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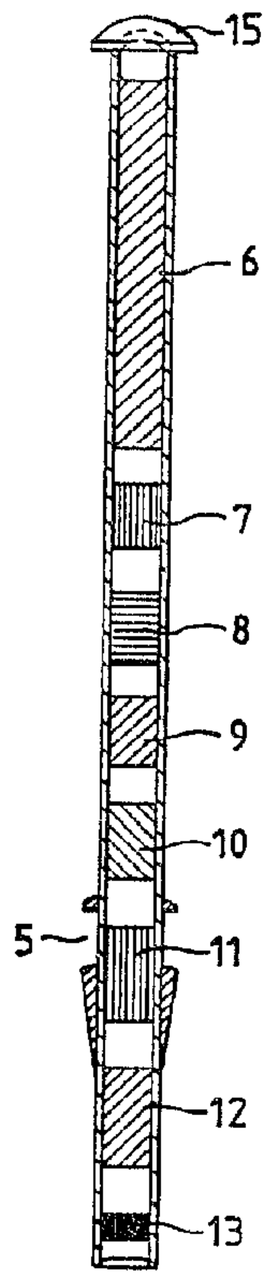
(72) Inventeur/Inventor:
Malmquist, Mats, SE

(73) Propriétaire/Owner:
Malmquist, Mats, SE

(74) Agent: SMART & BIGGAR

(54) Titre : METHODE ET MOYENS MIS EN OEUVRE POUR OBTENIR DES REACTIONS BIOCHIMIQUES
(54) Title: METHOD AND MEANS TO PERFORM BIOCHEMICAL REACTIONS



(57) Abrégé/Abstract:
The present invention relates to a method to perform biochemical reactions and a combination of a capillary (3) and a reaction vessel (1) for use in said method. The reagent capillaries (3) contain frozen reagents (6-13) separated from each other by air or an inert fluid. At use, the reagent capillaries (3) are placed in a reaction vessel (1) to be thawed and then the contents are centrifugated to the bottom of the reaction vessel (1). The invention is intended for use in all types of biochemical standard reactions and diagnostic tests in which the reagents, cannot be mixed in advandce. It is particularly suitable for CPR diagnostics but is also especially beneficial when handling radiactive reagents, e.g. labeled nucleotides, at sequencing reactions, etc.

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(71)(72) Applicant and Inventor: MALMQUIST, Mats [SE/SE]; Hurtigs gata 63, S-754 39 Uppsala (SE).

(74) Agents: ALDENBÄCK, Ulla et al.; Dr Ludwig Brann Patentbyrå AB, Box 1344, Drottninggatan 7, S-751 43 Uppsala (SE).

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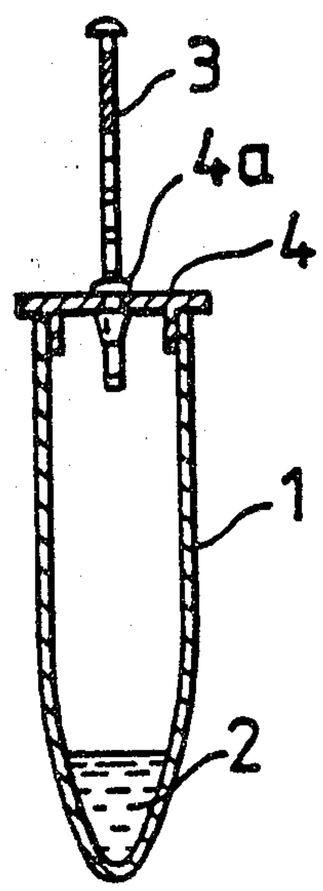

(57) Abstract

The present invention relates to a method to perform biochemical reactions and a combination of a capillary (3) and a reaction vessel (1) for use in said method. The reagent capillaries (3) contain frozen reagents (6-13) separated from each other by air or an inert fluid. At use, the reagent capillaries (3) are placed in a reaction vessel (1) to be thawed and then the contents are centrifugated to the bottom of the reaction vessel (1). The invention is intended for use in all types of biochemical standard reactions and diagnostic tests in which the reagents cannot be mixed in advandce. It is particularly suitable for PCR diagnostics but is also especially beneficial when handling radiactive reagents, e.g. labeled nucleotides, at sequencing reactions, etc.

