(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 November 2006 (23.11.2006)

PCT

(10) International Publication Number
WO 2006/123949 A2

(51) International Patent Classification: Not classified

(21) International Application Number: PCT/NZ2006/000116

(22) International Filing Date: 16 May 2006 (16.05.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
540060 16 May 2005 (16.05.2005) NZ

(71) Applicant (*for all designated States except US*): **LAB-TEK INTERNATIONAL LIMITED** [NZ/NZ]; 26 Ti Rakau Drive, Brookhaven, Christchurch (NZ).

(72) Inventor; and
(75) Inventor/Applicant (*for US only*): **SMITH, Stewart** [NZ/NZ]; 56 Dunster Street, Christchurch (NZ).

(74) Agents: **CABLE, Andrew, J.** et al.; Level 11, PricewaterhouseCoopers Centre, 119 Armagh Street, PO Box 2201, Christchurch (NZ).

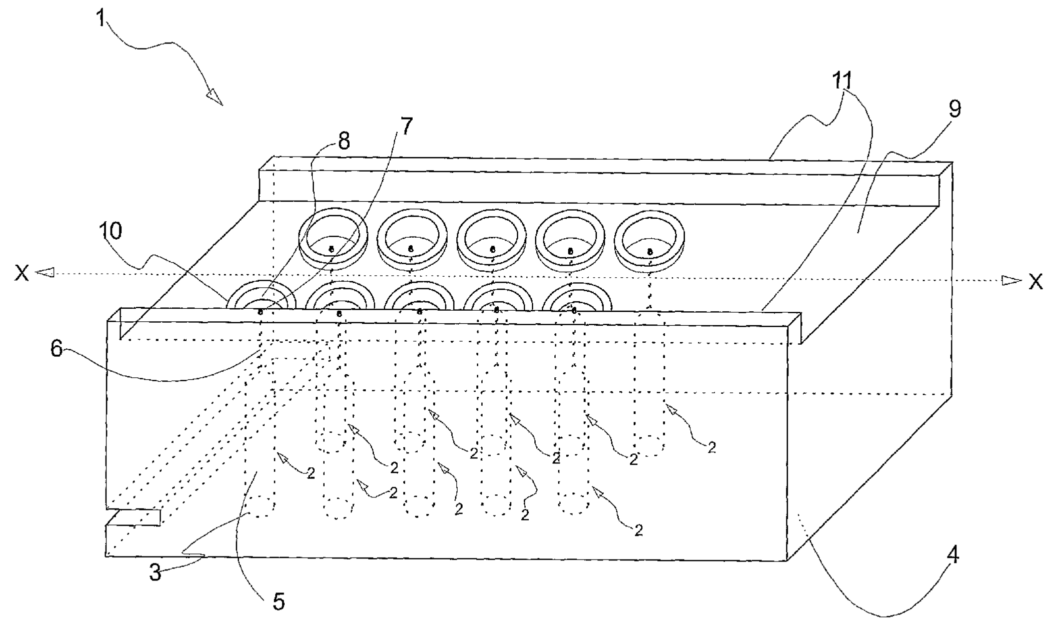
(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: SAMPLE HOLDER



(57) Abstract: A sample holder (1) for use in centrifugation of a sample fluid, the sample holder (1) including; at least one fluid passageway (2) extending through the sample holder (1) between an inlet (3) on an inlet face (4) and an outlet (8) on an outlet face (9), the fluid passageway (2) including a sample reservoir (5), characterised in that the fluid (2) passageway further includes a constricted portion (6) extending from the sample reservoir (5) to the outlet (8) on the outlet face (9).

WO 2006/123949 A2

TITLE: Sample Holder

TECHNICAL FIELD

The present invention relates to a sample holder, in particular it relates to a sample holder for the projection of cellular samples onto sample receiving devices via centrifugation.

BACKGROUND ART

Cytocentrifuges are used to isolate and flatten biological cell samples from fluids having such samples suspended therein. A sample fluid such as blood, hypocellular fluids and similar fluids containing cell specimens to be studied is placed in a sample mounting device or chamber.

During centrifugation the samples are subject to centrifugal force and are projected onto a microscope slide. As the centrifuge continues to spin the samples are flattened against the microscope slide and form a layer, ideally a single layer of cells thick on the microscope slide. The majority of the fluid is absorbed by filter paper placed around the sample area on the microscope slide.

After the centrifuge is stopped and the microscope slide is removed, reagents are used to stain the sample, whereby different reagents reveal different structures of the sample under analysis via a microscope. Such a method is used in the study of anti-Neutrophil Cytoplasmic antibodies, cervical screening and other processes that require a deposit of cells on a microscope slide in a single layer in order to effectively analyse.

Known prior art includes US patent 5,252,228, describing a device consisting of a sample chamber, slide and filter paper assembly placed inside a centrifuge. The sample fluid is placed in the sample chamber, exits the chamber during centrifugation and passes along a conduit to the filter paper and slide. While this device achieves the desired result it is limited to processing two samples per centrifugation, which means extended time to process a greater number of samples or requiring many such devices within the centrifuge which is limited by the size of the centrifuge and the power of the centrifuge. Also, the sample must be carefully placed in the device within a given orientation range to avoid spillage.

Further prior art devices include the Shandon Octospot ®, produced by Thermo Shandon Inc, which is capable of processing multiple samples. The Octospot ® consists of a mounting device with eight wells, a sheet of filter paper with eight apertures, a microscope slide, a clamping plate and a clamping mechanism for holding the clamping plate tight against the mounting device, having the filter paper and sample receiving device interleaved between. The Shandon device is placed horizontally in the centrifuge with the wells facing upwards to prevent the sample fluids leaking from the wells pre-centrifugation.

The centrifuge is then spun causing the fluid to be expelled from the wells onto the microscope slide. Thus, while capable of producing suitable sample slides the Shandon device poses difficulties in use as the device must be maintained horizontally throughout assembly to prevent the sample fluid leaking out. A further disadvantage of this device is absence of any sample well supporting structure. The wells are consequentially fragile necessitating extra care when preparing the device.

It is an object of the present invention to address the foregoing problems or at least to provide the public with a useful choice.

All references, including any patents or patent applications cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein; this reference does not constitute an admission that any of these documents form part of the common general knowledge in the art, in New Zealand or in any other country.

It is acknowledged that the term 'comprise' may, under varying jurisdictions, be attributed with either an exclusive or an inclusive meaning. For the purpose of this specification, and unless otherwise noted, the term 'comprise' shall have an inclusive meaning - i.e. that it will be taken to mean an inclusion of not only the listed components it directly references, but also other non-specified components or elements. This rationale will also be used when the term 'comprised' or 'including' is used in relation to one or more steps in a method or process.

Further aspects and advantages of the present invention will become apparent from the ensuing description which is given by way of example only.

DISCLOSURE OF INVENTION

According to one aspect of the present invention there is provided a sample holder for use in centrifugation of a sample fluid, said sample holder including;

at least one fluid passageway extending through the sample holder between an inlet on an inlet face and an outlet on an outlet face, said fluid passageway including a sample reservoir,

characterised in that

said fluid passageway further includes a constricted portion extending from said sample reservoir to the outlet on said outlet face.

As used herein, the term 'sample fluid' includes, but is not restricted to, fluids containing particulates, biological cells or biological cell components contained therein.

Preferably, the constricted portion is formed as a capillary tube. However, alternative configurations may be employed without departing from the scope of invention.

The key function of the constricted portion is to prevent the egress of the sample in the sample reservoir through the fluid passageway outlet solely under the force of gravity. Consequently, the degree of constriction required will be dependant on the characteristics of the sample, including viscosity, particulate constituents, and the like. The degree of constriction or impediment to fluid flow through the constricted portion may be varied by corresponding variations in the length, or cross-sectional size and shape and/or any combination of same. In the interests of clarity however, the constricted portion is hereon described with respect to a capillary tube embodiment, though as discussed above, this is for exemplary purposes only. Furthermore, although a plurality of fluid passageways may be formed through a sample holder providing means to centrifuge multiple samples simultaneously, for the sake of clarity the description herein refers to the features of a fluid passageway and associated interaction with other elements of a sample holder in the singular. It will be readily appreciated that not only may multiple fluid passageways be utilised, but that they need not all be identical in a common sample holder.

