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(54) Flow control in a containment device.

(57) In unvented containment devices used for DNA amplification, problems are encountered due to back pressure in the waste compartment due to incoming flow. This back pressure tends to stress the detection chamber and dislodge anchor sites for the target. Described herein is a containment device (10) in which a waste compartment (42) provided downstream from a detection site (40, 41) is provided with fold lines (74) which give the compartment a bistable configuration so that it can expand to relieve back-pressure which otherwise builds up in the device (10).

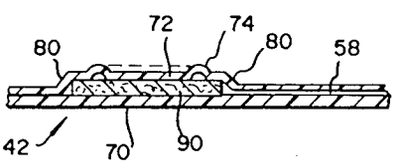


FIG. 2

EP 0 594 259 A1

This invention relates to containment devices used to process a liquid under contained conditions, including detection of analyte and collection of waste liquids.

It is known to do polymerase chain reaction (PCR) or other forms of DNA amplification in a containment device, using, for example, a flexible pouch. Such a technique is described in EP-A-0 381 501, wherein flow of target and reagents proceeds past a detection chamber and into a dead-end waste compartment.

Although such a device is very effective, the use of a dead-end waste compartment can create a problem on occasion. In particular, sufficient back-pressure from incoming flow can be created so as to interfere with the sequential reactions desired at the detection chamber. For example, back pressure tends to stress the detection chamber to the point that beads used to anchor the target can themselves become dislodged.

The most obvious solution to back-pressure caused by a dead-end waste compartment is to vent that compartment to the atmosphere. However, that is unacceptable since it defeats the first principle of PCR devices, namely that of keeping contained the amplified product.

Accordingly, prior to this invention it has been a problem to ensure that no undesirable back-pressure will be created by a waste compartment such as might interfere with optimum results.

It is therefore an object of the present invention to provide a containment device which avoids the above-noted problems.

More specifically, in accordance with one aspect of the present invention, there is provided a containment device for use in amplifying and detecting nucleic acid material comprising:-

an inlet port through which a sample is inserted into the device, the device being sealable after sample insertion to prevent leakage of nucleic acid material;

a reaction compartment in which amplification of nucleic acid material takes place;

a detection site for detecting the nucleic acid material;

flow means for allowing fluid flow from the reaction compartment to the detection site; and

a waste compartment downstream of the detection site and fluidly connected thereto to receive reagents and material after passage over the detection site, the waste compartment comprising a pair of opposing walls;

characterized in that at least one of the opposing walls is provided with fold lines along a crease so as to have a bi-stable configuration, said at least one wall being collapsed proximal to the other of the opposing walls in a first configuration, and expanded more distally away from the other of the opposing walls in a second configuration to relieve build-up of pressure in the waste compartment by the movement of said at least one wall from the first configuration to the second configuration.

Accordingly, the invention provides the advantageous feature of a containment device with a dead-end waste compartment which minimizes the build-up of back pressures as the waste compartment fills up without leaking the contents of the device to the atmosphere.

For a better understanding of the present invention, reference will now be made, by way of example only, to the accompanying drawings in which:-

Figure 1 is a plan view of one embodiment of a device constructed in accordance with the present invention;

Figure 2 is a fragmentary sectioned view taken generally along the line II-II of Figure 1;

Figure 3 is a sectioned view similar to that shown in Figure 2, but of a second embodiment of a device constructed in accordance with the present invention;

Figures 4 and 5 are plan views similar to that shown in Figure 1, but of a third and fourth embodiment of a device constructed in accordance with the present invention;

Figure 6 is a fragmentary plan view similar to that shown in Figure 5, but of a fifth embodiment of a device constructed in accordance with the present invention;

Figures 7A and 7B are sectioned views taken along the line VII-VII of Figure 6, before and after, respectively, sufficient liquid has entered the waste compartment to expand outward the creased opposing wall;

Figure 8 is a fragmentary sectioned view taken along the line VIII-VIII of Figure 6; and

Figure 9 is a fragmentary plan view similar to that shown in Figure 6, but of a sixth embodiment of a device constructed in accordance with the present invention.

The invention is hereinafter described in connection with certain preferred embodiments, in which a particular flexible device is processed by a certain processor for amplification and detection of DNA.

Additionally, the invention is useful regardless of the peculiar construction of the device and/or processor, and regardless whether the device is processed horizontally or while inclined, as long as there is a waste compartment which receives liquid from a detection site, with the risk of the build-up of back pressure in such compartment.

Still further, it is useful regardless of the liquid contents of the device - that is, this invention does not concern or require any particular chemistry or reaction, so long as the reaction is contained in a

closed device. Hence, the invention is independent of the particular liquid reaction occurring at the detection chamber and is not limited just to detection of nucleic acid materials.

