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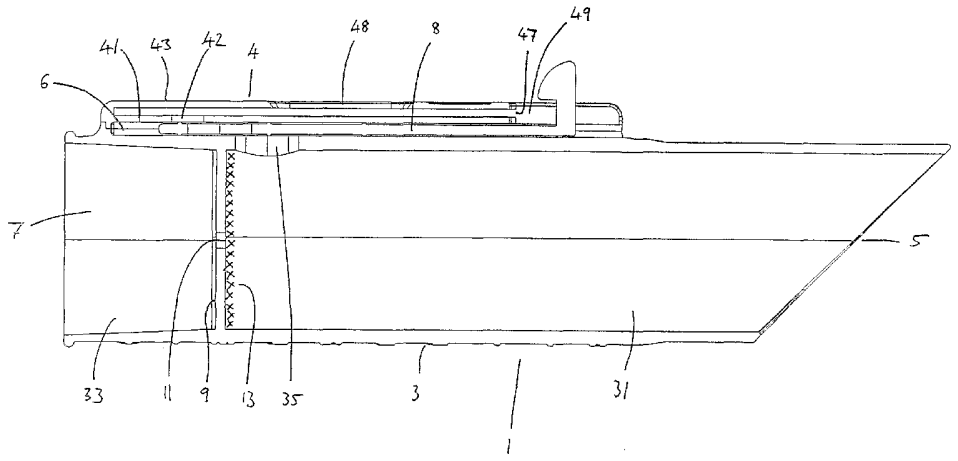
(54) Title: ASSAY DEVICE



(57) Abstract: The invention provides an assay device (1) comprising a sample chamber (31) for receiving a test solution, and a test chamber (45) for receiving detection means (60), such as a lateral flow test strip, capable of detecting a target analyte in the test solution. The device further comprises actuating means (8) movable to enable an aliquot of test solution to pass between a sample exit port (35) of the sample chamber and a sample entry port (42) of the test chamber (45). In preferred embodiments, the device is adapted for connection to a vacuum cleaner hose in order to draw air through the sample chamber (45), and comprises a filter (13) to retain solid material such as dust mite antigens within the test chamber (45).

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— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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Assay Device

Field of the Invention

The present invention relates to devices for the specific detection of analytes in samples of household material. In preferred embodiments the invention relates to devices for the detection of house dust mite allergens.

Background to the Invention

Today, approximately six million people in the UK are house dust mite sensitive; a figure which has been increasing for the past twenty-five years. Growing public concern surrounding allergic disease and allergens has resulted in an increasing demand for products which measure levels of contaminating antigen within the domestic environment. At present however, the majority of available tests are laboratory based and therefore not accessible to the general public. A number of devices have been described for collection of house dust mite allergen in the domestic environment, but disadvantages of these are that the dust collected must either be sent away for analysis, or the user may be exposed to the allergen when carrying out the test themselves.

Summary of the Invention

In certain embodiments, the present invention provides an integrated device for collection and testing of house dust for allergens, which is suitable for use in the domestic environment, requires no skilled operation, and which minimises contact between the user and the material collected between collection and testing. Embodiments of the invention also find application in other fields, which do not require the test material to be first collected from the environment, such as in the testing for particular

analytes in bodily fluids, such as blood or urine, or solid or semi-solid material such as faeces.

The present invention provides an assay device comprising a sample chamber for receiving a test solution, and a test chamber for receiving detection means capable of detecting an analyte in the test solution, the device further comprising actuating means movable to enable an aliquot of test solution to pass between a sample exit port of said sample chamber and a sample entry port of said test chamber.

Thus an aliquot of a test solution present in the sample chamber can be transferred to the test chamber without further user contact.

In preferred embodiments, a measured amount of test solution passes between the sample and test chambers. To facilitate this, the sample exit and entry ports may be offset from one another, so that it is not possible to open a fluid flow passage between them, whatever the position of the actuating means. In such an embodiment, the actuating means preferably comprises a fluid receptacle movable between a first position adjacent the sample exit port and a second position adjacent the sample entry port. The actuating means may be slidable between said first and second positions, preferably manually.

Thus in such embodiments, fluid communication is never established between sample and test chambers. Instead a measured amount of test solution is taken up by the fluid receptacle and moved between the exit and entry ports. The fluid receptacle may comprise or consist of a bore or aperture extending through said actuating means.

In an alternative embodiment, the actuating means may be movable to establish fluid communication between the sample exit and sample entry ports, e.g. by opening a fluid flow passage between them. The sample exit and entry ports may be aligned on opposite sides of the actuating means. In this case, the fluid communication between the chambers may be maintained only for a short period of time, to control the amount of test solution passing between them. The actuating means may be biased to a position at which it prevents fluid communication between the fluid exit and entry ports and movable to a position at which it allows fluid communication between the ports. Further alternative embodiments will be apparent to the skilled person.

The assay device may be of various types. In certain embodiments it may be of a "closed" type, in which the sample chamber is sealed to fluid flow at one end, and where a test sample may simply be added to the sample chamber.

Alternatively the device may be of "flow-through" type, in which a fluid is drawn through the device and one or more components of the fluid or carried by the fluid is retained in the sample chamber.

