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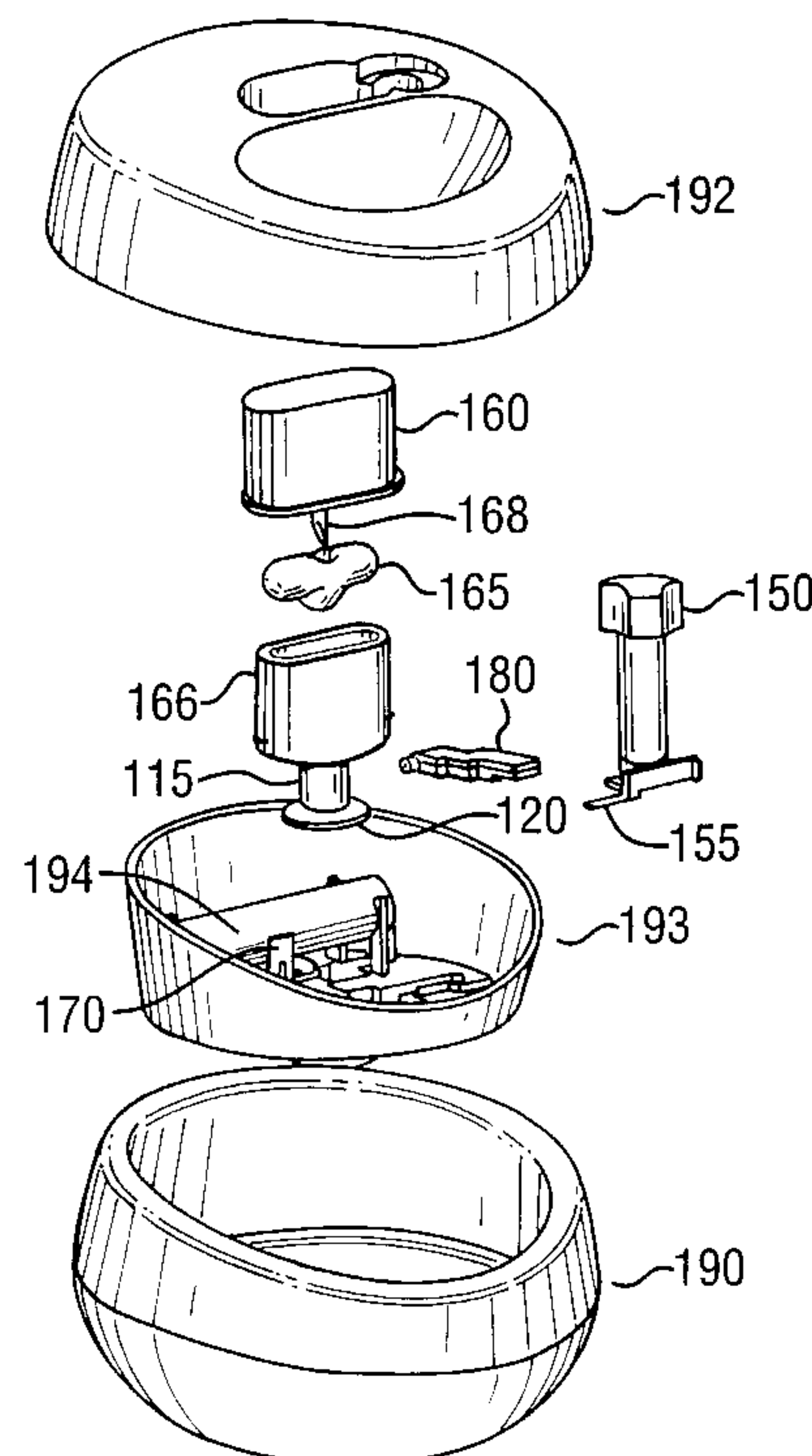
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(54) Titre : DISPOSITIFS POUR SEPARATION DE SPERMATOZOIDES MOTILES

(54) Title: DEVICES FOR MOTILE SPERM SEPARATION



(57) Abrégé/Abstract:

Device (10, 110) for separating motile sperm from a sample (100, 200), comprising a vessel (15, 115) having an inlet port (20, 120), an outlet port (35, 135) which is initially closed, a medium (40, 140) into which motile sperm in the sample (100, 200) can migrate into the vessel (15, 115) via the inlet (20, 120), and an actuator (50, 150), the operation of which opens the outlet (35, 135), thereby allowing the medium (40, 140) to flow out of the vessel (15, 115) through the outlet (35, 135). The medium (40, 140) in the vessel (15, 115) is initially prevented from flowing through the outlet (35, 135), thus allowing an incubation period which allows motile sperm sufficient time to migrate from the sample (100, 200) into the medium (40, 140) before the medium (40, 140) leaves the vessel (35, 135). The device (10, 110) preferably comprises spermatozoa detection means in communication with the outlet. In devices for separating sperm, capillary flow takes place through non-fibrous material, such as

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the space between sheets (81a and 81b; 181a and 181b) of closely-juxtaposed material. A device for separating motile and non-motile sperm includes a temperature sensor, a heat source (70, 170) and, optionally, temperature regulation means.

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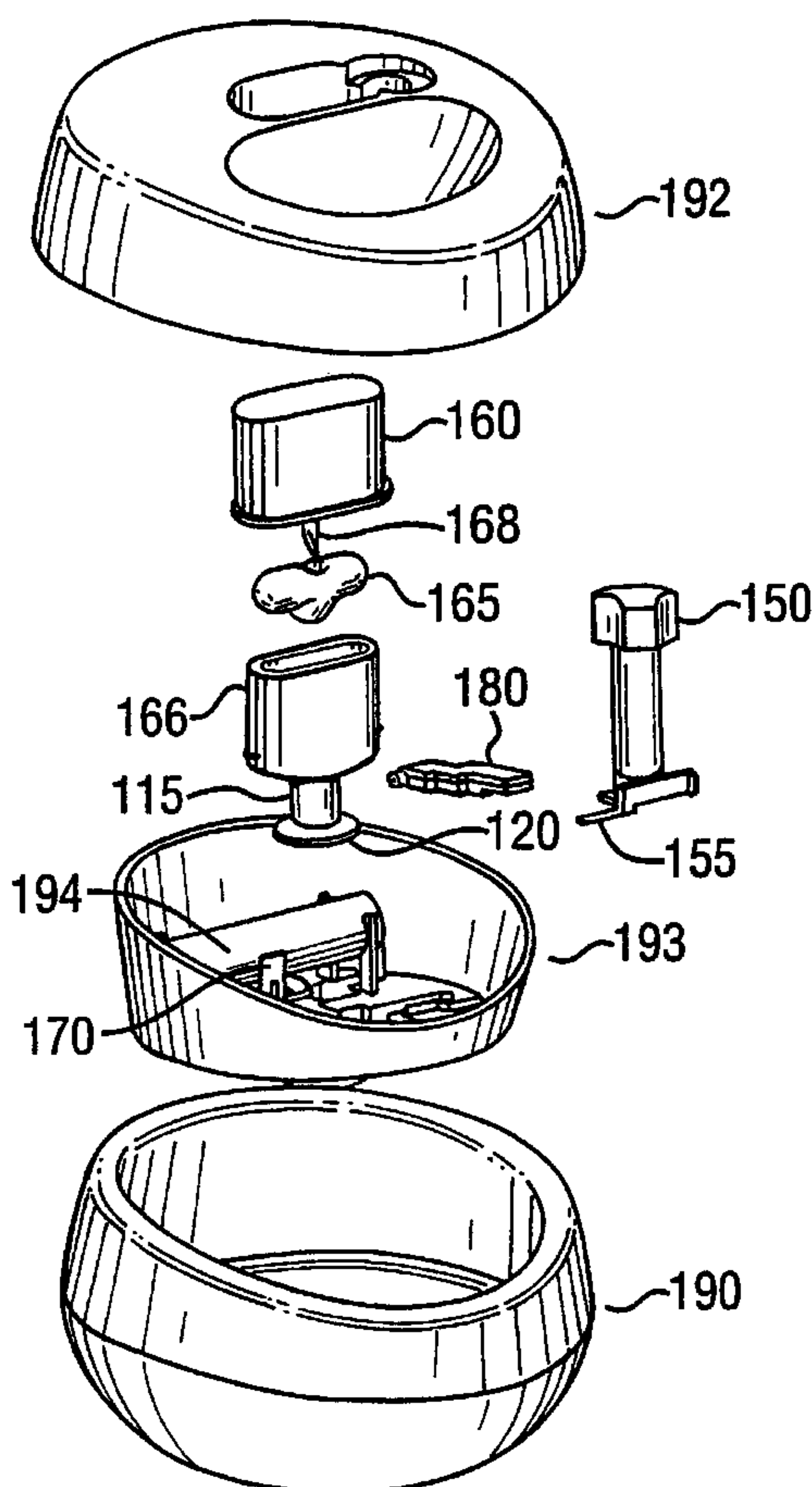
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(54) **Title:** DEVICES FOR MOTILE SPERM SEPARATION

(57) **Abstract:** Device (10, 110) for separating motile sperm from a sample (100, 200), comprising a vessel (15, 115) having an inlet port (20, 120), an outlet port (35, 135) which is initially closed, a medium (40, 140) into which motile sperm in the sample (100, 200) can migrate into the vessel (15, 115) via the inlet (20, 120), and an actuator (50, 150), the operation of which opens the outlet (35, 135), thereby allowing the medium (40, 140) to flow out of the vessel (15, 115) through the outlet (35, 135). The medium (40, 140) in the vessel (15, 115) is initially prevented from flowing through the outlet (35, 135), thus allowing an incubation period which allows motile sperm sufficient time to migrate from the sample (100, 200) into the medium (40, 140) before the medium (40, 140) leaves the vessel (35, 135). The device (10, 110) preferably comprises spermatozoa detection means in communication with the outlet. In devices for separating sperm, capillary flow takes place through non-fibrous material, such as the space between sheets (81a and 81b; 181a and 181b) of closely-juxtaposed material. A device for separating motile and non-motile sperm includes a temperature sensor, a heat source (70, 170) and, optionally, temperature regulation means.

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DEVICES FOR MOTILE SPERM SEPARATION

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention is in the field of male fertility tests, more specifically tests for separating and
5 detecting motile spermatozoa in a semen sample.

BACKGROUND ART

Approximately 15% of couples attempting to conceive fail to do so within one year of unprotected intercourse. Fertility specialists define these couples as being infertile. 40% of these cases result from male factors. In a substantial proportion of these, treatment is available
10 to ameliorate or relieve the condition which leads to infertility.

Other conditions also exist in which it is desirable to test for the presence or otherwise of viable spermatozoa in a sample. For example, vasectomies are now frequently carried out as a method of contraception, but it is necessary to confirm that ejaculate is free of viable spermatozoa after the operation.

15 A number of methods exist for assessing the motility and number of spermatozoa in a sample. One such method is microscopic analysis, which is typically carried out in a hospital or commercial laboratory. More recently, however, a number of proposals have been made for test kits which are intended to simplify the detection of spermatozoa.

WO97/40386 discloses a kit which is based on immunodetection of the 34kDa human
20 epididymal spermatozoa protein. Spermatozoa in a sample are washed three times by centrifugation in Dulbecco-phosphate buffered saline. The samples are then heat denatured at 95°C, centrifuged at 14000g, and the supernatants are then used for analysis.

WO95/29188 describes a test based on antibodies to the SP-10 antigen of human spermatozoa.

EP-A-0387873 discloses a kit which uses solid beads to which are bound antibody specific to
25 the human spermatozoon acrosome. The beads are mixed with a sample, incubated, separated, washed, and the number of spermatozoa bound to the beads is measured, preferably by examination with the aid of a microscope.

A disadvantage of these test kits is that they do not distinguish between motile and non-motile spermatozoa. This distinction is the most predictive indicator of male infertility. Moreover,

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they involve procedures which do not lend themselves to home use (*e.g.* centrifugation, microscopy), thus requiring implementation by a skilled practitioner.

These disadvantages are addressed by the device of WO00/09648 (Genosis Limited), which discloses an apparatus for separating motile spermatozoa from non-motile spermatozoa in a liquid sample, the apparatus comprising (i) a vessel having a sample receiving inlet, a filtered sample outlet and a sample separation filter mounted therebetween, the sample separation filter having a sample-receiving surface and an opposed surface, and the sample separation filter being effective substantially to prevent flow of the sample therethrough, but permitting passage of motile spermatozoa therethrough when said opposed surface of said sample separation filter is placed in contact with a liquid medium and (ii) means for supplying a liquid to said opposed surface of said filter.

Further improvements in sperm separation, detection and analysis are disclosed herein.

DISCLOSURE OF THE INVENTION

The present invention provides a device for separating motile spermatozoa from a liquid sample, the device having a sample separation vessel comprising:

- (a) a sample inlet port;
- (b) a sample outlet port, which is initially closed;
- (c) a sample separation medium into which motile spermatozoa in the sample can migrate via the sample inlet port; and
- (d) an actuator operable to open the sample outlet port, thereby allowing the sample separation medium to flow out of the vessel through the sample outlet port.

In this device, the separation medium in the vessel is initially prevented from flowing through the outlet port. This allows an incubation period which allows motile sperm sufficient time to migrate from the sample into the sample separation medium before it exits the vessel. On operation of the actuator, the outlet port is opened and the separation medium (including motile spermatozoa) can migrate through the outlet port and leave the vessel *e.g.* for analysis.

In order for motile spermatozoa in the sample to enter the separation medium, the inlet port and the separation medium must be in liquid communication. Preferably, however, such communication is initially prevented, thus preventing contamination of the medium via the inlet port before the device is used. This may also prevent the medium from escaping from the

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vessel via the inlet port, although this may be achieved anyway due to, for instance, viscosity or surface tension of the medium.

In this embodiment, therefore, the vessel comprises (e) a second actuator, operable to bring the separation medium into communication with a sample via the inlet port. This might be
5 achieved by storing the medium remotely from the inlet port until the second actuator is operated or, alternatively, by having an inlet port which is initially closed or sealed, which is opened using the actuator.

During use of this device, therefore, the second actuator is operated, thereby bringing the separation medium and the inlet port into communication. This allows motile spermatozoa in
10 the sample to migrate from the sample into the separation medium. After a period of incubation, the (first) actuator is operated, thereby allowing the separation medium (now containing motile spermatozoa) to leave the vessel. It is preferred that the first actuator cannot be operated until the second actuator has been operated.

It will be appreciated that feature (e) can operate independently from feature (d).

15 The vessel may be of circular cross-section *e.g.* conical or cylindrical.

The inlet and outlet ports can take a variety of forms. For instance, the inlet port might be an open end of a cylinder or cone. This open end can be placed in a sample and, when the separation medium and the sample are in communication, spermatozoa can enter the vessel via the open end. It may be desirable to cover the open end by a mesh or grid, to assist in retaining
20 sample separation medium within the vessel (via surface tension) without preventing the entry of spermatozoa from the sample.

The outlet port is preferably an opening, hole or aperture in the vessel's wall, which may continue into a pipe, tube or conduit leading away from the vessel.

The inlet and outlet ports are preferably arranged such that, during use, the outlet port is above
25 the inlet port. Spermatozoa thus have to swim against gravity in order to exit the vessel, enhancing the preferential migration of motile *vs.* non-motile sperm.

Any suitable actuators can be used in the device *e.g.* buttons, switches *etc.* Where the vessel is of circular cross-section, the (first) actuator is preferably a rotatable collar containing an opening. Rotation of the collar from a closed position aligns openings in the collar and vessel
30 wall, thereby allowing sample separation medium to leave the vessel via the outlet port.