Thus, sample fluid placed in the sample reservoir via the inlet, may pass to the outlet face via capillary action, through the capillary tube from the fluid reservoir. However, in the case of samples having a high particulate content, unaided movement of the sample fluid along the capillary tube may be restricted. This further enhances the sample holder's ability to retain the sample fluid while being handled prior to centrifugation, reducing the risk of spillage, or cross-contamination.

In use, the sample holder is typically used in conjunction with a sample slide and filter layer held in contact with the outlet face by a retaining means.

The present invention thus provides a sample holder assembly including

- a sample holder substantially as hereinbefore described,
- a retaining means adapted to releasably locate a filter layer and a sample slide against the outlet face,

said filter being interposed between the sample holder outlet face and the sample slide.

The sample fluid is inserted into the fluid passageway with the fluid inlet orientated upwards and retained in the sample reservoir before placement of the sample holder in a centrifuge with the fluid outlet facing outwardly from the axis of rotation. When the centrifuge reaches a predetermined rotational speed, the centrifugal effects on the sample fluid by the centripetal force overcomes the combined effects of the sample fluid's surface tension and the adhesion with the inner walls of the fluid passageway.

In the absence of any form of restraint, releasing the centripetal force would cause the fluid sample to move tangentially to direction of rotation. However the constraints of the fluid passageway walls cause the sample fluid to move outwards from the center of rotation along the capillary tube to the fluid outlet on the outlet face. At the outlet the fluid sample detaches from the sample holder and impacts the sample slide.

The sample slide is typically a transparent microscope slide (e.g. glass) with an opaque overlay or mask to delineate deposition regions on the sample slide configured to align with the respective fluid outlets to receive the centrifuged sample. It will be appreciated however, that a variety of sample slide types may be utilised, provided they may be detached from the sample holder to enable inspection of the centrifuged sample(s) located on their surface. Thus, the sample slide may be opaque, translucent, or transparent; a planar plate, block, disc; rigid or flexible, demarcated into regions (including the deposition regions) or unmarked and so forth.

Prior to microscope analysis, reagents are typically placed on the sample deposited on the deposition regions. To prevent cross contamination between multiple samples deposited on a single sample slide, a solvent-resistant barrier may be applied around each deposition region to aid retention of the reagent and sample in the deposition region.

In preferred embodiments said sample slide is coated with a solvent resistant material (e.g. PTFE, Teflon™) surrounding the deposition regions.

To ensure reliable sample collection, the sample slide requires accurate and repeatable positioning to ensure the deposition regions are aligned with the fluid passageway outlets. Conventional glass microscope slide are simple rectangular plates with no apertures. Consequently, such slides may be retained in the desired position by location means in the form of one or more raised portions or flanges formed on the outlet face positioned to abut the sample slide perimeter when positioned in the correct position. Non-conventional slides may be specifically adapted for secure placement on the sample holder by a number of different techniques, e.g., apertures to accept locating pins or projections located on the outlet face or sample slide.

Preferably, said capillary tube fluid outlet is formed within a well on the outlet face. Recessing the capillary tube outlet in a well permit an advantageous outlet configuration to be utilised positioned without being brought into direct contact with the sample slide. Numerous forms of well configuration may be utilised including cylindrical, ellipsoidal, parabolic or any other suitable configuration in which the fluid outlet is recessed below the outlet face. Preferably, the well is cylindrical with first and second opposing circular ends, said fluid outlet located substantially centrally at a first end, said second end forming an aperture in the outlet face.

In one embodiment, the fluid outlet is formed as a protrusion. The protrusion protrudes from the well surface adjacent the outlet to increase the tendency of sample fluid exiting the outlet to form a droplet.

Fluid is drawn in to a capillary tube if:

a) the adhesive force between the fluid and capillary walls is greater than the cohesive forces between fluid molecules; and

b) the adhesion is greater than the weight of the fluid to be drawn into the capillary tube.

Thus, depending on the above described characteristics, sample fluid may not be drawn, or be partially drawn, or fully drawn along the capillary tube. However, irrespective of the degree of ingress of the sample fluid into the capillary tube, the additional force provided by the centrifuge provides sufficient impetus to expel the fluid onto the sample slide.

In a preferred embodiment, after sample fluid is placed in the sample reservoir with the fluid inlet orientated upwards, the adhesive forces between the fluid and walls of the capillary tube are greater than the gravitational force of the fluid, thus preventing the fluid sample exiting the capillary tube outlet.

When the sample holder is subjected to an increasing rotational speed in a centrifuge, at a predetermined rotational speed (at least partially governed by the aforesaid fluid characteristics including viscosity and particulate content) the sample fluid will start to exit the fluid outlet. The cohesive forces between fluid molecules act to minimise the surface tension of the fluid, thus (according to Laplace's law which states *"The larger the vessel radius, the larger the wall* [surface] *tension required to withstand a given internal fluid pressure"*) acting to force the fluid into a substantially spherical droplet shape. In embodiments where the fluid outlet is not formed as a projection (e.g. the outlet is flush with the surrounding surface of the well), there would be an increased tendency of the sample fluid egressing the outlet to radially spread across the well surface. Such seepage of the sample fluid over the well surface causes variability in the size and type of any drop eventually formed or, in the alternative potentially causing the fluid to travel along the well surface until contacting the filter layer and/or sample slide. Such an uncontrolled release of the sample fluid hinders the effective placement of the sample particulate constituents on the sample slide for analysis.

The degree of adhesive force between a sample fluid droplet formed at a fluid outlet protrusion is governed by the properties of the sample fluid and the configuration of the protrusion. Higher viscosity fluids generate higher cohesion between molecules causing greater surface tension and a lower adhesive force with the protrusion surface. The protrusion aids the formation of a sample droplet due to the increased angular separation formed between the sample fluid and surface of the projection.

Theoretically, a steep (near vertical) protrusion of infinitesimal wall thickness at the fluid outlet would provide the optimum droplet-forming protrusion configuration. In practice, such a configuration would lack structural integrity, be vulnerable to damage during cleaning/handling and/or be unduly exacting to manufacture. Consequently, practical protrusion configurations may be regarded as having a finite wall thickness about the fluid outlet and some form of sidewall configuration inclined between the fluid outlet and the well surface adjacent the protrusion.

Decreasing the size and/or increasing the smoothness of the wall thickness at protrusion fluid outlet reduce the adhesion of the sample droplet to the protrusion and vice versa. Inclining the sides of the protrusion away from the fluid outlet increases the distance between the surface of the fluid droplet and the protrusion surface, further decreasing the adhesive force.

Thus it should be appreciated that the configuration and manufacturing material used to form the protrusion and capillary tube may be selected according to the viscosity and adhesive properties of a specific sample fluid, e.g. blood.

In a preferred embodiment the sample holder is constructed from Perspex, though glass, plastic, steel or any other suitable materials may also be employed.

According to a preferred embodiment, said protrusion includes a sidewall, inclined away from the fluid outlet towards from the well surface adjacent the protrusion. According to one aspect, said sidewall projects from the well surface adjacent the protrusion at an inclination angle between 0-90° (non-inclusive) and preferably between 45 - 90°.

In a preferred embodiment the projection configuration is conical or frustro-conical.

The inclination angle of the sidewalls may also be chosen to provide a sufficiently wide projection base to withstand cleaning of the sample holder between samples.

Preferably the sample holder may be constructed from appropriately solvent resistant materials in the event corrosive cleaning solvents are employed.

As previously described, the fluid sample droplet separates from the fluid outlet when the centrifuge reaches a predetermined rotational speed. After impacting the deposition region of the sample side, the sample fluid spreads outwards across the sample slide. The centripetal force applied by the sample slide on the deposited sample fluid not only causes the spreading of the sample fluid, but may also cause the particulates suspended in fluid to be at least partially flattened against the slide. Moreover, the centripetal force acts to distribute the particulates into a single layer across the deposition region, which is particularly desirable for subsequent microscope analysis.

The rate at which the sample fluid spreads across the deposition area governs the concentration of particulates deposited per unit area. Consequently, in some embodiments the rate of dispersion requires controlling to maintain high particulate concentrations in the deposition regions.

Thus, according to a further aspect of the present invention there is provided a choke about at least one fluid outlet, preferably located on the outlet face about a perimeter of at least one well. The choke provides a means of restricting the rate of sample fluid dispersion from the deposition region after impacting the sample slide.

