



- (51) International Patent Classification:
C12M 1/26 (2006.01) *G01N 1/30* (2006.01)
- (21) International Application Number:
PCT/ZA2012/000014
- (22) International Filing Date:
7 March 2012 (07.03.2012)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
2011/05403 22 July 2011 (22.07.2011) ZA
- (71) Applicant (for all designated States except US): **JOHNSON, Basil, F.** [ZA/ZA]; 31 Park Drive, #3 Ocean Dream, Umhlanga Rocks, Kwa Zulu Natal, 4319 Durban (ZA).
- (72) Inventor; and
- (75) Inventor/Applicant (for US only): **ANDRESEN, Constance, Wilson**; 31 Park Drive, #3 Ocean Dream, Umhlanga Rocks, Kwa Zulu Natal, 4319 Durban (ZA).

- (74) Agent: **EDWARD NATHAN SONNENBERGS**; 1 Richefond Circle, Ridgeside Office Park, Umhlanga, 4320, Kwa Zulu Natal, P O Box 3052, Kwa Zulu Natal, 4000 Durban (ZA).

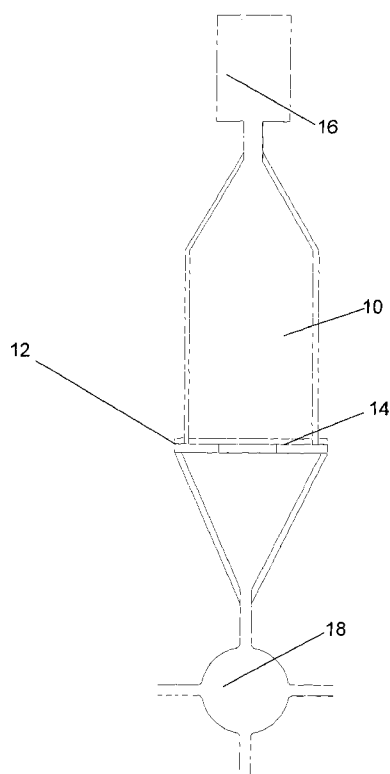
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG,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

- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,

[Continued on next page]

- (54) Title: DEVICE AND METHOD FOR RAPID AND ACCURATE ANALYSIS OF BODY FLUIDS

FIGURE 1



- (57) Abstract: The invention relates to a method of analysis of menstrual blood collected by a tampon or the like, which blood would include cells of clinical interest. Blood is deposited in a container (10) provided with a filter (12) having a pore size such that all cells of clinical interest in the blood specimen cannot pass through the filter while cells which are not of clinical interest, for example red and white blood cells are small enough to pass through the filter (12), subjecting the menstrual blood cells in the container to washings and back washings, and further subjecting the suspended cells to the approximately 18 Pap solutions after which the cells remaining in the container (10) may be deposited on a glass slide and analyzed by a cytologist. The invention also extends to the device used in Pap analysis.



LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG).

— *before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments (Rule 48.2(h))*

Published:

— *with international search report (Art. 21(3))*

DEVICE AND METHOD FOR RAPID AND ACCURATE BODILY FLUID ANALYSIS**TECHNICAL FIELD OF THE INVENTION**

The invention relates to device and method for rapid and accurate bodily fluid analysis.

BACKGROUND ART

The current Pap test requires the gynaecologist to take a specimen by scraping or smear from the cervix, using a spatula or swab and rubbing the material onto a slide. The slide is sprayed with a fixative to preserve the specimen and is then transported to the lab. The specimen is then placed into a slide holder (along with many other slides – as many as twenty or more, and from any other patients, and dipped either by hand or by automatic dipping machine into the various Pap Test solutions (approximately 18) each of which is contained in its own vessel. The vessels used are small, usually glass, and usually rectangular tubs.

During this process, cells fall or float off each patient's slide and settle to the bottom of the vessel and/or stick to other patient's slides. After several sets of slides have dipped into a vessel, a layer of specimen material, including cells, may be found at the bottom of the vessel. Moreover, with each dipping, the composition of the solution in each vessel is changed by carry over of solution from the preceding vessel and by material floating or falling off the slides.

This overall procedure is time consuming and can be wrought with many inaccuracies resulting from the above steps which cause variable output in terms of stained slides, with each set of slides also differing from each other in terms of staining.