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Another preferred form of (first) actuator acts to withdraw fluid into a barrel or tube *e.g.* it withdraws the plunger of a syringe. The plunger will initially block the outlet port but, on withdrawal of the plunger, sample separation medium can flow through the outlet port and into the internal space of the syringe. A suitable actuator for effecting withdrawal of the syringe
5 plunger is a rotatable knob which is attached to the plunger *e.g.* by a rack and pinion mechanism.

Where the device includes a second actuator, this is preferably a button. When pressed, sample separation medium stored within the device is released so that it reaches (*e.g.* by gravity) the inlet port. This can be achieved by, for instance, including a reservoir of medium within the
10 vessel, sealed by a foil wall, together with a foil cutter. Operation of the second actuator causes the cutter to pierce the foil, releasing the medium.

A preferred arrangement of reservoir is that disclosed in United Kingdom patent application 0021665.5, in which the separation medium is held within a hermetically sealed reservoir. The device will include a venting needle for venting the medium from the reservoir, the venting
15 needle comprising a fluid delivery portion and a reservoir-venting portion, the reservoir-venting portion being distal along the needle of the delivery portion, the delivery portion and the venting portion each defining a channel extending from the respective portion through the sidewall of the needle. The channels allow the delivery portion, in use, to deliver fluid from the reservoir, for example by venting air at one end and dispensing fluid at the other. In this
20 arrangement, the venting needle will preferably be at least partially a cannula having a cannulated point at the delivery portion for controlled, pipette-like delivery. In a needle with a proximal portion and a distal portion, the distal portion may comprise both the venting portion and the delivery portion. The needle preferably has a C-shaped section at the venting portion and the delivery portion. The two channels are preferably unitary, the channel extending from
25 the venting portion to the delivery portion.

In a venting position, the venting needle preferably extends through two wall portions of the reservoir such that the venting portion forms a passage through the first wall portion to allow the container to be vented with air and the delivery portion forms a passage through the second wall portion to allow fluid to be dispensed from the reservoir.

30 Using this sort of arrangement, the needle is operated by the second actuator (*e.g.* it is attached to a button or a screw-advance mechanism).

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The separation medium allows motile spermatozoa, in preference to non-motile sperm, to migrate therethrough. This can be achieved using any suitable buffer (*e.g.* HEPES, EBSS *etc.*), as motile spermatozoa will be able to migrate through the medium actively whereas, over the relevant time-scale, non-motile spermatozoa will at best enter passively by diffusion. It is preferred, however, to use a medium that enhances the migration of motile spermatozoa from the sample. Suitable media include cervical mucus [*e.g.* Keel & Webster (1988) *Fertil. Steril.* 49:138-143], polyacrylamide gel [*e.g.* Lorton *et al.* (1981) *Fertil. Steril.* 35:222-225], hyaluronic acid [*e.g.* Aitken *et al.* (1992) *J. Androl.* 13:44-54], or a cellulose derivative [*e.g.* international patent application PCT/GB00/03130 (Genosis Limited)] such as methylcellulose.

10 The medium may be in the form of a solution or a gel.

Advantageously, the separation medium also serves to 'wash' the sample, in order to remove components such as seminal plasma.

In addition to separating motile and non-motile spermatozoa, it is preferred that the device can detect motile spermatozoa after separation. Accordingly, the device of the invention preferably comprises spermatozoa detection means in communication with the vessel outlet port. The detection means may be integral with the device, or may be provided as a separate component for inserting into the device before, during or after sample separation.

Accordingly, the invention provides a device for separating and detecting motile spermatozoa in a liquid sample, the device having:

20 – a sample separation vessel comprising:

- (a) a sample inlet port;
- (b) a sample outlet port, which is initially closed;
- (c) a sample separation medium into which motile spermatozoa in the sample can migrate via the sample inlet port; and
- 25 (d) an actuator operable to open the sample outlet port, thereby allowing the sample separation medium to flow out of the vessel through the sample outlet port,

– spermatozoa detection means comprising:

- (i) an application zone in communication with the outlet port;
 - (ii) a detection zone, in which the presence of spermatozoa can be detected; and
 - 30 (iii) a reagent zone containing a reagent which is capable of reacting with spermatozoa to facilitate their detection in the detection zone,
- these zones being arranged to permit capillary flow of spermatozoa from the application zone to the detection zone.

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Preferably, the detection zone is a zone past which spermatozoa cannot flow (a 'trapping zone'). During operation, flow of spermatozoa is thus prevented beyond the trapping zone, and sperm are immobilised for detection. Preferably, the trapping zone utilises the principles disclosed in WO00/20866 – the zone is porous with a pore size such that spermatozoa cannot
5 enter. During flow into the trapping zone, therefore, sperm are captured at the entrance; as sperm concentration increases, the amount retained there also increases.

The trapping zone can be made from any suitable porous material (*e.g.* HDPR, nitrocellulose) through which spermatozoa cannot migrate. This requirement is reflected in the pore size of the trapping zone. The head of a human spermatozoon is typically 3-5µm in diameter, and tail
10 length is approximately 50-60µm. The pore size should be selected accordingly (*e.g.* nitrocellulose, with a nominal pore size between 5-8µm), and an appropriate pore size may be determined empirically by simple experimentation.

It will be apparent that any porous material encountered before the trapping zone must, in contrast to the trapping zone, have a pore size large enough to allow spermatozoa to move
15 relatively freely.

As is well known to those in the art, the nominal pore size of a porous material can be determined by hard particle challenge testing *i.e.* by determining the maximum diameter of spherical particles which can pass through the material. Alternatively, the pore size of a material may be determined by measuring its 'bubble point'. The bubble point is the pressure
20 required to force air through a (water) wet membrane, and correlates with the pore size as measured by particle retention (although at extremes of pressure and pore size, the correlation may be weaker). The bubble point is generally easier to measure than particle retention and is thus the preferred test when assessing pore size.

The entrance to the trapping zone is preferably narrow, such that sperm are focused to give a
25 sharper signal.

As a less-preferred detection zone, immobilised anti-sperm antibody may be utilised.

In one embodiment of the detection zone, detection is based on acrosin. The detection zone will contain a reagent for detecting acrosin (*e.g.* see Mortimer, *Practical Laboratory Andrology* (1994), page 90) and the reagent zone will include a reagent such as proteinase K
30 or the calcium ionophore A23187 [Perry *et al.* (1997) *J.Exp.Zool.* 279:284-290; Perry *et al.* (1997) *J.Exp.Zool.* 279:291-300 (1997); Perry *et al.* (1996) *Human Reprod.* 11:1055-1062;

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Perry *et al.* (1995) *Fertil.Steril.* 64:150-159] which causes the acrosomes to open. A preferred reagent for lysing acrosomes is a lysis buffer comprising 2% SDS, 100 µg/ml proteinase K in 10 mM Tris-HCl and 0.1 M EDTA. During migration, therefore, acrosin is released from the sperm and is detected downstream at the detection zone.

- 5 More usually, the reagent zone will contain a reagent which binds to intact spermatozoa or to one or more of the components thereof, and this binding reaction is used to generate a visual signal. Preferably, therefore, the reagent zone is a 'labelling zone', containing a label capable of binding to spermatozoa. This is preferably used in conjunction with a trapping zone.

10 The label within the labelling zone can be any suitable reagent that can bind to spermatozoa, preferably giving a visible signal. Because only motile spermatozoa will reach the outlet port, the label need not be specific for sperm.

The label is typically an antibody which can bind to spermatozoa and which has been suitably tagged. It is preferred to use a visible tag, such as colloidal gold (which is visible as a pink colour), although fluorescent, luminescent or radioactive tags can also be used. It will be
15 appreciated that the term 'antibody' may include polyclonal and monoclonal antibodies, as well as antibody fragments (*eg.* F(ab)₂, Fc *etc.*), provided that anti-sperm reactivity is retained.

Preferred labels recognise a surface antigen which is present on the majority of a population of spermatozoa, rather than a subset. Whilst any sperm antigen may be used (*eg.* P34H (WO97/40836), SP-10 (WO95/29188), see also EP-A-0387873), 'universal' antigens such as
20 CD59 may also used. As an alternative, a stain, such as eosin, may be used.

It is preferred that the label is not activated (*eg.* re-hydration of dehydrated label) until sample separation medium leaves the vessel. This can be achieved by arranging the labelling zone such that label therein is not activated until the (first) actuator has been operated. Label therefore remains static until motile spermatozoa have left the sample and entered the sample
25 separation medium.

The labelling zone may be arranged in any suitable position such that its label can contact spermatozoa in the detection zone (*eg.* sperm retained at the trapping zone). It may be upstream or downstream of the application zone, or may be integral therewith. If it is upstream, sample separation medium leaving the vessel should be able to contact the labelling
30 zone separately from its contact with the application zone, in order to activate the label; if it is downstream or integral, flow will automatically activate the label.

Where the detection means includes both a 'trapping zone' and a 'labelling zone', the pore size of the trapping zone will be such that free label (not bound to spermatozoa) can flow therethrough, whereas label which is bound to spermatozoa cannot. During flow into the trapping zone, therefore, bound label is captured at the entrance. As sperm concentration
5 increases, the amount of capture label also increases. It will be apparent that the label must be smaller than spermatozoa, such that free label is not retarded by the trapping zone.

Where the detection means utilises a label, it preferably includes a zone downstream of the detection zone which retains unbound label (the 'label control' zone). This will typically comprise immobilised antibody which can bind to unbound label (*e.g.* if the label is a murine
10 monoclonal antibody, the label control zone may utilise anti-mouse antibody). Label which passes through the detection zone (*e.g.* which is not captured on entry to the trapping zone) is thus retained within the label control zone, where it can be measured. A comparison of the amount of label in the detection zone and the label control zone allows semi-quantitative measurement of the amount of spermatozoa in the sample.

15 The application zone is where the sample separation medium leaving the vessel comes into contact with the spermatozoa detection means. It can be formed from any material suitable for allowing capillary flow of spermatozoa therethrough *e.g.* fibrous material, such as a pad of HDPE material, bonded polyester fibre, glass fibre, or the like.

It has been found, however, that spermatozoa tend to be retained within fibrous materials, thus
20 reducing sensitivity. It is therefore preferred to use a non-fibrous application zone. Suitable examples include capillary tubes, the space between two or more sheets of material juxtaposed in close proximity such that capillary flow can occur therebetween, or a series of parallel capillary channels or grooves. In such arrangements, if the labelling zone and the application zone are integral, this is preferably formed from more than one piece of material such that
25 label can be applied during assembly. Where juxtaposed sheets are used, for instance, label can be applied to one or more sheets, and these can then be brought into proximity to permit capillary flow. Similarly, where parallel channels or grooves are used the label can be applied to the channelled material and, optionally, covered by a further sheet.

In a similar arrangement, capillary flow takes place past a fibrous material rather than through
30 it. Whilst flowing past, liquid can enter the fibrous material (*e.g.* to re-hydrate a label

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impregnated therein), but the main capillary flow through the application zone of sperm is not within the fibrous material.

The application zone and the sample outlet port may be essentially unitary.

5 In preferred embodiments, flow of a sample to the detection zone is assisted by a downstream wick, to aid capillary movement.

During use of preferred devices, therefore, the migration path of motile sperm in the sample is: entry to the separation medium via the vessel's inlet port, exit from the separation medium via the vessel's outlet port (upon operation of the actuator after an incubation period), entry to the application zone, and capillary flow to the trapping zone, in which further migration is
10 prevented. A visible signal at the trapping zone indicates the presence of motile spermatozoa in the sample

The visible signal provided by the detection means is preferably external to the vessel, and more preferably one which is also visible from the exterior of the device.

15 The detection means is preferably in the form of a lateral flow test strip mounted on the outside of the vessel.

It will be appreciated that the vessel may include more than one sample outlet port, each of which may lead to separate detection means. This allows independent tests on the same sample *e.g.* an assay for motile sperm on one strip and an assay for acrosome reaction on another strip. This can also be achieved by using a single outlet port that leads downstream to several
20 separate detection means.

To enhance the separation of motile and non-motile spermatozoa (*e.g.* EP-A-0437508), it is preferred that the device should be operated at an essentially fixed temperature. This will typically be between 30°C and 44°C, preferably between 35°C and 39°C, and more preferably fixed at or around 37°C. Preferably, therefore, the device includes a temperature sensor.

25 The temperature sensor may give a simple signal to indicate when operating temperature has been reached. The device could be held in a user's hand, or inserted in a heated water bath, until the signal is given.

More preferably, however, the device also includes its own heat source, which can preferably be regulated *e.g.* using thermostatic control. The heat source should supply heat primarily to

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the region of the vessel which contains the sample separation medium. Power and control circuitry for the heat source may conveniently be concealed within an actuator button, or within the body of the device.

5 It will be appreciated that the inclusion of temperature regulation in the device can operate independently from the device's other features.

The device of the invention may be used with any suitable liquid sample, which is preferably a semen sample. The device may include a receptacle for the sample. Preferably, this is in the form of a cup or sloping surface onto which a sample can be deposited, after which it collects at the base (*e.g.* in a well). The sample inlet can then be placed into contact with the collected
10 sample. The receptacle may also include an overflow near its base. The receptacle may be integral with the device, or may be provided as a separate component to which the device may be attached before, during or after sample deposition and/or collection.

Advantageously, the interface between the receptacle and the vessel's inlet port (*e.g.* where the vessel attaches to the well) is of a defined area, thereby aiding assay reproducibility.

15 The device of the invention can be produced simply and cheaply. Furthermore, it can be used very easily, for instance by the home user. The invention thus provides an assay device which can be used at home as a basic screen of, for instance, male fertility.

The invention also provides corresponding processes. Thus the invention provides a process for separating motile spermatozoa from a liquid sample, the process comprising the steps of:

- 20 (a) providing a vessel having a sample inlet port, a closed sample outlet port, and a sample separation medium;
- (b) allowing spermatozoa in the sample to migrate into the sample separation medium over a period of between 5 and 60 minutes (preferably around 30 minutes); and
- (c) allowing the sample separation medium to leave the vessel by opening the sample
25 outlet port.

The process may initially comprise the step of opening the inlet port.

Preferably, the process also comprises steps for detecting spermatozoa in the medium once it has exited the vessel. Thus the invention provides a process for separating and detecting motile spermatozoa in a liquid sample, comprising the further steps of:

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- (i) allowing sample separation medium that leaves the outlet port to flow by capillary action into material capable of immobilising spermatozoa;
- (ii) contacting spermatozoa with a label; and
- (iii) detecting label that is retained at the entrance to said material.

5 The invention further provides a process for separating and/or detecting spermatozoa, wherein sperm flow by capillary action takes place through non-fibrous material.

The invention further provides a process for separating motile and non-motile spermatozoa, wherein said process takes place at an essentially fixed temperature, preferably around 37°C.

BRIEF DESCRIPTION OF DRAWINGS

10 **Figure 1** shows a device according to the invention. **Figure 2** shows this device after insertion into a receptacle. **Figures 3 to 6** show the operation of this device. **Figure 7** shows an overview of using this device.

Figure 8 shows a second device according to the invention, prior to operation. **Figure 9** shows the same device separated into its two constituent pieces, with **Figure 10** showing an exploded
15 view of the top piece. **Figure 11** shows the construction of the test strip assembly used in the device. **Figures 12 to 16** show an overview of using this device.