METHOD AND MEANS TO PERFORM BIOCHEMICAL REACTIONS

The present invention relates to a method to perform biochemical reactions and a combination of a capillary and a reaction vessel for use in said method.

The invention is applicable for all small volume biochemical reactions in which the reagents cannot be mixed beforehand. Particularly, the invention is intended for the PCR (Polymerase Chain Reaction)-technique.

The recently developed PCR-technique has led to great advances in a number of important diagnostic sectors, e.g. the diagnosis of many different diseases, determinations of paternity, forensic medicine, etc. When it is desired to detect RNA, a necessary preliminary stage is the conversion of the RNA into DNA by means of the enzyme reverse transcriptase. The diagnosis of AIDS is routinely made by the detection of antibodies to the HIV-virus in the blood by means of an ELISA (Enzyme Linked ImmunoSorbent)-test. A person may, however, be HIV-positive without antibodies being present if he/she, for instance, is in the early stages of the disease. In this case the ELISA test gives a negative result and the person concerned then risks unwittingly transmitting the infection to others. Therefore the need for a better, i.e. more sensitive, HIV test is very great. The diagnosis of other viruses, also, the culture of which previously took a long time, has been improved with the PCR technique.

As regards the practical procedure, PCR diagnosis comprises three stages:
1) preparation of the reaction mixtures, i.e. preparation of the samples to be tested;
2) the actual amplification, i.e. the chain reaction in which the DNA molecules are replicated exponentially; and
3) the detection of positive samples by means of electrophoresis or hybridisation.

A disadvantage of the PCR method which the present inventor aims to eliminate is that stage 1) is time-consuming and demanding

work, primarily because the reagents cannot be mixed in advance, and thus give rise to many sources of error. It is very important that stage 1) should be carried out with great care and precision because the amplification in stage 2) and the detection result in stage 3) depend absolutely on the reliability of stage 1).

During the various stages of preparing the reagents for a biochemical reaction, such as PCR mentioned above, there is a risk of cross-contamination between the different reaction vessels or test tubes.

While preparing for a PCR reaction there is also a risk of so-called "carry-over contamination" from the person who handles the sample. This applies especially to routine analysis to detect a specific DNA if the same person carries out all the stages before PCR reaction and also handles the PCR product. On skin, hair and laboratory clothing there may be remnants of PCR products from amplifications carried out previously which engender "false" positive resluts. The risk of false positive results increases the more sensitive the test. The test for HIV is very sensitive and it need scarcely be said that a false positive result causes needless distress to the individual notified of it.

The object of the invention was to deminish the contamination risk as well as the time required to prepare small reagent volumes for a specific biochemical reaction in which the reagents cannot be mixed in advance and the preparation is time-consuming.

This object is achieved by a method using a combination of a capillary and a reaction vessel, according to claims 1 and 6, respectively.

In DD-A1-225 788 a capillary is described, which contains several reagents separated by intermediate hydrophobic liquid, e.g. paraffines, oils, alkanes. In this capillary, reagent storage as well as sample reaction takes place. The sample is added to the capillary and then the capillary is melted at one end. The mixing

of the sample with the reagents is done in that a steel pin is put into the capillary and a magnet is moved in an upward and downward direction along the outside of the capillary. After a suitable incubation period the capillary is centrifugated to obtain the reaction solution and the hydrophobic liquid in two separate phases. To be able to analyze the reaction solution the capillary has to be cut at the sealed end and also at the boundary between hydrophobic liquid-reaction solution and thereafter the reaction solution is transferred to a cuvette or the like, for measurement of, for example, UV absorbance.