Preferably the choke is formed as a raised perimeter or flange portion about at least part of the outlet well on the outlet face. Preferably, the filter layer is provided with apertures substantially coterminous with the deposition regions. The choke preferably presses on the filter layer interposed between the sample holder and the sample slide thus restricting the egress of fluid from the deposition region to pass through the filter layer. Thus, the filter layer not only prevents the cross contamination of sample fluids between different deposition regions, it also slows the release of sample fluid liquid from the deposition region thus increasing the concentration of deposited particulates. Continued centrifugation allows the absorbent filter layer to absorb the liquid from the sample fluid leaving the particulates on the deposition region.

The use of the choke regulates the rate of liquid absorption and constrains the liquid path to optimize the deposition of particulates to a single layer.

It will be apparent the sample slide is preferably held firmly against the choke/filter layer to ensure the filter layer correctly absorbs the sample liquid and to avoid being dislodged during handling and/or centrifugation. It is also desirable that pressure is applied to the sample slide uniformly, to avoid possible damage to fragile sample slides.

According to a further embodiment of the present invention, the sample holder assembly retaining means includes a pressure plate and a clamping mechanism. Preferably, said pressure plate is adapted to be releasably engaged into forced contact with the sample slide by said clamping mechanism.

Preferably said pressure plate is a section of Perspex, glass, hardened plastic, steel or other substantially rigid materials or structure.

According to another aspect of the present invention the sample holder and the pressure plate are each provided with a cooperating portion of a male and female location means, wherein said male portion includes a projection, and said female portion includes a corresponding recess. Preferably, the filter layer includes a locating aperture to accommodate the insertion of the male projection. Optionally, the sample slide also includes a locating aperture capable of accommodating said male projection. The sample holder assembly may thus be assembled by inserting the male projection through locating apertures in the filter layer (and optionally the sample slide) before insertion into the female portion recess, whereupon the clamping mechanism is engaged to hold sample holder assembly components together.

The location means also ensures the correct positioning of the sample slide and filter layer in alignment with the fluid passageway chokes.

In alternative embodiment said location means includes said one or more raised portions or flanges formed on the outlet face, substantially as described above.

The clamping mechanism may be formed from any convenient means capable of releasably attaching the sample holder assembly components together under pressure, including clamps, springs, latches, straps, clips, eccentric cams, threaded fixtures/ levers and the like.

In one embodiment the clamping means includes an elongate flexible rod pivotally fixed at a first distal end and detachably fixable at a second distal end of one of either the sample holder or the pressure plate, the rod being configured to extend from the pivotal fixing about a protrusion located on the other of the sample holder or the pressure plate and thereafter being releasably latched at said second distal end.

Thus, a clamping mechanism pivotally attached to the sample holder (for example) may thus be pivoted aside while the filter layer and pressure plate are placed on the outlet face of the sample holder, whereupon the clamping means is pivoted until the rod bends around a protrusion on the pressure plate before being latched to the sample holder. The clamping mechanism preferably extends symmetrically about opposing sides of the sample holder and engages symmetrical protrusions located on opposing sides of the pressure plate. In one embodiment, an elongated pin extending through the pressure plate forms said symmetrical protrusions.

Thus, in operation said clamping mechanism positioned about said protrusion and latched to secure the sample holder assembly components together provides a compressive force between the sampled holder, filter layer and pressure plate in a direction substantially orthogonal to the plane of the sample slide.

In a further embodiment, the clamping mechanism is releasably latchable by laterally displacing engaging portions of the clamping mechanism orthogonally to the plane of said compressive force

It has been found that sample fluid placed in the sample reservoir with an inlet aperture of approximately 6 mm or less will not drain from the sample holder during normal handling (i.e. excluding sudden or violent accelerations/decelerations) even if inverted. However, to provide further security from possible leakage and/or if larger diameter inlet apertures are utilised, the present invention may be further provided with a removable cover releasably fixable over the fluid inlets of each fluid passageway.

In preferred embodiments when the sample holder is placed in a centrifuge the inlet is perpendicular to gravity and consequently sample fluid may drain from fluid inlets greater than a predetermined diameter. Sample holders formed with multiple fluid passageways risk cross-contamination from any such sample fluids draining down the inlet face. Thus the cover prevents sample fluid from exiting the inlet, preventing cross-contamination.

In some embodiments there is provided a recessed portion of the inlet face about the at least one inlet.

Preferably said cover is placed in intimate contact with the recessed portion of the inlet face. Preferably, said cover is fitted flush with the inlet face.

To enable the sample fluid to flow though the fluid passageway and exit via the fluid outlet during centrifugation, the cover cannot form a fluid-tight seal over the fluid inlets. Consequently, the inlets are provided with a fluid channel connecting the fluid inlets to a non-sealed inlet positioned to remain uppermost when the sample holder is rotated into position in the centrifuge with the fluid outlet face substantially vertical.

The channel may thus allow passage of atmospheric air whilst the sample fluid is being expelled from the sample holder during centrifugation or alternatively be used for the passage of cleaning fluid between samples.

According to one aspect of the present invention there is provided a method of preparing a sample for analysis using a sample holder assembly as hereinbefore described including the steps;

- placing a filter layer on the outlet face of the sample holder
- placing the sample slide on the filter layer,
- placing the pressure plate on the sample slide,
- latching the clamping mechanism to apply a compressive force between the sample holder and pressure plate,
- placing a sample fluid in the fluid reservoir,
- placing the sample holder assembly in a centrifuge; and
- operating the centrifuge to rotate at a predetermined rotational speed,

wherein sample fluid travels through said restricted portion to form a droplet on said projection until separating from the sample holder and impacting the deposition region on the sample slide.

Preferably, said method further includes the step of:

- restricting the flow of sample fluid liquid from the deposition region by constraining the available liquid path through said filter layer compressed between said choke and the perimeter of the deposition region on the sample slide.

Thus, it can be seen that the present invention provides a sample holder offering many advantages over the current state of the art.

- It provides a single layer of sample on the sample slide, therefore allowing effective and efficient analysis.
- The sample fluid remains in the capillary tube pre-centrifugation, regardless of the orientation of the sample holder, therefore reducing the risk of the sample fluid spilling and reducing the level of care required in utilising the device compared with the prior art.
- The choking means controls the speed of exit of the fluid from the deposition region and so controls the level of concentration of sample components within the deposition region.
- The sample holder and components therein are of robust construction and so offer greater durability than the prior art.

Aspects of the present invention have been described by way of example only and it should be appreciated that modifications and additions may be made thereto without departing from the scope thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Further aspects of the present invention will become apparent from the following description which is given by way of example only and with reference to the accompanying drawings in which:

Figure 1. shows a side perspective view of a sample holder according to a preferred embodiment;

Figure 2. shows a perspective view of the choke, projection and fluid outlet;

Figure 3. shows a plan view of the filter layer in a preferred embodiment.

Figure 4. shows an exploded assembly diagram of a sample holder assembly according to a preferred embodiment.

Figure 5. shows a side cross section of an assembled sample holder assembly according to a preferred embodiment.

Figure 6. shows the clamping mechanism in a latched and unlatched configuration.

Figure 7. shows a preferred embodiment of the clamping mechanism.

Figure 8a shows a plan view of the inlet face, recess, the channels and cover according to preferred embodiments.

Figure 8b shows an end view of the recess on the inlet face, the channels and cover according to preferred embodiments.

BEST MODES FOR CARRYING OUT THE INVENTION

With respect to Figures 1-8 there is provided a representation of the present invention in the form of a sample holder (1).

The sample holder (1) has a plurality of fluid passageways (2), each having an inlet (3) on the inlet face (4) connected to a sample reservoir (5) in which the sample fluid volume is placed. The sample reservoir (5) is connected to a constricted portion in the form of a capillary tube (6) that extends through the sample holder (1) to a fluid outlet formed as a protrusion (7) located within an outlet well (8) on the outlet face (9). A flanged periphery of the outlet well (8) forms a choke (10) configured to compress the filter layer (14) and provide a restrictive barrier to the flow of liquid from the sample fluid.

Locating means are provided by both a projection in the form of guiding pin (37) (shown in figure 4) and the flanged longitudinal edges (11) of the sample holder (1).