MODES FOR CARRYING OUT THE INVENTION

The device (10) shown in Figure 1 comprises: a cylindrical plastic vessel (15); a sample inlet port (20), covered by a nylon mesh (21) formed from 0.15mm strands spaced by 0.25mm; a
20 hole (30) in its side, shown in the open position; a solution (40) of EBSS supplemented with 0.88mg/ml hyaluronic acid and 0.45% BSA; a plastic rotatable collar (50), including a hole (51) which is shown aligned with hole (30) to form an outlet port (35); a button (60, shown depressed), which houses a battery and circuitry for powering a circumferential heat source (70) and attached to which is a solution reservoir (65, shown empty), a foil seal (66, shown
25 broken), and a hollow foil cutter (68); and an externally mounted test strip (80).

Figure 2 shows the Figure 1 device (10) attached to a semen receptacle (90) with sloped walls. A semen sample (100) has collected in the well (99) at the base of the receptacle (90), but some of it has overflowed into the receptacle overflow (95).

Figure 3 shows the device (10) immediately prior to use. A semen sample (100) has collected
30 in the well (99) and is in contact with the inlet port (20). The outlet port (35) is closed because

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the hole (51) in the collar (50) is not aligned with the hole (30) in the vessel wall. Solution (40) is held in reservoir (65) by foil seal (66), away from the inlet port (20).

To initiate the test, button (60) is depressed, as shown in Figure 4A. Foil cutter (68) pierces seal (66), releasing solution (40). Motile sperm in the sample (100) are now in liquid
5 communication with solution (40) and are able to migrate into it through the inlet (20), as shown in Figure 4B, whereas non-motile sperm and seminal plasma remain in sample (100). Button (60) also activates heat source (70), bringing the temperature of solution (40) to 37°C.

After a period of about 30 minutes, during which motile sperm have migrated into the solution (40), collar (50) is rotated, as shown in Figure 5, so that its hole (51) aligns with hole (30),
10 thereby opening the outlet port (35). Solution (40), now containing motile sperm, is free to leave the vessel and contact externally-mounted test strip (80).

As shown in Figure 6, the solution flows by capillary action through closely-juxtaposed plastic strips (81a & b) at the base of the test strip (80). Between the strips (81a & b) is an area (82) of dehydrated gold-labelled murine anti-CD59. As the solution passes area (82) between the
15 strips (81a & b), the antibody is re-hydrated and is able to bind to spermatozoa in the solution. Further downstream, the solution reaches nitrocellulose strip (83). The pore size of the strip (83) is too small to allow the spermatozoa to enter, so they are captured at its entrance (84). Free label continues to flow until it is captured downstream at a line (85) of immobilised anti-mouse antibody.

20 As shown in Figure 7, entrance (84) and line (85) are visible through windows. In Figure 7E, two lines are visible, indicating the presence of motile spermatozoa in the original sample. In contrast, only the control line (85) is visible in Figure 7F, indicating an absence of motile sperm in the original sample.

The device (110) shown in Figures 8 and 9 comprises an upper piece (191) and a lower piece
25 (190) which fit together. The base of lower piece (190) contains a well (199) into which a semen sample (200) is deposited. Upper piece (191) includes a recessed window (113), a button (160) and a rotatable knob (150). Knob (150) and button (160) are shaped such that knob (150) cannot be rotated until button (160) has been depressed.

In Figure 10, the internal components of upper piece (191) are shown in an exploded view.
30 Upper piece (191) is formed from a top piece (192) which engages a seating (193). On the

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bottom of button (160) is a needle (168) and, when button (160) is operated, needle (168) pierces reservoir (165) which, prior to operation, contains a solution (140) of EBSS supplemented with 0.88mg/ml hyaluronic acid and 0.45% BSA. Reservoir (165) sits in plastic housing (166), which has a neck portion (115) and a head portion (120). The side of neck (115) contains a hole (130) which engages the tube portion (186) of test strip assembly (180).
5 The base of head portion (120) is covered by a circular nylon mesh (121) formed from 0.15mm strands spaced by 0.25mm. In the assembled device (110), mesh (121) is in contact with sample (200) within well (199). Knob (150) is attached via a rack and pinion mechanism to plunger (155) which, prior to use, passes through tube (186) and towards hole (130).
10 Seating (193) contains a battery (194) which powers heat source (170). When assembled, heat source (170) surrounds neck (115) circumferentially, except in the region of hole (130).

Exploded and assembled views of test strip assembly (180) are shown in Figure 11. In the assembled device (110), tubular portion (186) communicates directly with hole (130) and, prior to use, plunger (155) engages and fills tube (186), thereby preventing liquid flow
15 therethrough. As plunger (155) is withdrawn by operation of knob (150) in the direction of the arrow in Figure 11, tube (186) opens to form, together with hole (130), an outlet port (135) through which liquid can flow. Tube (186) and plunger (155) therefore operate in the manner of syringe. Liquid flows through tube (186) into the capillary space between clear plastic housings (181a; 181b) and passes under a pad (182) containing dehydrated gold-tagged
20 murine anti-CD59. As liquid passes pad (182), the antibody is re-hydrated and can pass into the liquid, where it is able to bind to spermatozoa. The liquid continues to flow towards and into nitrocellulose strip (183), aided by a wick (188). The pore size of strip (183) is too small to allow the spermatozoa to enter, so they are captured at its entrance (184). Antibody can bind captured spermatozoa at entrance (184) and form a pink line. Any free antibody continues to
25 flow until it is captured downstream at a line (185) of immobilised anti-mouse antibody.

The device is used as illustrated in figures 12 to 16:

- In figure 12, a semen sample (200; *e.g.* obtained by masturbation) is placed into lower piece (190) and collects in well (199) whilst lower piece (190) rests on a flat surface.
- After 30 minutes, upper piece (191) is assembled with lower piece (190) as shown in
30 figure 13, to form device (110).
- Button (160) is then depressed, as shown in figure 14. This releases solution (140) and also activates heat source (170), bringing the temperature of solution (140) to 37°C.

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- After around 30 minutes, to allow entry of sperm into solution (140) and also temperature equilibration, LED (116) indicates that knob (150) should be rotated, as in figure 15.
- This withdraws plunger (155) and allows motile sperm from sample (200) which have swum into medium (140) to pass into the tube portion (186) of test strip (180). The solution (140) flows through the test strip (180) by capillary action towards wick (188). Sperm in the solution are retained at the entrance (184) of nitrocellulose strip (183). Free gold-tagged antibody continues to flow until it is captured downstream at a line (185) of immobilised anti-mouse antibody. The line at (184) in figure 16 indicates a positive result. The line at (185) indicates that the test has operated correctly.

10

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

CLAIMS

1. A device for separating and detecting motile spermatozoa in a liquid sample, the device having:
 - a sample separation vessel comprising:
 - 5 (a) a sample inlet port;
 - (b) a sample outlet port, which is initially closed;
 - (c) a sample separation medium into which motile spermatozoa in the sample can flow via the sample inlet port; and
 - (d) an actuator operable to open the sample outlet port, thereby allowing the sample
 - 10 separation medium to flow out of the vessel through the sample outlet port.
 - spermatozoa detection means comprising:
 - (i) an application zone in communication with the outlet port;
 - (ii) a detection zone, in which the presence of spermatozoa can be detected; and
 - (iii) a reagent zone containing a reagent which is capable of reacting with
 - 15 spermatozoa to facilitate their detection in the detection zone,
- these zones being arranged to permit capillary flow of spermatozoa from the application zone to the detection zone.
2. The device of claim 1, wherein the zones are arranged to permit capillary flow of spermatozoa from the application zone to the detection zone via the reagent zone.
- 20 3. The device of claim 1 or claim 2, wherein the detection zone is a spermatozoa-trapping zone capable of immobilising spermatozoa, and the reagent zone is a labelling zone containing a label capable of binding to spermatozoa.
4. The device of any preceding claim, wherein the vessel additionally comprises (e) a second actuator, operable to allow the separation medium to come into communication with a
- 25 sample via the inlet port.
5. The device of claim 3 or claim 4, wherein the trapping zone is porous with a pore size such that free label can flow therethrough, whereas label which is bound to spermatozoa cannot.
6. The device of claim 3, claim 4 or claim 5, wherein the detection means includes a zone downstream of the detection zone which retains label which is not bound to spermatozoa.
- 30 7. The device of any preceding claim, wherein the application zone is non-fibrous.

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8. The device of any preceding claim, wherein the device includes a temperature sensor.

9. The device of claim 8, which includes its own heat source and, optionally, temperature regulation means.

10. A device for separating and detecting motile spermatozoa in a liquid sample, the device
5 having:

– a sample separation vessel comprising:

(a) a sample inlet port;

(b) a sample outlet port, which is initially closed;

(c) a sample separation medium into which motile spermatozoa in the sample can
10 flow via the sample inlet port; and

(d) an actuator operable to open the sample outlet port, thereby allowing the sample separation medium to flow out of the vessel through the sample outlet port.

– spermatozoa detection means comprising:

(i) an application zone in communication with the outlet port;

15 (ii) a trapping zone capable of immobilising spermatozoa; and

(iii) a labelling zone containing a label capable of binding to spermatozoa,

these zones being arranged to permit capillary flow of spermatozoa from the application zone to the trapping zone.

11. A device substantially as hereinbefore described with reference to the accompanying
20 drawings.

12. A device for separating and/or detecting spermatozoa, wherein sperm flow by capillary action takes place through non-fibrous material.

13. A device for separating and/or detecting motile and non-motile spermatozoa, wherein the device includes a temperature sensor, a heat source and, optionally, temperature regulation
25 means.