This known capillary solves the problem of preparing reagents which cannot be prepared in advance. However, because of the above mentioned handling stages, there is no time savings nor reduction of contamination compared to conventional pipetting techniques.

The invention will now be described in greater detail below with reference to the accompanying drawings in which

Fig. 1 is a diagrammatic view if a reaction vessel including a reagent capillary containing reagent;

Fig. 2 is a diagrammatic view of an alternative embodiment of a reaction vessel including an alternative embodiment of a reagent capillary;

Fig. 3 is a plan view of the embodiment shown in Fig. 2;

Fig. 4 shows the reagent capillary depicted in Fig. 1 on a larger scale; and

Fig. 5 shows the reagent capillary depicted in Fig. 2 on a larger scale.

According to one aspect of the present invention, there is provided a method to perform biochemical reactions in which the reagents cannot be mixed in advance, wherein a reagent capillary (3) is used being filled with reagents separated from each other, characterized in that the reagent capillary (3) is inserted in a first bore (4a) in the lid (4) of a reaction vessel (1), in that the reaction vessel (1) with inserted capillary (3) is centrifugated to bring the contents of the reagent capillary (3) in the bottom of the reaction vessel (1), and in that the sample to be reacted is added to the reaction vessel (1).

According to another aspect of the present invention, there is provided a combination of reagent capillary and reaction vessel, characterized in a reagent capillary (3) comprising different reagent solutions (6-13) in predetermined volumes separated from each other by air or an inert fluid, and a reaction vessel (1) comprising a lid (4) provided with one or more bore(s) (4a), the reagent capillary (3) being intended to be inserted into the bore (4a) in the lid (4) of the reaction vessel (1) at use.

Fig. 1 shows a ready-prepared reaction vessel 1 according to the present invention. Inside the reaction vessel 1, for instance an

Eppendorf tube, is placed a reagent capillary 3. The reagent capillary 3 is provided with different reagents, which can be of any suitable type for a desired reaction. In the bottom of the reaction vessel 1 there may be water or buffer 2 for subsequent dilution of the reagents.

Fig. 2 shows an alternative and preferred embodiment of a reaction vessel 1 and a reagent capillary 3. The reaction vessel 1 is provided with a lid 4 having a bore 4a. The bore 4a is covered by a permeable membrane 4b. The bore 4a fitted with a membrane is located centrally in the lid 4 in the shown embodiment but this is not a critical feature. In fact it is possible to provide the lid with several bores to be able to put in more than one reagent capillary as desired. The bore 4a forms a stop collar for the reagent capillary 3 in accordance with Fig. 5, which is described in greater detail below.

The reagent capillary 3 depicted in Fig. 4 is designed to be inserted into the reagent vessel 1 shown in Fig. 1. The reagent capillary 3 is provided with different reagents 6-13 for a specific biochemical reaction. The amount of each reagent is calculated and intended only for this specific reaction. If a PCR reaction is to be performed the reagent solutions 6-13 comprise PCR buffer, dCTP, dGTP, dATP, dTTP, two or more oligonucleotides, all of the reagents being calculated for a specific PCR reaction, and termostable DNA polymerase. Between the reagents there is air or an inert fluid. Naturally, the mutual order is optional.