The issued Andresen Patent Nos. 5 247 941 and 5 327 897 describes a method for collection and analyzing blood by introducing into a cavity of the person, for example the vagina in the case of a woman, of a device which has an interior capable of receiving and holding blood which is present in the cavity. For example, the device may be a tampon inserted by a woman into the vagina for collecting the blood during menstrual flow. The blood thus collected can be subjected to analysis, for example Pap Rest analysis.

This method and device for collecting blood as set forth in the Andresen Patent Nos. 5 247 941 and 5 327 847 provide a greater accuracy for analysis than does the ordinary Pap smear or scraping test. However, the very simplicity of the method and device of the Andresen patents leads to the problem of a need for a more rapid and possibly automated analysis system

as described because the method and device do not require the gynaecologist or nurse examiner, and the laboratories would be flooded with collections that would be requiring analysis. Still further, greater accuracy of analysis is required and will lead to better results because of the large number of collections obtained for analysis.

DISCLOSURE OF THE INVENTION

It is accordingly a primary object of the present invention to provide a method for rapid and accurate analysis of blood, particularly blood collected by tampons and the like during menstrual flow.

It is another object of the present invention to provide a device for receiving a tampon or the like containing menstrual blood flow and fluid for rapid and accurate treatment in a device in which all of the stages of a Pap Test can be carried out up to the point of analysis of cells deposited on a glass slide. It is yet another object of the present invention to provide a device for sequential contact with Pap solutions for rapid and accurate analysis of cells. Other objects and advantages of the present invention will be apparent, from a reading of the specification.

SUMMARY OF THE INVENTION

With the above and other objects in view, the present invention mainly comprises a method of analysis of menstrual blood flow wherein menstrual blood collected by a tampon or the like, which blood would include cells of clinical interest, is deposited in a container provided with a filter having a pore size such that all cells of clinical interest in the blood specimen cannot pass through the filter while cells which are not of clinical interest, for example red and white blood cells are small enough to pass through the filter, subjecting the menstrual blood in the container to washings and back washings, for example with saline solution or balanced salt solution, e.g. Ringer's solutions, and further subjecting the suspended cells to the approximately 18 Pap solutions after which the cells remaining in the container may be deposited on a glass slide and analyzed by the cytologist. The analysis may be by way of standard slide examination or utilizing computers.

The filter used in the container of the invention is preferably a 12-micron pore size filter. Cells of clinical interest are substantially larger than 25 microns and cannot go through the pores of the filter. On the other hand, the cells that are not of clinical interest, for example red and white blood cells which have a size of about 7 microns, are small enough to go through the filter pores and are removed from the specimen along with the blood plasma. This is accomplished by repeated washings and back washings.

Although 12-micron pore filters are preferred, it is possible to use filters in the range of 5-12 microns. When filters of 5 – 8 micron size are used, 7-micron red blood cells and 12-micron white blood cells can still be forced through the filters by pressure.

This same container, which now contains only the cells of clinical interest is then subjected to the approximately 18 Pap solutions which are generally sequentially introduced into the container in an amount to fill the same, then removed until the final cells are stained in accordance with the Pap process, after which the cells are evenly deposited on a glass slide with or without a filter.

By proceeding in accordance with this method, cells of clinical interest in any specimen are entrapped because these cells cannot enter or leave, so that there is no loss or gain of cells of clinical interest. All other cells and fluids are washed out so that at the end only a clean suspension of cells of clinical interest is obtained which cells are processed through the Pap solutions, in suspension, reducing overall time, increasing quality and resulting in uniformity of analysis. The overall process results in a saving in labour, materials, time, and of course therefore in costs.

The increased quality and greater uniformity of test results by proceeding in accordance with the method of the present invention are achieved as a result of many advantages derived from proceeding in accordance with the method of the invention.

Thus, for example, the washings and Pap solutions used according to the present invention cannot result in either the loss of cells of clinical interest or the contamination of the cells in the container by another patient's cells. Thus, cells of clinical interest cannot get in or out of the container and other cells cannot get into the container.

The time required for contact of the cells with the various Pap solutions is substantially reduced as compared to proceeding in accordance with the normal Pap procedure because the cells are in suspension rather than being flat on a slide. Furthermore, the amount of solution is greatly reduced since only a few ml of solution are required to fill the container.