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FIG. 1

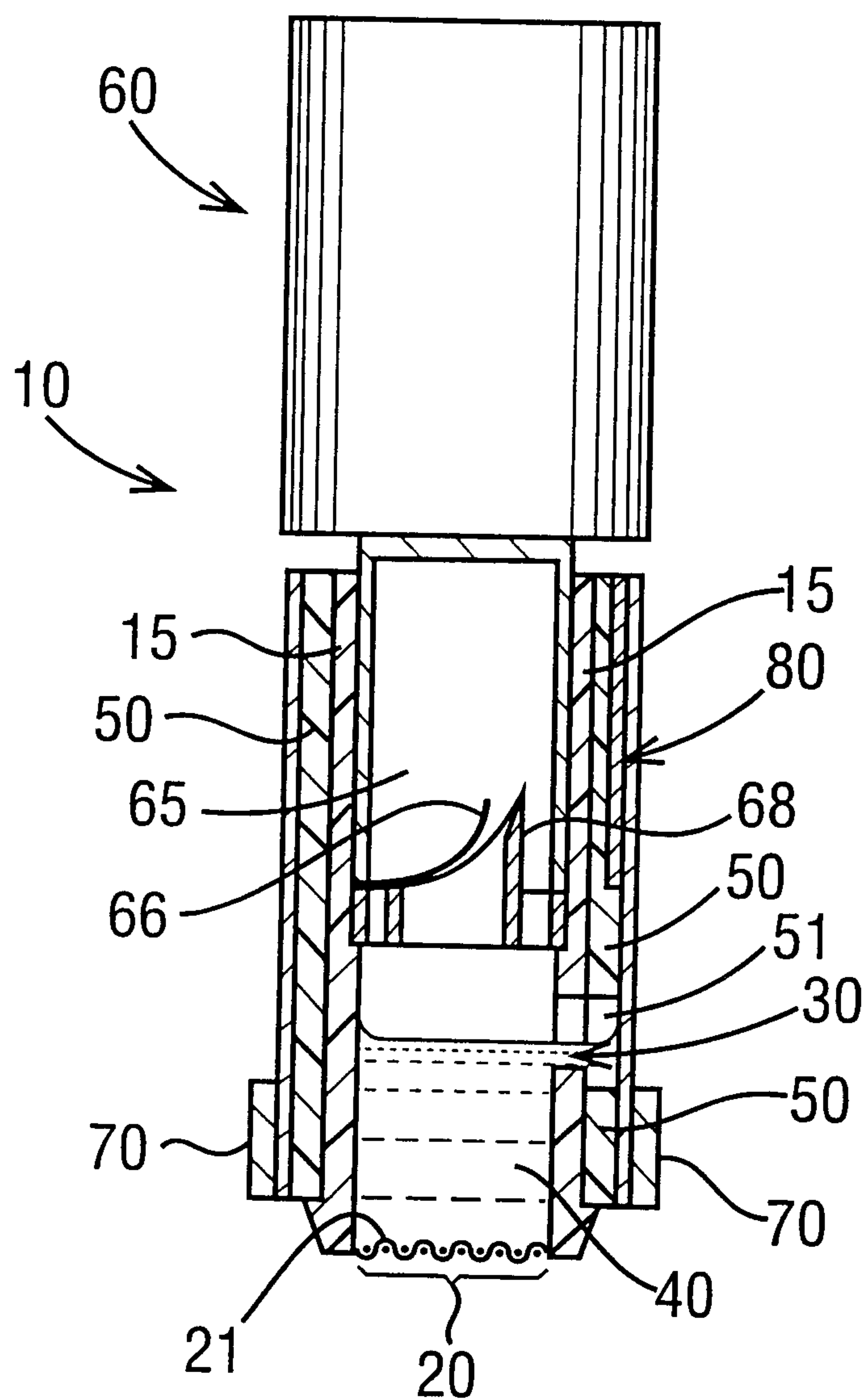


FIG. 2

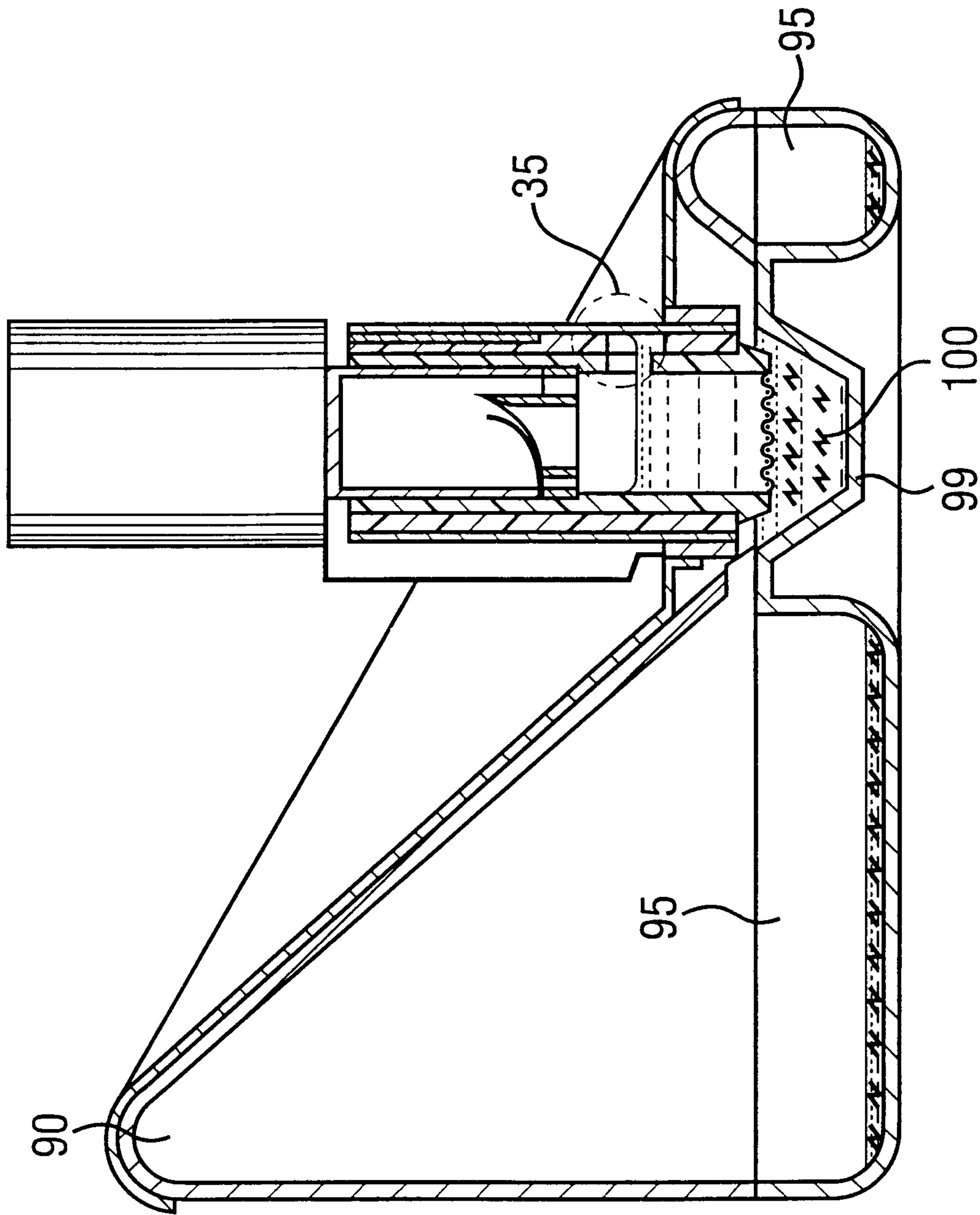
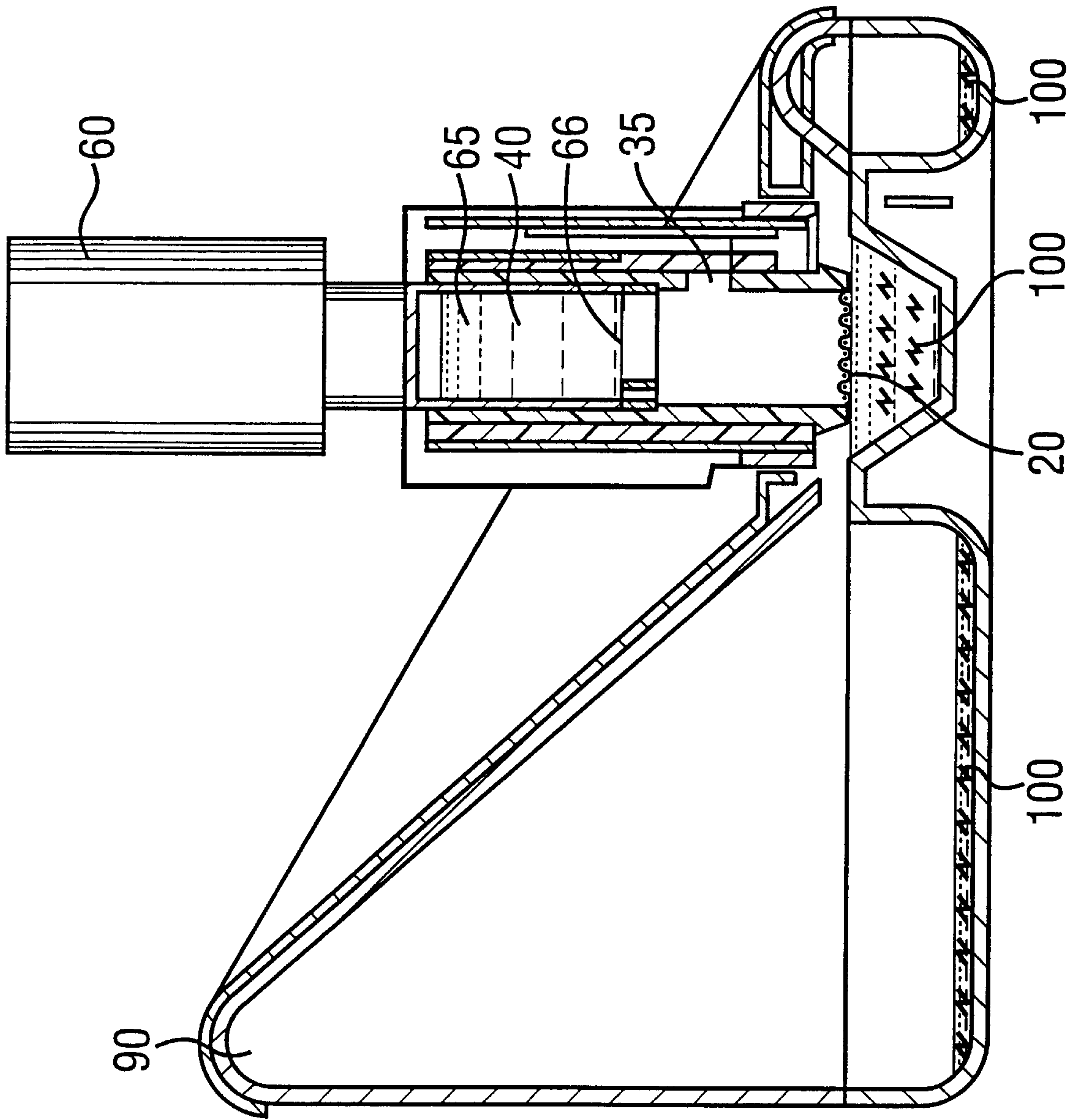
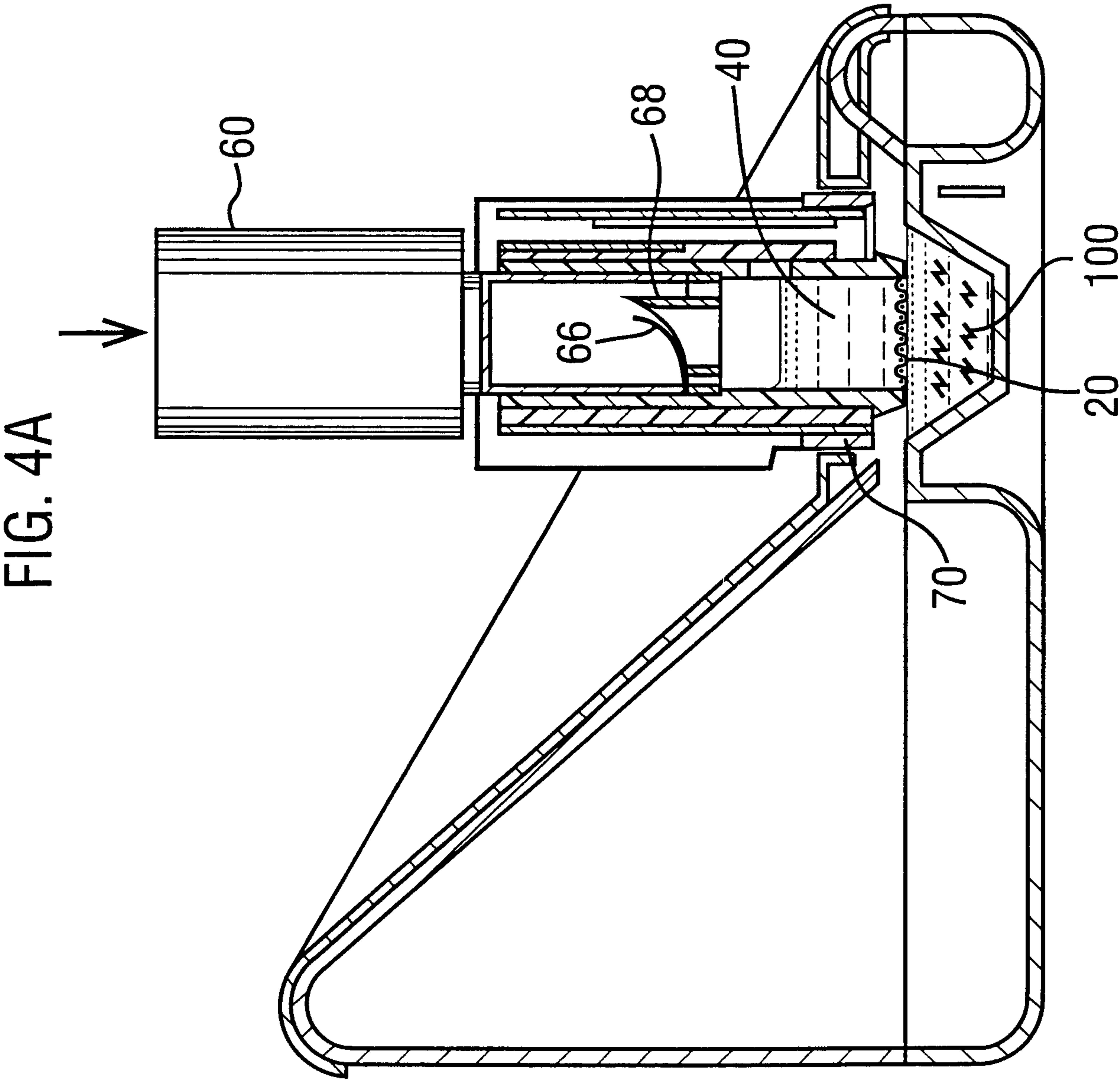


