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(54) **DEVICE FOR PREPARING SAMPLES FOR ANALYSIS, SUPPORT, SYSTEM FOR PREPARING SAMPLES FOR ANALYSIS, METHOD FOR PREPARING SAMPLES, AND METHOD FOR ANALYSING A SAMPLE**

(57) The present invention relates to a device adapted to dosing and preparing samples for analysis and adapted to housing at least reagents and magnetizable particles, characterized in that it comprises at least a first chamber adapted to storing in a leak-tight manner first reagents and the magnetizable particles and which in turn comprises first sealing means for hermetically sealing said first chamber and first sealing means' opening means, at least a second chamber adapted to storing in a leak-tight manner second reagents which in turn comprises second sealing means for hermetically sealing said second chamber and second sealing means' opening means, at least one main chamber adapted to housing samples and for allowing the reaction of the reagents and at least the first chamber and second chamber opening into said main chamber through the sealing means, respectively, sample suction means and at least one inlet-outlet port. The invention also relates to a support adapted to housing the device, a system comprising the device and the support, a method for preparing samples using the device and a method of analysis comprising a step for preparing samples as indicated in said method for preparing samples.

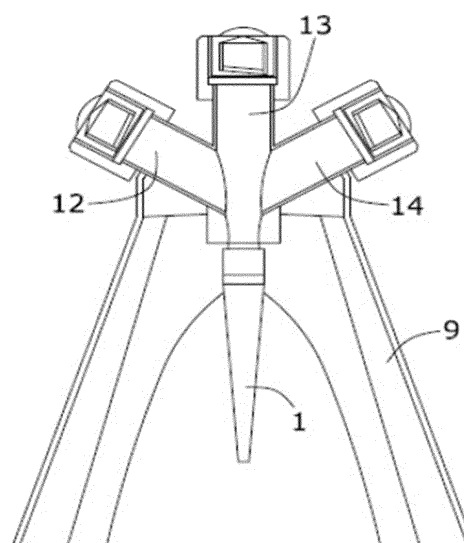


FIG. 7

Description

Object of the Invention

[0001] The present invention relates to a device adapted to preparing samples for analysis which allows dosing reagents in a reaction body with a single chamber which allows handling samples and reagents from the outside thereof; the device assures maintenance of reagent sterility conditions until the reagent is exposed to the sample and minimizes the risk of sample handling by users. The invention also relates to a support for the device, a system comprising the device and the support, a method for preparing samples using the system and a method of analysis comprising a step for preparing samples as indicated.

Background of the Invention

[0002] There are devices in the state of the art which allow handling biological samples, extraction and concentration of molecular fractions (DNA and RNA), proteins, antibodies, peptides, etc., as well as adding amplification reagents in an easy and safe manner.

[0003] However, the main technical problem of these devices of the state of the art is that it is not possible to perform a preparation completely avoiding contact between the user performing the analysis with one of the reagents, with the subsequent risk of contaminating the reagents during the analytical process, and even with the sample itself, which can constitute an intrinsic biological risk, without using auxiliary equipment such as, for example, electronic systems, pumps, etc., which allow the movement and/or dosing of the different samples or reagents. Furthermore, there is an additional problem of losses when transferring samples or reagents from one pipette to another or to different containers, and such losses in the case at hand, in which volumes in the order of microliters (μL) are handled, are costly, being able to affect the yield of the reaction/reactions, repeatability and, in general, robustness of the analytical method.

Description of the Invention

[0004] The present invention solves the problems described above by means of a device according to claim 1, a support according to claim 11, a system according to claim 13, a method for preparing samples according to claim 14 and a method of analysis according to claim 15. The dependent claims define the preferred embodiments of the invention.

[0005] The first inventive aspect relates to a device adapted to dosing and preparing samples for analysis and adapted to housing at least reagents and magnetizable particles.

[0006] This device can be used for packaging or dosing molecular reagents, for example, for aliquoting, extracting and preparing samples for analysis by means of meth-

ods of molecular analysis, including, among others, PCR (Polymerase Chain Reaction), Q-PCR/RT-PCR, immuno-PCR, pyrosequencing, isothermal nucleic acid amplification, etc.,

characterized in that it comprises:

- at least a first chamber adapted to leak-tight storing first reagents and the magnetizable particles which in turn comprises

o first sealing means for hermetically sealing said first chamber,
o first sealing means' opening means

[0007] The device has a first independent storage chamber leak-tight closed in a manner for storing the reagents of one or several of the following components: lysis buffer, capture buffer, resuspension buffer, washing buffer and amplification buffer. This chamber can also store magnetizable particles if it is going to perform a process for concentrating/capturing target analytes: nucleic acids, proteins, antibodies, peptides etc. The magnetizable particles consist of synthetic polymers incorporating a magnetizable material core and are chemically modified to allow binding thereof with a specific substance, for example: silene, peptides, antibodies, proteins, nucleic acids, etc. Such particles can be concentrated from a suspension by means of exposing them to a magnetic field, therefore being an alternative to centrifugation. Depending on their functionalization with specific sequences, the magnetizable particles in the present invention allow capturing nucleic acids, proteins, antibodies, peptides, etc., from the sample to analyse. To magnetize these particles, means capable of generating a magnetic field which can be incorporated by the user must be used.