As shown in Figure 1, reaction cuvettes 10 useful with the present invention comprise those having an inlet port 22 for patient injection of sample liquid, which connects via a passageway 21 to a PCR reaction compartment 26. A seal 46 temporarily blocks flow out of compartment 26. When seal 46 is broken, liquid feeds via a passageway 44 to a detection chamber 40 having sites 41 comprising, preferably, beads anchored in place which will complex with any targeted analyte passing them from compartment 26, and then with reagents coming from the other reagent compartments. Those other compartments are compartments 30, 32, 34 and optionally additional compartments 36, each feeding via passageways 48, 50, and 52, to chamber 40. Each of those passageways is temporarily sealed at 56, and contains an appropriate reagent liquid (and possibly, residual air).

The details of the chemicals useful in all the compartments, and of the sites 41, are explained in more detail in the aforesaid EP-A-0 381 501. However, since the time of the invention of EP-A-0 381 501, the number of necessary compartments has been simplified. Hence compartments 26, 30, 32, and 34 preferably comprise:

Compartment 26, in addition to the patient liquid later added by the user, can include all the conventional reagents needed for PCR amplification, kept in place by temporary seal 25. This includes primers which are bound to one member of a binding pair, the other member of which appears in compartment 30 described below. A useful example of the binding member attached to a primer is biotin. (Seal 25 is burst by injecting sample.) Alternatively, the reagents can be injected with the sample, so that seal 25 is eliminated.

Compartment 30 comprises, preferably, an enzyme bound to a complexing agent, such as avidin, which is a member of a binding pair, the other member of that pair being bound to a targeted analyte in the reaction compartment 26 as described above. Hence, a useful reagent in compartment 30 is strep-avidin horseradish peroxidase (hereinafter, strep-avidin HRP).

Compartment 32 preferably comprises a wash solution as the reagent.

Compartment 34 preferably comprises a signal precursor, and any dye stabilizing agent which may be useful. Thus, for example, a useful reagent solution in compartment 34 is a solution of a leuco dye which is a conventional substrate for the enzyme of compartment 30.

The remaining compartments 36 are preferably eliminated, along with their passageways, but can be optionally added. Hence, if a second wash is desired prior to adding the leuco dye of compartment 34, then such wash is provided by compartment 34 and the leuco dye is moved to compartment 36, and so forth.

Compartment 40 feeds to compartment 42 via passageway 58. Compartment 42 is the waste-collecting compartment to which the invention is particularly applicable, as described hereinafter.

Roller 60 exemplifies the exterior pressure means used to burst sequentially each of the compartments to advance the contents of the respective compartment to detection chamber 40 sequentially. Roller 60 advances along path 62 having width "A".

Distances P1, P2, and so forth, between the exit locations for each burstable compartment are preferably equal.

Sealing of port 22 occurs by folding over the upper left corner of the cuvette, Figure 1, to crimp off passageway 21, as is described in US-A-5 154 888.

In accordance with the present invention, waste compartment 42 is intended to receive all excess liquids flowing past the detection sites in compartment 40, without creating back-pressure due to the absence of an outlet. This is achieved by forming waste compartment 42 comprising opposing side walls 70, 72, Figure 2, which provide the major interior surface area of the compartment (in contrast to side walls 80), that is, at least 51% of the total surface area. At least wall 72 has therein sufficient fold lines 74 to provide wall 72 with a bi-stable configuration. The fold lines are formed in at least one of the opposing walls of the pair 70, 72, as to project a bead out of the plane of that opposing wall. The fold lines and the bead can either be a continuous, closed loop, or a majority fraction of a closed loop, for example, at least 50% of the loop which would be formed if the fold lines and bead extended all the way around. Further, the fold lines and bead can either be at the perimeter of the waste compartment, or just inside that perimeter.

As shown in Figure 1, fold lines 74 form a closed loop, which most preferably traces a pattern, Figure 1, which is congruent with the overall shape and inside the perimeter of compartment 42 as determined by the side walls 80. Walls 80 connect walls 70 and 72, Figure 2, to form the sealed enclosure of the compartment except for incoming passageway 58. As shown, that shape is roughly a rectangle. Other shapes will be readily apparent.

The bi-stable configuration will be readily apparent. Initially, wall 72 is collapsed as shown in the solid lines, so it is proximal to wall 70. However, as liquid moves into compartment 42, wall 72

snaps outwardly along fold line 74, to occupy the phantom position, thus relieving any back-pressure which is created. In actuality, back-pressure first builds up to a point sufficient to snap wall 72 outwardly, at which point the pressure in compartment 42 becomes negative until more liquid comes in.

Optionally, more than one fold line can be present (not shown), to provide, for example, concentric shapes which in turn allow for greater expansion of the wall; for example, there could be included another fold line inside that of line 74, tracing a concentric rectangle.