FIG. 3





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FIG. 4B

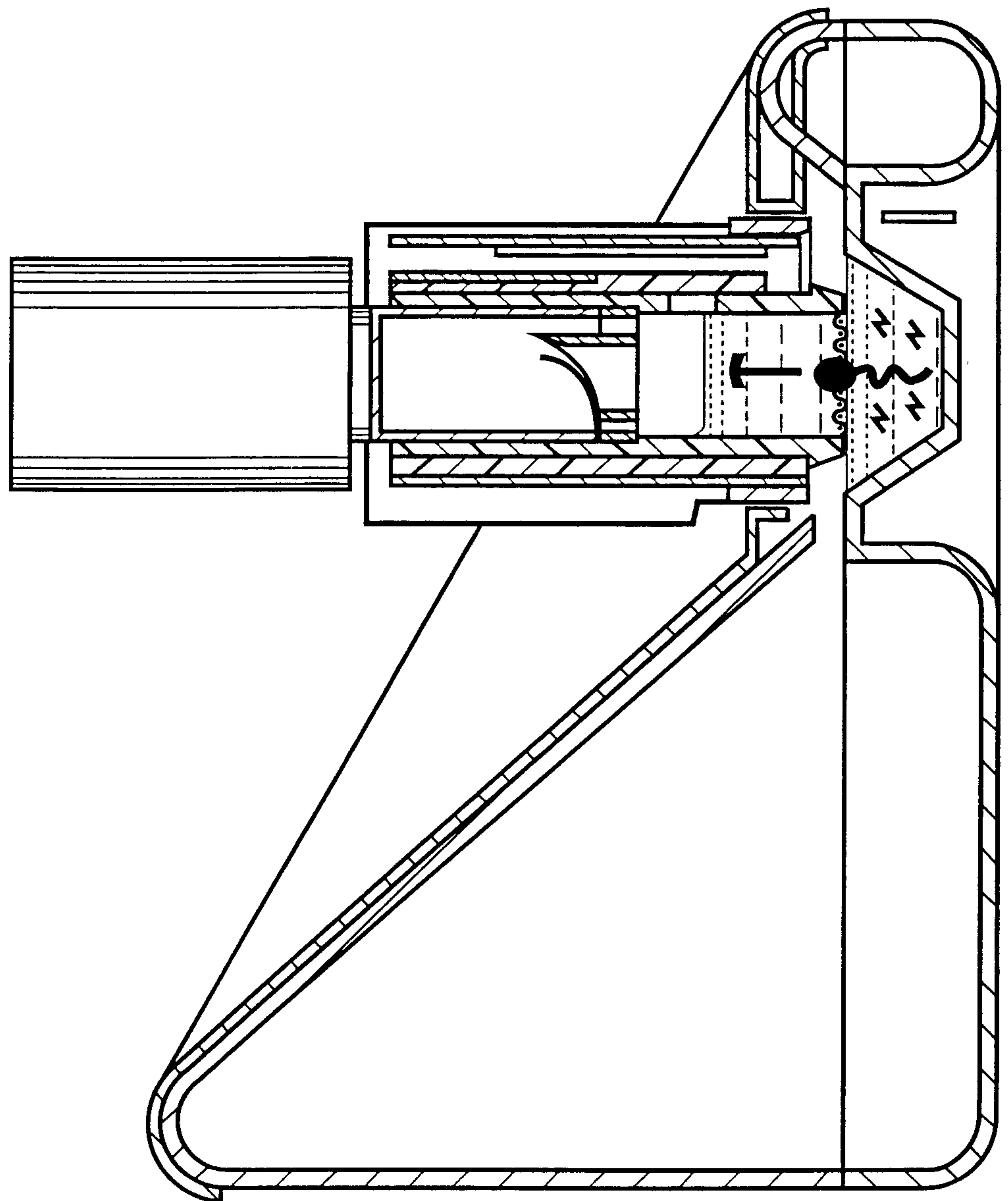
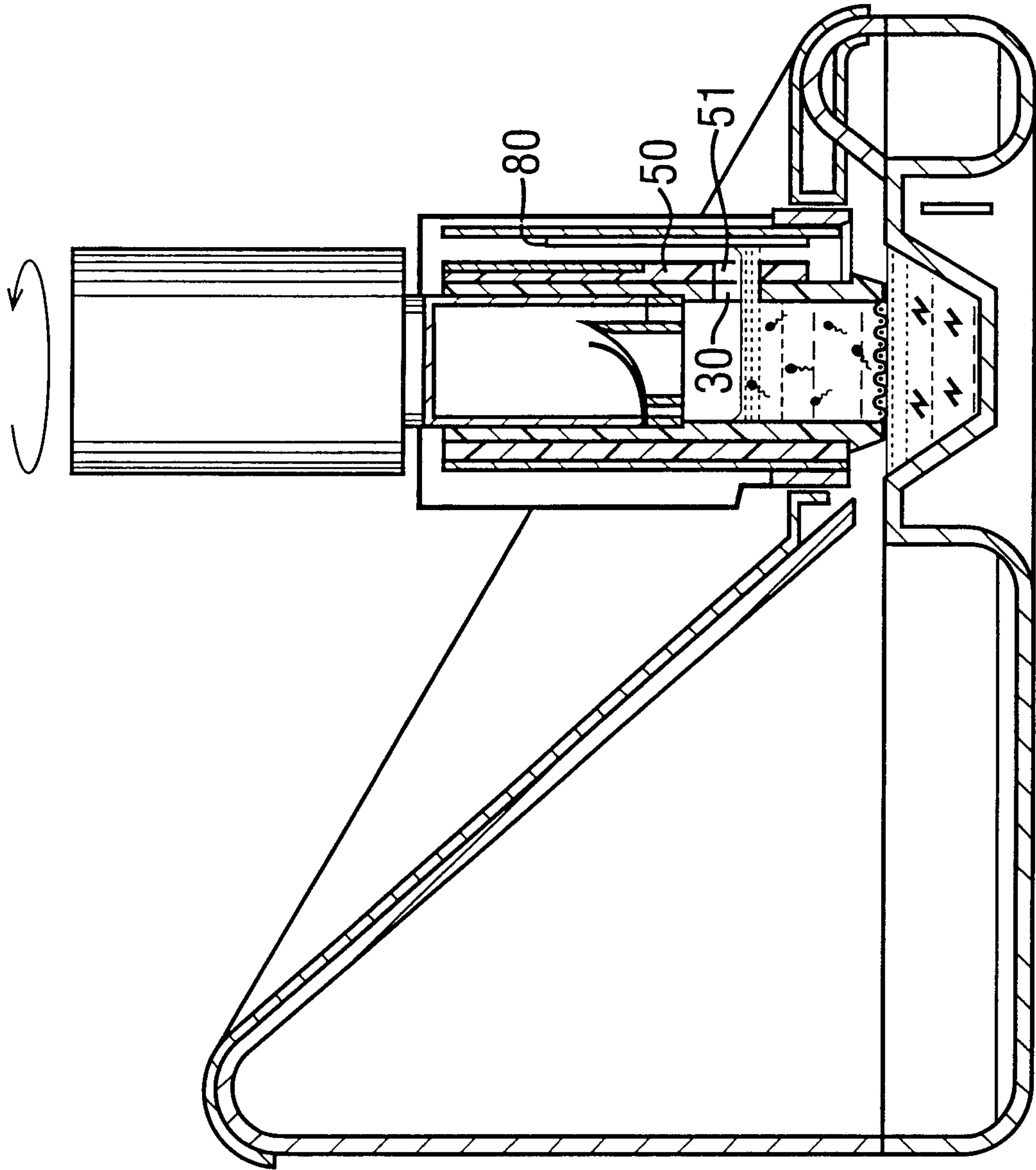
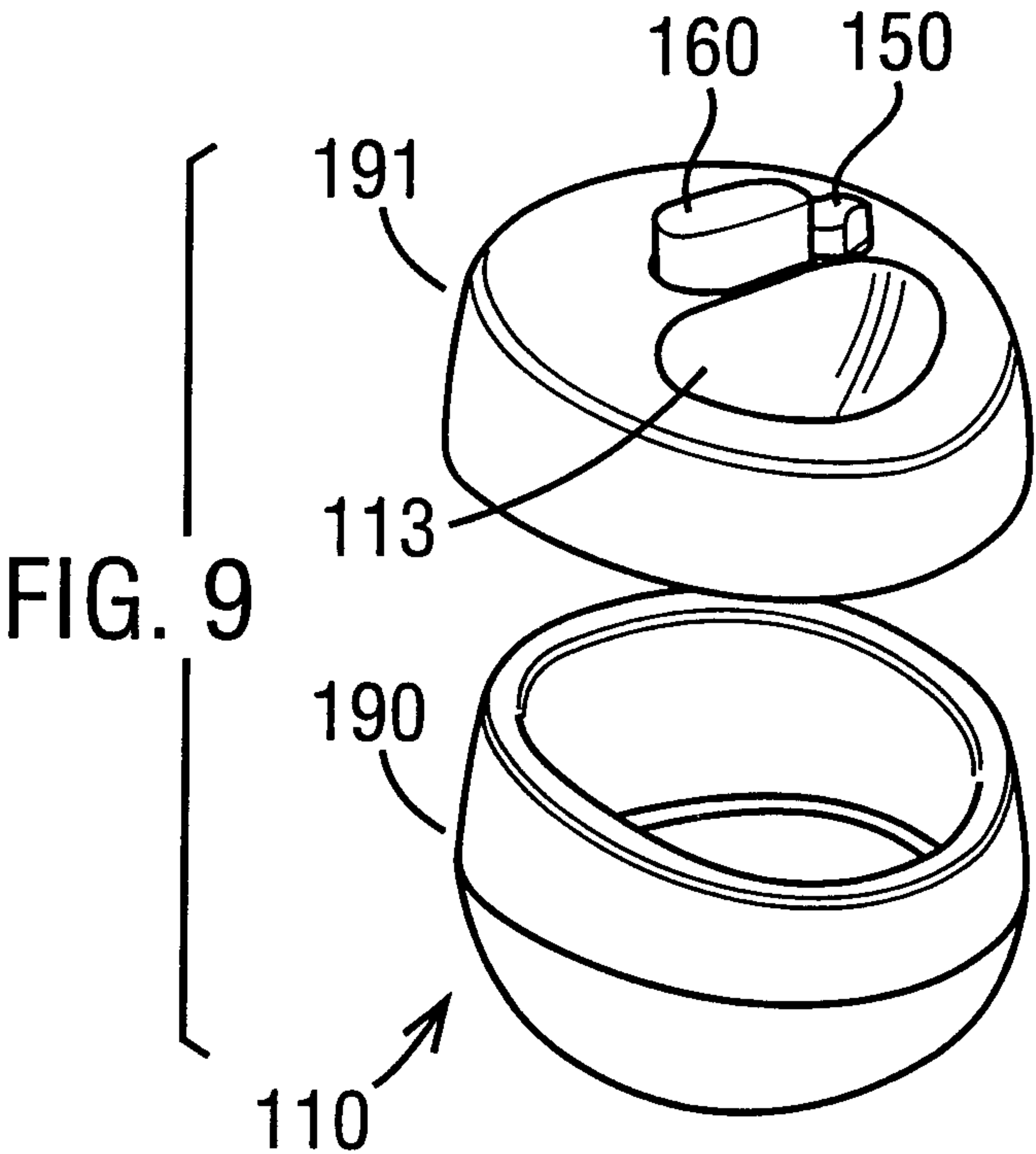
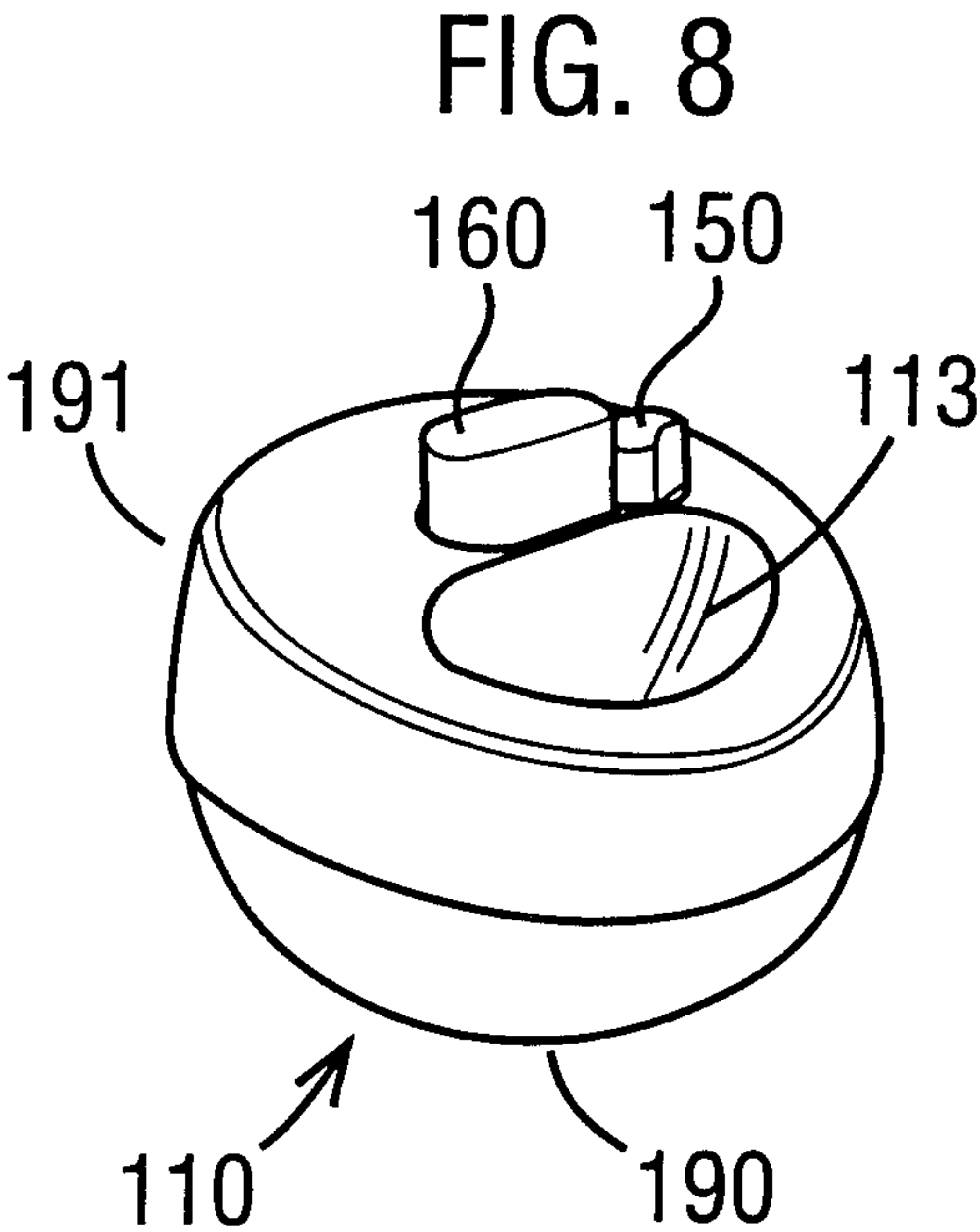
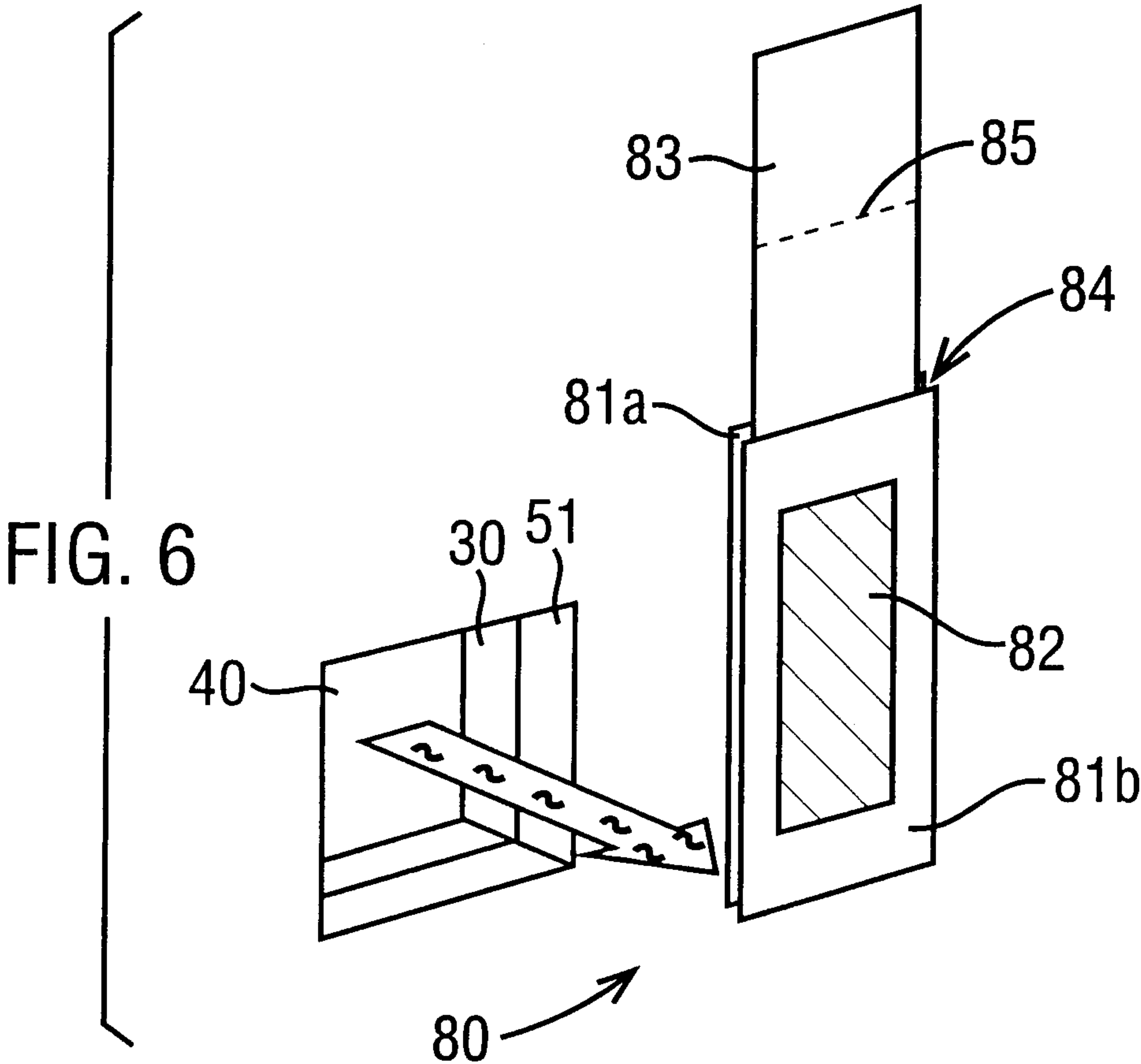