Thus in certain embodiments of the "flow-through" type, the sample chamber comprises retention means positioned between a fluid inlet and a fluid outlet of the device for retaining a desired component of a fluid in the sample chamber. The device preferably possesses a fluid inlet and a fluid outlet which together define a fluid flow passage through which a desired fluid may be passed or drawn through the sample chamber, and a desired component of the fluid retained on contact with the retention means. The desired component of

the fluid will typically constitute or comprise the analyte to be tested for.

In a preferred embodiment, the retention means is a filter. The skilled person will be able to select suitable materials and pore sizes depending on the nature of the fluid and/or the component to be retained.

In a preferred embodiment, the flow-through device is used for detection of house dust mite allergens. Thus the filter is preferably capable of retaining airborne solid material, e.g. dust mites or their faeces. Dust mite faecal pellets are around 0.3 - 50 micrometers in diameter, and a cellulose based material having a pore size ranging from 10 to 50 micrometers has been found to provide a good balance between obtaining satisfactory airflow through the device while retaining enough test material on the filter to give a strong positive signal in an immunoassay for the Der p1 antigen.

Subsequently, the retention means may be contacted with a suitable solvent or other liquid in order to solubilise or suspend the retained material. Thus the nature of the solvent will depend upon the nature of the retained material and the analyte. The solvent may comprise a surfactant or detergent and/or one or more enzymes to aid solubilisation or suspension. For example, for solubilisation of Der p1 antigen, a suitable extraction fluid may comprise an aqueous buffer such as phosphate buffered saline, a surfactant such as Tween 20, and/or an enzyme such as chitinase (see e.g. W93/18404).

Preferably the sample chamber is sealable following addition of the solvent or sample solution, e.g. by means of a cap or lid.

Preferably, the retention means is substantially impermeable to the solvent to be added, to prevent flow of the solvent from the sample chamber. In a preferred embodiment the retention means is permeable to air, but impermeable, or substantially impermeable to water.

Additionally or alternatively the fluid outlet may be sealable prior to addition of the solvent to the sample chamber, e.g. by means of a cap or lid.

In flow-through devices utilising retention means as described, the sample exit port of the sample chamber is typically located between said the retention means and the fluid inlet.

The fluid may be passed or drawn through the sample chamber by any suitable means. In a preferred embodiment, the fluid is air, and is drawn through the sample chamber by a standard vacuum cleaner. Thus the fluid outlet of the assay device is preferably adapted for connection to the hose of a standard vacuum cleaner. The device may comprise an attachment unit having an internal taper to enable a snug fit with hoses of different diameters. The attachment unit may be located between the fluid outlet and the retention means.

Whether the device is of a flow-through type or of simple closed form, means may be provided for agitating the test solution within the sample chamber. Preferably the agitating means is provided with a handle located outside

the sample chamber for manipulation by a user, and suitable agitating means, for example a blade, spatula or stirrer element, located inside the chamber for agitation of the test solution. This facilitates mixing of reagents in the sample chamber, or solubilisation, disruption or dispersion of a material suspected to contain a test analyte in a solvent. Thus for example, a lid may be provided to seal the sample inlet or fluid inlet, with a suitable agitating device extending therethrough. The agitating device may be movable laterally (to stir the sample) and/or axially (e.g. by a piston-type action, to disrupt a sample) relative to the sample chamber.

Additionally or alternatively the device may be provided with means to prevent suspended solid material from passing through the sample exit port, while allowing substantially free flow of liquid (typically the test solution). The device may therefore comprise sieving means adapted to trap solid material adjacent or against the retention means or the bottom surface of the sample chamber, while allowing liquid to flow through or around it. For example, said sieving means may comprise an axially movable plunger having a foraminous head (e.g. comprising a web of mesh) fitting flush with the internal walls of the sample chamber, similar to the sort found in conventional cafetière-type coffee makers.

In all embodiments herein described, the device is preferably an integral unit comprising both sample chamber and test chamber. The term "integral" as used herein is not intended to mean that the sample chamber and detection chamber must be formed in one piece, although such a construction is possible. Rather, the a test unit comprising the test chamber, and a unit comprising the

sample chamber remain attached throughout one use of the device from sample collection to obtaining a signal from detection means housed within the test chamber. The device may be constructed such that the test unit is reversibly detachable from a unit comprising the sample chamber. The test unit may be detachable in order to remove the detection means after testing, and/or to insert fresh detection means into the test chamber. Either or both of the units may be re-usable after a test has been performed. For example, after use, a filter located within the sample chamber may be changed for a fresh one, and a test strip (see below) housed in the test chamber replaced. Forming the two units separately may facilitate such operations, and also the cleaning of the device. Alternatively, all portions of the device may be disposable.

The detection means used in the device may be of any suitable type. It may be integral with the test chamber or removable. In preferred embodiments the detection means generates a visible signal in the presence of the target analyte. The intensity of the signal may correlate with the concentration of the target analyte in the test solution.

In preferred embodiments, the detection means uses capillary flow to bring the test solution into contact with reagents for detection of the analyte. A preferred format for the detection means is a test-strip format of the kind used in lateral flow assays. These typically comprise a solid material that conducts fluid by capillary flow, such that a fluid sample to be tested for the presence of an analyte of interest can pass from one part of the strip to another. Additionally, the solid material supports the various reagents required for detection of the analyte of interest, according to the design of the particular assay. A reagent

may remain immobilised on the solid material or may be solubilised by the sample and/or any buffer solution used allowing it to pass through the device. Such strips take many formats, but commonly the various reagents of the assay are located on the solid support material in distinct zones. The zones are suitably positioned with respect to one another to enable the reactions of the assay to proceed. The zones may be present on a single absorbent component of the solid support or on different absorbent components of the support, arranged (typically abutting or partially overlapping) to conduct fluid through the device as required. Suitable materials for the solid support and absorbent components include nylon, glass fibre and nitrocellulose.