Figure 2 shows an enlarged view of the outlet well (8). The capillary tube (6) extends outwards from the surface of the well (8) to form a protrusion (7). Under centrifugation any sample fluid contained in the capillary tube (6) is forced from the protrusion (7) to form a droplet which detaches from the protrusion (7) at a predetermined rotational speed.

The angle of inclination (12) of the outer surface of the protrusion (7) determines the degree of contact between the droplet and the protrusion (7) and thus the extent of the droplet's adhesion to the protrusion (7).

Figure 3-5 show a filter layer (14) with a guiding hole (15) through which the guiding pin (37) may pass, together with ten sample inlet (16), each aligned with an outlet well

(8), when the filter layer (14) is guided into position on the sample holder by the guiding means in the form of flanged portions (11) and a guiding pin (37).

Figure 4 shows a cross section along the axis X-X of a sample holder assembly indicated by arrow (24) including the sample holder (1), filter layer (14) with guiding hole (15), sample slide (19) with ten deposition regions (20), the pressure plate (17) with loading pin (18) and guiding hole (13), guided by the guiding pin (37) and flanged longitudinal edges (11) of the sample holder (1) into a position so that each deposition region (20) on the sample slide (19) and sample hole (16) in the absorbent material align with an outlet well (8) on the sample holder (1).

Figure 5 shows a sample holder assembly as indicated by arrow (24) including the sample holder (1) with the filter layer (14), sample slide (19) and pressure plate (17) guided into place on the outlet face (9) by the guiding pin (37) and the flanged longitudinal edges (11).

Figure 6 shows a clamping mechanism indicated by arrow (21) in an unlatched configuration (solid lines) and the clamping mechanism (21') in a latched configuration (dotted lines). The attaching portion (23) is pivotally attached to a recess (25) at a first distal end of the sample holder (1), wherein arcuate portions (22, 22') are then extended over a projection in the form of a loading pin (18) and the latching portions (23, 23') latched to the sample holder at a second distal end. This presses the pressure plate (17) against the sample holder (1) with sample slide (19) and filter layer (14) interleaved therebetween. Thus the flanged periphery (10) compresses the filter layer (14) and therefore restricts absorption of the sample fluid by the filter layer (14). The clamping mechanism in the latched position (21') can be unlatched by laterally increasing the separation between the latching portions (23') as indicated by arrows (26', 27') by squeezing the tabs (28) together as indicated by arrows (26, 27), thus unlatching the unlatching portions (23') from the sample holder (1).

Figure 7 shows two sample holder assemblies (24, 24') placed on opposing sides of a centrifuge (29) to ensure balance. Spring loaded clips (30) are utilized to retain the sample holder assemblies (24, 24') in an upright position, perpendicular to the direction of centripetal acceleration (31) as indicated by arrow (32). The central axis of the centrifuge (26) is shown by axis Y-Y.

Figure 8a shows a plan view of the inlet face (4) of the sample holder (1), the cover (34) and air holes (36). Figure 8b shows an end view of the channels (33) on the inlet face (4), of the sample holder (1), the channels (33) facilitate the entry of air into the inlet (3), and reservoir (5) and thus prevent a vacuum from forming in the reservoir (5) when sample fluid is expelled from the fluid outlet onto the sample slide (19). The cover (34) is placed in the recessed portion (35) of the inlet face (4) thereby preventing sample fluid contained in the reservoir (5) from exiting via the inlet (3).

In operation, each sample fluid volume is placed in the separate inlets (3) of the sample holder (1). The clamping mechanism (21') is applied as shown in Figure 6 to the sample holder assembly assembled as shown in Figure 5. The cover (34) is then placed on the recessed portion of the inlet face (4) as shown in figure 8 to prevent sample fluid from exiting the reservoir via the inlet (3). The sample holder assembly is then placed in a centrifuge (27) as shown in Figure 7 and rotated at said predetermined rotation speed, causing the sample fluid to exit the capillary tube (6) at said predetermined rotation speed and thereupon impact with the sample slide (19). The impact and centrifugation causes the sample fluid constituent particulates within the sample fluid to be at least partially flattened and spread to form a single layer on the sample slide (19). The liquid from the sample fluid is absorbed by the filter layer (14). The clamping mechanism is then unlatched as shown in Figure 6, the filter layer (14) removed discarded and the sample slide (19) removed for analysis.

Claims:

1. A sample holder for use in centrifugation of a sample fluid, said sample holder including;

   at least one fluid passageway extending through the sample holder between an inlet on an inlet face and an outlet on an outlet face, said fluid passageway including a sample reservoir,

   characterised in that

   said fluid passageway further includes a constricted portion extending from said sample reservoir to the outlet on said outlet face.

2. The sample holder as claimed in claim 1, wherein the constricted portion is formed as a capillary tube.

3. The sample holder as claimed in claim 1 or claim 2, wherein the constricted portion prevents the egress of the sample in the sample reservoir through the fluid passageway outlet solely under the force of gravity.

4. The sample holder as claimed in claim 1 or claim 2, wherein the degree of constriction to fluid flow through the constricted portion is varied by variations in the length, cross-sectional size, shape and/or any combination of same.

5. The sample holder as claimed in any one of claims 1 - 4, wherein said outlet face includes location means to locate a sample slide in contact, or immediately adjacent with said outlet face.

6. The sample holder as claimed in claim 5, wherein said location means includes one or more raised portions or flanges formed on the outlet face positioned to abut the sample slide perimeter when correctly positioned.

7. The sample holder as claimed in claim 5, wherein said location means includes one or more locating pin(s) or projections located on the outlet face or sample slide.

8. The sample holder as claimed in any one of claims 2 - 7, wherein said capillary tube fluid outlet is formed within a well on the outlet face.

9. The sample holder as claimed in claim 8, wherein said well is a cylindrical, ellipsoidal, or parabolic configuration with the fluid outlet recessed below the outlet face.

10. The sample holder as claimed in claim 9, wherein the well is cylindrical with first and second opposing circular ends, said fluid outlet located substantially centrally at a first end, said second end forming an aperture in the outlet face.

11. The sample holder as claimed in any one of claims 1-10, wherein the fluid outlet is formed as a protrusion.

12. The sample holder as claimed in any one of claims 8-10, wherein the fluid outlet is formed as a protrusion protruding from the well surface adjacent the outlet.

13. The sample holder as claimed in claim 12, wherein said protrusion includes a side wall, inclined away from the fluid outlet towards the well surface adjacent the protrusion.

14. The sample holder as claimed in claim 13, wherein said sidewall projects from the well surface adjacent the protrusion at an inclination angle between 0-90° (non-inclusive)

15. The sample holder as claimed in claim 14, wherein said inclination is between 45 - 90°.

16. The sample holder as claimed in any one of claims 11-15, wherein the projection configuration is conical or frustro-conical.

17. The sample holder as claimed in any one of claims 1-16, further including a choke about at least one fluid outlet.

18. The sample holder as claimed in claim 17, wherein, said choke is located on the outlet face about a perimeter of at least one well.

19. The sample holder as claimed in any one of claims 17-18, wherein the choke is formed as a raised perimeter or flange portion about at least part of the outlet well on the outlet face.

20. A sample holder assembly including:

- a sample holder as claimed in any one of claims 1-19,
- a retaining means adapted to releasably locate a filter layer and a sample slide against the outlet face, said filter being interposed between the sample holder outlet face and the sample slide.

21. A sample holder assembly as claimed in claim 20, further including a sample slide with one or more deposition regions.

22. A sample holder assembly as claimed in claim 21, wherein said the filter layer is provided with apertures capable of alignment substantially coterminous with the deposition regions.

23. A sample holder assembly as claimed in claim 21, wherein at least one said filter layer aperture are capable of alignment substantially coterminous with a choke formed as a raised perimeter or flange portion about at least part of at least one fluid on the outlet face.

24. A sample holder assembly as claimed in any one of claims 20 - 23, wherein said retaining means includes a pressure plate and a clamping mechanism, said pressure plate being adapted to be releasably engaged into forced contact with the sample slide by said clamping mechanism.

25. A sample holder assembly as claimed in claim 24, wherein said sample holder and the pressure plate are each provided with a cooperating portion of a male and female location means, wherein said male portion includes a projection, and said female portion includes a corresponding recess.