[0008] Furthermore, the chamber is leak-tight closed by means of sealing means preventing the loss of reagents or preventing the reagents from falling uncontrollably, or preventing them from coming into contact with the outside, thus preventing the risk of unwanted contaminations or changes in the physical and chemical parameters of the reagents housed therein: dehydration, wetting, oxidation, etc., entry of organic and inorganic biological contaminant particles, etc., which would result in reagent alteration, affecting functionality, being able to completely or partially compromise method reliability. Opening means are used to enable the reagents to exit the chamber.

- at least a second chamber adapted to leak-tight storing second reagents which in turn comprises

o second sealing means for hermetically sealing said second chamber,
o second sealing means' opening means

[0009] Similarly to the first chamber, the second cham-

ber is an independent storage chamber leak-tight closed for storing the reagents of one or several of the following components: lysis buffer, capture buffer, resuspension buffer, washing buffer and amplification buffer. It can also optionally house magnetizable particles.

- *at least one main chamber adapted to housing samples or matrices and for allowing the reaction of the reagents and at least the first chamber and second chamber opening into said main chamber through the sealing means, respectively,*

[0010] The main chamber of the device, or reaction chamber, is made of a sterilized material and allows holding the liquid sample. The material used for forming the main chamber corresponds to conventional materials for such devices for dosing and preparing samples, comprising any polymer material, preferably thermoplastic material. The following materials are mentioned by way of example: polyethylene (PE), high density polyethylene (HDPE), low density polyethylene (LDPE), polystyrene (PS), polytetrafluoroethylene (PTFE), PTFE perfluoroalkoxy (PFA), polypropylene (PP), polymethylpentene (PMP), neoprene, perbunan, etc.

[0011] The reagents are added in said reaction chamber either simultaneously or sequentially, according to the practice for which the device is used.

[0012] The reagents housed in at least one of the storage chambers, depending on the practice to be performed, contact the sample for analysis in this reaction chamber. It can also be used as an individual housing.

[0013] The chambers adapted to storing reagents contact the reaction chamber upon acting on the opening means, preventing direct contact of the handler with the sample, drastically minimizing exposure to a possible biological risk and the reagents, preventing the risk of test contamination.

- *sample suction means*

[0014] The sample prepared with the device is introduced in the main chamber or reaction chamber using these suction means.

- *and at least one inlet-outlet port*

[0015] The suction means are used to introduce the sample such that the volume of air inside the body is expelled by compressing said suction means. The inlet-outlet port is then submerged in the liquid sample. Finally, the suction means are released and the sample is introduced through the inlet-outlet means.

[0016] Once the sample for analysis has reacted with the reagents, it is placed in the tube to be used for analysis which, in the case of performing PCR, Q-PCR/RT-PCR or immuno-PCR, is inside a thermal cycler for polymerase chain reaction and specific nucleic acid sequence amplification depending on the reagents added in the

mixture and the nature of the sample to be analyzed. This is achieved by means of expelling the sample from the main chamber of the device through the inlet-outlet port.

[0017] The advantage of this device is that it allows using any variant of the molecular analysis technique, such as for example: Q-PCR/RT-PCR, immuno-PCR, isothermal amplification, pyrosequencing, etc., in clinical laboratories, with staff who are not highly skilled, therefore generalizing technique accessibility and competitiveness on different markets, preferably on the health product market. Furthermore, the fundamental difference with respect to devices of the state of the art is that it comprises a single reaction chamber which allows maximally reducing losses as well as standardizing the inter- and intra-analysis test method.

[0018] *The second inventive aspect relates to a support for a device such as the one of the first inventive aspect, furthermore comprising a removable element for suctioning the sample. The support comprises extraction means for extracting the removable element.*

[0019] This support for the device has a configuration such that the main chamber of the device for preparing samples is immobilized when the support is coupled thereto. This immobilization allows the reagents to fall from the storage chambers by gravity and remain perfectly positioned. Furthermore, the support comprises extraction means for extracting the removable element to enable separating the two elements, i.e., the device and the removable element. These extraction means provide the advantage that the user does not have to touch the removable element which contacts a sample that may be contaminated.

[0020] *The third inventive aspect relates to a system adapted to dosing and preparing samples for analysis by means of a method of analysis, characterized in that it comprises a device according to the first inventive aspect, and a support according to the second inventive aspect.*

[0021] *The fourth inventive aspect relates to a method for preparing samples for analysis which comprises preparing samples by means of the device according to the first inventive aspect, or a system according to the second inventive aspect.*

[0022] *The fifth inventive aspect relates to a method for analyzing a sample which comprises preparing said sample by means of the method of the fourth inventive aspect.*

[0023] All the technical features described in this specification (including the claims, description and drawings) can be combined in any combination, with the exception of the combinations of such mutually exclusive features.

Description of the Drawings

[0024] The foregoing and other advantages and features of the invention will be more clearly understood from the following detailed description of a preferred embodiment in reference to the attached drawings provided

only by way of an illustrative and non-limiting example:

Figure 1 shows the elements used in the state of the art for preparing samples for analysis.

Figure 2 shows an embodiment of the device including branches for housing storage chambers.

