(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 503 209 A1

(12) **EUROPEAN PATENT APPLICATION**
published in accordance with Art. 158(3) EPC

(43) Date of publication:
02.02.2005 Bulletin 2005/05

(21) Application number: **02724714.7**

(22) Date of filing: **08.05.2002**

(51) Int Cl.7: **G01N 30/00**, G01N 31/20, G01N 33/53, G01N 33/48, G01N 37/00, G01N 35/02

(86) International application number:
PCT/JP2002/004458

(87) International publication number:
WO 2003/096008 (20.11.2003 Gazette 2003/47)

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR

(71) Applicant: **Hitachi High-Technologies Corporation**
Tokyo 105-8717 (JP)

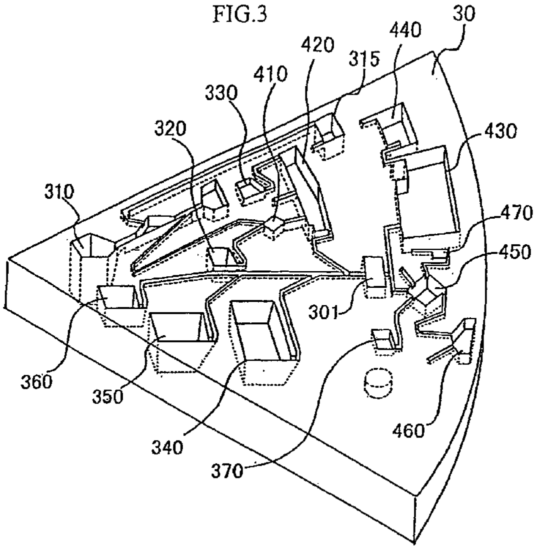
(72) Inventors:
- **NAGAOKA, Yoshihiro, Mechanical Eng. Res. Lab. Tsuchiura-shi, Ibaraki 300-0013 (JP)**
- **WATANABE, Naruo, Mechanical Eng. Res. Lab. Tsuchiura-shi, Ibaraki 300-0013 (JP)**
- **TAKENAKA, Kei, Mechanical Eng. Res. Lab. Tsuchiura-shi, Ibaraki 300-0013 (JP)**
- **OHASHI, Tomoki, Mechanical Eng. Res. Lab. Tsuchiura-shi, Ibaraki 300-0013 (JP)**
- **MIYAHARA, Yuji, c/o Design & Manufacturing Group Hitachinaka-shi, Ibaraki 312-8504 (JP)**

(74) Representative: **Beetz & Partner Patentanwälte**
Steinsdorfstrasse 10
80538 München (DE)

(54) **CHEMICAL ANALYZER AND GENE DIAGNOSING APPARATUS**

(57) In a chemical analysis apparatus for extracting a specific substance, such as nucleic acid, from a sample containing a plurality of chemical substances, solutions remaining in valves are prevented so that a reagent in an earlier step does not contaminate the subsequent steps. Predetermined quantities of solutions are carried and supplied by centrifugal force without providing valves for controlling the flow of the solutions. After perforating the lids of ventilation holes 272, 273, 274 communicated with a detection container 450 and disposal containers 460 and 470 in an analysis disc 2, the analysis disc 2 is rotated to supply and carry a predetermined quantity of the sample.

FIG.3



EP 1 503 209 A1

Description

BACKGROUND OF THE INVENTION

1. Technical Field

[0001] The present invention relates to a chemical analysis apparatus for extracting a specific chemical substance, such as nucleic acid, from a biological sample, such as blood or urine. An extracted chemical substance such as nucleic acid is mixed with a reagent for detection, and the mixture is analyzed. The invention also relates to a genetic diagnostic apparatus equipped with the chemical analysis apparatus.

2. Background Art

[0002] As an example of a chemical analysis apparatus for extracting and analyzing a specific chemical substance, such as nucleic acid, from a sample including. a plurality of chemical substances, JP Patent Publication (PCT Translation) No. 2001-527220 (WO99/33559) discloses an integrated fluid manipulation cartridge. This device includes a reagent such as a lysing solution, a washing solution, or an eluent, and a capturing component for capturing nucleic acids. A sample including nucleic acid is injected into the cartridge and mixed with the lysing solution, and the mixture is passed through the capturing component. Further, the washing solution is passed through the capturing component, and then the eluent is passed through the capturing component. The eluent is brought into contact with a PCR reagent after passing through the capturing component and caused to flow toward a reaction chamber.

[0003] As an example of the method of extracting nucleic acids that is employed in the above-mentioned first prior art, JP Patent Publication (PCT Translation) No. 8-501321 (WO95/01359) discloses a method of purifying and separating a nucleic acid mixture by chromatography. In this method, the nucleic acid mixture is adsorbed on a mineral substrate of silica gel, for example, from an aqueous adsorption solution containing a high concentration of salts. The substrate is washed by a washing solution, and nucleic acids are eluted with a solution having a low concentration of salts. The silica gel is fixed inside a hollow cylindrical column, into which a solution of the nucleic acid mixture to be separated is poured and passed through the mineral substrate by suction or centrifugation.