26. A sample holder assembly as claimed in claim 25, wherein said filter layer includes a locating aperture to accommodate the insertion of the male projection.

27. A sample holder assembly as claimed in claim 25, wherein said sample slide includes a locating aperture capable of accommodating said male projection.

28. A sample holder assembly as claimed in any one of claims 24 - 27, wherein the clamping mechanism includes at least one of the group including: clamps; springs; latches; straps; clips; eccentric cams, threaded fixtures/ levers and/or any combination or permutation of same.

29. A sample holder assembly as claimed in any one of claims 24 - 27, wherein said clamping means includes an elongate flexible rod pivotally fixed at a first distal end and detachably fixable at a second distal end of one of either the sample holder or the pressure plate, the rod being configured to extend from the pivotal fixing about a protrusion located on the other of the sample holder or the pressure plate and thereafter being releasably latched at said second distal end.

30. A sample holder assembly as claimed in claim 29, wherein the clamping mechanism extends substantially symmetrically about opposing sides of the sample holder and engages protrusions located on opposing sides of the pressure plate.

31. A sample holder assembly as claimed in claim 30, wherein said protrusions are formed by an elongated pin extending through the pressure plate.

32. A sample holder assembly as claimed in any one of claims 24 - 31, wherein the clamping mechanism is releasably latchable by laterally displacing engaging portions of the clamping mechanism orthogonally to the plane of said compressive force

33. A sample holder assembly as claimed in any one of claims 20 - 32, further including a removable cover releasably fixable over the fluid inlets of each fluid passageway.

34. A sample holder assembly as claimed in claim 33, wherein said cover is locatable in intimate contact with a recessed portion of the inlet face.

35. A sample holder assembly as claimed in an one of claims 33 - 34, wherein the fluid inlets are provided with a fluid channel connecting the fluid inlets to a non-sealed inlet positioned to remain uppermost when the sample holder is rotated into position in the centrifuge with the fluid outlet face substantially vertical.

36. A method of preparing a sample for analysis using a sample holder assembly as claimed in any one of claims 24 - 35, said method including the steps of;

- placing a filter layer on the outlet face of the sample holder
- placing the sample slide on the filter layer,

- placing the pressure plate on the sample slide,
- latching the clamping mechanism to apply a compressive force between the sample holder and pressure plate,
- placing a sample fluid in the fluid reservoir,
- placing the sample holder assembly in a centrifuge; and
- operating the centrifuge to rotate at a predetermined rotational speed,

wherein sample fluid travels through said restricted portion to form a droplet on said projection until separating from the sample holder and impacting the deposition region on the sample slide.

37. The method as claimed in claim 36, further includes the step of:

    - restricting the flow of sample fluid liquid from the deposition region by constraining the available liquid path through said filter layer compressed between said choke and the perimeter of the deposition region on the sample slide.

38. A sample holder substantially as hereinbefore described, with reference to, and as shown in the attached drawings.

39. A sample holder assembly substantially as hereinbefore described, with reference to, and as shown in the attached drawings.

40. A method of sample holder assembly substantially as hereinbefore described, with reference to, and as shown in the attached drawings.

41. The method for preparing a sample for analysis substantially as hereinbefore described, with reference to the examples, and as shown in the attached drawings.

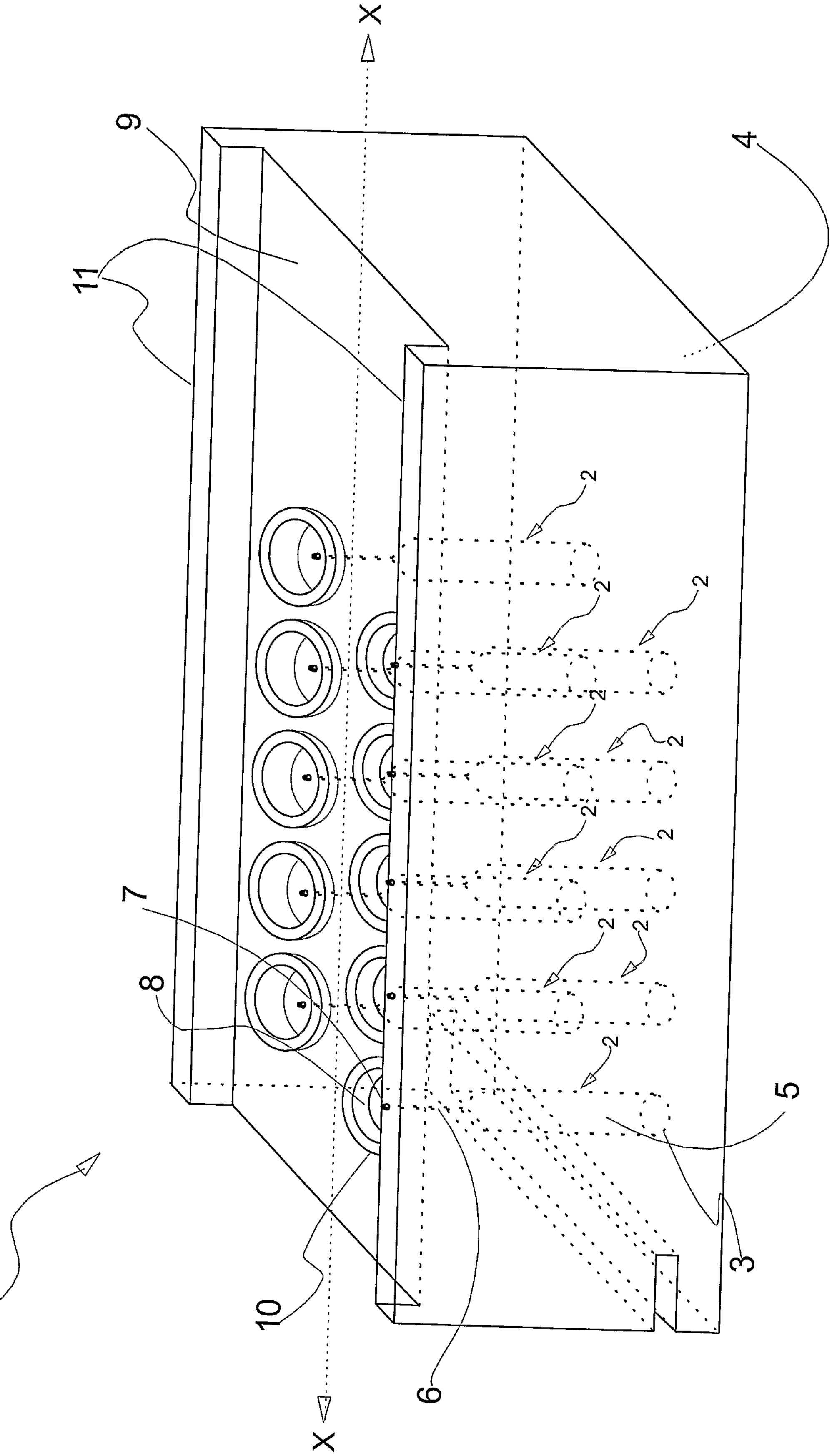


Figure 1.