Optionally, an expansion pad 90 is included, which when wetted tends to expand, further aiding in the process of pushing wall 72 to its outward position where it is distal to wall 70. Such pad can be any conventional sponge, such as a commercially available cellulose sponge dried to a compressed state.

As a further alternative embodiment, Figure 3, both walls of the waste compartment can have the fold lines so that both walls have a bi-stable configuration. Parts similar to those previously shown bear the same reference numeral, to which the distinguishing suffix "A" is appended.

Thus, waste compartment 42A is constructed as in the embodiment of Figure 2, except that wall 70A has a fold line 74A' which is similar to fold line 74A of wall 72A. The solid line positions are of course the collapsed configuration where the two opposing walls are proximal, whereas the phantom positions are the expanded configurations in which the walls are distal to each other. Greater expansion is possible when both walls are so provided. As before, optional pad 90A can be present, preferably adhered to one or the other of walls 70A or 72A if present.

The paths traced by passageways 44, 48, 50 and 52 need not be as shown, nor need they extend so far away from path 62 of roller 60. Instead, the passageways can be disposed so that the majority of their path length (at least one-half) is within path 62 of the roller, Figure 4. Parts similar to those previously described bear the same reference numeral, to which the distinguishing suffix "B" is applied.

Thus, cuvette 10B has inlet port 22B and all the compartments 26B, 30B, 32B, 34B, 36B, 40B and 42B as described with reference to the previous embodiments with passageways 44B, 48B, 50B and 52B, respectively, providing flow means connecting the upstream compartments with compartments 40B and 42B. Waste compartment 42B has the fold line 74B to allow at least wall 72B to snap outward to relieve back-pressure. However, unlike the previous embodiments, passageways 48B and 50B have a majority of their paths extending parallel and closely adjacent to the path of passageway 44B providing the flow means from compartment 26B, so that application of the roller pressure along path 62B having a width "A", will cause the roller to at some point compress each of the noted passageways along at least half of their length. Such coverage by the roller allows for better positive control of the emptying of each respective passageway. That is, as long as the roller is pinching off each passageway, including passageway 44B, which occurs up to point "X," there can be no "back-flow" into that passageway such as might disturb proper sequential delivery of reagents to the detection sites.

Optionally, each of the compartments 30B, 32B, 34B and 36B can be provided with a side-fill port 100, such that the filling proceeds by filling each compartment out to line 102, eliminating any air, and thereafter heat-sealing the opposing walls together at 104 through the liquid, as is conventional. This ensures that no air bubbles will be pushed by the external roller into compartment 40B where they might interfere with the liquid-phase reactions which occur.

However, each passageway in the embodiment of Figure 4 has a substantial length from its respective burstable compartment, to the location where it joins the other passageways just upstream of compartment 40B. This is the feeder portion of each passageway. It is not necessary that this be so. Rather, the feeder portion length of the passageway from its compartment to the junction location with other passageways can be minimized to the extent that the length is less than the maximum diameter of the burstable compartment from which it extends, Figure 5. Parts similar to those previously described bear the same reference numeral, to which the distinguishing suffix "C" is applied.

Thus, cuvette 10C has inlet port 22C and all the compartments 26C, 30C, 32C, 34C, 40C and 42C as described with reference to the previous embodiments, with passageways 44C, 48C, and 50C, respectively, providing the flow means connecting the upstream compartments with compartments 40C and 42C. Waste compartment 42C has the fold line 74C to allow at least wall 72C to snap outward to relieve back-pressure.

However, unlike the previous embodiments, each passageway 48C and 50C has a junction with passageway 44C such that the length L of the passageway from its respective burstable storage compartment, to the junction, is less than the maximum dimension D of its storage compartments. (As shown, that dimension is measured from the future exit aperture of the compartment to an opposite point closest to the next upstream compartment, due to the tear-drop shape of the compartments.)

In fact, most preferably L is less than one-half of D for a respective compartment. Such an arrangement further minimizes back-flow of reagent from an upstream compartment into the passageway length L, prior to expulsion of the contents of the storage compartment through length L. This in turn minimizes undesired side-reactions which might occur between reagents in path length L rather than in compartment 40 where they are desired.

As before, preferably roller path 62C covers the majority of the path lengths of the passageways.

Optionally, an air vent path 200 can be provided from reaction compartment 26C back into a sealed portion of the pouch, for example, to dead storage area 202 of the pouch, to minimize build-up of back-pressure such as might inhibit ingestion of sample from port 22C along passageway 21C. However, as with all flow lines and compartments, path 200 is also sealed from leakage to the atmosphere to provide positive containment against leakage of amplified nucleic acid material which could cause carry-over contamination.

Inlet port 22C and passageway 200 are preferably closed and sealed, following sample injection, by folding over the corner as with the previous embodiments, all as described in the aforesaid US-A-5 154 888.

