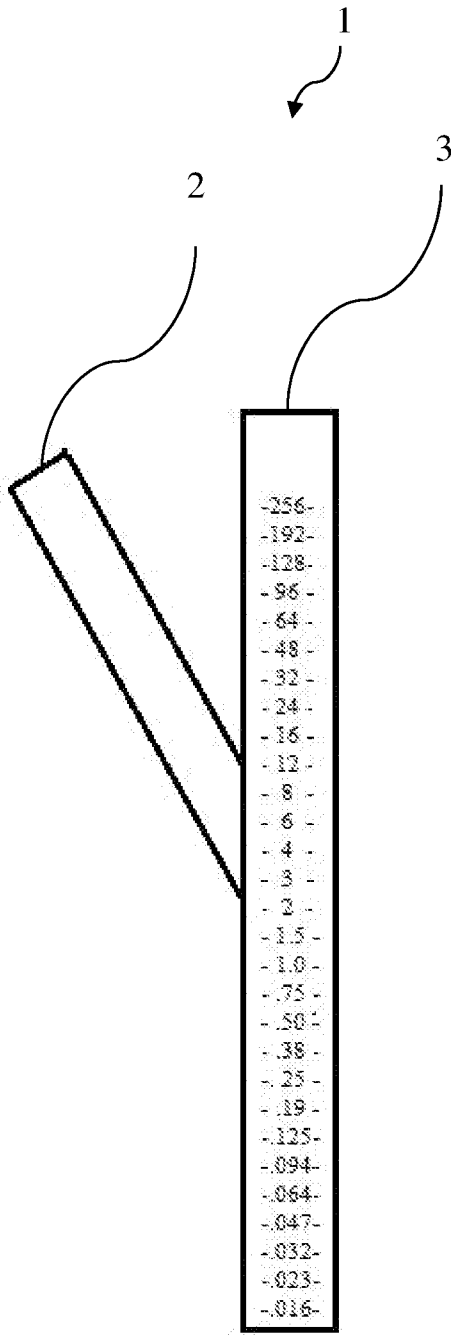


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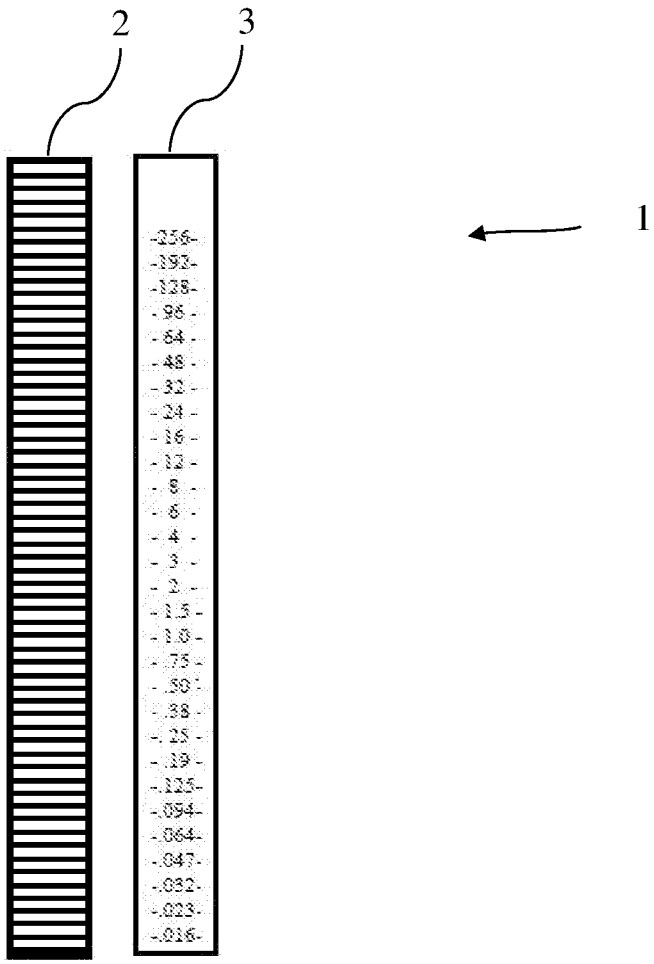


FIGURE 5

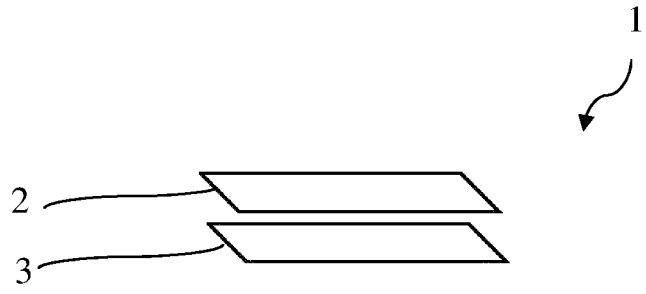


FIGURE 6