Figure 2

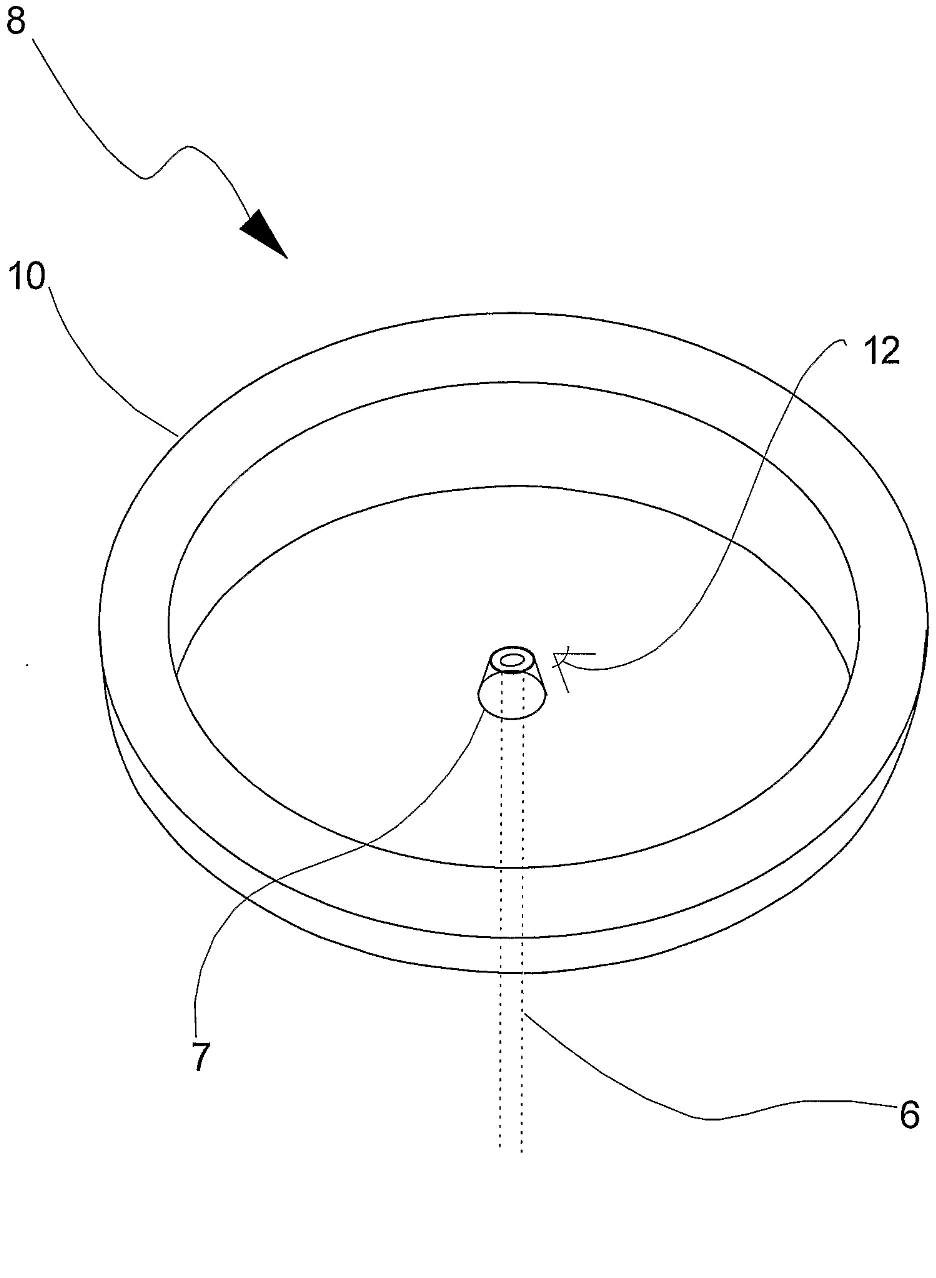


Figure 3

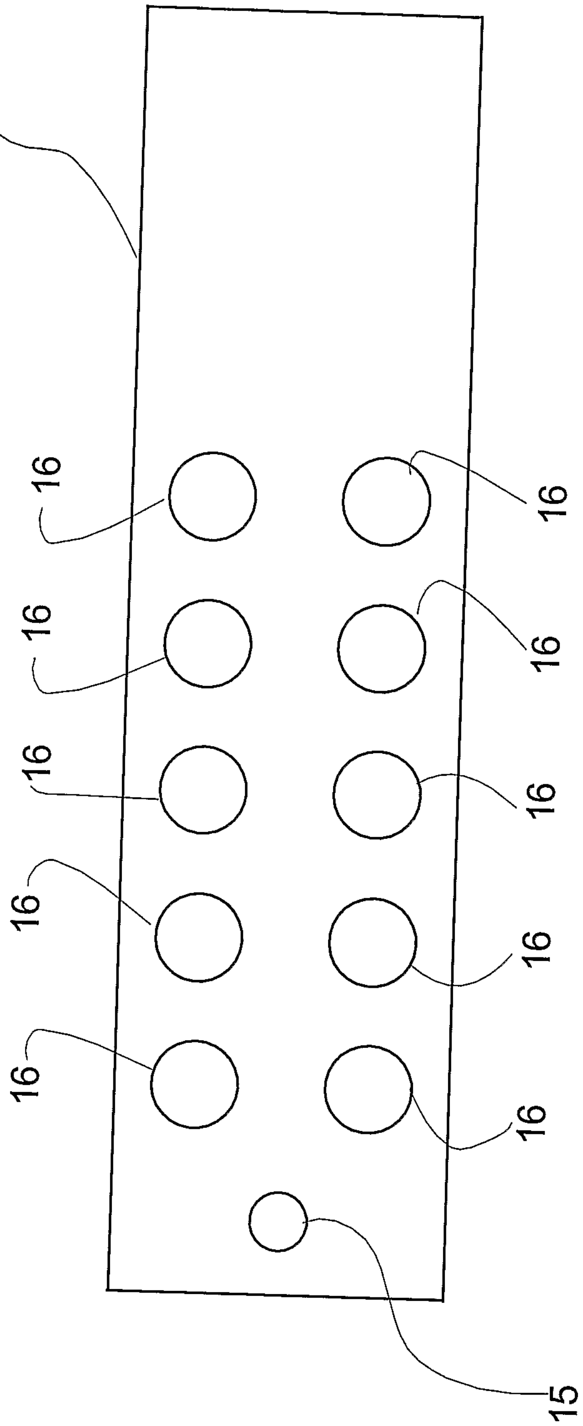
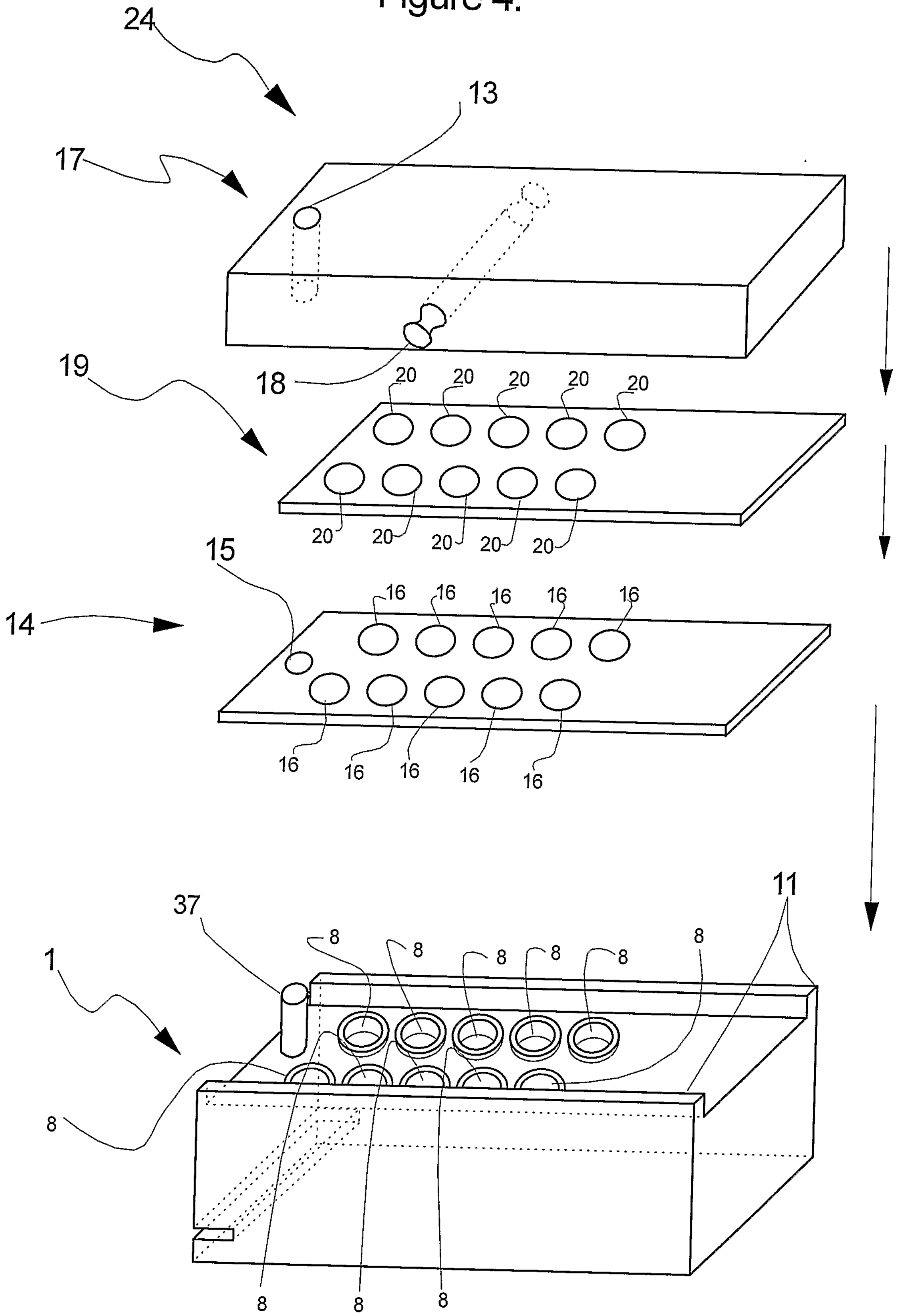


Figure 4.