Figure 3 shows an embodiment of the device including a removable suction element with a frustoconical tubular shape.

Figure 4 shows an embodiment of the device including a removable suction element with a frustoconical tubular shape and three branches for housing the storage chambers.

Figure 5 shows two views of the support for housing the device. Figure 5A shows a section view and Figure 5B shows a plan view thereof.

Figure 6 shows an embodiment of the support in a section view including an additional enclosure.

Figure 7 shows an embodiment of the device with three branches coupled in the support.

Detailed Description of the Invention

[0025] In one embodiment, the device comprises at least a first branch (12) housing at least the first chamber (2) and at least a second branch (13) housing at least the second chamber (3). These two branches (12, 13) open into the main chamber (4). This design allows housing the chambers in an individual and spatially separated manner such that they are independent of the main reaction chamber (4). Figure 2 shows this way of positioning chambers (2, 3) with respect to the main chamber (4).

[0026] In one embodiment of the invention, the main chamber is prepared with an elastic material, such as plastic, for example, therefore allowing the suction means (15) to be the main chamber (4) itself. The operating principle is such that by pressing on the body of the main chamber (4), the inner volume of air is expelled allowing subsequent sample suction.

[0027] In one embodiment of the invention, the suction means are formed with a nipple in an additional branch without the main chamber (4) having to be elastic.

[0028] In one embodiment, the device incorporates a removable element (1), preferably in a frustoconical tubular shape, for sample suction. Figure 3 shows the removable element (1) as a tip fitted in the reaction chamber of the device. It is easier to perform the liquid sample suction step through this tip.

[0029] In one embodiment, the end of the removable element (1) fitting with the inlet-outlet means (5) of the device has a configuration which allows fitting thereof with a complementary fitting area envisaged for such purposes in a support for said device.

[0030] In one embodiment of the device, filtering means (16) are incorporated in the removable element (1) which allow filtering solid particles that can contaminate the sample before being prepared for reaction with the reagents in the main chamber (4).

[0031] In one embodiment, the device incorporates means capable of generating a magnetic field, for example a magnet, which allow immobilizing the magnetizable particles in the main chamber (4), making expelling the rest of the reagents easier, if necessary. This case can be, for example, the need to immobilize the magnetizable particles once these particles have captured specific sequences of DNA, RNA, proteins, peptides, antibodies, etc.; this allows expelling the rest of the reagents and excess liquid sample from the main chamber or reaction chamber.

[0032] In one embodiment, the device as shown in Figure 4 includes a third branch (14) housing a third chamber (10) with third sealing means (11) and third opening means (19). In this embodiment, it is possible to house more reagents in the third chamber (10), such as a washing buffer, for example. With the same operating principle as the preceding embodiments, the third branch (14) is arranged equidistant together with the first branch (12) and the second branch (13). The third chamber (10) also opens into the main chamber (4) and releases its content when the third sealing means (11) have been opened with the third opening means (19). Figure 4 also shows the removable element (1).

[0033] In one embodiment, the sealing means (7, 8, 11) are manufactured from a material the composition of which comprises aluminum and polypropylene and are heat sealable to their respective chambers (2, 3, 10).

[0034] In one embodiment, the sealing means (7, 8, 11) opening means' (17, 18, 19) are punches the punching end of which is arranged facing the wall of the sealing means (7, 8, 11). The forward movement of these opening means (17, 18, 19) causes the sealing means (7, 8, 11) to break, allowing the reagents to exit towards the reaction chamber or main chamber.

[0035] In a particular embodiment, the chambers (2, 3, 10) have an outer elastic area which the user presses on to act on the punches, causing them to move and break the sealing means with the subsequent opening of the chambers (2, 3, 10).

[0036] In one embodiment, the sealing means (7, 8, 11) opening means' (17, 18, 19) are blades the cutting end of which is arranged facing the wall of the sealing means (7, 8, 11). The forward movement of these opening means (17, 18, 19) causes the sealing means (7, 8, 11) to break, allowing the reagents to exit towards the reaction chamber or main chamber.

[0037] In a particular embodiment, the chambers (2, 3, 10) have an outer elastic area which the user presses on to act on the blades, causing them to move and break the sealing means with the subsequent opening of the chambers (2, 3, 10).

[0038] One embodiment of the device includes sealing means' (7, 8, 11) opening means in the form of windows that open by means of rotating the end of the branches (12, 13, 14).

[0039] In one embodiment, the sealing means (7, 8, 11) break by tearing.

[0040] In one embodiment, the sealing means (7, 8, 11) break by detachment.

[0041] The device for dosing and preparing samples described above can cooperate with a complementary support which, in one embodiment, comprises securing means (20) for securing the removable element (1).

[0042] In one embodiment of the support, the securing means (20) have a configuration which allows the fitting thereof with the end area of the removable element (1) coupled with the inlet/outlet port (5).

[0043] In a particular embodiment, said fitting configuration has a deformable toothed area in the securing means (20), as depicted in Figure 5B, fitting with the surface at said end of the removable element (1). This configuration of the securing means (20) allows extracting the removable element (1) by means of rotating the device such that the user never contacts the removable element (1). Once the removable element (1) has been extracted, it is possible to position the device for preparing samples, in the example of amplifying specific sequences of DNA, RNA, proteins, antibodies, peptides, etc., in a tube inside a thermal cyclor to denature the DNA samples.