[0004] WO00/78455 further discloses a microstructure and a method for examinations based on amplification. The disclosed apparatus, using the nucleic-acid mixture purification and separation method according to the above-mentioned JP Patent Publication (PCT Translation) No. 8-501321 (WO95/01359), passes a DNA mixture through a glass filter as a mineral substrate, and then passes it through a washing solution and an eluent, thereby collecting only DNA. The glass filter is provided on a rotatable structure, and reagents, such as the washing solution and eluent, are stored in individual reagent reservoirs inside the same structure. Each reagent is moved by the centrifugal force created by rotation of the structure, and the reagents are passed through the glass filter by opening a valve provided on a micropath connecting each reagent reservoir and the glass filter.

[0005] JP Patent Publication (PCT Application) No. 2001-502793 (WO98/13684) discloses an apparatus and method for chemical analysis. The apparatus comprises a disc-shaped member which has a chamber, paths, a reservoir, and analysis cells. A blood sample is introduced into the centrifugal chamber and centrifuged to separate blood cells from serum. Only the serum is caused to flow into a reaction chamber having beads, the surface of which has been coated with a reagent. Then, a washing solution flows into the reaction chamber, to which an eluting solution further flows. Thereafter, the eluting solution is moved from the reaction chamber to the analysis cells.

[0006] In the first prior art, namely that regarding the integrated fluid manipulation cartridge according to JP Patent Publication (PCT Translation) No. 2001-527220 (WO99/33559), when the individual reagents are delivered by pump, the valve or the like provided on the micropath connecting each reagent chamber and the capturing component is opened, thereby passing the reagent through the capturing component. Of the reagents that have passed through the capturing assembly, the washing solution is caused to flow to a waste chamber while the eluent is caused to flow to the reaction chamber by controlling the valve or the like provided on the path between the capturing component and each chamber. When a plurality of reagents are delivered by pump, the reagents remain on the walls of the paths, particularly when there is an obstacle such as a valve. Once such liquids are left, they never move, and it is possible for one reagent to cause contamination at the connecting point. Further, when the washing solution and the elution fluid that have passed through the capturing component are caused to flow to separate chambers by switching the valve or the like, the washing solution that has first flowed to the waste chamber can contaminate the path upstream of the value or the like used for switching to the reaction chamber, possibly resulting in the washing solution mixing with the elution liquid.

[0007] According to the second prior art, namely that regarding the purification and separation method disclosed in JP Patent Publication (PCT Translation) No. 8-501321 (WO95/01359), the nucleic acid mixture is introduced into the hollow cylindrical column in which silica gel is fixed. After passing the nucleic acid mixture through the silica gel by centrifugal force, a plurality of reagents are passed through, thereby collecting only nucleic acids. This publication, however, does not disclose the method of introducing the individual reagents into the hollow column or the method of collecting the washing solution and the elution fluid that have been passed through the silica gel.

[0008] According to the third prior art, namely that regarding the structure disclosed in WO00/78455, the individual reagents pass through the glass filter when moved by centrifugal force as the valve provided on a micropath connecting each reagent reservoir and the glass filter is opened. While the valve is made of wax that melts when heated, there is a possibility that a reagent that has passed through could remain at the valve and contaminate the collected DNA. Specifically, the DNA mixture or the washing solution may remain in the valve, and it is possible for the remaining DNA mixture or the washing solution to flow into the glass filter as the elution liquid is passed through the glass filter by centrifugal force.

[0009] According to the fourth prior art, namely that regarding the apparatus known from JP Patent Publication (PCT Translation) No. 2001-502793 (WO98/13684), when the blood serum is separated, the disc-shaped member revolves around a central axis outside of the disc-shaped member (revolution), and when the serum is guided to the reaction chamber, the disc-shaped member rotates about a central axis within itself (rotation). Thus, individual rotating mechanisms are required for the revolution and the rotation, which complicates the apparatus. Further, when the washing solution and the elution liquid are guided to the reaction chamber, a piston in a cylinder provided inside the disc-shaped member is driven, which further complicates the apparatus.

SUMMARY OF THE INVENTION

[0010] It is an object of the invention to solve at least one of the above-described problems and thus provide an inexpensive chemical analysis apparatus for analyzing a specific chemical substance in a liquid sample with high accuracy. Another object of the invention is to provide a genetic diagnostic apparatus comprising the chemical analysis apparatus.

[0011] The above objects can be achieved by the invention in which an eluting solution carrier is provided for carrying an eluting solution after a specific chemical substance has been eluted from a captor, and an eluting solution disposal portion is provided in communication with the eluting solution carrier for disposing of part of the eluting solution. In the communication passage communicating the eluting solution carrier with the eluting solution disposal portion, a connecting portion connecting to the eluting solution carrier is located more towards the center of rotation than a connecting portion connecting to the eluting solution disposal portion.