In accordance with the method of the invention, fresh solution is used each time with the solution sent to drain after each use, so that each specimen is stained in the same consistent way. Furthermore, at the end of the Pap process, the cells are evenly deposited on the glass slide, with or without the filter.

The general procedure according to the present invention is as follows:

The tampon used during menstrual flow, after removal from the woman, is placed in a vial containing fixative solution. The vial is sealed and transported to the laboratory. At the laboratory the vial is shaken, inverted, swirled, or mildly sonicated to move cells from the tampon into the fixative solution and to create a cell suspension in the fixative solution. The tampon may be removed and discarded, alternatively the vial's reciprocal cap may be punctured and the cell suspension extracted from the vial, following which the vial containing the tampon may be discarded.

The cell suspension is poured into a disposable plastic syringe with a filter holder containing a 5-12 micron pore filter, preferably a 12 micron pore filter. The cells are washed and back washed by passing solutions through the filter until the blood and mucous have been removed. After the last washing, the cells are subjected to the standard Pap solutions, left on the filter and the filter is clipped to a glass slide and analyzed by the cytologist.

For large scale analyses the procedure is as follows:

Each of the tampons is inserted into a separate vial containing a fixative solution and the vials are placed into machines which mildly sonicate the same to move cells from the tampon into the fixative solution and to create a cell suspension in the fixative solution. The cell suspension is aspirated by the machine into a modified disposable plastic syringe with a filter holder containing 12 micron filter. The vial and tampon are removed and discarded by the machine.

The machine automatically works the modified disposable plastic syringe to wash and backwash the cells until the blood and mucous have been removed. The machine then automatically moves the modified disposable plastic syringe to expose the cells sequentially to each and all of the solutions used in the standard Pap method. This can be done either by moving the syringe to each solution in turn, or by moving each solution in turn to the syringe. In either case, a solution is used once and then sent to drain.

After the standard Pap method treatment has been completed, the machine can either place the filter on a glass slide or centrifuge the cells onto a glass slide. In either case, the cells are ready to be read by the cytologist without ever having been touched by any hands, completely automatically.

In accordance with another embodiment, the cell suspension after the standard Pap Test has been completed is introduced into a currently commercially available cell counter/sorter (laser microscope) which reads the cells, separates abnormal and suspicious cells, and puts them on a slide for the cytologist. The machine also prints out a report. This possibility does not exist in the case of the ordinary Pap smear test because it is not possible by such test to obtain a sufficient number of cells in suspensions. This is only possible with the collection of menstrual blood by a tampon or the like and by proceeding as set forth herein.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is illustrated by way of example in the accompanying drawings which form part of this invention and in which:

Figure 1 is a schematic illustration of a device according to the invention in which a vial or cylinder is provided with a single filter;

Figure 2 is schematic illustration of another embodiment of the invention in which a vial or cylinder is provided with two filters;

Figure 3 is a schematic illustration of a device for sonicating specimens in the original collection container;

and

Figure 4 is a schematic illustration of a device wherein liquids used in processing the cells are controlled by valves.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Figure 1 illustrates a cylinder 10 provided with a filter 12 at one end thereof so that treating liquid may be introduced through the filter and discharged through the same filter. The filter 12 is held by filter holder ring 14. The treating liquid is alternately driven in and out of the cylinder 10 through the filter 12 by means of pressure/vacuum pump 16 with control by means of solenoid valve 18.

In the embodiment of Figure 2, the cylinder 10 is provided with two filters 12, one at each end of the cylinder, each held by a filter holder ring 14. Pump 16 provides suction for drawing the treating liquid in one direction through the filters 12, with valves 18 opening and closing as needed.

As illustrated in Figure 3, a collection container 20 containing the specimen 22 is sonicated by means of sonicator 24 to move the cells into suspension in the treating liquid. By means of aspirator 26 and pump 28 the liquid 22 containing the suspended cells is aspirated, with the aid of

pump 28 into cylinder 30 provided with a filter 32 which is held by filter holder ring 34. The thus aspirated suspended specimen is then subjected to treatment with the Pap solutions.