The distinct zones provided by the different absorbent components may include a sample application zone, one or more reaction zones, one or more indicator or detection zones and an absorbent zone (for example, into which excess sample and/or reagents can flow, for removal from the reaction, indicator and/or detection zone(s)). The actual nature, number and arrangement of zones provided by the components will vary according to the nature of the target analyte and the requirements of the design of a particular assay.

Test-strips of this kind typically rely on immunological detection of the analyte, in which the target analyte is brought by capillary flow into contact with a first antibody specific for the target analyte in a zone of the strip to form an immunological complex. The first antibody is typically labelled, e.g. with a gold conjugate. Detection of the complex may be carried out at an upstream region of the strip, e.g. with an immobilised second antibody specific

for a different epitope of the target analyte, which causes localisation of the labelled first antibody in sufficient density for the label to become visible.

Other suitable examples of detection means will be apparent to the skilled person, depending upon the requirements of the particular assay.

Where a visible signal is generated by the detection means, the test chamber preferably comprises a window for viewing that signal so that the detection means need not be removed from the device.

The present invention further comprises a kit comprising an assay device, detection means, and/or extraction fluid as herein described, optionally further comprising instructions for use.

Brief Description of the Drawings

Figure 1 is an exploded view of a flow-through-type device adapted for connection to a vacuum cleaner.

Figure 2 is a cross-section of the device shown in Figure 1.

Figure 3 shows the device of Figures 1 and 2 connected to the hose of a vacuum cleaner.

Figure 4 is a cross-section of a closed-type device.

Figure 5 is a cross-section of a closed-type device having an integral homogenising rod.

Detailed Description of Embodiments of the Invention

Referring firstly to Figures 1 to 3, a flow-through type assay device 1 for detection of allergens in the faeces of house dust mites comprises a substantially cylindrical collection unit 3 having an air inlet 5 and an air outlet 7. A partition 9 is located approximately one-fifth of the way between the outlet 7 and inlet 5 with a central hole 11 to allow passage of air through the collection unit.

The partition 9 divides the collection unit into an upper sample chamber 31 and a lower attachment unit 33 for attachment to the hose 50 of a vacuum cleaner as shown in Figure 3. The inner walls of the attachment unit 33 are tapered towards partition 9 to enable a snug fit with vacuum cleaner hoses of different widths.

A cellulose filter 13 having pores of various sizes ranging from 10 to 50 micrometers is supported on the sample chamber side of partition 9. A sample exit aperture 35 extends through the wall of sample chamber 31 adjacent filter 13. Surrounding sample exit aperture 35 on the exterior surface of collection unit 3 is an elongate rectangular U-shaped mounting portion 37, open at the end nearest inlet 5, which forms a mounting for receiving a complementarily-shaped elongate test unit 4 by a snap-fit.

Test unit 4 comprises a base portion 41 and a cover 43, which together define a test chamber 45. Cover 43 has a longitudinal slot 49 formed at the end nearest air inlet 5. Test chamber 45 opens into slot 49 via an aperture 47. Test chamber 45 is sized to receive a test-strip assay device 60, which is inserted through aperture 47.

A sample inlet aperture 42 extends through base portion 41 to the test chamber 45 at the end of the test unit nearest

outlet 7, and is spaced axially towards the outlet 7 of collector unit 3 from sample outlet aperture 35 of the collection unit 1.

When test unit 4 is attached to mounting portion 37, its base 41 is spaced from the exterior surface of the collector unit 3 to define a channel 6 which slidingly receives a rectangular actuator 8. Actuator 8 is movable manually in channel 6 by means of a grip portion 81 mounted on stem 83 which extends outwardly of cover 43 through slot 49. A sample-receiving aperture 85 extends through actuator 8 at the end remote from grip 81.

Actuator 8 is slidable in channel 6 between a first position, in which sample-receiving aperture 85 is aligned with sample exit aperture 35 of the collector unit 3, and a second position in which sample-receiving aperture 85 is aligned with sample entry aperture 42 of test unit 4. Transverse ridges 87 standing proud of actuator 8 may engage with complementary grooves (not shown) of channel 6 in order to stabilise actuator 8 in either or both of these positions, and may also function as fluid seals, to prevent leakage of fluid along channel 6 either side of actuator 8.

In use, a test-strip assay device 60 for detection of a target analyte is inserted into test chamber 45, such that its sample application point is located adjacent sample entry aperture 42. Actuator 8 is positioned so that sample-receiving aperture 85 is aligned with sample exit aperture 35, and attachment unit 33 is connected to the hose of a conventional vacuum cleaner as shown in Figure 3.

The vacuum cleaner is then used to draw air through collection unit 3 from an article or area suspected to be

contaminated with dust mites, and solid airborne material is retained on filter 13. The device is then removed from the vacuum cleaner hose and fluid outlet 78 capped with a snap-fit lid (not shown).