FIG. 5





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FIG. 7A

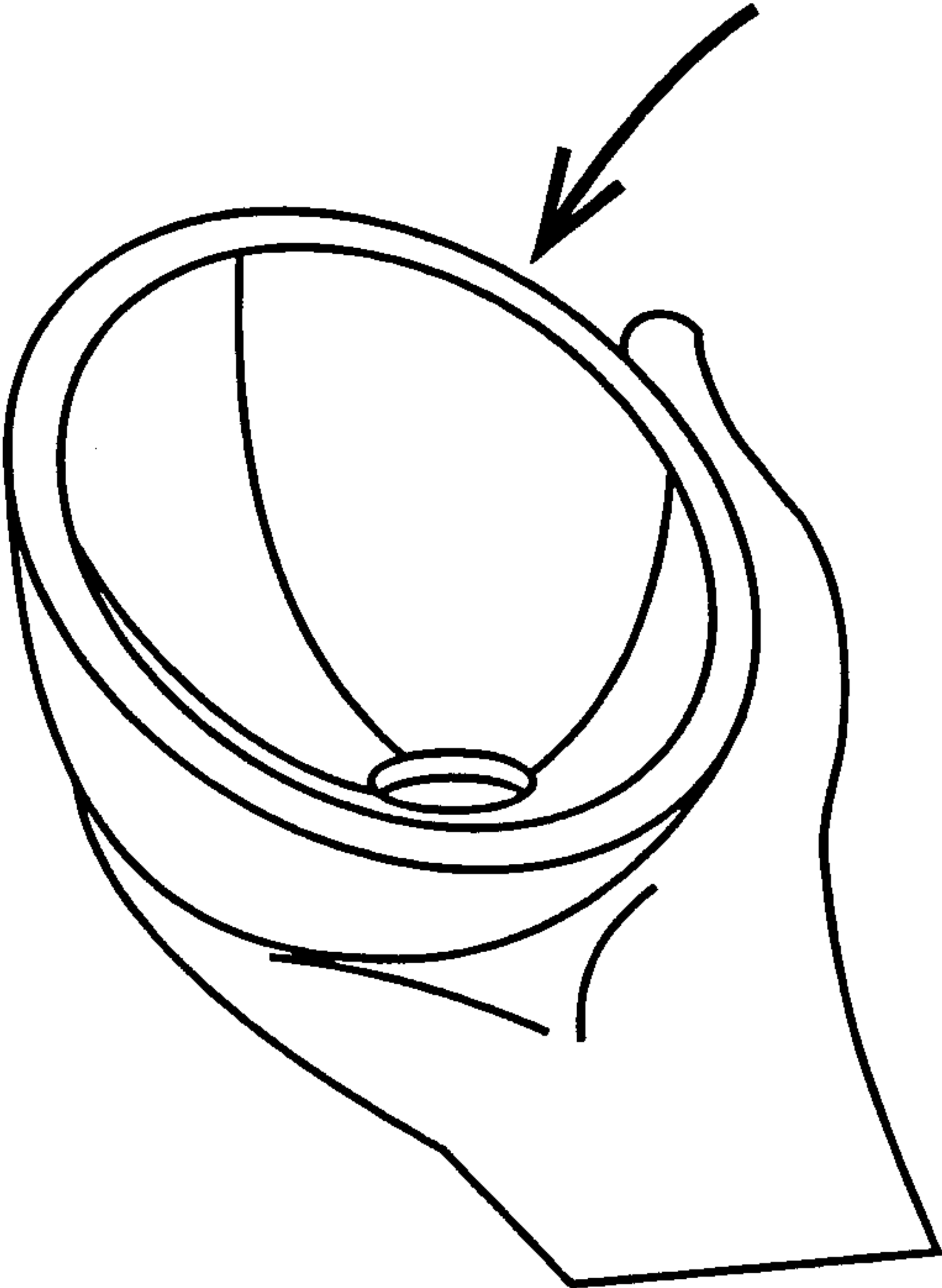


FIG. 7B

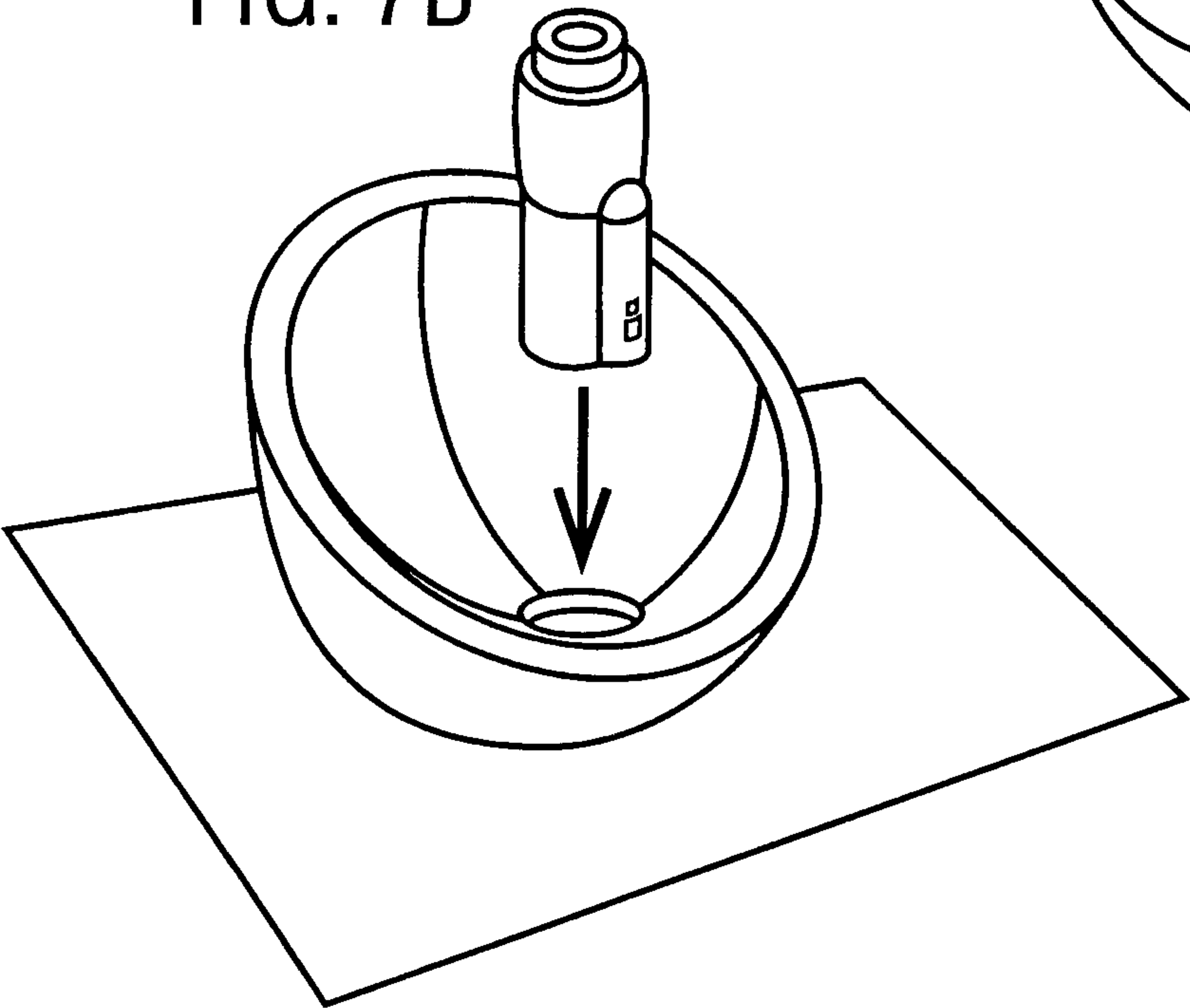
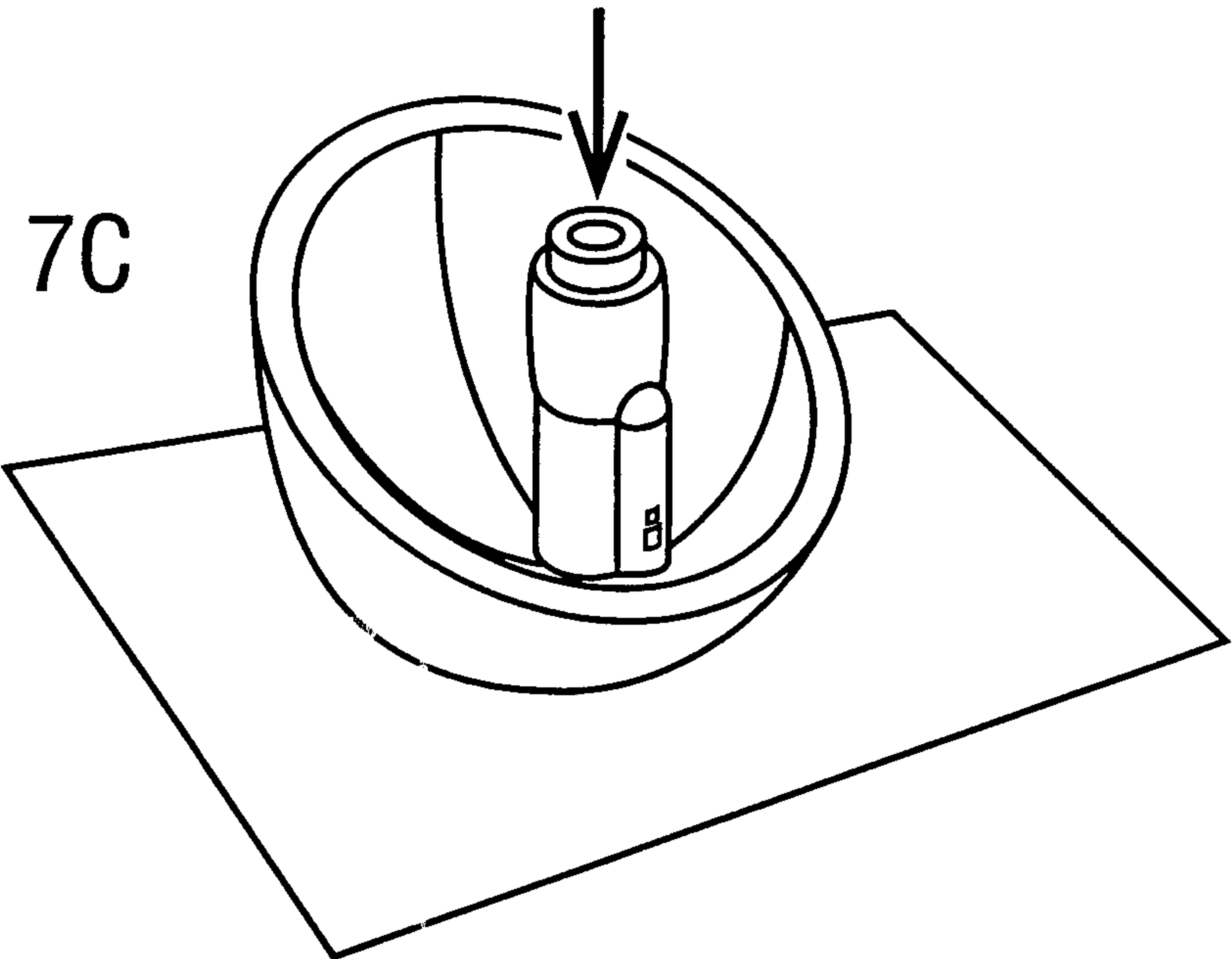
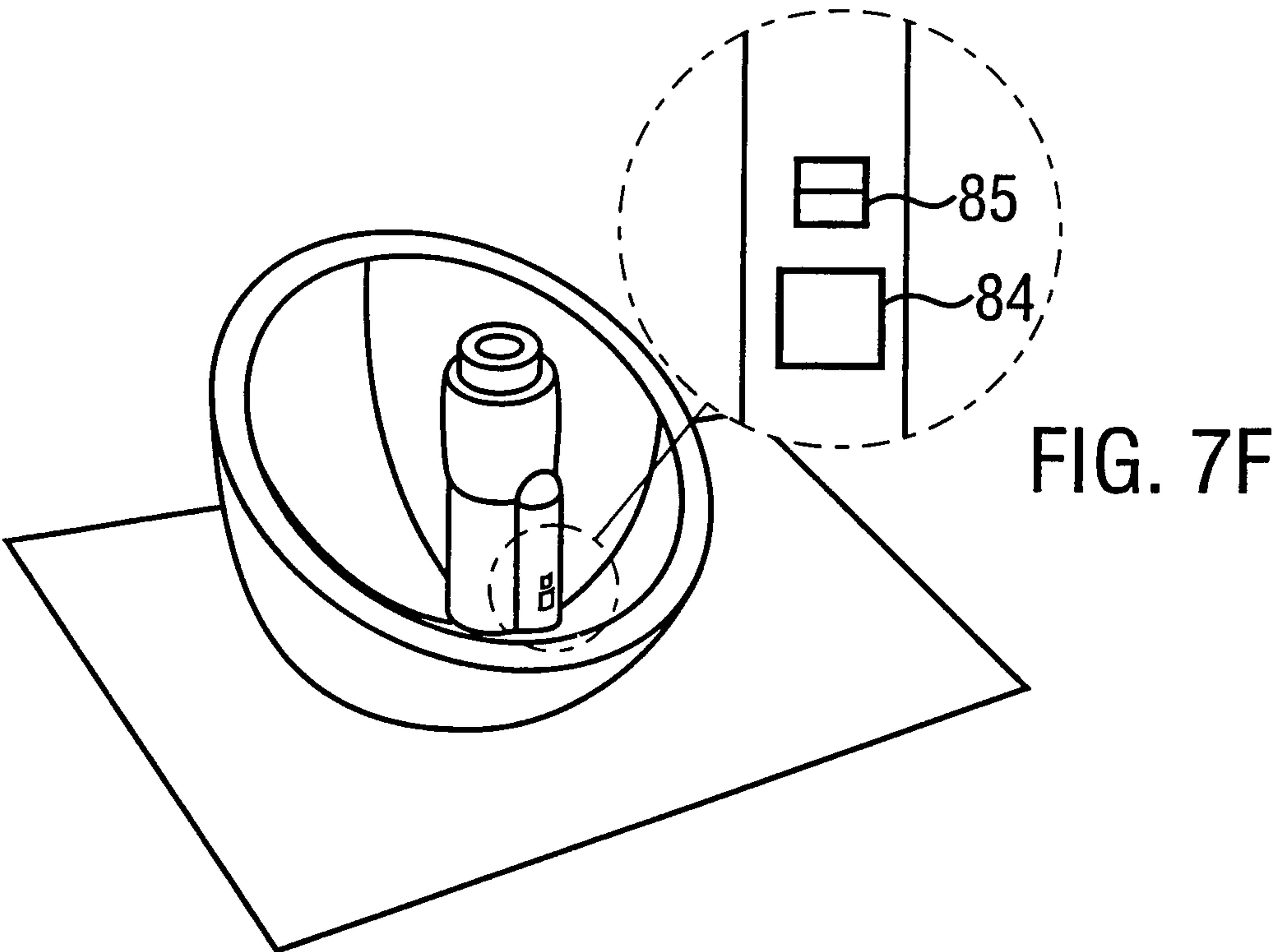
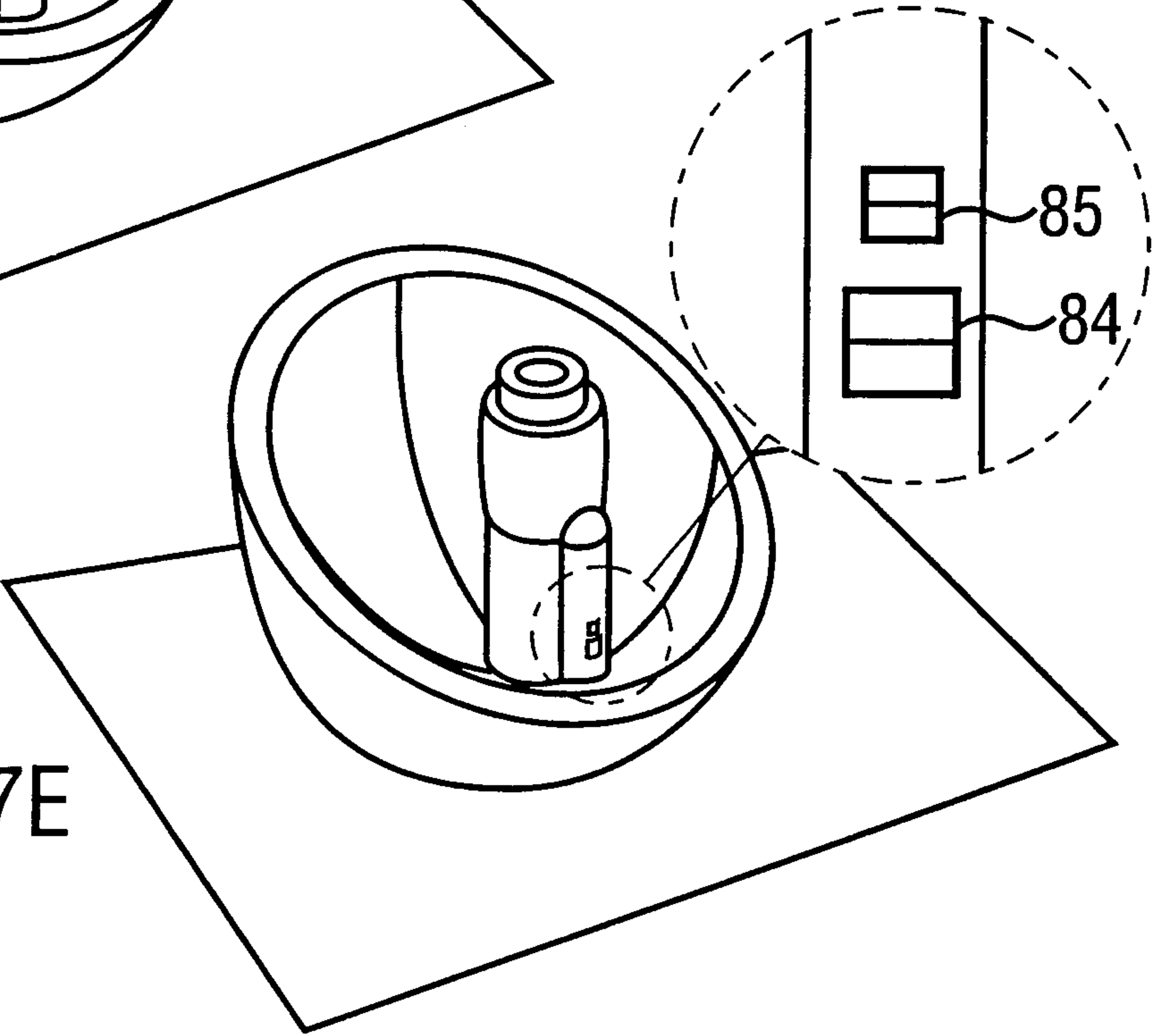
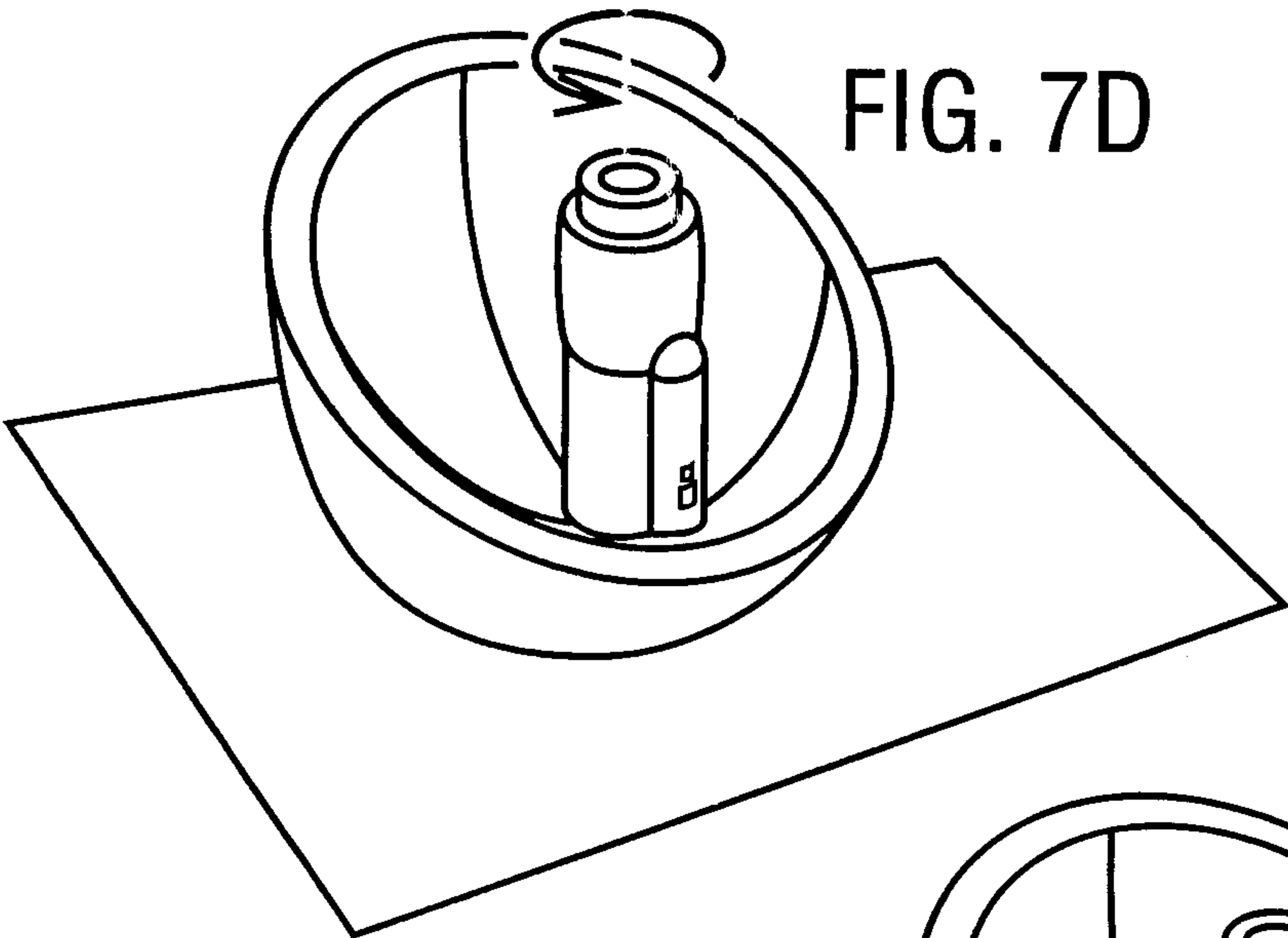