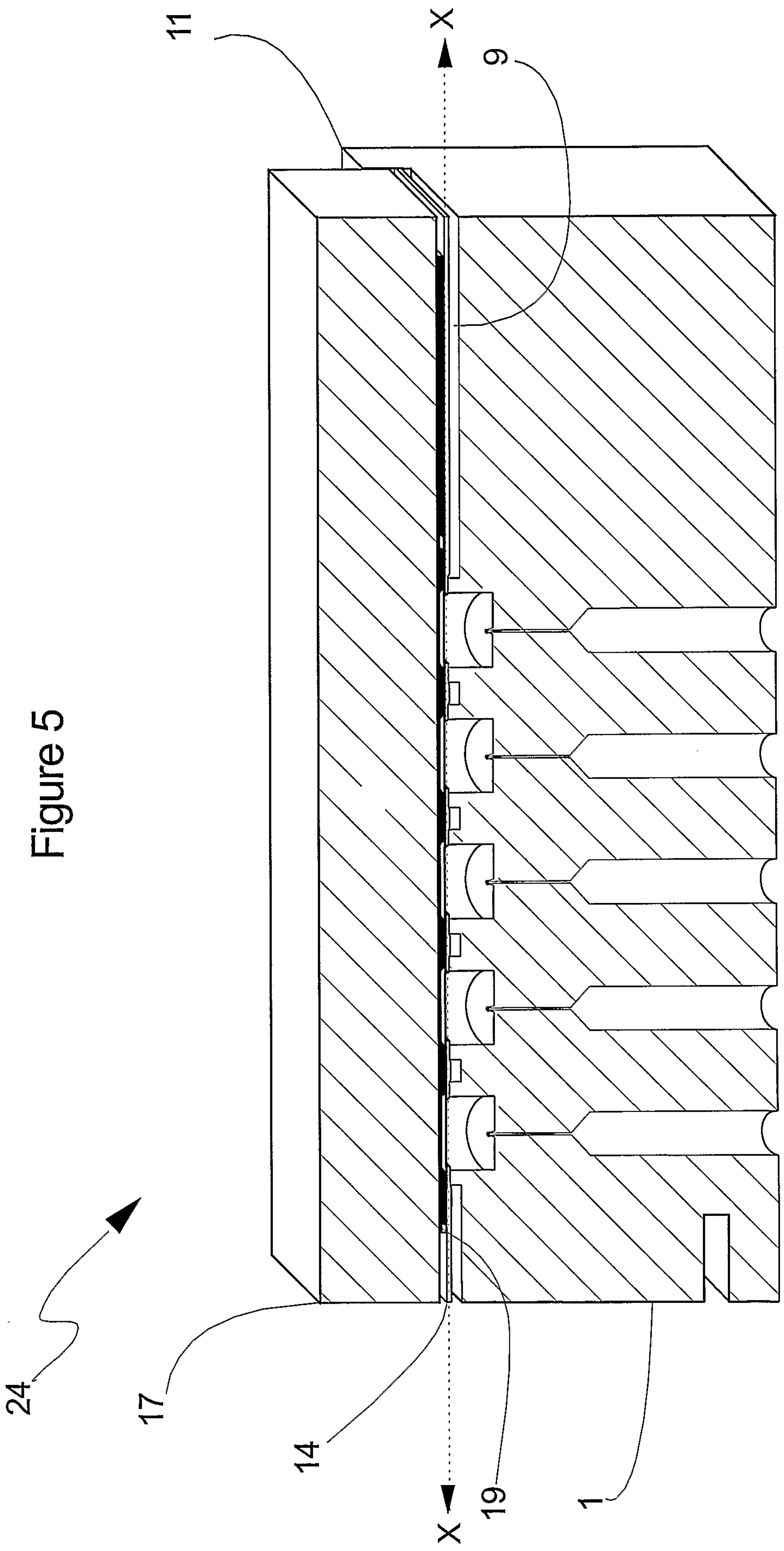


Figure 5

Figure 6

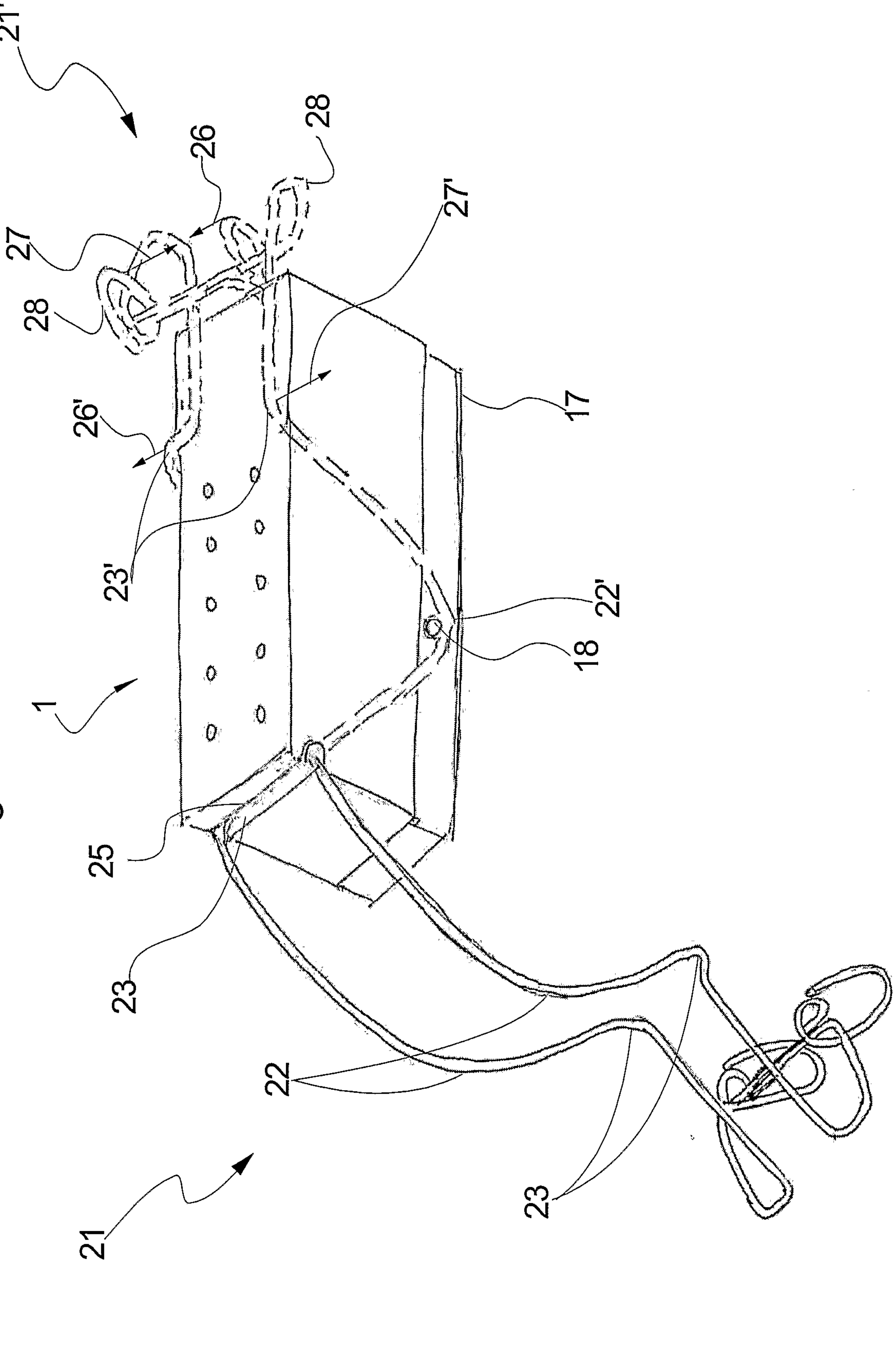