[0044] In one embodiment, the height of the support (9) is essentially greater than the height of the removable element (1) to allow housing the device with the removable element (1) for suctioning the sample.

[0045] In one embodiment, the support contains housing means (21) for housing the means (22) capable of generating a magnetic field if they are not located in the device and not incorporated into the system by the user. Figure 6 shows a section view of the support. These housing means (21) are in the position of the support coinciding with the position of the main chamber (4) of the device for preparing samples when it is arranged. The magnetizable particles can thus be magnetized inside the main chamber (4).

[0046] Figure 7 shows a view of an embodiment of the system comprising one of the embodiments of the device and the support. Specifically, the embodiment of the device comprises sealing means' (7, 8, 11) opening means (17, 18, 19) in the form of blades.

Claims

1. A device adapted to dosing and preparing samples for analysis and adapted to housing at least reagents and magnetizable particles,
characterized in that it comprises:

- at least a first chamber (2) adapted to leak-tight storing first reagents and the magnetizable particles which in turn comprises

- o first sealing means (7) for hermetically sealing said first chamber,
- o first sealing means' (7) opening means

(17),

- at least a second chamber (3) adapted to leak-tight storing second reagents which in turn comprises

- o second sealing means (8) for hermetically sealing said second chamber,
- o second sealing means' (8) opening means (18),

- at least one main chamber (4) adapted to housing samples or matrices and for allowing the reaction of the reagents and at least the first chamber (2) and second chamber (3) opening into said main chamber (4) through the sealing means (7, 8), respectively,
- sample suction means (15),
- and at least one inlet-outlet port (5).

2. The device according to claim 1, **characterized in that** it comprises at least one removable element (1) for suctioning the sample, preferably with a frusto-conical tubular shape adapted to being fitted in the inlet-outlet means (5).

3. The device according to claim 2, **characterized in that** the removable element (1) comprises filtering means (16).

4. The device according to any of the preceding claims, **characterized in that** it comprises

- at least a first branch (12) housing at least the first chamber (2), and
- at least a second branch (13) housing at least the second chamber (3).

5. The device according to any of the preceding claims, **characterized in that** the sample suction means (15) are the main chamber (4) which is made of an elastic material.

6. The device according to any of the preceding claims, **characterized in that** it comprises means (6) capable of generating a magnetic field.

7. The device according to any of the preceding claims, **characterized in that** it comprises at least a third chamber (10) adapted to hermetically housing third reagents and which in turn comprises

- third sealing means (11) for hermetically sealing said third chamber (10),
- third sealing means' (11) opening means (19),

and opening into the main chamber (4) through the third sealing means (11).

8. The device according to any of the preceding claims, **characterized in that** it comprises at least a third branch (14) housing at least the third chamber (10).
9. The device according to any of the preceding claims, **characterized in that** the sealing means (7, 8, 11) are manufactured from a material consisting of aluminum and polypropylene and are heat sealable to their respective chambers (2, 3, 10).
10. The device according to claim 9, **characterized in that** the sealing means' (7, 8, 11) opening means (17, 18, 19) are punches.
11. A support (9) for a device according to any of claims 2 to 10, comprising extraction means (20) for extracting the removable element (1).
12. The support (9) according to claim 11, **characterized in that** it has housing means (21) for housing means (22) capable of generating a magnetic field.
13. A system adapted to dosing and preparing samples for analysis by means of a method of analysis, **characterized in that** it comprises:
- a device according to any of claims 1 to 10,
 - and a support (9) according to any of claims 11 to 12.
14. A method for preparing samples for analysis which comprises preparing samples by means of the device according to any of claims 1 to 10, or a system according to claim 13.
15. A method for analyzing a sample which comprises preparing said sample by means of the method of claim 14.

