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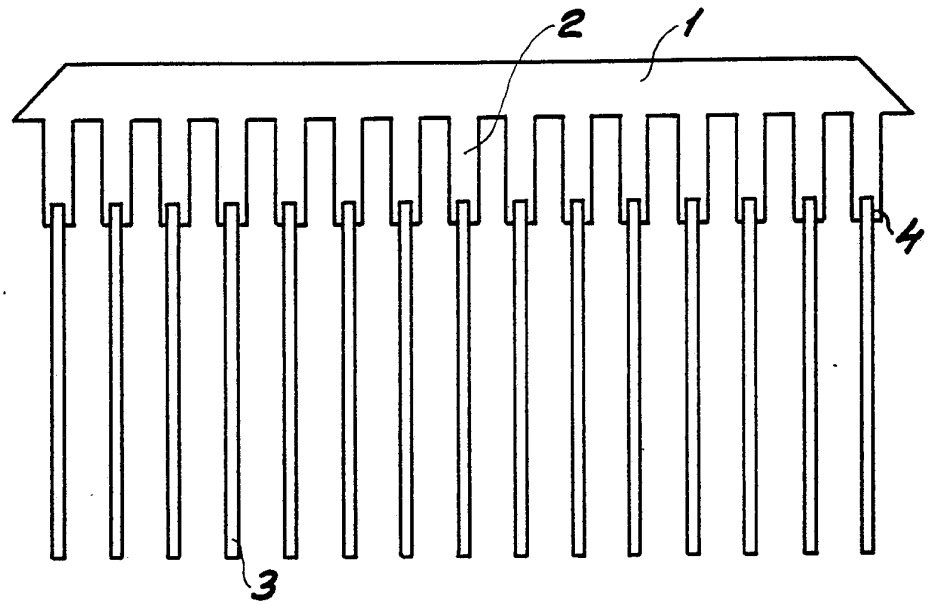
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(54) Title: METHOD FOR DETECTING ANTIBODIES IN BLOOD, AND MEMBRANE STRIP AND MEMBRANE BATTERY FOR USE WHEN CARRYING OUT THE METHOD



(57) Abstract

A method for detecting antibodies in blood utilizes a coagulated blood sample on a carrier, such as a blood spot on a piece of filter paper which is introduced into a container together with an elution and incubation liquid known per se and a foil strip coated with antigenes corresponding to the antibody type/types which it is/are desired to detect. After incubation and coulouring detection for positive or negative reaction is performed. The invention also relates to a membrane strip or membrane battery and elution and incubation liquid for use in the method and a test kit comprising the equipment necessary for carrying out the test.

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Method for detecting antibodies in blood, and membrane strip and membrane battery for use when carrying out the method

The present invention relates to a method for detecting the presence of antibodies in blood by immunoblotting-technique (Western blot) using a membrane coated with screened antigenes corresponding to the antibodies to be detected. The invention further relates to a membrane strip and a membrane battery for use when carrying out the method.

A number of methods for detecting antibodies and antigenes are known. Antigenes can e.g. be separated and detected by gel electrophoresis, e.g. SDS. By this method it has been possible to decompose an almost arbitrary mixture of proteins into the individual components. However, further analysis of the separated proteins has been limited due to their relative inaccessibility within the gel-matrix employed.

To overcome this disadvantage the gel matrix has been replaced by a membrane onto which the proteins could be fixated in immobilised state.

The advantages of the so-called blotting technique include i.a. antigene concentration on the surface of a membrane in immobilised state, so that the individual separated antigenes are easily accessible for further identification.

An analysis method based on immunoblotting-technique can be used for characterization of both antibodies and antigenes. Examples of antibodies which can be detected in this way include all kinds of retrovirus, such as HIV-I, HIV-II, HTLV-I and HTLV-II. Other examples are

malaria, schistosomasis, filariasis, African trypanosomiasis and laishmaniasis. Further, allergy producing substances can be detected.

The normal immunoblotting-technique employs blood samples which are pretreated for isolating serum. The isolated serum is contacted with a membrane in the form of a strip, width typically 4-5 mm, and length 120 mm, and which is coated with screened antigenes corresponding to the antibodies to be detected. After suitable incubation time the membrane is removed and tested for positive reaction. Testing may be by any known method, such as colouring.