Extraction fluid is added to the filter through fluid inlet 5, which is then also capped with a cylindrical lid (not shown). Enough extraction fluid is added to cover sample exit aperture 35 when the device is stood upright on the fluid outlet end, so that an aliquot of the extraction fluid enters the sample-receiving aperture 85 of actuator 8. The assay device may be agitated to disperse, disrupt or dissolve the target analyte in the extraction fluid. After suitable incubation time, the actuator is slid from the first position to the second position in which sample-receiving aperture 85 is adjacent sample entry aperture 42 of test device 4. The aliquot of sample held by sample-receiving aperture 85 is thus brought into contact with the sample application point of test-strip 60. Analyte is conducted along the test-strip by capillary action in conventional manner. The test strip typically provides a visible signal correlating to the presence, absence or amount of target analyte in the aliquot of sample. The signal may be viewed through a window 48 located in the upper surface of cover 43.

Further embodiments of the invention are shown in Figures 4 and 5, which show closed format assay devices rather than the flow-through type device of Figures 1 to 3, and in which the same numbering will be used where possible.

The device of Figure 4 is squared off at fluid inlet 5, rather than having the sloped inlet of the device described above intended for use with a vacuum cleaner. Inlet 5 is

sealable with a snap-fit cap 15. The device has a flat integral base 17 instead of fluid outlet 7, and lacks partition 9 and filter 11. Thus the entire interior of the collection unit constitutes a sample chamber 31.

In use, a solid or liquid test material to be assayed for the presence or absence of a test analyte may be added to the sample chamber 31. Where the material is solid, a solvent will typically be added to the sample chamber to disperse or dissolve the analyte to be tested for. Further reagents may be added if desired. Assay is performed as for the flow-through device described above.

The device of Figure 5 has a homogeniser rod 19 extending through a slot (not shown) in cap 15, having at opposite ends a handle portion 21, located outside the sample chamber and a stirring loop 23 located inside the sample chamber for agitation of a sample, to facilitate mixing of liquid components, or disruption, dispersion or dissolution of solid components in the liquid sample. Homogeniser rod 19 may be slidable in the slot of cap 15 in order that it may be moved axially within sample chamber 31.

The device may be reusable, but in preferred embodiments is disposable after one use. Thus the device described enables collection of dust or other material, and testing for a target analyte, with minimal user contact. In particular, there is no user contact with the solution of test analyte after addition of extraction fluid to the sample chamber as there is no need to remove sample from the chamber or testing. This is important for individuals who are sensitive to the material collected, e.g. a user with dust mite sensitivity may suffer symptoms if exposed to collected dust.

Examples of Assays

A flow-through device as described above was used for assay of the house dust mite allergen Der p1 found in dust mite faeces.

A lateral flow test strip was prepared as follows:

40nM Gold conjugated monoclonal anti-Der p1 antibody (prepared by British BioCell, Cardiff) was dissolved in 10% Trehalose / PBS to give a final concentration of gold of $OD_{0.5}$.

This was used to coat a 2cm x 3cm glass fibre pad as follows ; 0.1ml Gold Conjugate (OD_{10}), 1.0ml Trehalose (10%) and 0.9ml PBS / 1% BSA / 0.5% Tween 20 were mixed together added to the pad and allowed to dry for at 37^0C in a dessicator oven for 4 hours.

1mg/ml Capture Antibody mAb anti-Der p1, diluted in PBS, was striped on the test strip (Millipore HF135MC100 - medium flow cellulose acetate strip) in order to detect the antigen/gold conjugate complex. A second positive control line was added to detect excess monoclonal using an anti IgG capture line

It was determined that 200µL of sample when added to the strip resulted in all the gold conjugate being removed from the fibre pad within 30 mins. The sample-receiving aperture of the actuator was sized accordingly.

Undiluted capture antibody (1mg/ml) detected antigen over the following range 2.5, 25 and 250ng/ml). A graded colour response was observed over this dilution range.

Lateral flow kinetics of the test strip were tested using 25ng/ml of purified test antigen.

Time (mins)	Sample progression
0	200ul of sample added to sample pad
0.5	Sample leaves gold conjugate pad
1.5	Sample reaches capture line
3.5	Sample reaches top pad
7.5	Begins to wet top pad
10.0	1/3 of top pad saturated
25.0	2/3 of top pad saturated
32.5	4/4 of top pad saturated

A colour comparison card was constructed for quantification of test strip results. 3 colour intensities covering the range of 2.5, 25 and 250 ng/ml Der p1 antigen were made by directly striping appropriately diluted samples of gold conjugated antibody onto a test strip. The results of the test strip after 30 mins was then compared to the comparison card to determine the level of antigen present in the sample

Environmental sampling was performed by attaching the collection device to a Dyson® vacuum cleaner and sampling an area the size of an A4 sheet (for beds) and an A3 sheet (for carpets). Vacuuming was performed for 1 min in each case. The sample collected on the filter was then re-suspended in 10 ml of diluent (phosphate buffered saline/1% Tween 20) and left for 5 mins. 200ul of dissolved sample was then applied to the test strip and the results determined after 30 mins by comparison of the test strip colour to the comparison colour card.

Table 1. Comparison of Der p1 ELISA assay data and visual estimation of dipstick intensity for the same samples collected from different environmental sources.