[0012] Alternatively, the objects can be achieved by the invention in which an eluting solution carrier for carrying an eluting solution that has passed through the captor has an eluting solution outlet for discharging part of the eluting solution and a disposal opening for disposing of liquids other than the eluting solution, the eluting solution outlet being located more towards the center of rotation than the disposal opening.

[0013] Alternatively, the objects can be achieved by the invention comprising:

an eluting solution carrier for carrying an eluting solution after the specific chemical substance has been eluted from the captor;
a waste liquid disposal passage for disposing of liquids other than the eluting solution from the eluting solution carrier; and
an eluting solution disposal passage for disposing of part of the eluting solution from the eluting solution carrier,

wherein a connecting portion between the eluting solution carrier and the eluting solution disposal passage is located more towards the periphery than an innermost portion of the eluting solution disposal passage.

[0014] Alternatively, the objects can be achieved by the invention comprising:

an eluting solution carrier for carrying an eluting solution after the specific chemical substance has been eluted from the captor; and
a detection reagent supply passage for supplying a detection reagent to the eluting solution carrier,

wherein a connecting portion between the eluting solution carrier and the detection reagent supply passage is located more towards the center of rotation than an innermost portion of the eluting solution disposal passage.

[0015] Alternatively, the objects can be achieved by the invention in which carriers are provided individually for a sample solution that has passed through the captor and an eluting solution, wherein a ventilation hole for an eluting solution carrier is opened after the sample solution passed through the captor, so that the eluting solution can pass through the captor.

[0016] Alternatively, the objects can be achieved by the invention comprising:

an eluting solution carrier for carrying an eluting solution after the specific chemical substance has been eluted from the captor; and
a detection reagent container for supplying a detection reagent to the eluting solution carrier,

wherein a detection reagent controller for controlling the flow of the detection reagent is located upstream of a detection reagent outlet for supplying the detection reagent to the eluting solution carrier, and an eluting solution disposal passage for disposing of part of the eluting solution from the eluting solution carrier is provided, wherein the detection reagent is caused to flow to the eluting solution carrier after part of the eluting solution has been disposed from the eluting solution disposal passage.

[0017] Particularly, the reagent controller may comprise an openable ventilation hole and a hole-opening mechanism.

[0018] The reagent controller may be a reagent dispenser.

[0019] The objects can be achieved by the invention comprising:

an eluting solution carrier for carrying an eluting solution that has passed through the captor; and
a flow passage for the flow of the solution from the eluting solution carrier,

wherein a flow passage entrance connecting the eluting solution carrier and the flow passage is located more towards the center of rotation than a flow passage exit on another end of the flow passage,

wherein after the reagent that has passed through the captor during the rotation of the rotary structural member flowed through the flow passage, the rotary structural member is stopped, rotated again, and then stopped again, and thereafter the eluting solution is caused to pass through the captor.

[0020] Further, the objects can be achieved by a genetic diagnostic apparatus comprising the above chemical analysis apparatus.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021]

FIG. 1 shows the overall structure of a genetic analysis apparatus according to the invention.
FIG. 2 shows the structure of an analysis disc according to the invention
FIG. 3 shows the structure of a flow passage portion according to the invention.
FIG. 4 shows the flow of analysis operation according to the invention.
FIG. 5 shows the individual operations during analysis and illustrates their correspondence to the individual figures.
FIG. 6 illustrates the operation of the flow passage portion during separation of blood serum according to the invention.
FIG. 7 illustrates the operation of the flow passage portion during separation of blood serum according to the invention.
FIG. 8 illustrates the operation of the flow passage portion during separation of blood serum according to the invention.
FIG. 9 illustrates the operation of the flow passage portion when blood serum is mixed with a lysis solution according to the invention.
FIG. 10 illustrates the operation of the flow passage portion when blood serum is mixed and reacted with a lysis solution according to the invention.
FIG. 11 illustrates the operation of the flow passage portion during binding according to the invention.
FIG. 12 illustrates the operation of the flow passage portion during washing according to the invention.
FIG. 13 illustrates the operation of the flow passage portion during washing according to the invention.
FIG. 14 illustrates the operation of the flow passage portion during washing according to the invention.
FIG. 15 illustrates the operation of the flow passage portion when the flow of the eluting solution is controlled according to the invention.
FIG. 16 illustrates the operation of the flow passage portion during elution and an operation to maintain a certain quantity of eluting solution according to the invention.
FIG. 17 illustrates the operation of the flow passage portion during amplification according to the invention.
FIGs. 18(a) and 18(b) are cross-sectional views of the reagent inlets and ventilation holes of each reagent container according to the invention.
FIG. 19 shows a circuit diagram of a positioning mechanism according to the invention.
FIG. 20 shows a timing chart for a positioning operation according to the invention.
FIG. 21 shows the overall structure of another example of the genetic analysis apparatus according to the invention.
FIG. 22 shows another example of the analysis operation procedure according to the invention.