It is not necessary that the fold line of the waste compartment providing the bi-stable configuration be spaced inside the perimeter, or that the fold line crease form a completely closed loop. An alternative to these is shown in Figures 6 to 8, where parts corresponding to those previously described bear the same reference numeral to which the suffix "D" is appended.

Thus, cuvette 10D, Figure 6, is constructed as described in the previous embodiments, except that waste compartment 42D has a fold line 74D, Figure 6, in opposing wall 72D, Figure 7A, forming a crease or bead which does not join itself to form a closed loop, and it is at the periphery of the compartment, rather than spaced inside. Thus, fold line 74D is formed into parts 174 and 176 which are a majority fraction of the periphery, or a majority of what would be a closed loop if it did extend to join both parts 174 and 176 together. ("Majority" as applied to fold line 74D means, at least 50%, since amounts less than this are unlikely to allow wall 72D, Figure 7A, to move far enough out when liquid L enters, Figure 7B.)

When liquid enters compartment 42D, wall 72D eventually pops out from its collapsed configuration or position, Figure 7A, to its expanded, second configuration or position, Figure 7B, due to its bi-stable construction. Only the portion 178 of wall 72D which is pinch-sealed to opposing wall 70D, Figure 8, remains un-expanded.

Side wall 80D is unaffected by the in-flowing liquid. That is, as in the previously described embodiment, it does not expand sideways from its original position shown in Figure 7B, as indeed it cannot since it is sealed at 180 to opposing wall 70D.

All of the periphery, for example, at portions 180, Figure 7A, of compartment 42D is sealed shut permanently by sealing wall 72D to wall 70D at those locations, except for passageway 58D, Figures 6 and 8.

Yet another example is shown in Figure 9, wherein the same reference numerals are used for similar parts, with the exception of the distinguishing suffix "E". Thus, as in previous embodiments, cuvette 10E features a waste compartment 42E having fold lines 74E in one of its paired opposite walls 72E which forms the major interior surface area of the compartment. However, in this case the fold lines form a beaded crease generally in the shape of an "H", comprising a cross-member 190 and legs 192 and 194. The linear extent of the crease, defined as $(L_1 + 4 \times L_2)$, is such as to comprise at least 50% of what would exist if lines 74E formed a closed loop around the periphery. The expansion of wall 72E outward will, of course, peak along cross-member 190, when liquid enters compartment 42E.

Claims

1. A containment device (10; 10A; 10B; 10C; 10D; 10E) for use in amplifying and detecting nucleic acid material comprising:-

an inlet port (22; 22B; 22C) through which a sample is inserted into the device (10; 10A; 10B; 10C; 10D; 10E), the device (10; 10A; 10B; 10C; 10D; 10E) being sealable after sample insertion to prevent leakage of nucleic acid material;

a reaction compartment (26; 26B; 26C) in which amplification of nucleic acid material takes place;

a detection site (40, 41; 40B; 40C) for detecting the nucleic acid material;

flow means (44; 44B; 44C) for allowing fluid flow from the reaction compartment (26; 26B; 26C) to the detection site (40, 41; 40B; 40C); and

a waste compartment (42; 42A; 42B; 42C; 42D; 42E) downstream of the detection site (40, 41; 40B; 40C) and fluidly connected thereto to receive reagents and material after passage over the detection site (40, 41; 40B; 40C), the waste compartment (42; 42A; 42B; 42C; 42D; 42E) comprising a pair of opposing walls (70, 72; 70A, 72A; 70B, 72B; 72C; 70D, 72D; 72E);

characterized in that at least one of the opposing walls (72; 70A, 72A; 72B; 72C; 72D; 72E) is provided with fold lines (74; 74A, 74A'; 74B; 74C; 74D, 174, 176; 74E) along a crease so as to have a bi-stable configuration, said at least one wall (72; 70A, 72A; 72B; 72C; 72D; 72E) being collapsed proximal to the other of the opposing walls (70; 70A, 72A; 70B; 70D) in a first configuration, and expanded more distally away from the other of the opposing walls (70; 70A, 72A; 70B; 70D) in a second configuration to relieve build-up of pressure in the waste compartment (42; 42A; 42B; 42C; 42D; 42E) by the movement of said at least one wall (72; 70A, 72A; 72B; 72C; 72D; 72E) from the first configuration to the second configuration.

2. A device according to claim 1, wherein both opposing walls (70A, 72A) have the fold lines (74A, 74A') and the bi-stable configuration.

3. A device according to claim 1 or 2, wherein the waste compartment (42; 42A; 42B; 42C; 42D; 42E) further comprises compartment side wall portions connected to the opposing walls (70, 72; 70A, 72A; 70B, 72B; 72C; 70D, 72D; 72E) so as to give to the compartment a predetermined shape when viewed in plan, and wherein the fold lines (74; 74A, 74A'; 74B; 74C; 74D, 174, 176; 74E) form a shape congruent with but smaller than the predetermined shape.