The present invention is a further development and simplification of the known immunoblotting-technique, and involves significantly shortened analysis time. Furthermore, the analysis is made on whole blood in stead of serum, e.g. a blood sample which has been transferred onto a carrier and has coagulated.

Thus, the invention relates to a method of the kind stated in the introductory part of claim 1, being characterized in that a carrier with the blood sample which has coagulated after application onto the carrier, is introduced into a container together with the membrane in the form of a strip coated with the screened antigenes and an elution and incubation liquid, and that the membrane after a preset incubation time is removed and tested in a way known per se.

According to the invention it has proved expedient to use as a carrier a piece of filter paper on which the blood sample is sucked up. It is known to use a filter paper for sucking up a blood sample, however, according to the known processes it has been necessary first to elute serum from the blood sample and then test the

content of antibodies in the serum in a subsequent step.

With the present invention it is possible to carry out elution and incubation in one single step, which entails a significant simplification and shortening of the analysis time. Contributing to the obtained shortening of the analysis time is also the fact that with the method according to the invention it is possible to use a membrane in the form of a strip with extremely small dimensions. Preferred dimensions of the membrane strip used are: width 1.1-1.8 mm and length 10-100 mm. This considerably reduces the price of the process, and also the time necessary for elution and transfer of antibodies onto the membrane is shortened considerably, e.g. from 3-4 hrs. to 1-1 1.5 hrs.

The preferred membrane strip for use when carrying out the method according to the invention is a polyvinylidendifluoride foil, cut into strips of the stated dimensions, i.e. preferably a width of 0.1-1.8 mm and a length of 10-100 mm, e.g. 70-75 mm. Examples of other suited membrane materials are nitrocellulosis or polyamide. Each strip can be marked with an identification code, such as a sticker with a journal number or a bar code directly on the film strip.

For carrying out the test use can be made of an arbitrary container capable of containing the membrane and the carrier with the blood sample as well as elution and incubation liquid. If a large number of tests are to be carried out simultaneously it is expedient to use a block with a number of cells into which a corresponding number of membranes and carriers with blood sample can be introduced.

An expedient embodiment of such a test block with a number of cells is shown in the drawing in which

Fig. 1 is a perspective view of a block according to the invention,

Fig. 2 is a cross-section of a part of the same block,

Fig. 3 is a perspective view of one of the two lids of the block, and

Fig. 4 is a cross-section similar to that of Fig. 2, in which the membrane, carrier with blood sample and elution and incubation liquid have been introduced.

Fig. 5 is a membrane battery with 15 membrane strips, coated with similar or different antigenes,

Fig. 6 is a membrane battery of the same type as shown in Fig. 5, each membrane strip, however, being divided into 3 sections.

Fig. 7 is a side-view of the membrane battery shown in Fig. 6,

fig. 8 is a side and partly sectional view of a test block with a number of chambers suited for introduction of the membrane battery shown in Fig. 7,

Fig. 9 is a top view of the test block shown in Fig. 8,

Fig. 10 is a section through a test block chamber along the line a-a in Fig. 8, and

Fig. 11 is a carrier strip for a number of pieces of filter paper with blood samples, suited for introduction into the chambers of the test block

shown in Figs. 8-10.

The test block consists of a plate 1 of an autoclavable material, such as POM. A number of parallel through-going holes 2 has been bored in the plate, into which holes through-going tubes 3 projecting in excess of both sides of the plate 1, have been inserted. In the embodiment shown in the drawing the thickness of the plate 1 is 30 mm. The diameter of the holes 2 is 10 mm$^{\varnothing}$. 80 mm long tubes, projecting 25 mm in excess of both sides of the plate 1, have been introduced into the holes, so that the openings 6 of the tubes are free of the surface of the plate, and each tube end is provided with an annular siliconelastomer seal 9. The openings 6 can be closed with two lids 4 ad 5 provided with recesses 7 corresponding to the tube openings.

Fig. 4 shows a crosse-section of a tube into which a piece of filter paper with blood sample 8 and a membrane strip 10 have been introduced. The two tube openings 6a, 6b are closed with lids 4, 5.

In the following the invention will be described in connection with detection of antibodies to AIDS-virus, but it will be understood that the process is equally suited in connection with numerous other antibodies or antigenes.