DESCRIPTION OF THE INVENTION

(Embodiment 1)

[0022] An embodiment of the chemical analysis apparatus according to the invention will be described by referring to FIGs. I to 17.

[0023] FIG. 1 is an overall view of a genetic analysis apparatus 1 according to the invention. The genetic analysis apparatus 1 includes a carrier disc 12 rotatably supported by a motor 11, a plurality of sectored analysis discs 2 positioned via a protrusion 121 on the carrier disc 12, a perforator 13 for controlling the flow of solutions, two optical devices for heating and detection, namely an upper optical device 14 and a lower optical device 15, and a positioning sensor 16, which will be described later (FIG. 19). The carrier disc 12 includes a carrier disc optical window 122 for the lower optical device 15.

[0024] FIG. 2 shows the structure of the analysis disc 2, which comprises an upper cover 20 and a fluid passage portion 30 joined together. The upper cover 20 includes a sample inlet 210, a plurality of reagent inlets 220, 230, 240, 250, 260, and 270, a plurality of ventilation holes 212, 222, and 223, and a plurality of lidded ventilation holes 221, 231, 241, 251, 261, 271 272, 273, and 274. The flow passage portion 30 includes a positioning hole 710, containers to be described later, and passages. The analysis disc 2 is positioned when the positioning hole 710 fits with the protrusion 121 of the carrier disc 12.

[0025] FIG. 3 shows an example of the fluid passage portion 30, which comprises passages that are used when blood serum is separated from whole blood, nucleic acids contained in a virus in the blood serum are extracted, a solution of the extracted nucleic acids is quantitatively determined, and a detection reagent is added to analyze the solution.

[0026] Hereafter, the operation for extraction and analysis of viral nucleic acids will be described for a case where whole blood is used as the sample. The flow of the extraction and analysis is depicted in FIGs. 4 and 5. The fluid states in the flow passage portion 30 will be described step by step by referring to FIGs. 6 to 17.

[0027] An operator dispenses a reagent via each of the reagent inlets 220, 230, 240, 250, 260, and 270 on the upper cover 20 of the analysis disc 2 into each of reagent containers 320, 330, 340, 350, 360, and 370, and closes their lids. Depending on the number of analyses, the reagents are injected into as many analysis discs as necessary. The analysis discs are then mounted on the carrier disc 12.

[0028] Then, whole blood collected by a vacuum blood-collecting tube, for example, is introduced via the sample inlet 210 into the sample container 310 (FIG. 6).

[0029] After the whole blood 501 is introduced, the carrier disc 12 is rotated by the motor 11. The whole blood introduced into the sample container 310 moves towards the periphery on account of the centrifugal force created as the carrier disc 12 is rotated. The whole blood thus fills a blood cell storage container 311 and a blood serum quantitative determination container 312, and excess whole blood flows via an narrow overflow passage 313 and an wide overflow passage 314 into a whole-blood disposal container 315 (FIG. 7). The whole-blood disposal container 315 has a ventilation passage 318 for disposing of whole blood. Further, there is a ventilation hole 212 for disposing of whole blood in the upper cover 20 at a position corresponding to an innermost portion of the whole-blood disposing ventilation passage 318, allowing free passage of air. The connecting portion between the narrow overflow passage 313 and the wide overflow passage 314 increases in size suddenly and is located at the innermost side of the thin overflow passage 313 (radius position 601). Thus, the whole blood just fills the thin overflow passage 313 and does not flow continuously beyond the connecting portion. Accordingly, no liquid can exist beyond the radius position 601 towards the center of the disc, and the liquid level of the blood serum determination container 312 is also flush with the radius position 601. The whole blood also flows into a blood serum capillary tube 316 branching off from the blood serum quantitative determination container 312, where the innermost portion of the whole blood is also located at the radius position 601.

[0030] As the rotation is further continued, the whole blood 501 is separated into blood cells and blood serum (centrifugal separation). As a result, the blood cells 502 are moved to the blood-cell storage container 311 on the peripheral side, so that the blood-serum quantitative determination container is filled only with the blood serum 503 (FIG. 8).