4. A device according to claim 1 or 2, wherein the fold lines (74; 74A, 74A'; 74B; 74C) form a crease which is a closed loop.

5. A device according to claim 4, wherein the loop forms a shape which is concentric with the overall shape of the waste compartment (42; 42A; 42B; 42C).

6. A device according to claim 1 or 2, further including at least one storage compartment (30B, 32B, 34B, 36B; 30C, 32C, 34C)) and an associated fluid passageway (48B, 50B, 52B; 48C, 50C) extending between the storage compartment (30B, 32B, 34B, 36B; 30C, 32C, 34C) and the detection site (40B; 40C) along a curved path, at least half of the fluid passageway being parallel to and closely adjacent to at least half of the flow means (44B; 44C) so that a roller applied to transfer the contents of the reaction compartment (26B; 26C) and each storage compartment (30B, 32B, 34B, 36B; 30C, 32C, 34C) can be moved along in a path (62B; 62C) which will also cover at least half passageway (48B, 50B, 52B; 48C, 50C) and flow means (44B; 44C).

7. A device according to claim 6, wherein each passageway (48C, 50C) exiting from each of the compartments (30C, 32C, 34C) joins the others at a location upstream from the detection site (40C), the length (L) of the passageway (48C, 50C) from each storage compartment (30C, 32C, 34C) to the location being less than the maximum dimension (D) of the storage compartment (30C, 32C, 34C) so that the opportunity for back-flow of reaction material within the passageway length is minimized.