Source of sample	Quantification of Der p1 antigen using ELISA (ng/ml)	Visual estimation of Der p1 levels using rapid test
Bed 1	1,470	High
Bed 2	1,650	High
Carpet 1	460	Medium
Carpet 2	410	Medium
Carpet 3	100	Low
Carpet 4	30	Low
Control	0	Not detected

Summary of detection ranges for environmental and laboratory prepared samples

	Environmental sample range (ng/ml)	Purified antigen range (ng/ml)
High	800 +	250 +
Medium	200 - 800	25-200
Low	30-200	2.5-20
Not detectable	< 15	< 1.0

The detection range, as determined by using the colour card, was 10 - fold higher for environmental dust samples as compared to that obtained for purified Der-P1 antigen solutions. This was not surprising as the environmental sample contains large amounts of particles and debris that alter the lateral flow kinetics of the test strip.

CLAIMS:

1. An assay device comprising a sample chamber for receiving a test solution, and a test chamber for receiving detection means capable of detecting a target analyte in the test solution, the device further comprising actuating means movable to enable an aliquot of test solution to pass between a sample exit port of the sample chamber and a sample entry port of the test chamber.

2. An assay device according to claim 1, wherein the fluid exit and sample entry ports are offset relative to one another.

3. An assay device according to claim 1 or claim 2, wherein the actuating means comprises a fluid receptacle movable between a first position adjacent the sample exit port and a second position adjacent the sample entry port.

4. An assay device according to claim 3 wherein the fluid receptacle comprises a bore extending through said actuating means.

5. An assay device according to any one of claims 1 to 4, wherein the sample chamber comprises retention means positioned between a fluid inlet and a fluid outlet, the fluid inlet and fluid outlet defining a fluid flow passage through the sample chamber.

6. An assay device according to claim 6, wherein the sample exit port is located between said retention means and said fluid inlet.

7. An assay device according to claim 5 or claim 6, wherein the retention means is a filter.

8. An assay device according to claim 7, wherein the filter means is permeable to air and substantially impermeable to water.

9. An assay device according to any one of claims 5 to 8, wherein said fluid outlet is adapted for connection to suction means for drawing fluid through the sample chamber.

10. An assay device according to any one of claims 1 to 9, wherein said detection means operates by capillary flow.

11. An assay device according to claim 10, wherein said detection means is a test strip.

12. An assay device according to claim 11, wherein the test chamber comprises a window for viewing a signal produced by said test strip.

13. An assay device according to any one of claims 1 to 12, further comprising means for agitating a test solution within the sample chamber.

14. An assay device according to claim 13 wherein said means for agitating a test solution extends through a cap adapted to seal the sample chamber.