[0031] During the above-described sequence of blood serum separation operation, the ventilation holes 221, 231, 241, 251, 261, and 271 in the upper cover 20 for each reagent container are closed by the lids, and thus are airtight. Although the individual reagents tend to flow out via the peripheral side of the reagent containers due to centrifugal force, the airtightness of the containers lowers the pressure inside them, which balances the centrifugal force and prevents the reagents from escaping. However, as the rotation speed increases and the centrifugal force becomes greater, the pressure inside each reagent container gradually decreases further, and once the pressure drops below the saturation vapor pressure of the reagent, bubbles are formed. Accordingly, a flow passage structure (backward passages 322, 332, 342, 352, 362, and 372) is adopted, as shown in FIG. 6, whereby the reagent flowing out of each reagent container from the peripheral side can be brought back towards the center of rotation, thus controlling the pressure reduction in the reagent containers and preventing the generation of bubbles. This way, the individual reagents are held inside the reagent containers and do not flow during the blood-serum separating operation.

[0032] After the analysis disc is rotated for a predetermined period of time and the blood serum separating operation is finished, the analysis disc 2 comes to a stop. Part of the blood serum 503 in the blood-serum quantitative determination container 312 moves into the capillary tube 316 due to capillary phenomena by surface tension up to a mixing portion entrance 411, where the mixing portion 410 and the blood-serum capillary tube 316 are connected. Thus, the blood-serum capillary tube 316 is filled with the blood serum.

[0033] Thereafter the motor 11 is rotated as the perforator 13 perforates the lid of the ventilation hole above each of the reagent containers one by one, thus moving the individual reagents by centrifugal force. As shown in the cross-sectional view of the analysis disc in FIG. 18(a), the upper cover 20 has reagent inlets (240, 250, 260) and ventilation holes (241, 251, 261) above the individual reagent containers, and each ventilation hole is covered by a lid. By perforating the lid with the perforator 13, air is allowed to enter into the reagent container. Further, as shown in FIG. 18(b), filters 242, 252, and 262 are provided between the ventilation holes and the reagent containers in order to prevent contamination of the perforator 13.

[0034] Hereafter, the operation after the completion of blood serum separation will be described.

[0035] A lysis solution 521 for lysing the membrane protein of a virus in blood serum is dispensed into a lysis solution container 320. After the lid of the lysis solution ventilation hole 221 is perforated by the perforator 13, the motor is rotated, so that the lysis solution 521 flows from the lysis solution container 320 to the mixing portion 410 via the lysis solution backward passage 322 by centrifugal force. As the innermost portion of the blood serum (which is located at the radius position 601 upon completion of separation of blood serum) in the blood-serum quantitative determination container 312 is located more towards the center of rotation than the mixing-portion entrance 411 (at the radius position 602), the blood serum in the blood serum quantitative determination container 312 and that in the blood serum capillary tube 316 flow into the mixing portion 410 via the mixing-portion entrance 411 by the head difference due to centrifugal force (FIG. 9). The mixing portion 410 includes a member for mixing the blood serum and the lysis solution. Examples of the member include a porous filter made of resin, glass or paper, fibers, and silicon or metal projections made by etching or machining.

[0036] The blood serum and the lysis solution are mixed in the mixing portion 410 and caused to flow into a reaction container 420 (FIG. 10). The reaction container 420 has a reaction-container ventilation passage 423, and the reaction-container ventilation hole 222 is disposed in the upper cover 20 at a position corresponding to the innermost portion of the reaction-container ventilation passage 423, thus allowing free passage of air into and out of the reaction container 420. Because a branching portion 317 (radius position 603) branching from the blood serum quantitative determination container 312 to the blood serum capillary tube 316 is located more towards the center than the mixing-portion entrance 411 (radius position 602), all of the blood serum in the blood serum capillary tube 316 is caused to flow out to the mixing portion 410 by a siphon effect. At the same time, the blood serum in the blood serum quantitative determination container 312 is caused to flow to the blood serum capillary tube 316 due to centrifugal force, so the blood serum flows into the mixing portion 410 until the liquid level of the blood serum in the blood serum quantitative determination container 312 reaches the branching portion 317 (radius position 603). When the liquid level of the blood serum has reached the branching portion 317, air enters the blood serum capillary tube 316 and the flow stops when the tube is empty. Thus, the blood serum that exists in the blood serum quantitative determination container 312, in the thin overflow passage 313, and in the blood serum capillary passage 316 between the radius positions 601 and 603 upon completion of blood serum separation is caused to flow into the mixing portion 410 and mixed there with the lysis solution.

[0037] Thus, by designing the blood serum quantitative determination container 312, the thin overflow passage 313, and the blood serum capillary passage 316 between the radius positions 601 and 603 to have a predetermined volume (required quantity of blood serum), the blood serum to be used for analysis can be quantitatively determined even when the ratio of blood serum with respect to whole blood is different for each blood sample. For example, when the blood cell storage container has a volume of 250 μl and the required blood serum volume is 200 μl, if 500 μl of whole blood is dispensed, 50 μl of whole blood overflows into the whole blood disposal container 315, the remaining 450 μl is separated into blood serum and blood cells, and 200 μl of the separated blood serum flows into the mixing portion 410. Namely, the device according to the invention can analyze a whole blood sample containing 200 μl or more of blood serum with respect to 450 μl of whole blood. With regard to whole blood with a small ratio of blood serum, the volume of the blood cell storage container can be increased to increase the volume of the whole blood sample.