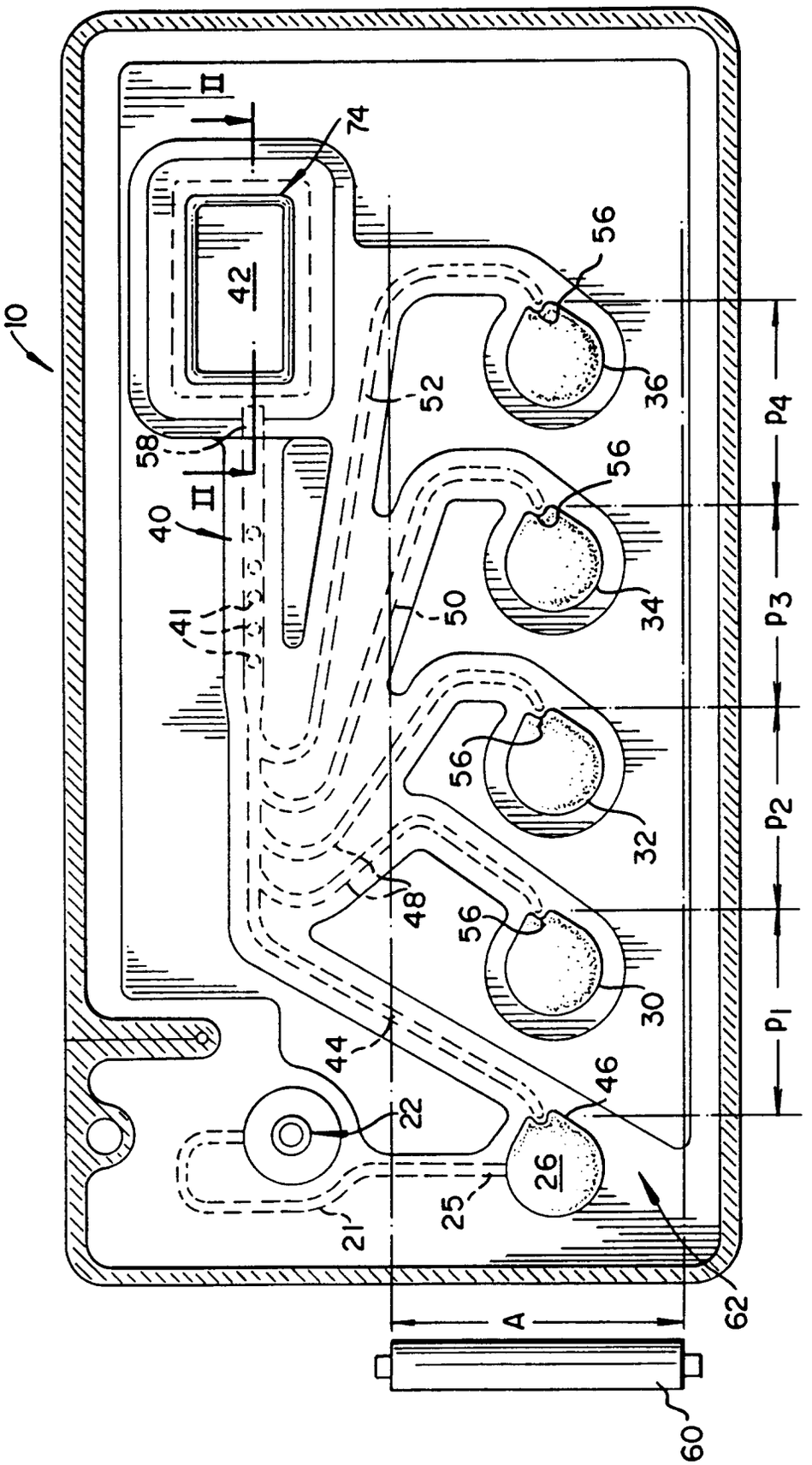

10
II
74
42
58
II
40
41
52
50
48
44
22
21
25
26
46
56
36
34
32
30
62
A
60
P4
P3
P2
P1

FIG. 1

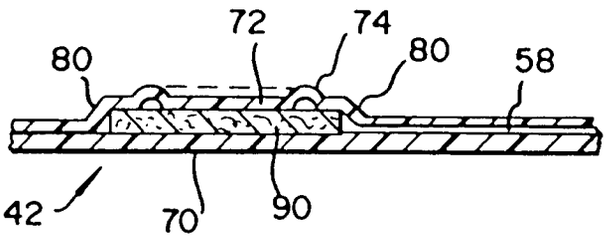
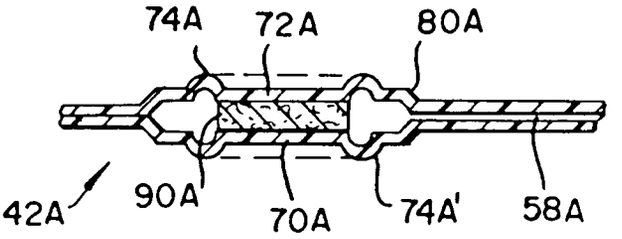