As illustrated in the embodiment of Figure 4, reservoirs 36A, 36B, 36C, etc., each containing a different Pap solution used in the Pap method is alternately supplied by means of control valves 38 and 40 to the cylinder for treatment of the cells therein. Each liquid, alcohol, stain, etc., is passed into the cylinder and then withdrawn from the cylinder. Supply 42 illustrates the possibility of various treating solutions entering the system from a different entrance.

In carrying out the method of the present invention, the tampon after removal from the woman is placed into a vial containing a fixative solution. The fixative is used in cytology to maintain the existing form and structure of the cells, particularly the nuclear detail. The fixative solution may be, for example, 10% neutral buffered formalin or dilute methyl or ethyl alcohol, or it may be a mixture of alcohol and wax, which forms a thin, protective coating over the cells and preserves the same until they are processed in the cytology laboratory.

At the laboratory, the vial containing the tampon in the fixative solution is shaken or sonicated to create a cell suspension in the solution. The tampon is removed and discarded, alternatively the vial's reciprocal cap may be punctured and the cell suspension extracted from the vial, following which the vial containing the tampon may be discarded.

The cell suspension is introduced into the disposable plastic syringe provided with a filter holder containing a 5 – 12 micron filter, preferably 12-micron filter. The cells are washed and backwashed until blood and mucous have been removed leaving only the cells of clinical interest on the filter. These cells are then subjected to the Pap stain solutions for visualization of cellular changes. The stain is basically composed of a blue nucleus stain and orange, red and green cytoplasmic counter stains. The nuclear stain demonstrates the chromatin patterns associated with normal and abnormal cells, while the cytoplasmic stains help to indicate cell origin. The cells are generally subjected to about 18 or more different staining solutions, after which the filter is placed up on a glass slide or the cells, are centrifuged onto the glass slide, after which the cytologist can read the slide.

While normal pap testing provides only about ninety percent accuracy, the above method provides accuracy approaching one hundred percent.

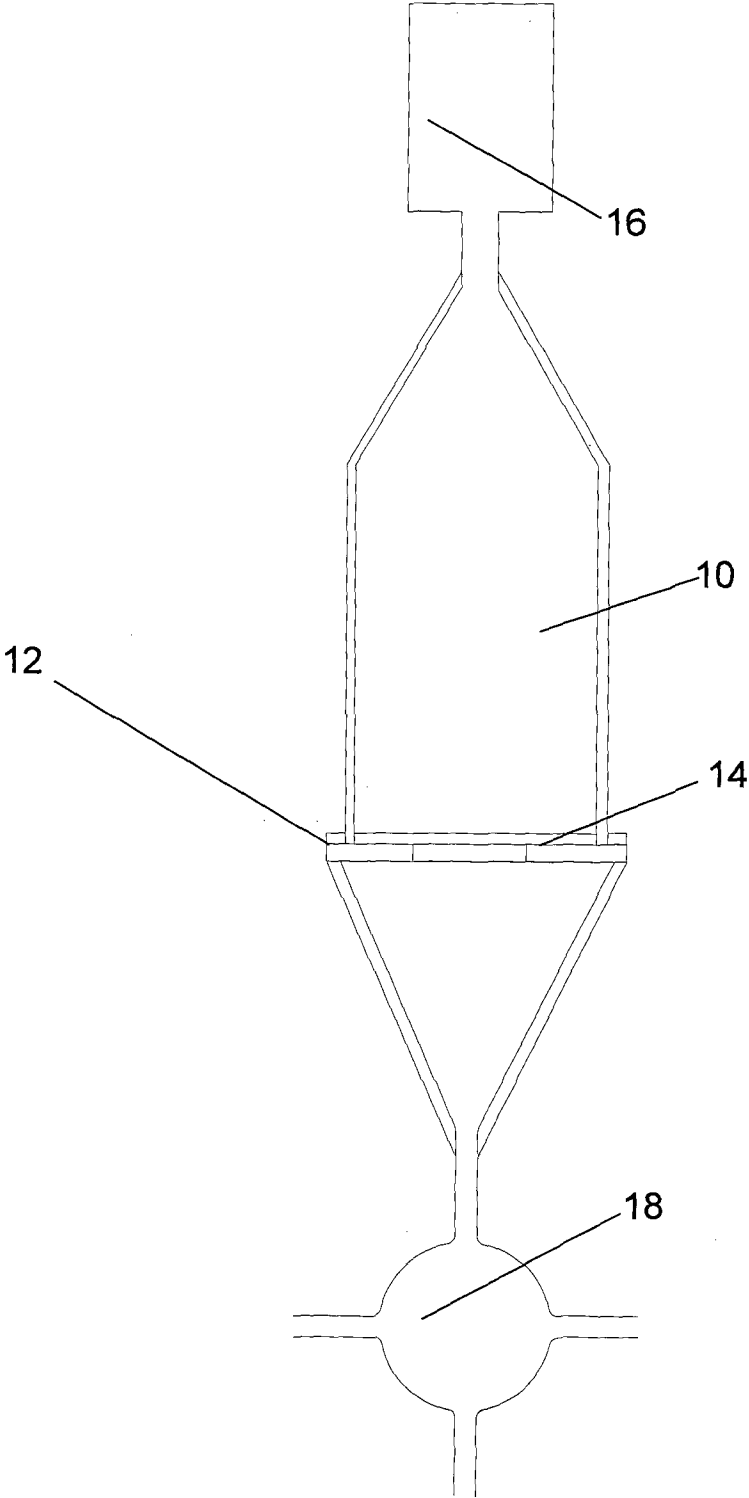
CLAIMS:

1. A method of Pap analysis characterised in that the method comprises the steps of;
introducing a collected menstrual blood specimen into a container provided with a filter having a pore size sufficiently large enough to permit cells not of clinical interest to pass there through, and sufficiently small enough to prevent cells of clinical interest from passing there-through,
subjecting the thus collected specimen to washings through the filter to remove blood, mucous and other cells not of clinical interest from the container while retaining cells of clinical interest on the filter;
subjecting the cells collected on the filter to Pap solutions;
and
making the cells treated by the Pap solution available for analysis by a cytologist on a slide or by computer analysis.
2. A method of Pap analysis as claimed in claim 1 characterised in that the filter has a pore size of approximately 5-12 microns.
3. A method of Pap analysis as claimed in claim 2 characterised in that the filter has a pore size of approximately 12 microns.
4. A method of Pap analysis as claimed in any of claims 1 to 3 characterised in that collected menstrual blood is contacted with a fixative solution, the fixative solution and menstrual blood are shaken or sonicated to suspend cells from the blood specimen in the fixative solution and the resultant fixative solution containing the suspended cells is introduced into the container provided with the filter, after which the washings and Pap solution treatment steps proceed.
5. A method of Pap analysis as claimed in claim 4 characterised in that the cell suspension is aspirated into the container provided with the filter.
6. A method of Pap analysis as claimed in either of claims 4 or 5 characterised in that the menstrual blood is collected by means of a tampon which is introduced into the fixative solution and after shaking or sonication to suspend the cells in the fixative solution, the tampon is removed and discarded.

7. A method of Pap analysis as claimed in any of claims 1 to 6 characterised in that the container is provided with a single filter and the specimen is washed and back –washed through the same filter.
8. A method of Pap analysis as claimed in any of claims 1 to 6 characterised in that the container is provided with filters at each end and the washing is effected by one way passage through the filters.
9. A method of Pap analysis as claimed in any of claims 1 to 8 characterised in that the container containing the filter with cells of chemical interest thereon is sequentially filled and emptied with each of the pap solutions for staining the cells in a manner such as to permit cytological examination thereof.
10. A device for Pap analysis characterised in that the device comprises;
a container provided with openings at two ends,
at least one filter secured in said container, said filter having a pore size sufficiently small enough to prevent cells of clinical interest for Pap analysis from passing there-through, but sufficiently large enough to permit cells not of such clinical interest to pass there through,
and
means for passing washing fluid through the filter to remove cells not of clinical interest from the container, the cells of clinical interest being retained by the filter.
11. A device for Pap analysis as claimed in claim 10 characterised in that the container is provided with filters at both ends.
12. A device for Pap analysis as claimed in either of claims 10 or 11, characterised in that the device includes means for controlling fluid flow through the filter and means for closing and opening the openings at each end of the container for the washing of the specimen contained therein and removal of fluid and cells not of clinical interest through the filter.