[0038] In the reaction container 420, the blood serum and the lysis solution that have been mixed react with each other. The liquid level in the reaction container 420 after the mixture of blood serum and lysis solution has flowed into the reaction container 420 is located more towards the periphery than the innermost portion (radius position 604) of the reaction solution passage 421. Thus, the mixture cannot go beyond the innermost portion of the reaction flow passage and is therefore retained in the reaction container 420 during rotation.

[0039] The lysis solution acts to elute nucleic acids from a virus or bacterium in blood serum by lysing their membranes. Further, the lysis solution facilitates the adsorption of the nucleic acids on a nucleic acid binding member 301, which is referred to as a captor by the invention. Examples of the reagents include guanidine hydrochloride for lysing and adsorbing DNA, and guanidine thiocyanate for RNA. The nucleic acid binding member may be made of a porous member of quartz or glass, or a fiber filter.

[0040] After the blood serum and the lysis solution are retained in the reaction container 420, the motor 11 is stopped, and the lid of an additional solution ventilation hole 231 is perforated by the perforator 13 in order to supply air to an additional solution container 330. As the motor 11 is rotated again, additional solution 531 flows out of the additional solution container 330 to the reaction container 420 via an additional solution passage 332, by centrifugal force. As a result, the liquid level of the mixture in the reaction container is shifted towards the center of the disc (FIG. 11). As the liquid level reaches the innermost portion (radius position 604) of the reaction solution passage 421, the mixture flows beyond the innermost portion of the reaction solution passage into the nucleic acid binding member 301 via a merging passage 422. The additional solution may be the lysis solution mentioned above, for example.

[0041] Depending on samples, the mixture has good wettability against the wall surface, so that the mixture may flow within the reaction solution passage 421 due to capillary action when the disc is stationary. In such a case, no additional solution 531 is required.

[0042] As the mixture of lysis solution and blood serum passes the nucleic acid binding member in the above-described manner, the nucleic acids are adsorbed on the nucleic acid binding member. The mixture further flows into a waste liquid storage container 430 via a waste liquid passage 431. A plurality of containers and passages are provided downstream of an eluting solution passage 451, and perforations are created in a later step to supply air into these containers. However, when the mixture passes the nucleic acid binding member 301, the containers are sealed, so that the mixture does not flow into the eluting solution passage 451. The waste liquid container 430 is communicated via a pressure control passage 432 to a pressure control container 440. The pressure control container 440 is provided with a pressure control ventilation passage 441, and a pressure control ventilation hole 223 is provided in the upper cover 20 at a position corresponding to the innermost portion of the pressure control ventilation passage 441. Thus, air can freely enter and exit the pressure control container 440.

[0043] Then, the motor 11 is stopped, and the lid of a first washing solution ventilation hole 241 is perforated by the perforator 13 in order to supply air to a first washing solution container 340. As the motor 11 is rotated again, a first washing solution 541 flows out of the first washing solution container 340 due to centrifugal force. The first washing solution 541 further flows into the nucleic acid binding member 301 via a first washing solution backward passage 342, and washes unwanted components, such as protein, that have attached to the nucleic acid binding member 301 (FIG. 12). The first washing solution may be the above-mentioned lysis solution, or such solution with a reduced salt concentration.

[0044] The waste liquid after the washing flows via the waste liquid passage 431 into the waste liquid storage container 430, as did the mixture.

[0045] The same washing operation is repeated several times. For example, after the first washing solution, the lid of a second washing solution ventilation hole 241 is perforated by the perforator 13 to supply air to a second washing solution container 350, with the motor stopped. Then, the motor 11 is again rotated, and unwanted components such as salts that are attached to the nucleic acid binding member 301 are washed. The second washing solution may be ethanol or an aqueous solution of ethanol.

[0046] The same washing operation may be repeated further if necessary.

[0047] During the washing step, as each washing solution flows to the waste liquid storage container 430 via the waste liquid passage 431, part of the eluting solution passage 451, particularly the areas near the branching portion connecting to the waste liquid passage, may possibly be contaminated. As will be described later, because the nucleic acids eluted from the nucleic acid binding member 301 pass the eluting solution passage 451, it is desirable to wash the eluting solution passage 451 as well.

[0048] In the first embodiment shown in FIGs. 6 to 17, two kinds of washing solutions, that is, the first washing solution 541 and a second washing solution 551, are used for washing. In the following, the example of washing the eluting solution passage 451 with the second washing solution will be described.