FIG. 2

FIG. 3



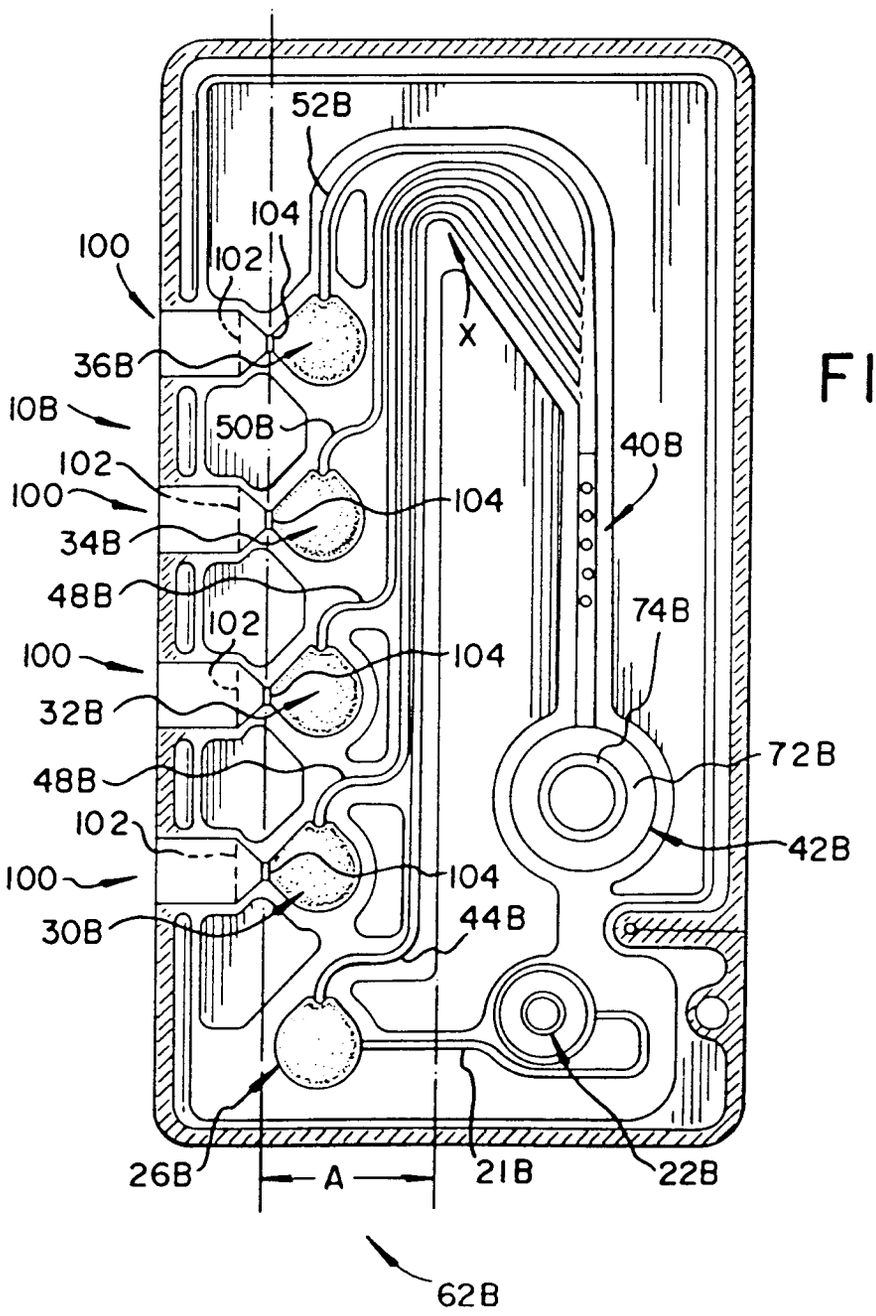
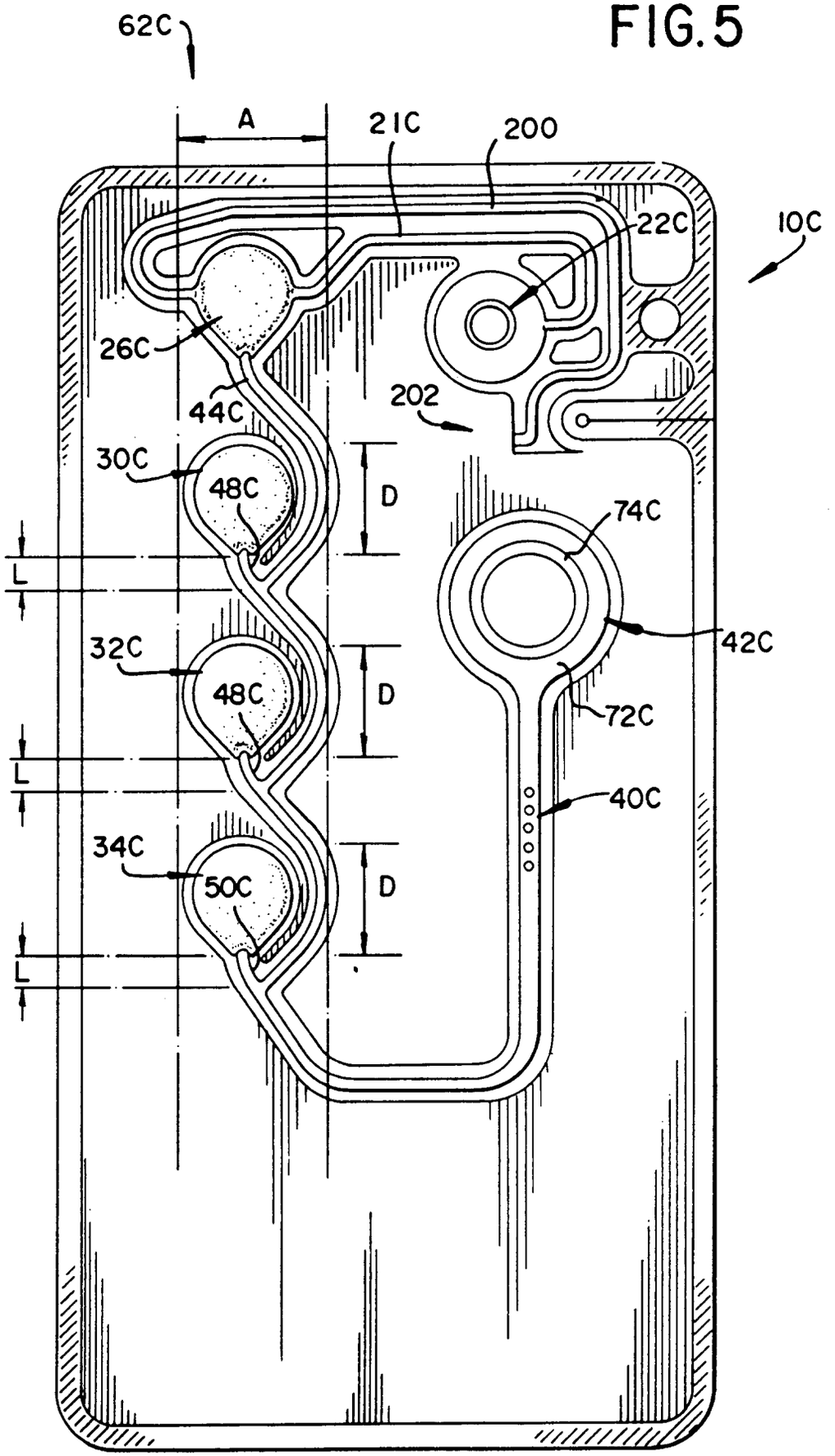