FIG. 7C

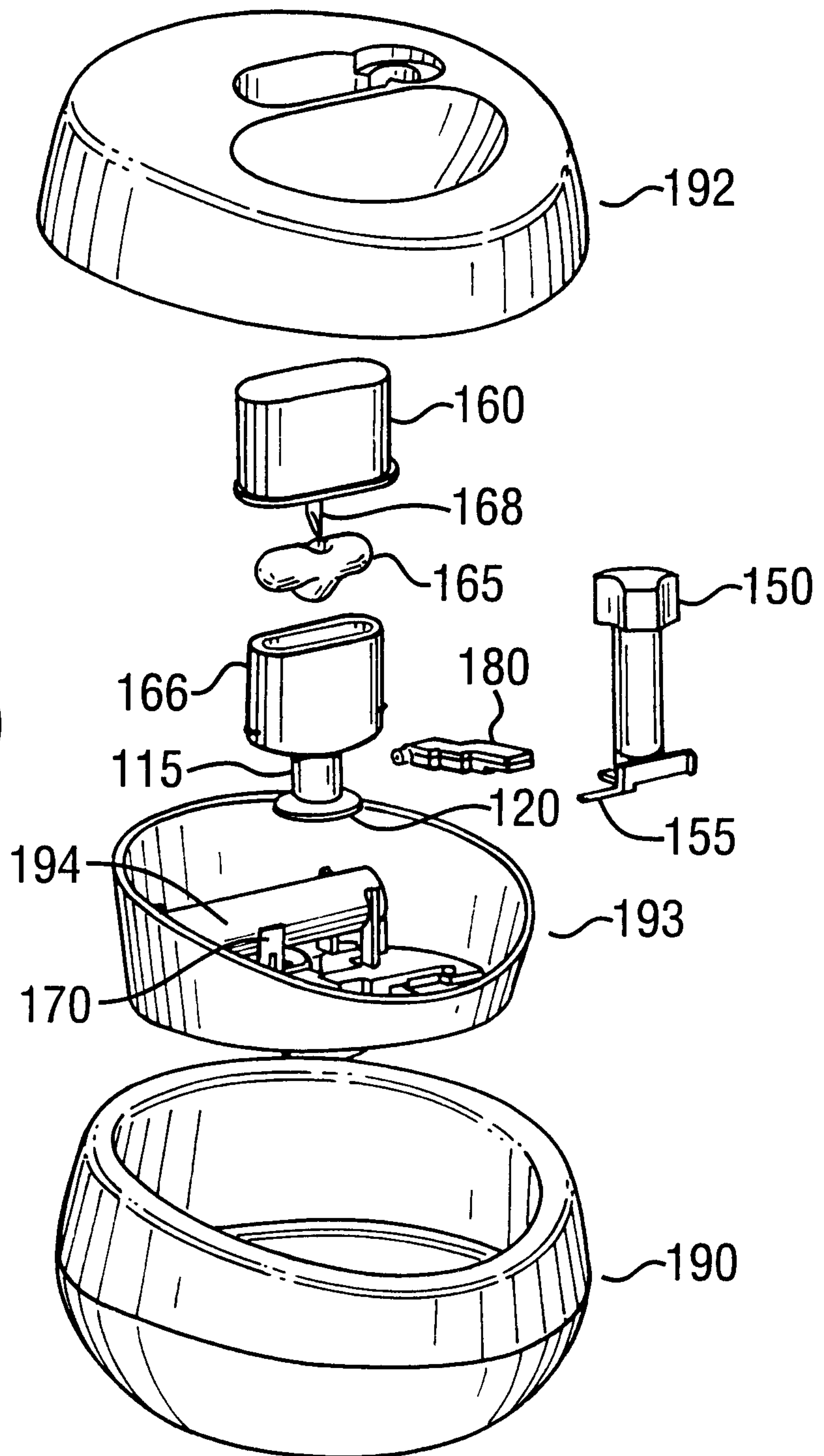


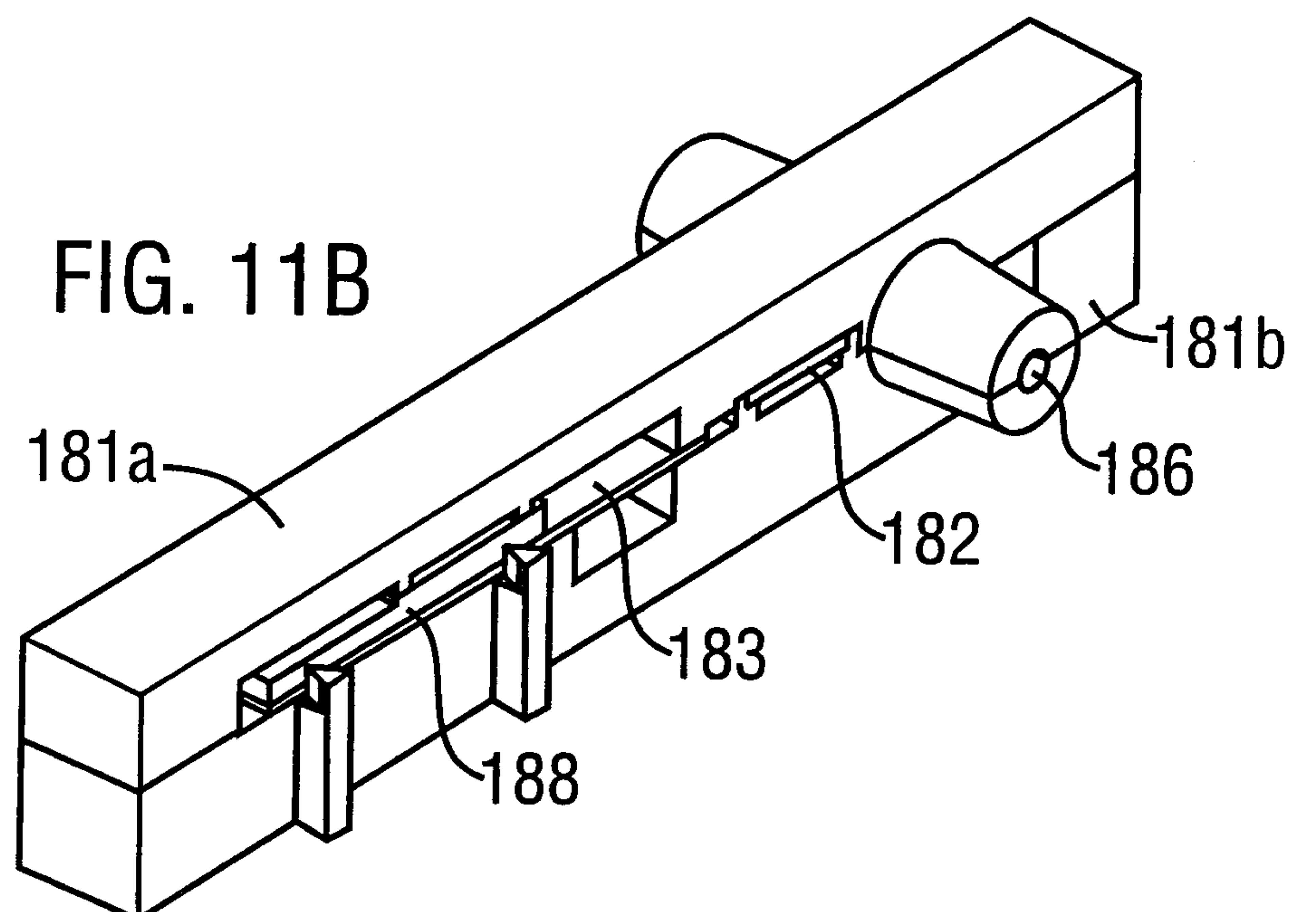
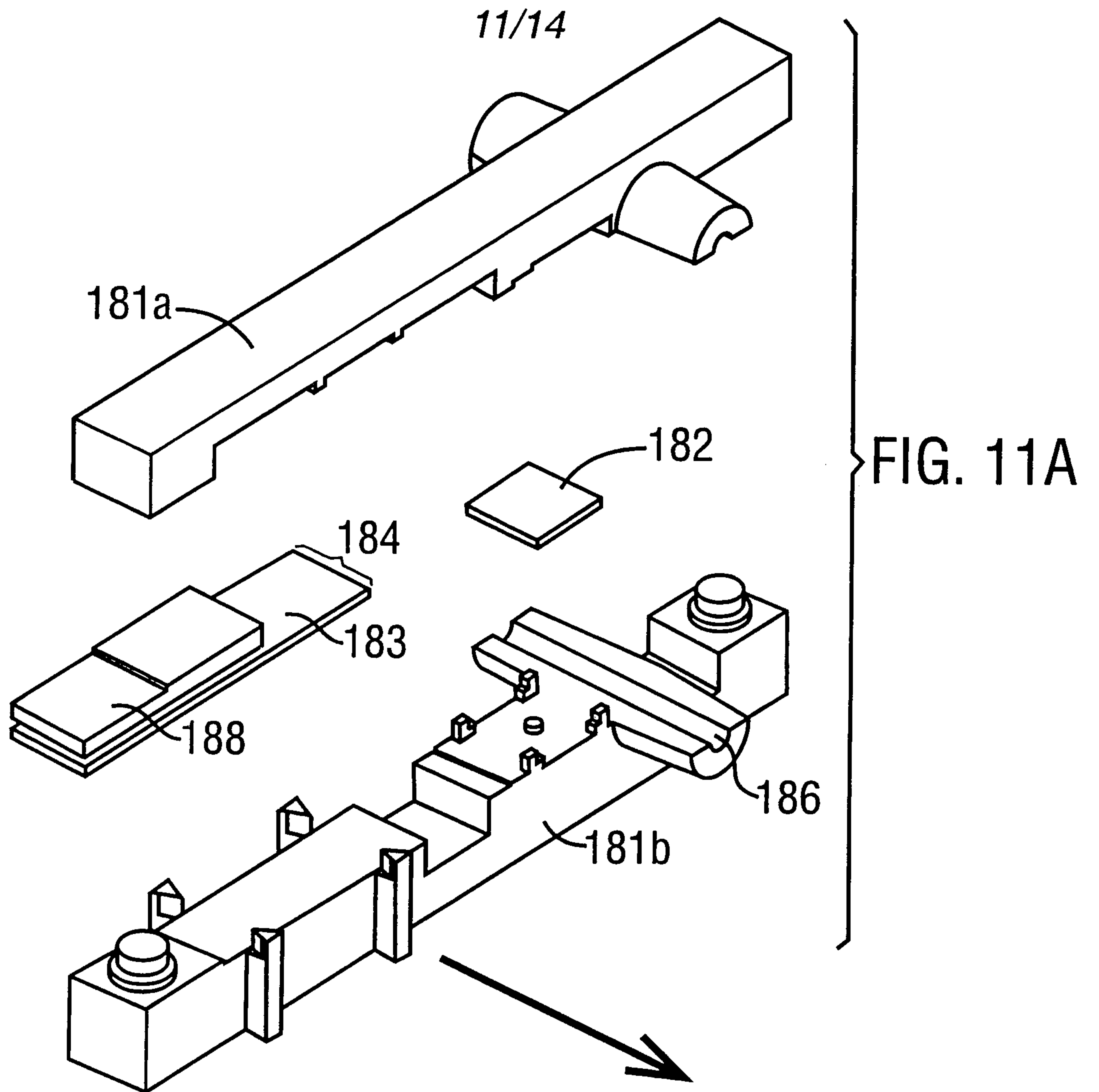
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FIG. 10