[0049] FIG. 13 shows the state in which all of the first washing solution has passed through the waste liquid passage 431. The waste liquid has overflowed from the waste liquid storage container 430 to the pressure control container 440 via the pressure control passage 432. The motor is once stopped, and the lid of the second washing solution ventilation hole 241 is perforated by the perforator 13 to deliver air into the second washing solution container 350. The lid of a detection container ventilation hole 272 is perforated in order to communicate a detection container 450 with the outside. The lid of a final washing-solution ventilation hole 273 is perforated in order to communicate a final washing solution disposal container 460 with the outside.

[0050] As the motor 11 is rotated again, the second washing solution 551 flows out of the second washing solution container 350 due to centrifugal force. The second washing solution 551 further flows into the nucleic acid binding member 301 via a second washing solution backward passage 352, and washes the first washing solution that has attached to the nucleic acid binding member 301 (FIG. 14). The second washing solution that has passed the nucleic acid binding member 301 tends to flow into both the detection container 450 and the waste liquid storage container 430. However, the second washing solution cannot enter the waste liquid storage container 430, due not only to a head difference (h1) that is encountered when the solution is to enter the pressure control passage 432, but also to another head difference (h2) that is required to push air from the pressure control passage 432 into the pressure control container 440. On the other hand, the solution can flow into the detection container 450 virtually resistance-free, because of the ventilation hole created by the above perforation operation. Thus, the second washing solution, after passing through the nucleic acid binding member 301, flows via the eluting solution passage 451 into the detection container 450, which is referred to as an eluting solution carrier by the invention. At the same time, the areas near the branching portion connecting to the waste liquid passage 431 that have been contaminated by the mixture or the first washing solution are washed.

[0051] As the second washing solution enters the detection container 450 and as soon as its liquid level reaches the innermost portion (radius position 605) of the washing solution disposal passage 452, the second washing solution begins to flow into the final washing solution disposal container 460. Because the connecting portion (radius position 606) between the washing solution disposal passage 452 and the detection container is located more towards the periphery than the innermost portion (radius position 605) of the passage, once the solution flows into the final washing solution disposal container 460, all of the solution in the detection container 450 tends to be drained due to a siphoning effect. However, minute amounts of the solution that have remained in the nucleic acid binding member 301, for example, could flow into the detection container 450 after drainage is complete. If that happens, the rotation is once stopped, and then re-started after the washing solution disposal passage 452 is filled by capillary flow with the solution that has remained in the detection container 450, so that the solution remaining in the detection container 450 is drained out to the final washing solution disposal container 460 again by a siphoning effect. Accordingly, with regard to the final washing solution, it is preferable to repeat the procedure of rotation and stop twice after creating the ventilation hole.

[0052] After the nucleic acid binding member 301 is thus washed so that only the nucleic acids are adsorbed thereon, a step of eluting the nucleic acids is carried out.

[0053] Specifically, the lid of an eluting solution ventilation hole 261 is perforated by the perforator 13 to supply air to the eluting solution container 360, with the motor stopped. Further, the lid of an eluting solution-disposal ventilation hole 274 is perforated to communicate an eluting solution disposal container 470, which is referred to as an eluting solution disposal portion by the invention, with the outside. The motor 11 is rotated again, and the eluting solution flows to the nucleic acid binding member 301 (FIG. 15). The eluting solution is a solution for eluting nucleic acids from the nucleic acid binding member 301, and it may be water or an aqueous solution with pH adjusted between 7 and 9. The solution is preferably heated to 40°C or higher for facilitating elution. The heating may be carried out by irradiating the eluting solution container 360 from above with light by means of the upper optical device 14 shown in FIG. 1.

[0054] After passing through the nucleic acid binding member 301, the eluting solution flows into the detection container 450 via the eluting solution passage 451. As the eluting solution disposal container 470 is communicated with the outside by perforation as described above, the eluting solution flows out to the eluting solution disposal container 470 via an eluting solution disposal passage 471. Because the connecting portion (radius position 607) between the eluting solution disposal passage 471 and the detection container 450 is located more towards the center than the connecting portion (radius position 608) connecting to the eluting solution disposal container 470, the eluting solution that exists in the detection container 450 beyond the radius position 607 towards the center is drained to the eluting solution disposal container 470 by a siphoning effect. Thus, a certain quantity of the eluting solution containing nucleic acids can be carried in the detection container 450 (FIG. 16).

[0055] Thereafter, with the motor stopped, the lid of a detection solution ventilation hole 271 is perforated by the perforator 13 to supply air to the detection solution storage container 370. The motor 11 is rotated again, and a detection solution 571 flows to the detection container 450 (FIG. 17). The detection solution is a reagent for amplifying and detecting nucleic acids, and it includes deoxynucleoside triphosphate, DNA synthetic enzyme, or fluorescence reagent, for example. Depending on the method of amplification, the detection solution may be heated from above through the detection container 450 with the upper optical device 14.

[0056] Next, the lower optical device 15 is transported below the detection container 450 to detect the amount of fluorescence, for example.