Example 1

Into each hole 2 of a block as shown in the drawing is introduced a strip of a polyvinylidendifluoride film provided with a reinforcement rib, which film in a manner known per se has been coated with screened HIV antigenes. The strips have a length of 70 mm and a width of 1.6 mm. The strips used are each labelled with stickers provided with printed on journal number. Then

pieces of filter paper with applied blood samples from the persons to be tested are cut to dimensions of 5 x 5 mm. These pieces are introduced into each separate one of the tubes shown, which are all filled three-quarters with an elution and incubation buffer having a pH-value of 7.2 and containing PBS, 0.1% Tween 20, 1% (w/w) rabbit serum, 0,05 N NaN_3. The tube openings are closed by lids 4, 5 and the entire battery is introduced in a rotating apparatus so that the liquid in each cell washes over the introduced strips with screened antigenes. After about 1 h the elution and incubation liquid is poured out, wash is with distilled water and incubation with HRP (Horse Radish Peroxidase Conjugated igG) for 3/4 h, followed by washing in distilled water and colouring with TMBS/DONS substrate. After completed colouring the strips are removed and detection for positive or negative reaction is performed.

Example 2

Use is made of a membrane battery as shown in Fig. 5 comprising a number of membrane strips 3 being attached at a binding site 4 to flaps 2 on a carrier strip 1 of a water-repelling plastic material. The membrane strips 3 are coated with similar or different antigenes corresponding to the antibodies to be detected.

The membrane battery is introduced in a block with the same number of holes or through-going borings and at the same interspaced distance as the membrane strips, corresponding to a row in the block shown in Figs. 1-4. Elution and incubation liquid and pieces of filter paper with dried up blood samples from the persons to be tested are introduced simultaneously into the said holes or through-going borings.

The test is carried out as described in eksample 1.

Example 3

Figs. 6 and 7 show a membrane battery consisting of a holder or rib 11 with a number of flaps 12 provided with a corresponding number of membrane strips 13 of a plastic material, such as polyvinylindendiflouride. Each membrane strip is divided into three sections 13a, 13b and 13c, each being coated with an antigene different from the antigenes on the other two sections, but corresponding to the antibodies to be detected. Each membrane strip is thus adapted for simultaneous detection of 3 different antigenes from a blood sample from the same person. The rib 11 is shaped as a tube so that the membrane battery may easily be fished out by means of a holder tool (not shown) comprising pins for sticking into the tubular rib.

Use is made of a test block with appertaining membrane battery as shown in fig. 8-10. The battery consists of a block 21 having a number of oblong grooved chambers or cells 22, being open at the top. The distance between the cells 22 is adjusted according to the distance between the flaps 12 and the membrane strips 13 with antigenes. The block 21 is provided with a longitudinal recess 23, adapted for receiving the ribs 11 with flaps 12 and strips 13 of the membrane battery.

Further, the block 21 is provided with another longitudinal recess 24, adapted for receiving a rib 31 on a holder for filter paper with blood samples, see fig. 11.

The said holder comprises a rib 31 with a number of flaps 32 with pieces of filter paper 33 being coated with blood samples. Fig. 10 shows a section through a cell 22 with inserted holder for blood samples 31, 32, 33 and holder for membrane strips 11, 12, 13.

Elution and incubation liquid 14 is introduced into the cells, and the culturing and aftertreatment are carried out under the same conditions as stated in example 1. After wash and colouring the membrane battery is removed and the test result read.

It will be understood that the examples are only illustrative and that the method can be varied in various ways, as will be readily appreciated by a person skilled in the art. As mentioned, the method may be used not only for detecting antibodies in blood, but also for detecting antigenes in various liquids. In the latter case use may be made of strips with screened polyclonal antibodies or monoclonal antibodies.

Patent Claims

1. Method for detecting antibodies in a blood sample by immunoblotting-technique (Western blot) using a membrane coated with screened antigenes corresponding to the antibodies to be detected, characterized in that a carrier with the blood sample which has coagulated after application onto the carrier is introduced into a container together with the membrane in the form of a strip coated with the screened antigenes and an elution and incubation liquid, and that the membrane after a preset incubation time is removed and tested in a manner known per se.

2. Method according to claim 1, characterized in that use is made of a carrier consisting of an absorbing filter material, such as a piece of filter paper.