15. An assay device substantially as herein described with reference to any one of Figures 1 to 5.

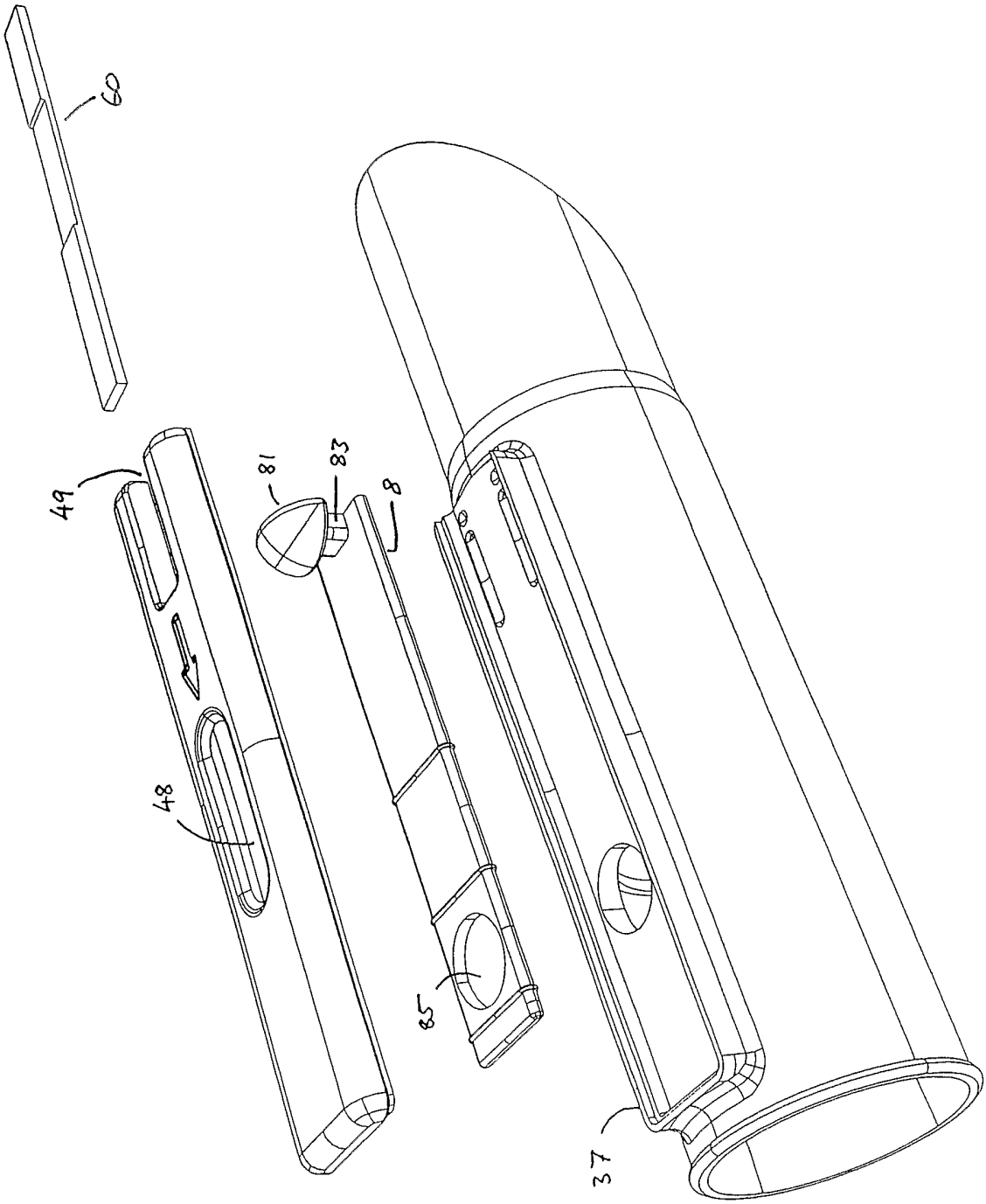

FIGURE 1

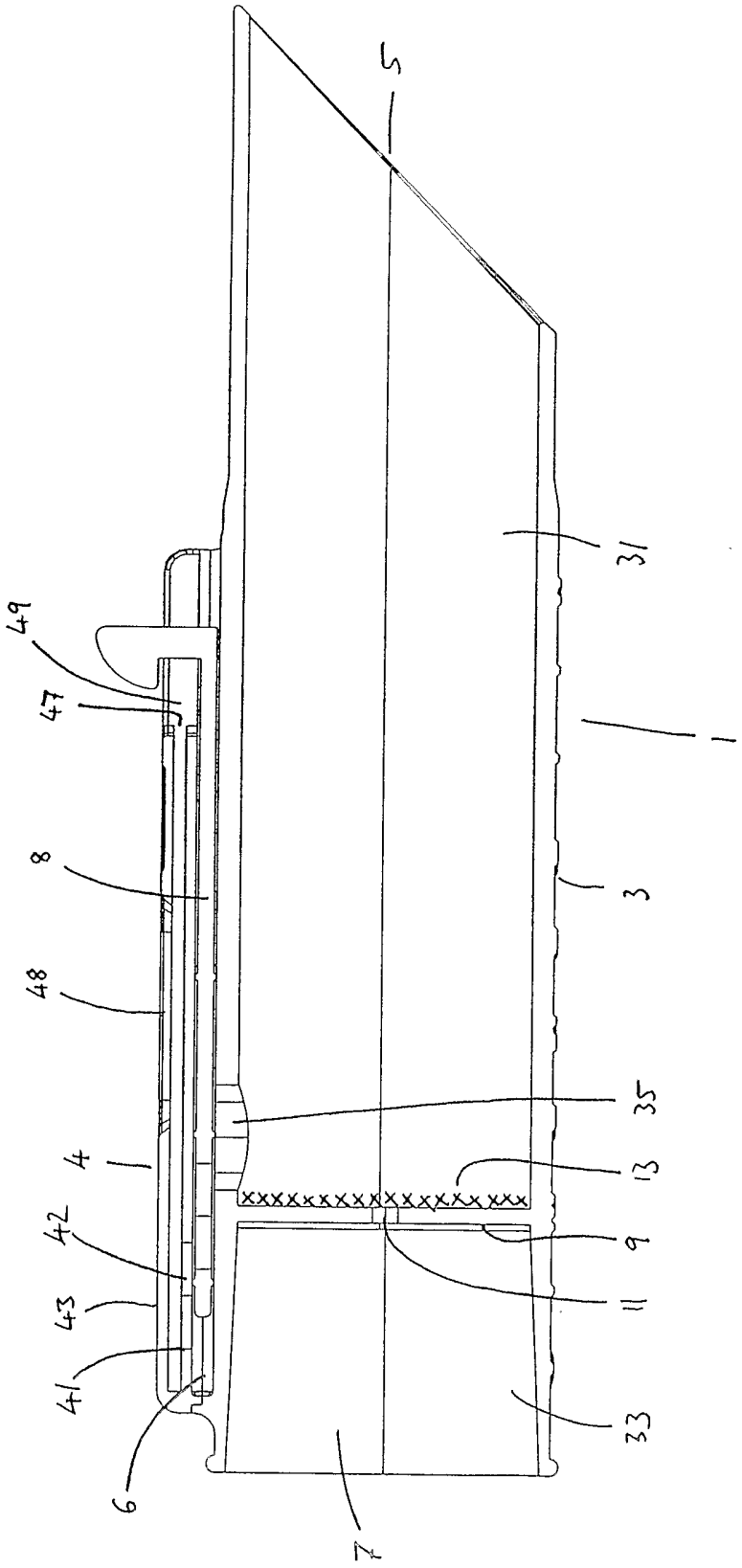

FIGURE 2

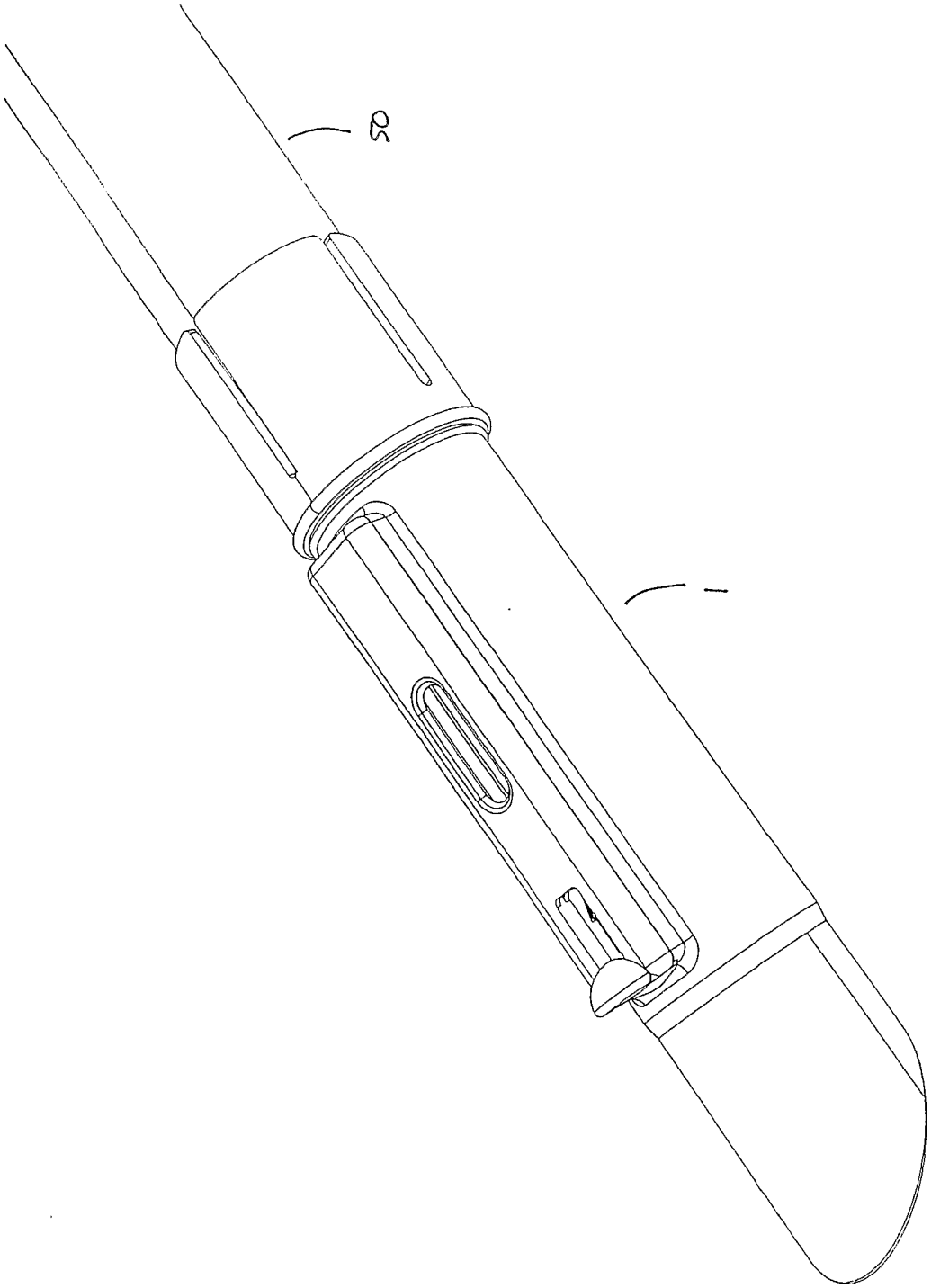

FIGURE 3

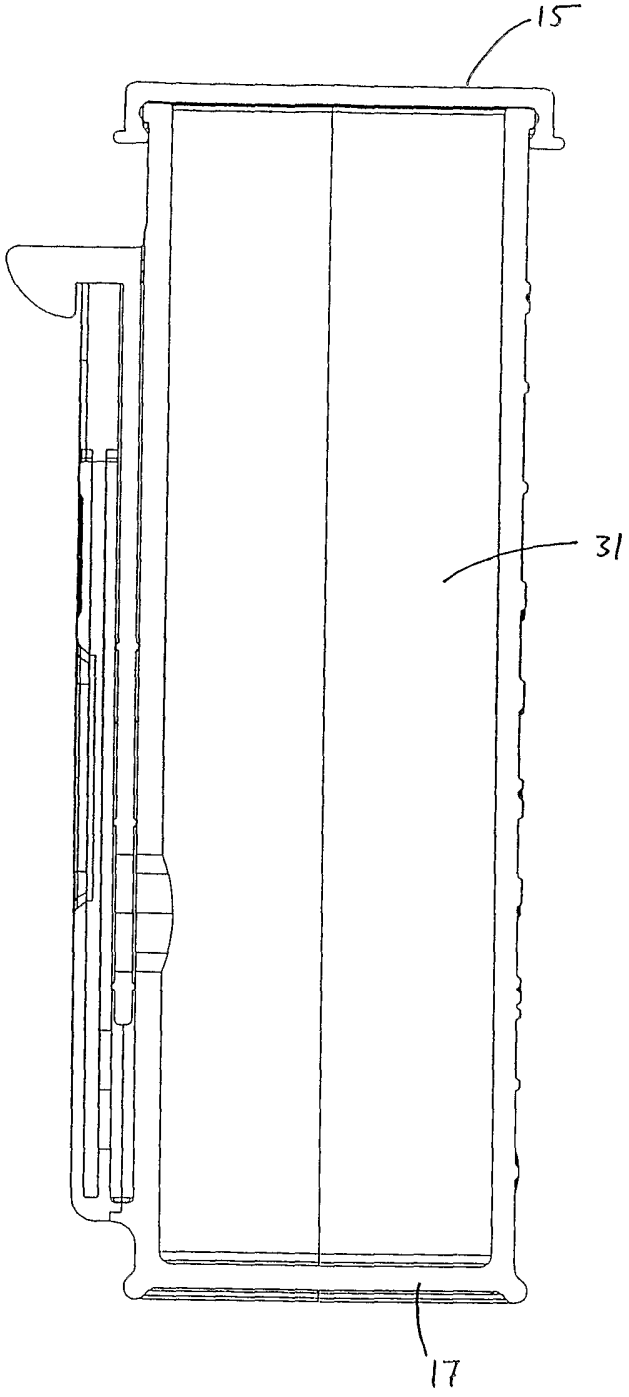

FIGURE 4

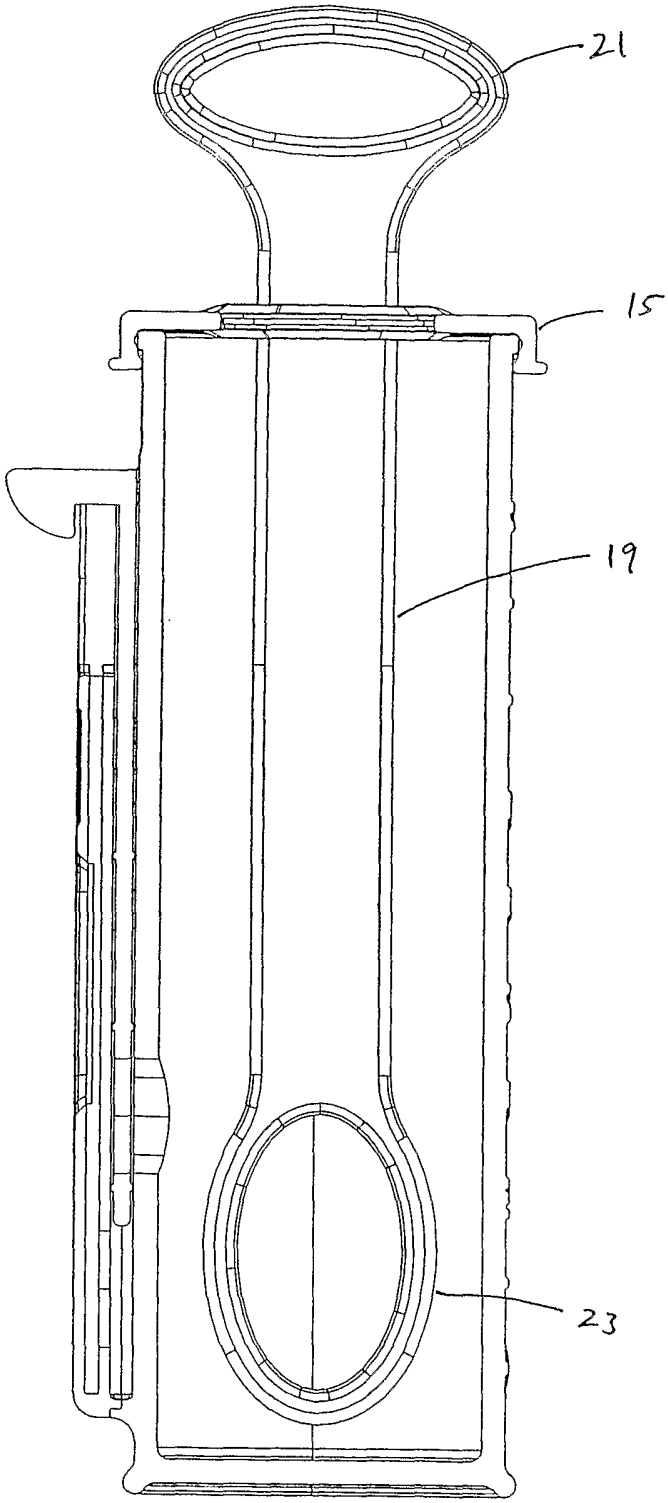

FIGURE 5

INTERNATIONAL SEARCH REPORT

PCT/GB2004/001085

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N1/24 B01L3/00 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2002/173047 A1 (BAUTISTA LORRAINE ET AL) 21 November 2002 (2002-11-21)	1
Y	paragraph '0042! - paragraph '0053!	1
A	paragraph '0066! - paragraph '070A!; figures 2,3,6	5-7, 10-13

Y	GB 2 371 768 A (MESOSYSTEMS TECHNOLOGY INC) 7 August 2002 (2002-08-07)	1
A	page 15, line 32 -page 18, line 15 page 24, line 32 -page 26, line 19; figures 1,5-9	5,9

Y	US 5 679 535 A (JOYCE PATRICK JOSEPH ET AL) 21 October 1997 (1997-10-21)	1
A	column 3, line 60 -column 9, line 67	5-9

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
19 July 2004	28/07/2004

Name and mailing address of the ISA	Authorized officer
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