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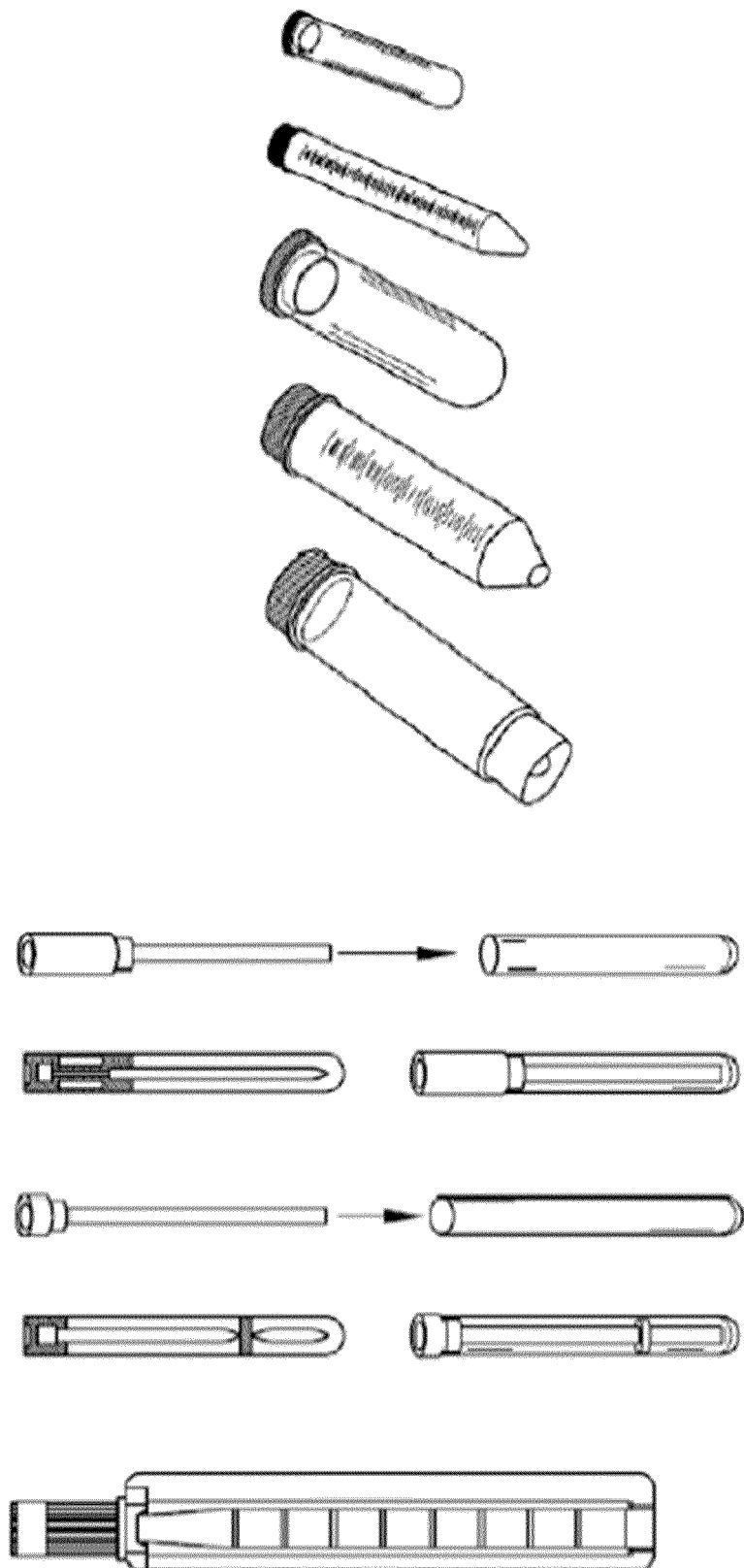
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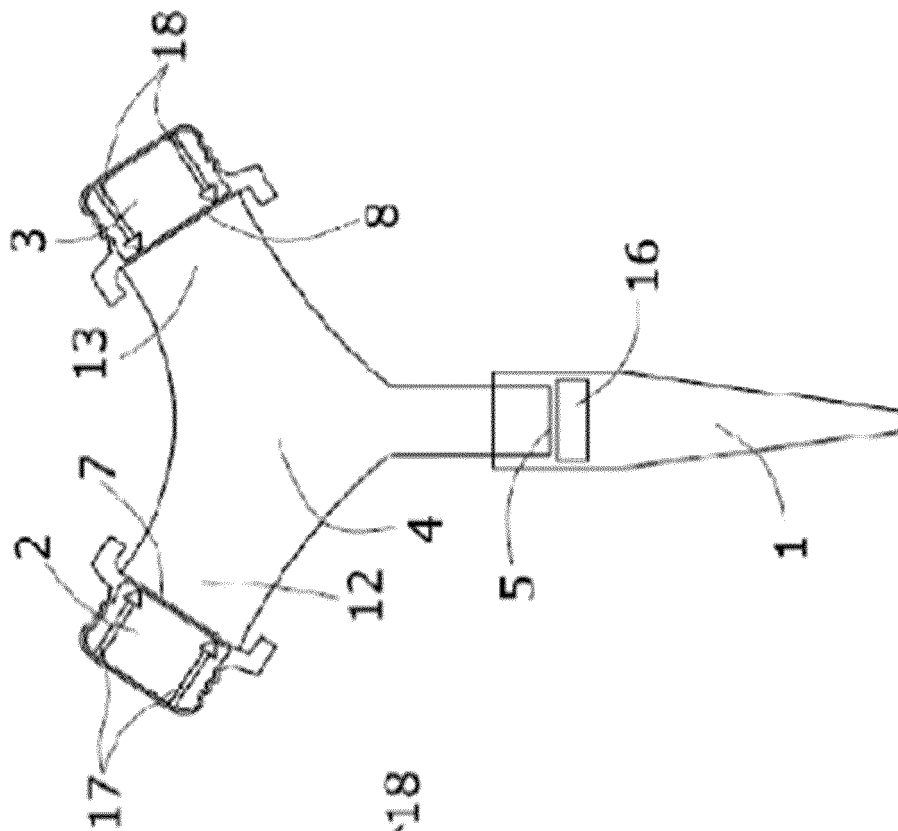