FIG. 4

FIG.5


62C
A
21C
200
22C
10C
26C
44C
202
30C
48C
D
74C
L
32C
48C
D
42C
72C
L
40C
34C
50C
D
L

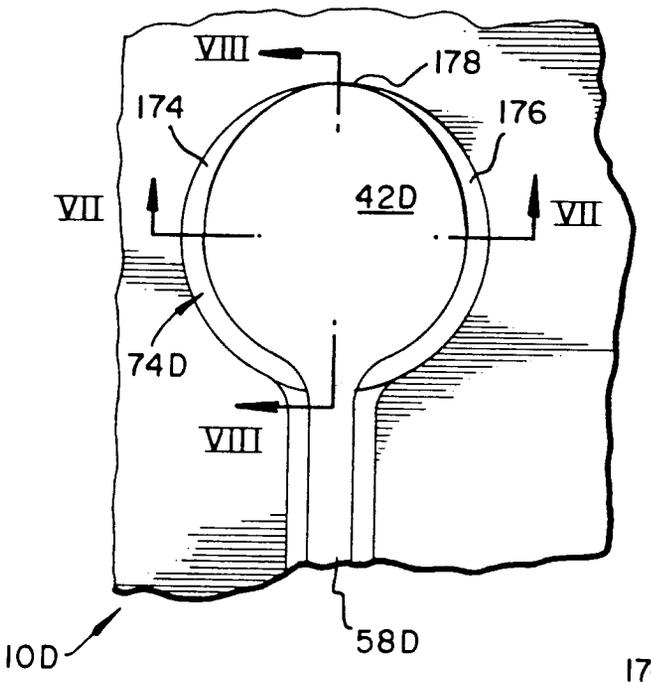


FIG. 6

FIG. 7A

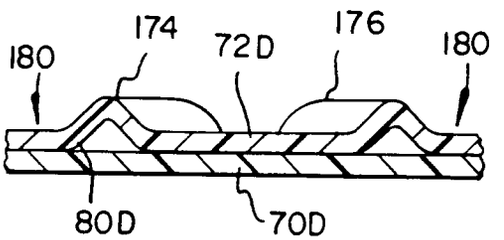


FIG. 7B

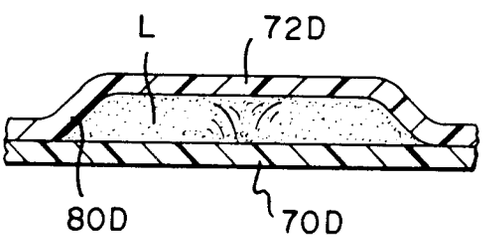
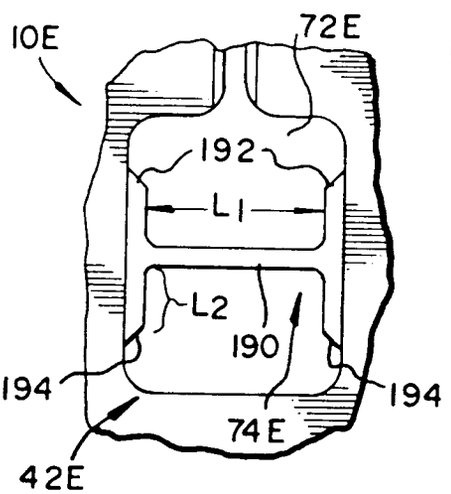


FIG. 8



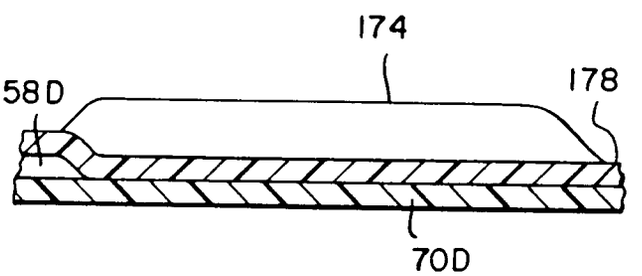


FIG. 9

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 93 20 2961

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
D,A	EP-A-0 381 501 (EASTMAN KODAK) * column 17, line 2 - line 13; figures 2,5,19 * * column 17, line 42 - line 49 * * column 18, line 36 - line 45; figure 9 * * column 19, line 28 - line 37 * * column 21, line 14 - line 18 *	1	B01L3/00
A	EP-A-0 287 170 (PROCTER & GAMBLE COMPANY) * column 1, line 34 - line 50 * * column 2, line 4 - line 10 *	1	

TECHNICAL FIELDS SEARCHED (Int.Cl.5)

B01L
B65D

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	7 February 1994	Hocquet, A

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)