A modified reagent capillary is depicted in Fig. 5. This reagent capillary is designed to be inserted into a reaction vessel according to Fig. 2. The reagent capillary differs from the reagent capillary shown in Fig. 4 in that there is an annular locking groove 5 on the lower part of the capillary intended to be snapped into the bore 4a. Moreover, a protective cover 15 is fitted over the upper end of the capillary. The reaction vessel according to Fig. 2 and the reagent capillary according to Fig. 5 are stored separately until use. Upon use, the lower end of the

capillary 3 is pushed through the permeable membrane 4b in the lid 4 of the reaction vessel 1, whereupon the locking groove 5 engages with the stop collar formed by the bore 4a. The protective cover 15 protects the contents of the reagent capillary 3 from contamination during the process of insertion and pushing into the reaction vessel 1. When the reagent solutions in the reagent capillary 3 have thawed, they are then centrifuged down and mixed with one another and, where applicable, with the diluent 2 at the bottom of the vessel 1. After centrifuging, the lid 4 may be opened without having to remove the capillary 3 from the lid. The advantage of this is that material can readily be added to or extracted from the reaction vessel if desired.

After producing the reagent capillaries, i.e. by aspirating the different reagents with air or inert fluid in between, either manually or automatically, they may be packed separately or placed in a reaction vessel in kits for performing a specific biochemical reaction. Of course, this packaging takes place under sterile conditions.

An alternative method of producing the capillaries would be to aspirate the reagents into capillaries with air or inert fluid between the reagents, freeze the capillaries, cut the capillaries in the air sections, and to place the desired capillary pieces in one common outer capillary having an inner diameter corresponding to the outer diameter of the capillary pieces. This would allow combining of the reagents in any desired way.

The reagent capillaries with or without the reaction vessels are stored in the frozen state until use. For use the reagent capillary is thawed and the contents thereof are centrifuged down in the reaction vessel, being mixed with each other and with diluent if any. If a PCR reaction is to be performed, all the reagents, including heat stable DNA polymerase, are now in the reaction vessel and the only further addition needed before the amplification is of the sample, e.g. blood.

Preferably the sample is added by using a dosing system described in applicants pending swedish patent application SE 91 0726-0. Briefly, the sample is drawn up into a capillary, of the same type as reaction capillary 3, by the capillary effects. Thereafter the sample capillary is inserted in an unoccupied bore 4a in the lid 4 of the reaction vessel 1 which thereafter is once again centrifugated.

For fast detection of the results of the amplification reaction, the present inventor suggest using material for the reaction vessels that does not or only slightly absorb UV light. If ethidium bromide is added after the reaction it would then be possible to detect whether or not DNA has been amplified by viewing the vessels under UV light with the naked eye. Preferably the ethidium bromide addition is made in the same manner as the above described sample addition.

It should be appreciated the the shown reagent capillaries 3 have been prepared for a specific sample volume and a specific biochemical reaction. Other reactions require different volumes and number of reagents.

According to the present invention numerous factors are obviated, e.g. pipetting, changing pipette-tips, changing gloves, repeated opening and closing of the reaction vessel, whereby the number of sources of error is substantially reduced and the tests are more reliable, quicker and cheaper. The problem of false positive results with PCR is thus appreciably reduced.

Thus the invention offers the biotechnical industry the chance to supply a new type of "kits", i.e. complete sets containing reagents for a specific reaction. Large numbers of such kits are on the market today; they usually consist of Eppendorf tubes containing different reagents suitable for about 100 standard reactions. For each reaction a certain volume is mixed from each tube. With the aid of reagent capillaries one kit can contain, e.g. 500 capillaries, each ready to use for the reaction it is

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designed for. The advantage of kits based in the reagent capillaries desribed in the present application is that the user does not have to pipette the reagent and is able, instead, to select the appropriate reagent capillary for the relevant reaction with fingers of tweezers. The simplification of the work is obvious, above all in regard to the handling of radioactive reagents, as there is no risk of contaminating pipettes, less risk of radioactive waste and shorter periods of exposure for the staff. In addition to the economic and operational advantages of reaction capillaries in PCR technology, there is the saving in time and the benefits of worker protection in many biotechnological sectors.