3. Method according to claim 1 or 2, characterized in that use is made of a membrane strip having a width of 1-1.8 mm and a length of 10-100 mm.

4. Membrane strip for use when carrying out the method according to claims 1-3, characterized in that it is coated with antigenes screened according to molecular weight, corresponding to the antibodies to be detected.

5. Membrane battery for use when carrying out the method according to claims 1-3, characterized in that it consists of a number of membrane strips being attached to a common carrier strip, each strip having been coated with each separate one of the antigenes for which it is desired to test the person.

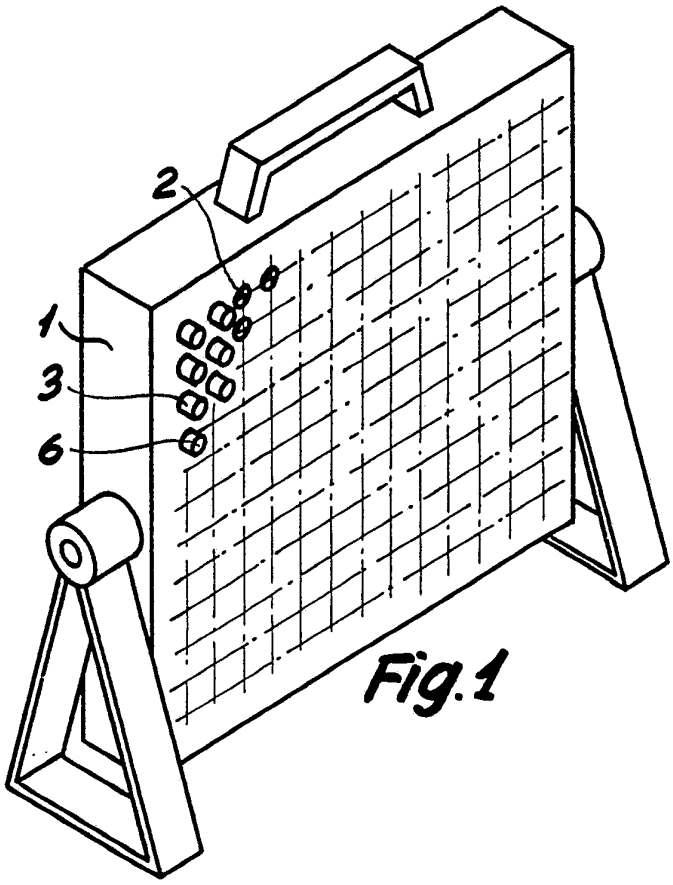


Fig.1

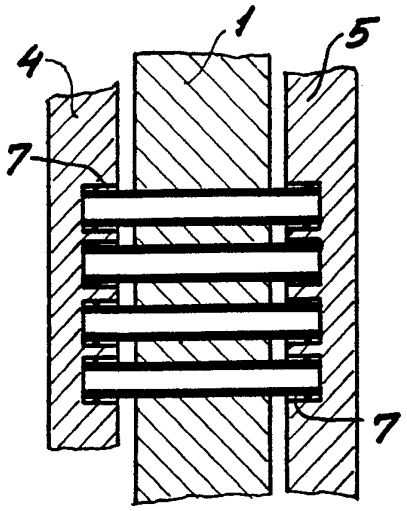


Fig.2

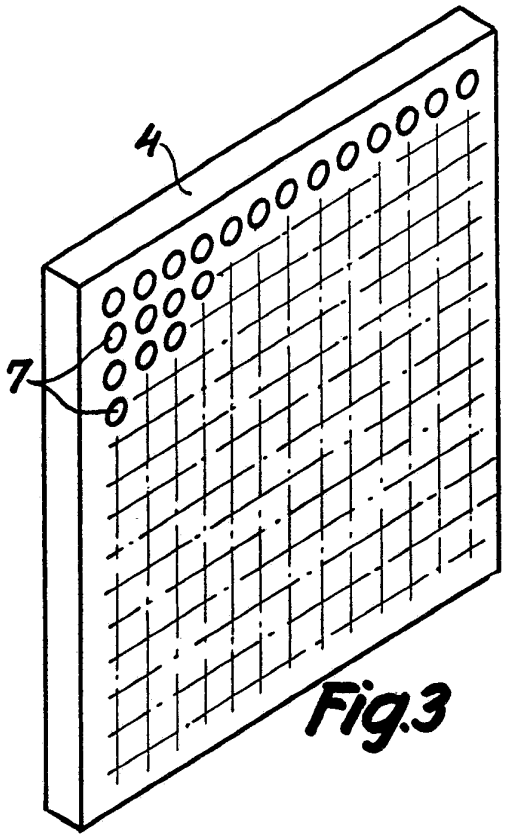


Fig.3

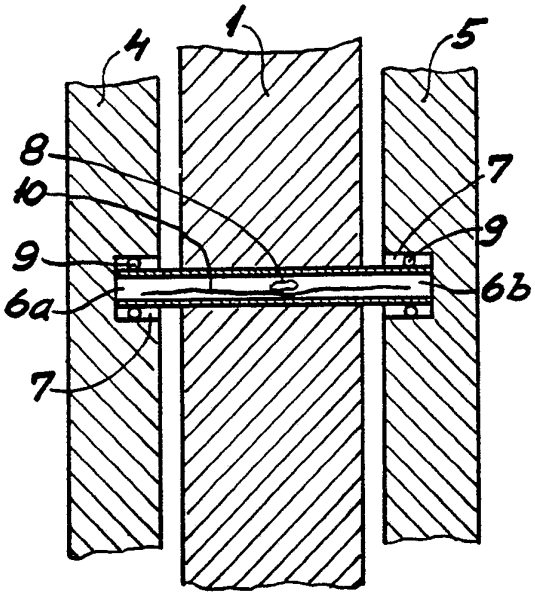


Fig.4