FIG.3

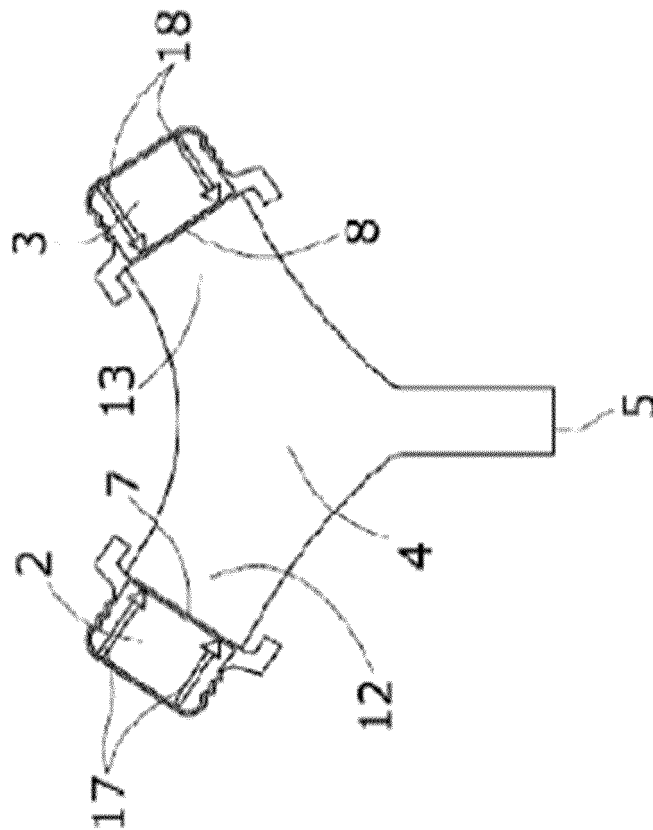


FIG.2

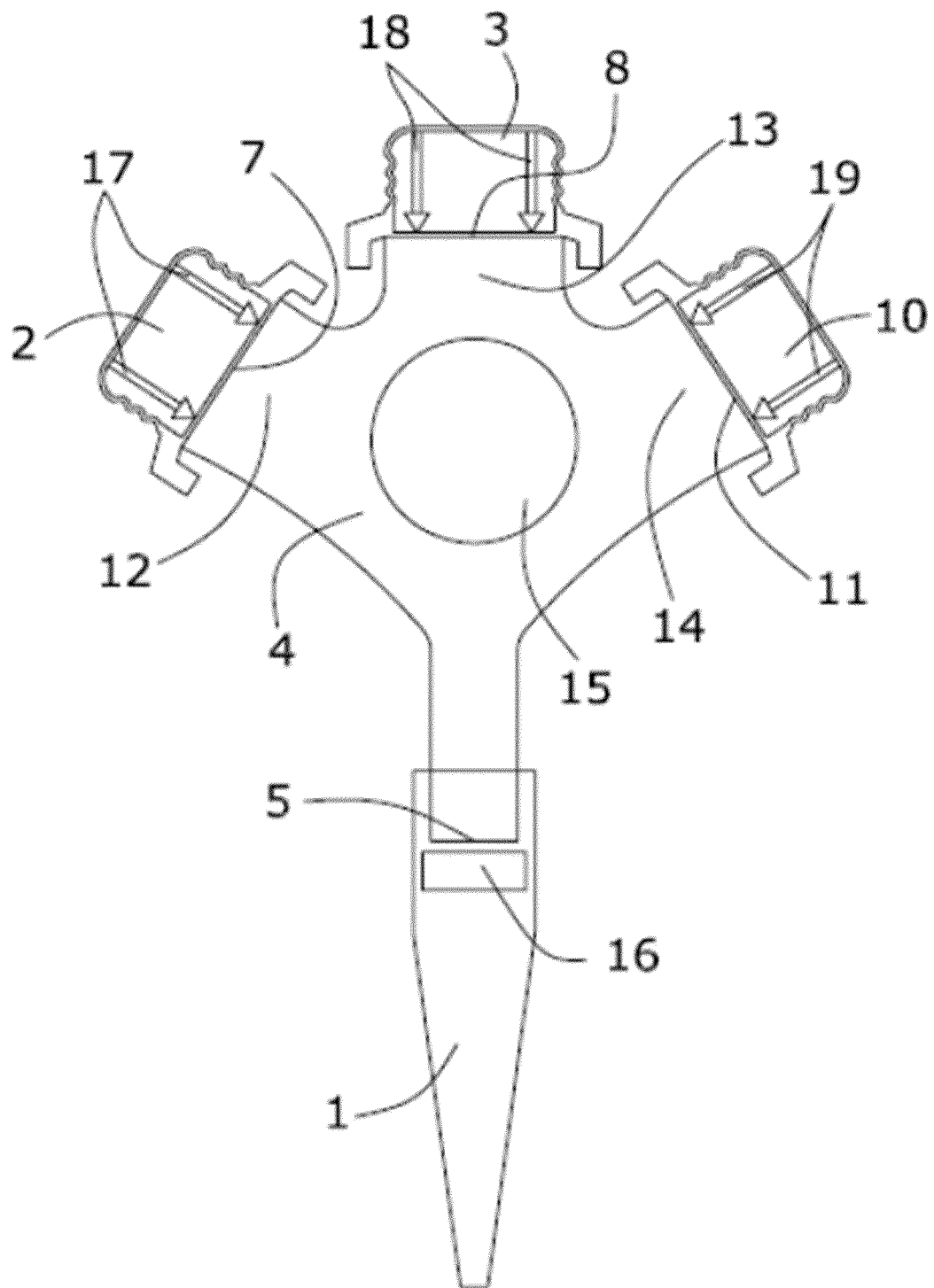


FIG.4

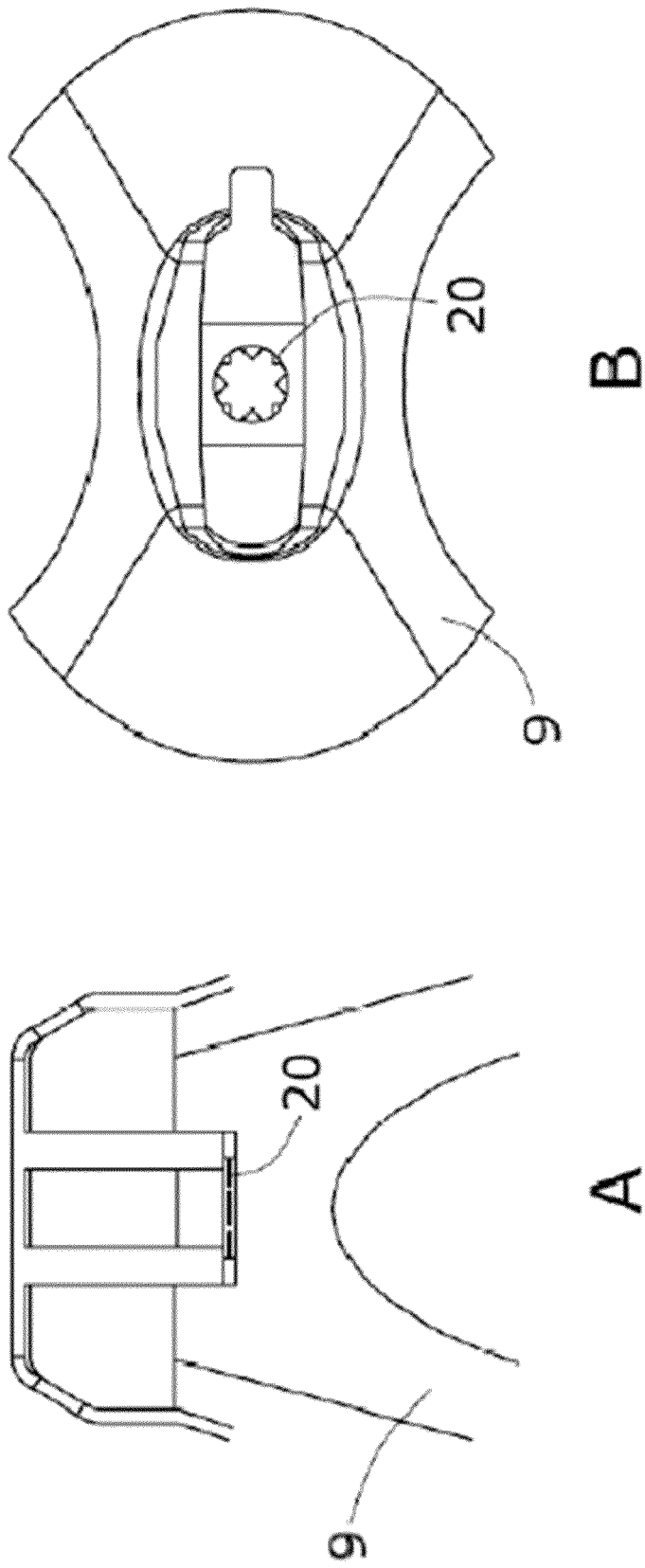


FIG. 5

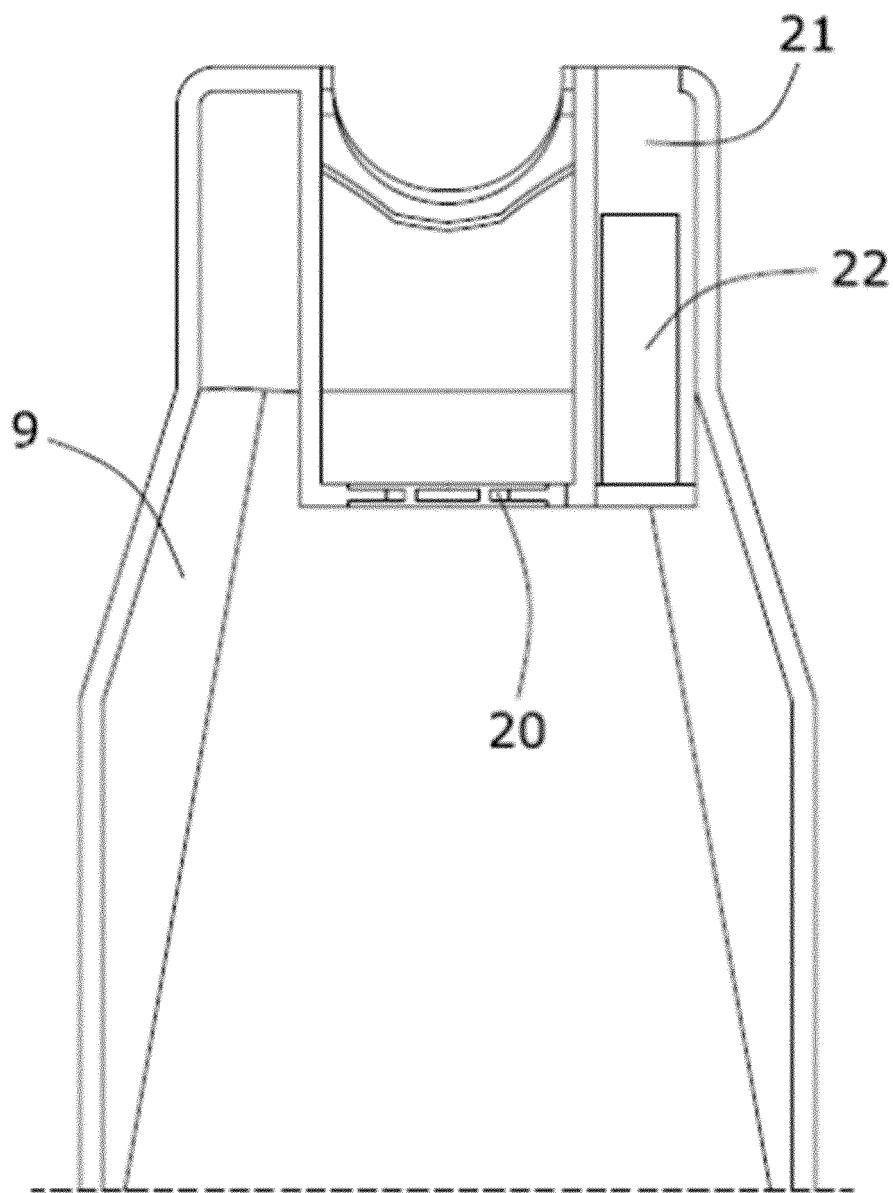


FIG.6

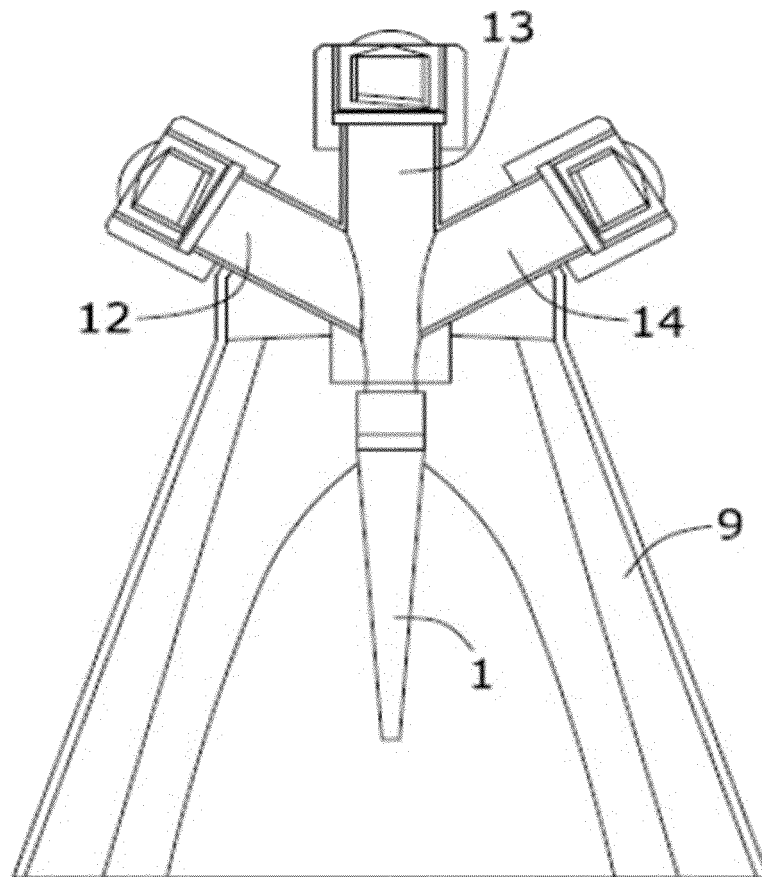


FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/ES2013/070054

A. CLASSIFICATION OF SUBJECT MATTER

B01L3/00 (2006.01)

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)

B01L

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)

EPODOC, INVENES, WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02058845 A2 (BIOPREVENTIVE LTD ET AL.) 01/08/2002, Page 10, paragraphs segundo and tercero; page 18, paragraph segundo; page 22, paragraph tercero; figures 1 and 2.	1-15
A	US 2002173046 A1 (HAFEZ AHMED ET AL.) 21/11/2002, Paragraphs [0003], [0004], [0013] and [0014]; figures 1-7; abstract.	1-15
A	US 2005123456 A1 (EICHACKER LUTZ) 09/06/2005, Paragraph [0007]; figures 1, 3, 5 and 9; abstract.	1-15
A	US 2007269355 A1 (MALMQVIST MATS) 22/11/2007, Abstract and figures 3, 4, 5 and 7.	1-15

☐ 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
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INTERNATIONAL SEARCH REPORT

International application No.

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