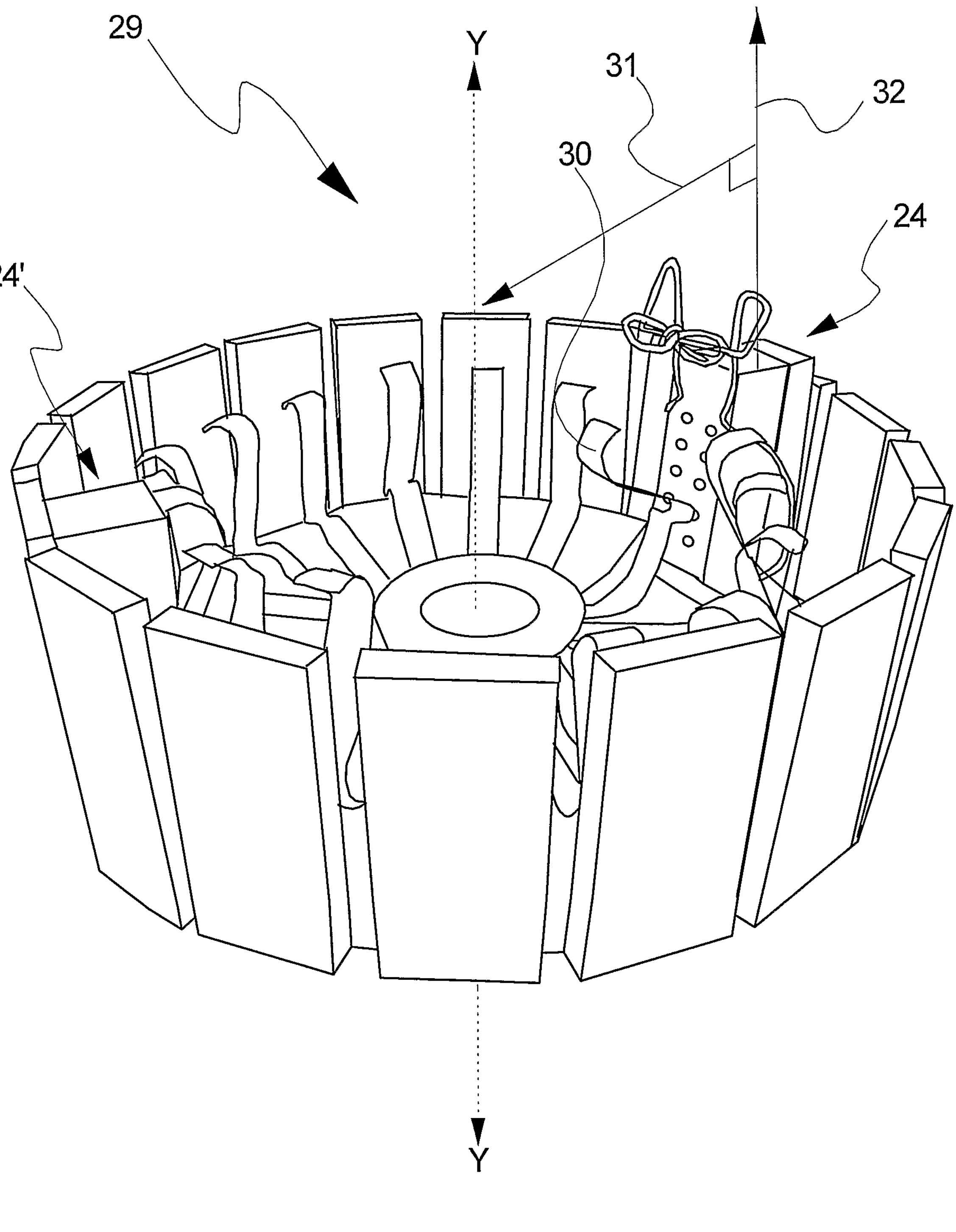


Figure 7



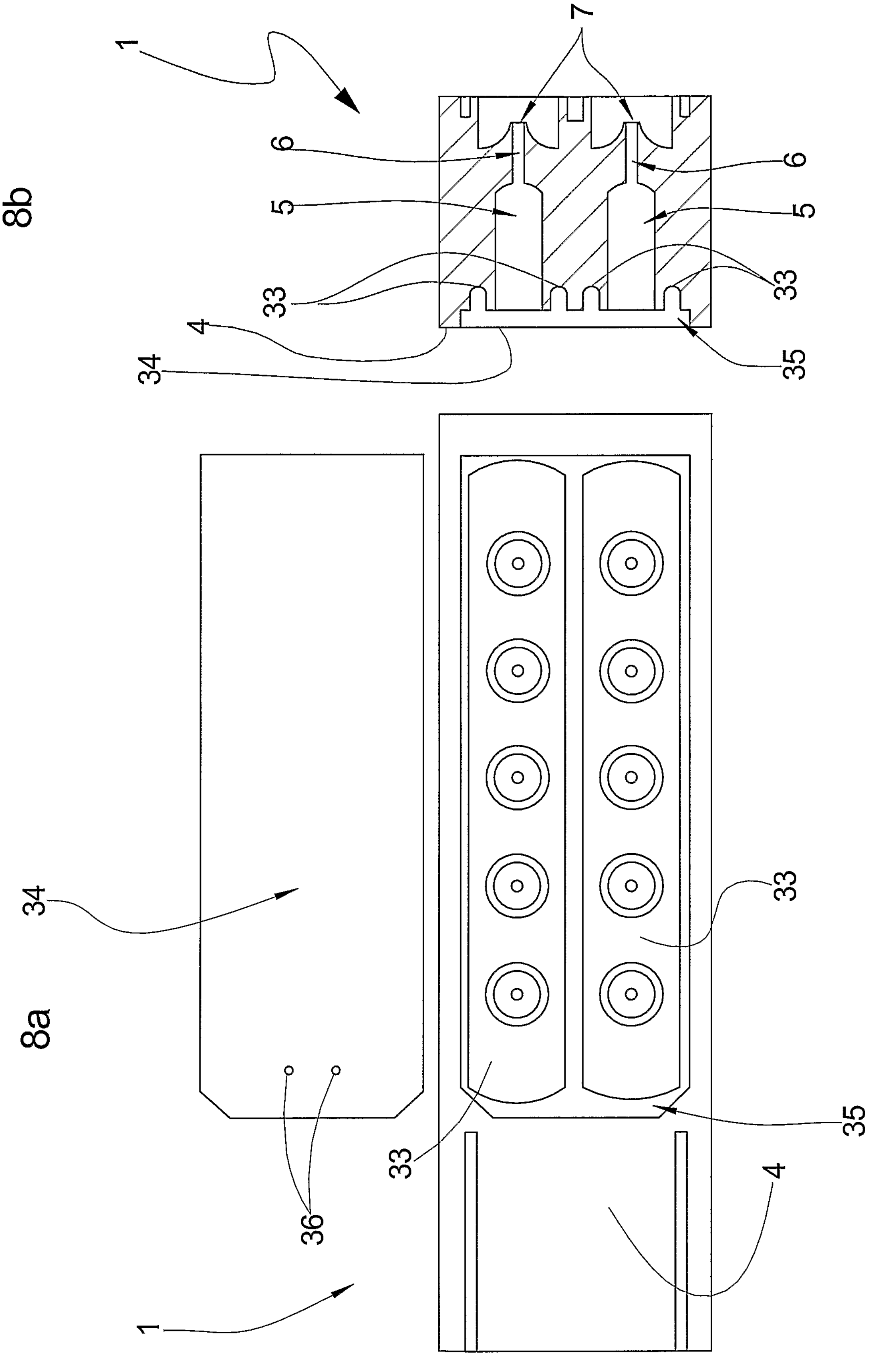


Figure 8

8a

8b