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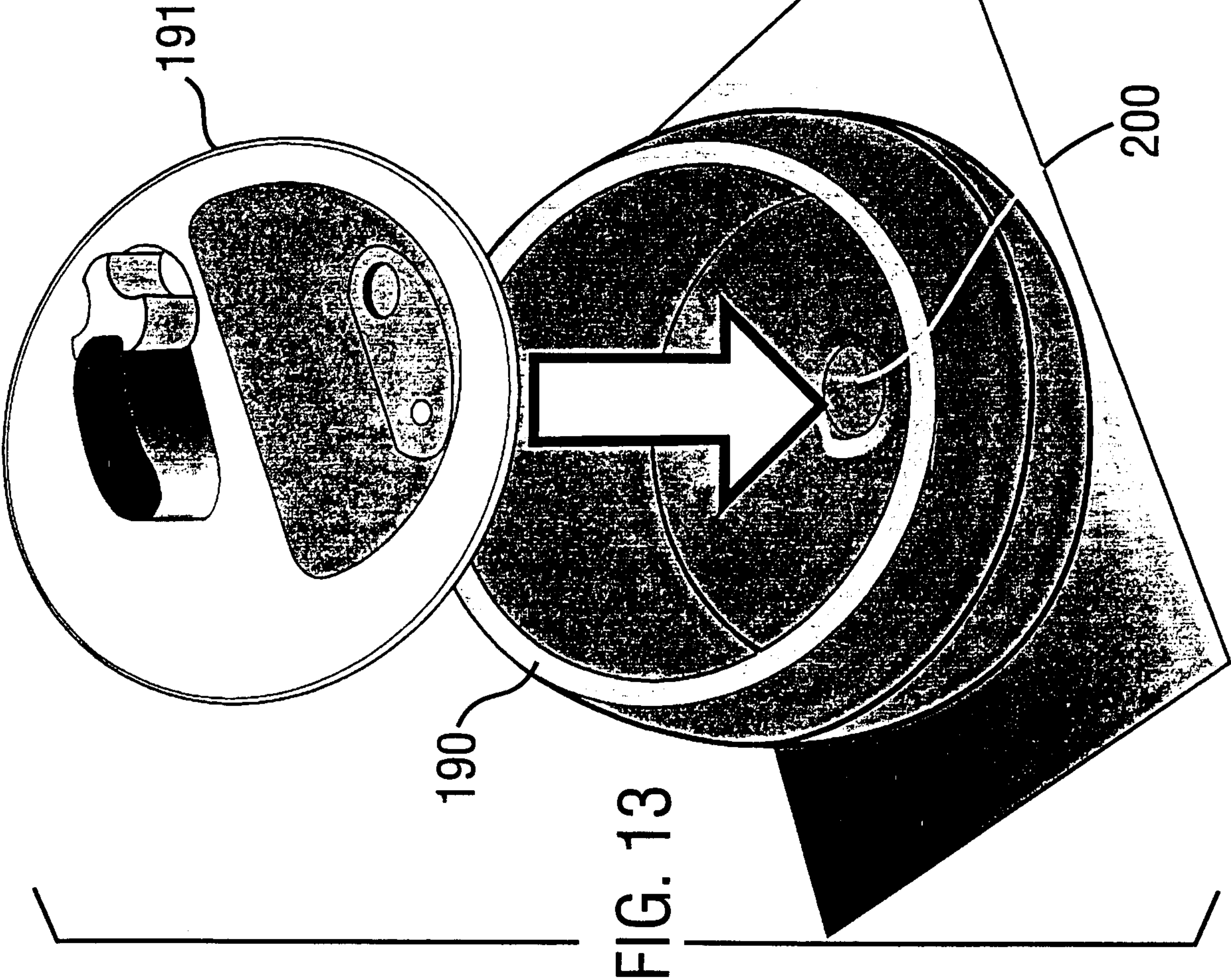


FIG. 13

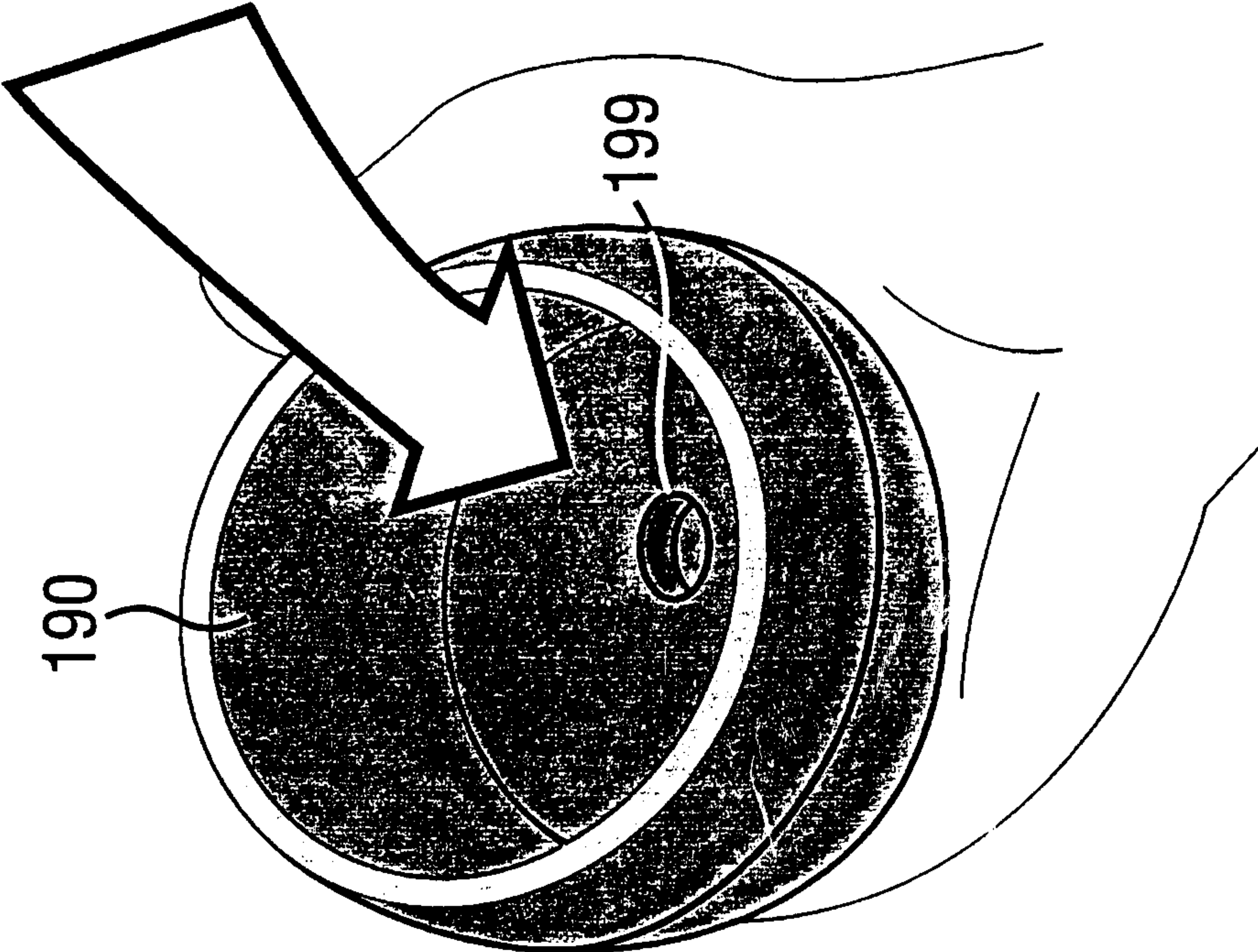


FIG. 12

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FIG. 14

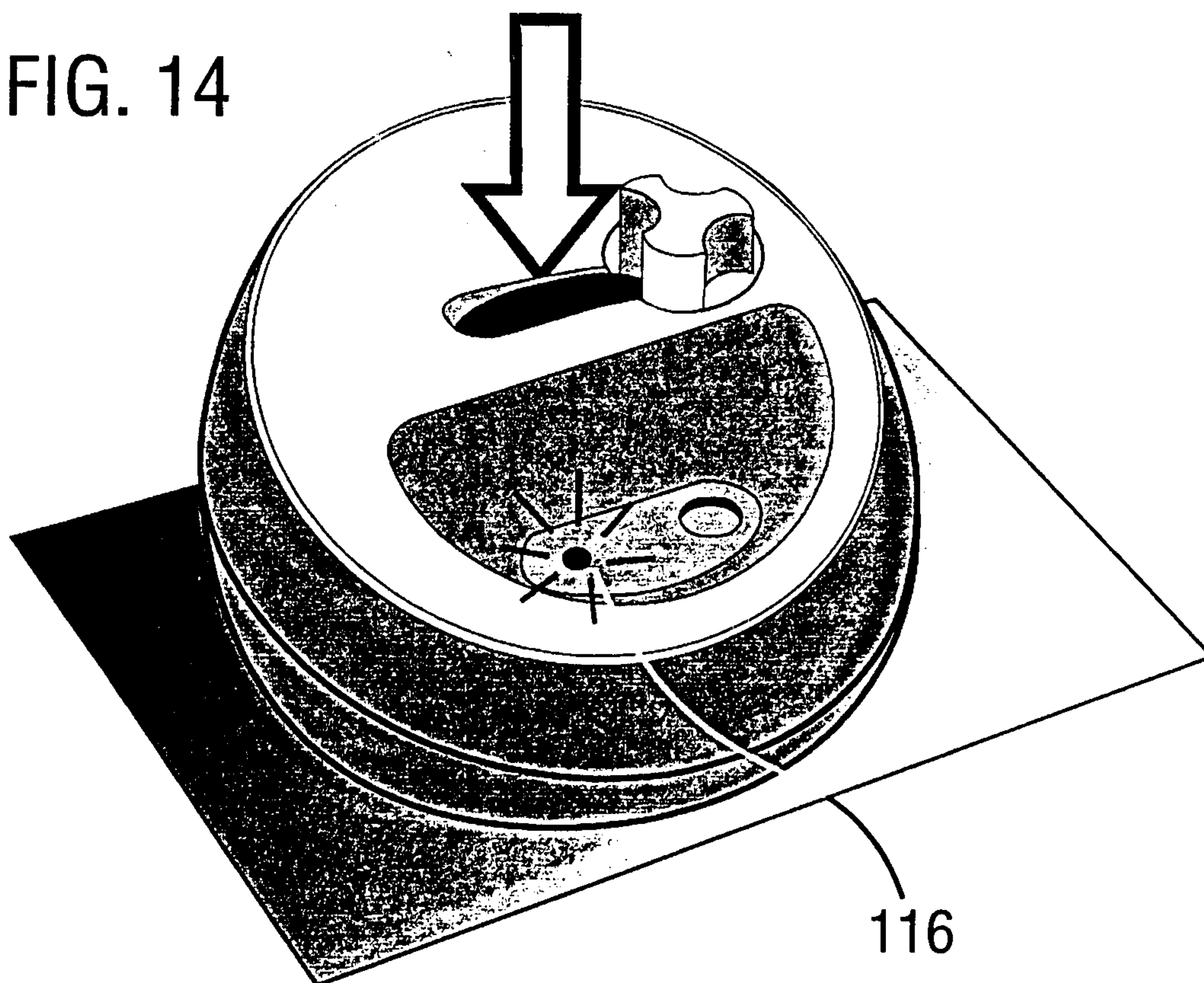
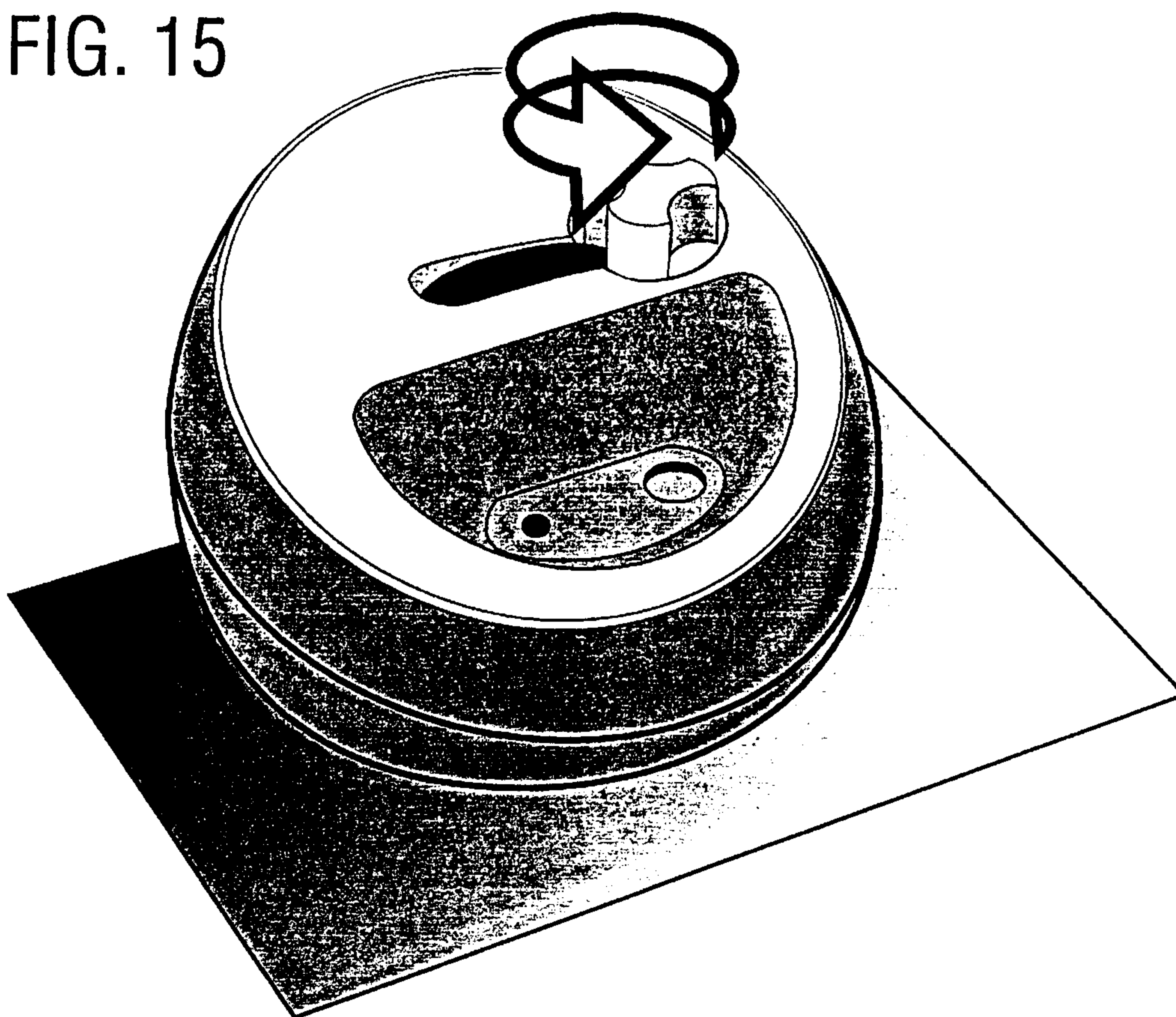


FIG. 15



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FIG. 16