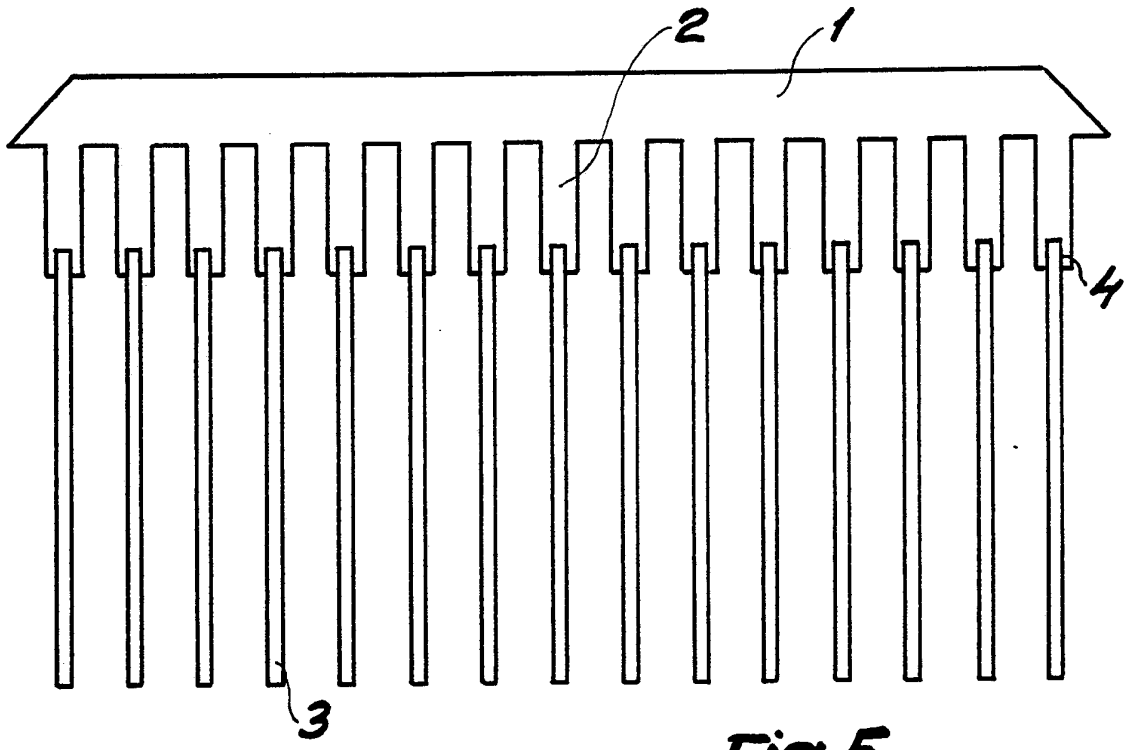


Fig. 5

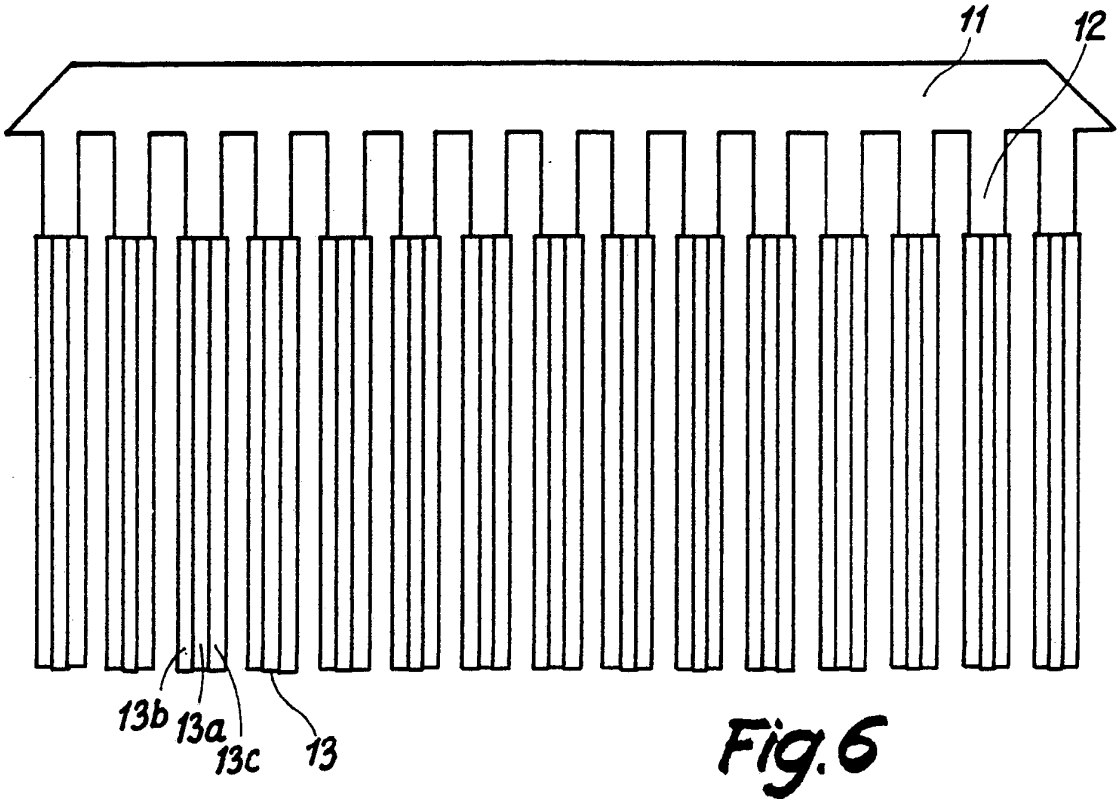


Fig. 6

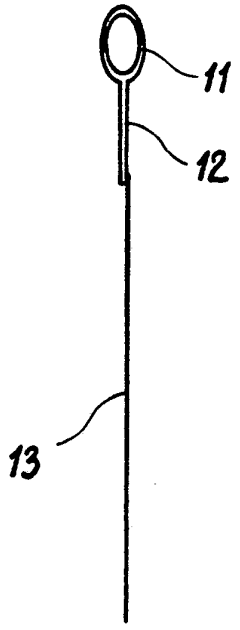


Fig. 7

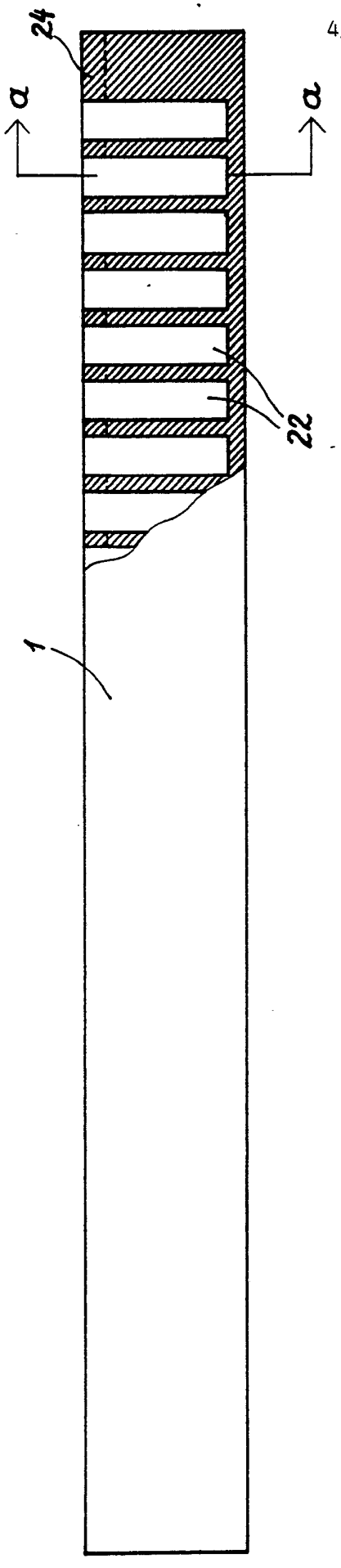


Fig.8

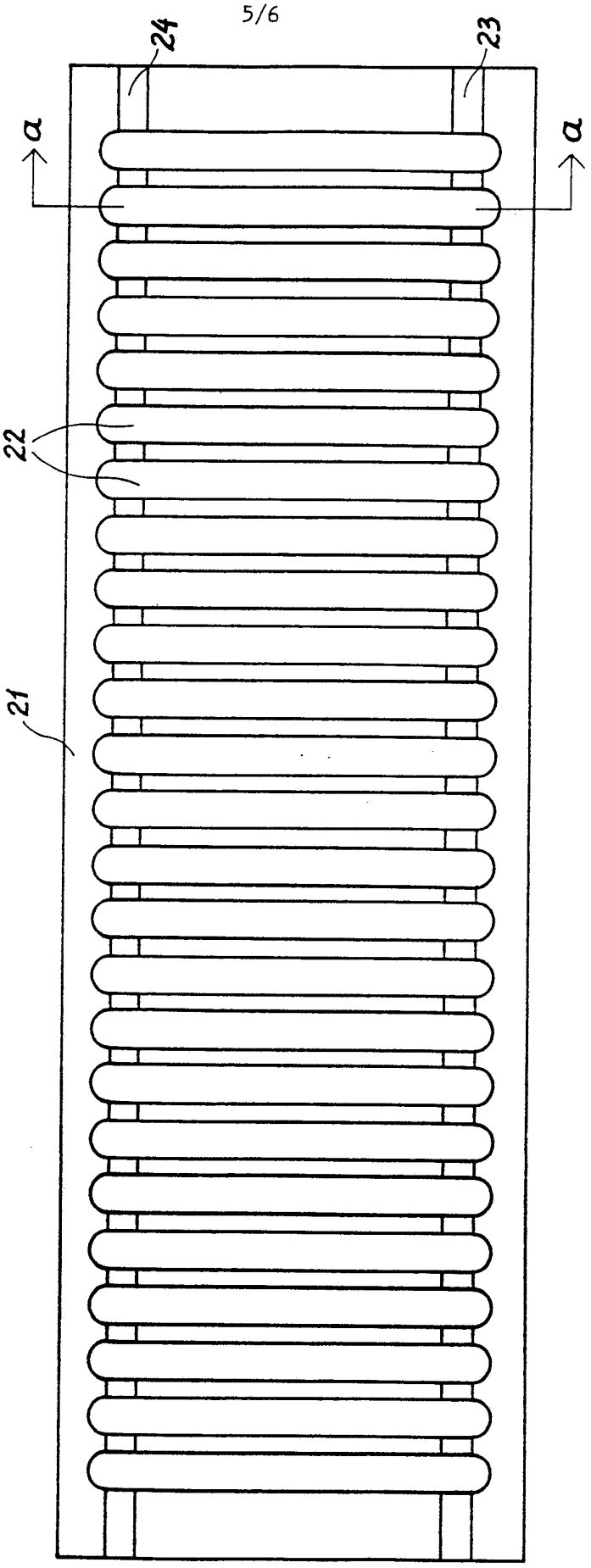
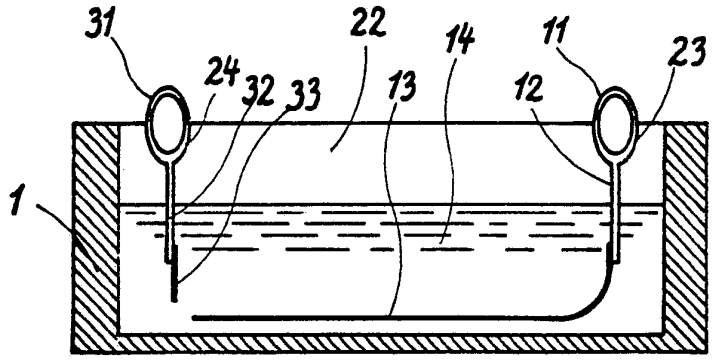