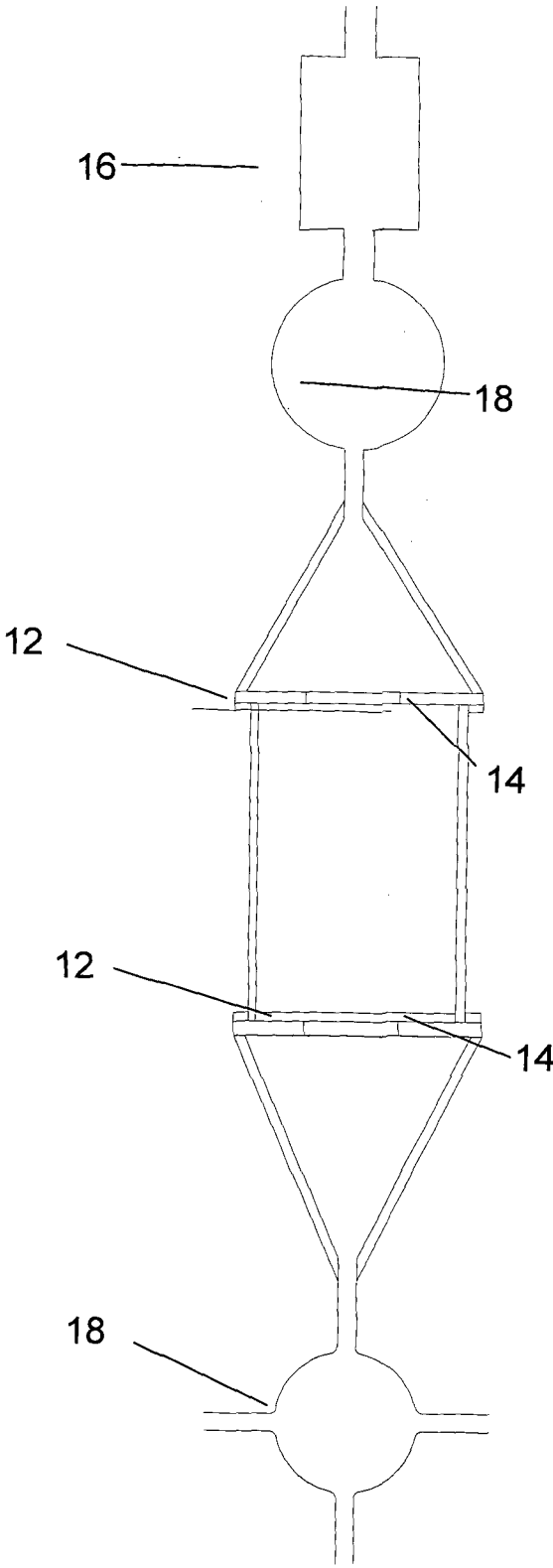
1/3

FIGURE 1



2/3

FIGURE 2



3/3

FIGURE 3

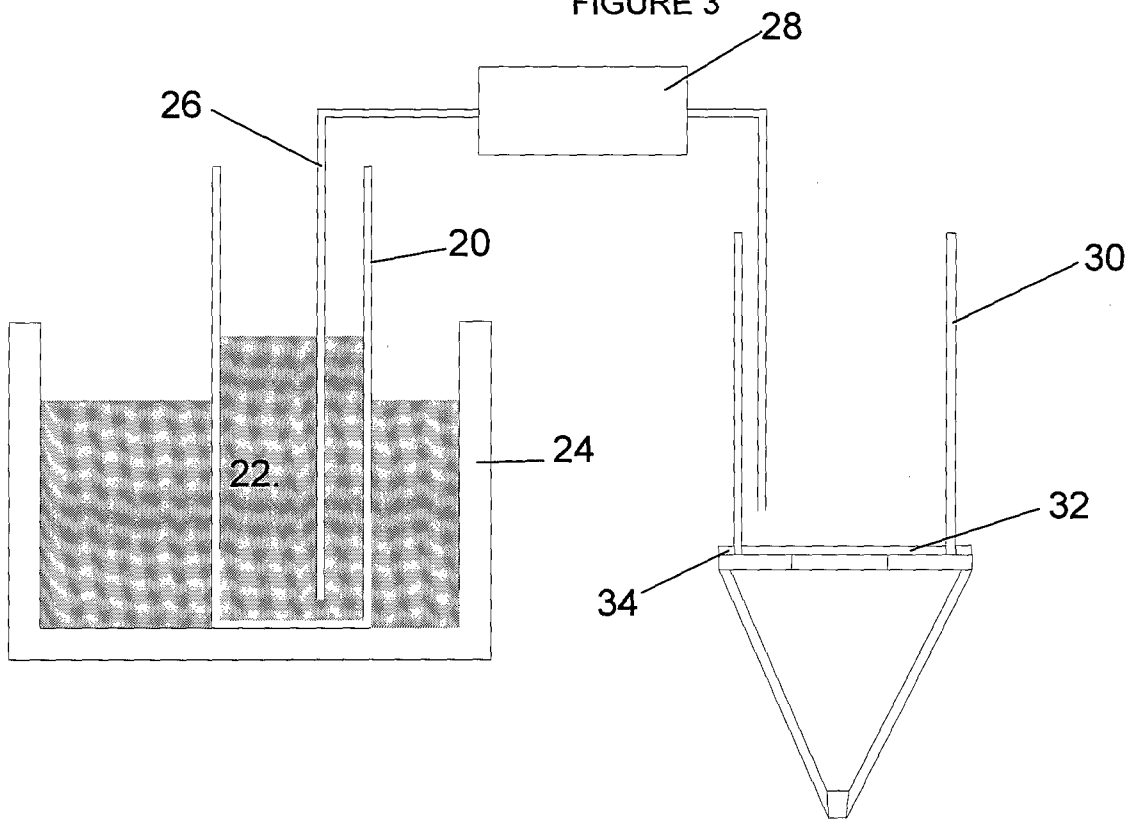
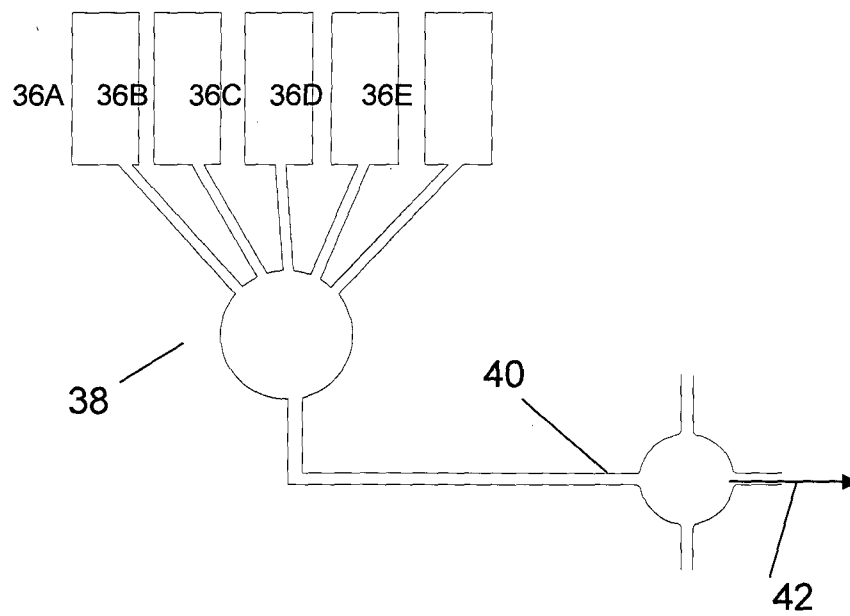


FIGURE 4



INTERNATIONAL SEARCH REPORT

International application No.

PCT / ZA 2012/000014

A. CLASSIFICATION OF SUBJECT MATTER IPC: C12M 1/26 (2006.01); G01N 1/30 (2006.01) According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C12M, G01N Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPODOC, WPI, X-FULL		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0483506 B1 (MICROBYX CORPORATION) 30 July 1997 (30.07.1997) Whole document	1-12
☐ Further documents are listed in the continuation of Box C.		☒ See patent family annex.
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 16 November 2012 (16.11.2012)		Date of mailing of the international search report 03 December 2012 (03.12.2012)
Name and mailing address of the ISA/AT Austrian Patent Office Dresdner Straße 87, A-1200 Vienna Facsimile No. +43 / 1 / 534 24-535		Authorized officer KÖNIG H. Telephone No. +43 / 1 / 534 24-339

PCT / ZA 2012/000014