CLAIMS

1. Method to perform biochemical reactions in which the reagents cannot be mixed in advance, wherein a reagent capillary (3) is used being filled with reagents separated from each other, **characterized** in that the reagent capillary (3) is inserted in a first bore (4a) in the lid (4) of a reaction vessel (1), in that the reaction vessel (1) with inserted capillary (3) is centrifugated to bring the contents of the reagent capillary (3) in the bottom of the reaction vessel (1), and in that the sample to be reacted is added to the reaction vessel (1).

2. Method according to claim 1, **characterized** in that a capillary containing the sample is inserted in a second bore (4a) in the lid (4) of the reaction vessel (1), and in that a second centrifugation is performed.

3. Method according to claim 1 or 2, **characterized** in that the reagent solutions (6-13) are frozen and in that they are thawed before centrifugation.

4. Method according to claim 1, 2 or 3, **characterized** in that a protective cover (15) is fitted on the upper end of the reagent capillary (3) before the lower end thereof is inserted in the bore (4a), the lower end being so far down inserted in the reaction vessel (1) until a locking groove (5) on the capillary engages with the edges of the bore (4a).

5. Method according to claim 4, **characterized** in that the reagent capillary (3) is inserted through a permeable membrane (4b) covering the bore (4a).

6. Combination of reagent capillary and reaction vessel, **characterized** in a reagent capillary (3) comprising different reagent solutions (6-13) in predetermined volumes separated from each other by air or an inert fluid, and a reaction vessel (1) comprising a lid (4) provided with one or more bore(s) (4a), the

reagent capillary (3) being intended to be inserted into the bore (4a) in the lid (4) of the reaction vessel (1) at use.

7. Combination according to claim 6, characterized in that the reagent solutions (6-13) are frozen and in that they are thawed before the reagent capillary (3) is inserted into the bore (4a).

8. Combination according to claims 6 or 7, characterized in that the reagent capillary (3) is provided with a locking groove (5) at its lower end and a protective cover (15) at its upper end.

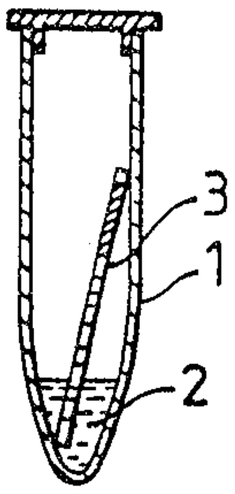
9. Combination according to claims 6, 7 or 8, characterized in that the bore (4a) is covered by a permeable membrane (4b).

10. Combination according to one or more of the claims 6-9, characterized in that the reagent solutions (6-13) comprise nucleic acid(s) and/or enzyme(s) for a specific reaction.

11. Combination according to claim 10, characterized in that the reagent solutions (6-13) comprise PCR buffer, dCTP, dGTP, dATP, dTTp, two or more oligonucleotides, all of the reagents being calculated for a specific PCR reaction, and termostable DNA polymerase.

12. Combination according to one or more of the claims 5-11, characterized in that the reaction vessel (1) is made of a material that does not or only slightly absorb UV light.