Fig.9



snit a-a

Fig.10

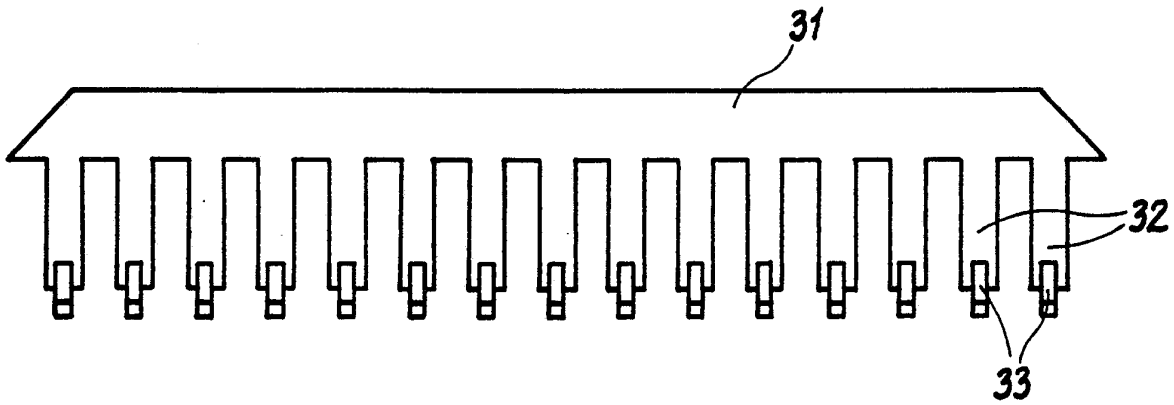


Fig.11

INTERNATIONAL SEARCH REPORT

International Application No PCT/DK89/00025

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC 4

G 01 N 33/543, 33/531

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC 4	G 01 N
US Cl	436; 435

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Clin. Chem. Vol. 27, No. 8, (1981), pages 1421-1423, (K. Miyai et al.) "Two-site Immunoenzymometric Assay for Thyrotropin in Dried Blood Samples on Filter Paper", see page 1422, "Assay Procedures".	1-4
Y	EP, A1, 0 217 583 (VXR, INC.) 8 April 1987 see in particular claims 1-4 and 16 & SE, 8604010 JP, 62156560 US, 4794076	1-4
A	Clin. Chem. Vol. 32, No. 11, (1986), pages 2079-2082, (D. Tsao et al.) "Two-Site Enzyme Immunoassay for Alpha-Fetoprotein in Dried-Blood Samples Collected on Filter Paper", see page 2080 "Assay Procedure".	1, 2
Y X	WO, A1, 87/03965 (CELLDYNAMICS AG) 2 Juli 1987 see pages 3-10 & EP, 0262150	1, 3 5

.../...

* Special categories of cited documents: [14]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1989-05-23	1989 -05- 24
International Searching Authority	**Signature of Authorized Officer**
Swedish Patent Office	Carl Olof Gustafsson

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y X	GB, A, 2 099 578 (CIBA-GEIGY AG) 8 December 1982 see in particular pages 6-7 and & EP, 0063810 JP, 58009070 CA, 1200761 AU, 560790	1, 3 5
Y	Electrophoresis, Vol. 7, 1986, pages 1-18 (U. Beisiegel) "Protein blotting", see paragraph 7.2.5	4
A	EP, A2, 0 174 247 (DE WECK, ALAIN LADISLAS) 12 March 1986	1-5