Fig. 1



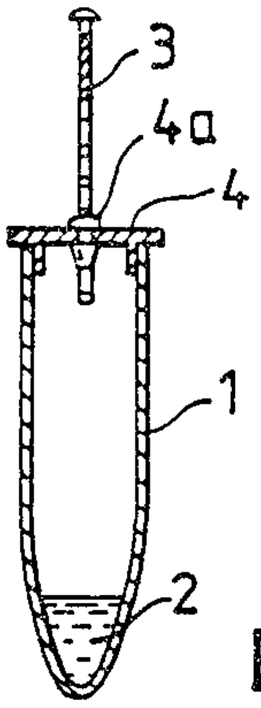


Fig. 2

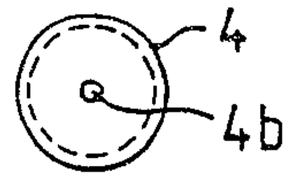


Fig. 3

Fig. 4

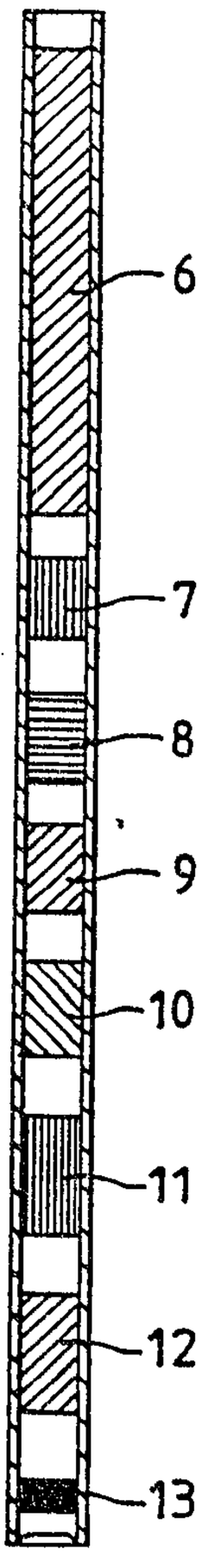
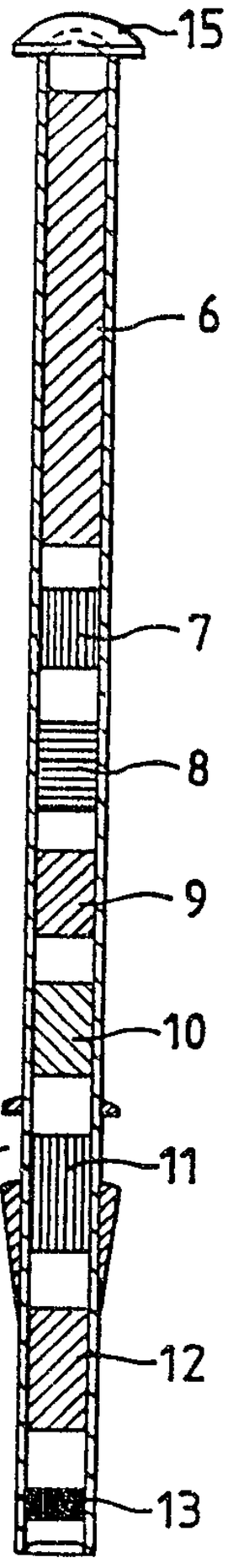
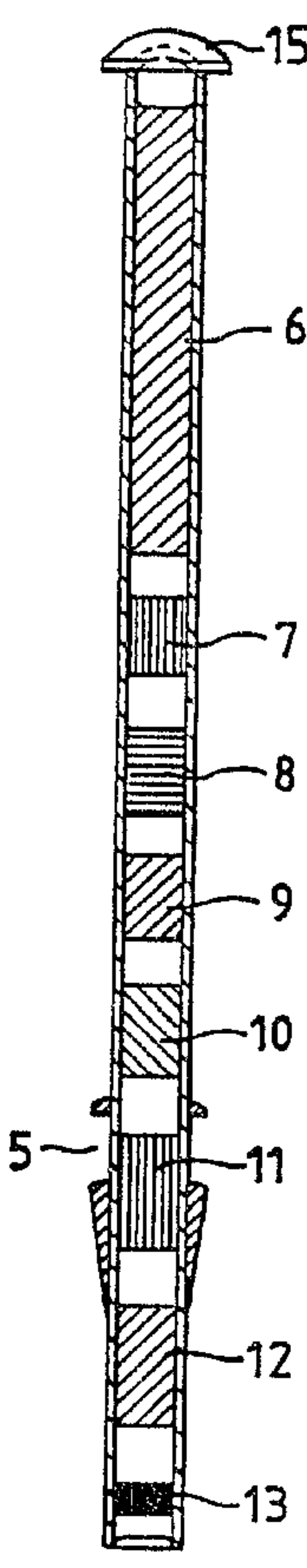


Fig. 5

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