[0057] The carrier disc 12 must be stopped at predetermined positions during perforation, heating, and detection. As shown in FIG. 19, the carrier disc 12 is provided with a positioning protrusion 17. This allows the rotation position of the carrier disc to be detected by a position detector 16, so that the rotation of the motor 11, the rotation and vertical movement of the perforator 13, and the rotation, irradiation, and detection by the upper and lower optical devices 14 and 15 can be controlled by a controller 18.

[0058] FIG. 20 shows the operational timing of the perforator 13, for example. The rotational speed of the carrier disc 12 is lowered after the whole blood or each reagent has been moved, and a slow rotation speed for positioning is maintained. As the position detector 16 detects the positioning protrusion 17, the carrier disc 12 is stopped. The perforator 13 is then lowered to perforate the lid of the ventilation hole for each reagent storage container, and then raised again. After perforation, the carrier disc 12 rotates at such a slow speed that the reagents do not flow out of the individual reagent storage containers after perforation. The carrier disc 12 comes to a stop at the position for the next analysis disc, that is, after rotating 60° in the case where six analysis discs are mounted. The same perforation operation is repeated. The location of the analysis disc can be known by irradiating light from above with the lower optical device through a flow passage optical window 490 and examining the reflected light. After all of the analysis discs have been perforated, the carrier disc is rotated at high speed to cause the reagents to flow.

[0059] In accordance with the present embodiment, there is no need to provide a valve in flow passages for controlling the flow of the sample and each reagent. Thus, the problem of solution remaining at the valve portion in the course of the flow passages does not occur, and the contamination by reagents in the pre-process can be prevented. Accordingly, specific components in a liquid sample, such as nucleic acids, can be extracted with high purity and analyzed accurately.

(Embodiment 2)

[0060] While in Embodiment 1 the flow of the reagents was controlled by opening ventilation holes with a perforator, a reagent dispensing mechanism can be used. Specifically, as shown in FIG. 21, after dispensing predetermined reagents from individual reagent bottles 400 into the reagent storage containers shown in FIG. 3 with a reagent dispenser 19, the analysis disc is rotated to cause the reagents to flow. The procedure is illustrated in FIG. 22.

[0061] In accordance with the present embodiment, there is no need to provide a valve in flow passages for controlling the flow of the sample and each reagent. Thus, the problem of solution remaining at the valve portion in the course of the flow passages does not occur, and the contamination by reagents in the pre-process can be prevented. Accordingly, specific components in a liquid sample, such as nucleic acids, can be extracted with high purity and analyzed accurately.

Claims

1. A chemical analysis apparatus comprising a rotatably supported structural member, the structural member comprising a captor for capturing a specific chemical substance in a sample, and a plurality of reagent containers for carrying liquids to be passed through the captor, the apparatus further comprising:

   an eluting solution carrier for carrying an eluting solution after the specific chemical substance has been eluted from the captor; and
   an eluting solution disposal portion communicated with the eluting solution carrier for disposing of part of the eluting solution,

   wherein a connecting portion between a communication passage communicating the eluting solution carrier with the eluting solution disposal portion and the eluting solution carrier is located more towards the center of rotation than a connecting portion between the communication passage and the eluting solution disposal portion.

2. A chemical analysis apparatus comprising a rotatably supported structural member, the structural member comprising a captor for capturing a specific chemical substance in a sample, and a plurality of reagent containers for carrying liquids to be passed through the captor,
   wherein an eluting solution carrier for carrying an eluting solution that has passed through the captor has an eluting solution outlet for discharging part of the eluting solution and a disposal opening for disposing of liquids other than the eluting solution, the eluting solution outlet being located more towards the center of rotation than the disposal opening.

3. A chemical analysis apparatus comprising a rotatably supported structural member, the structural member comprising a captor for capturing a specific chemical substance in a sample, and a plurality of reagent containers for carrying liquids to be passed through the captor, the apparatus further comprising:

   an eluting solution carrier for carrying an eluting solution after the specific chemical substance has been eluted from the captor;
   a waste liquid disposal passage for disposing of liquids other than the eluting solution from the eluting solution carrier; and
   an eluting solution disposal passage for disposing of part of the eluting solution from the eluting solution carrier,

   wherein a connecting portion between the eluting solution carrier and the eluting solution disposal passage is located more towards the periphery than an innermost portion of the eluting solution disposal passage.

4. A chemical analysis apparatus comprising a rotatably supported structural member, the structural member comprising a captor for capturing a specific chemical substance in a sample, and a plurality of reagent containers for carrying liquids to be passed through the captor, the apparatus further comprising:

   an eluting solution carrier for carrying an eluting solution after the specific chemical substance has been eluted from the captor; and
   a detection reagent supply passage for supplying a detection reagent to the eluting solution carrier,

   wherein a connecting portion between the eluting solution carrier and the detection reagent supply passage is located more towards the center of rotation than an innermost portion of the eluting solution disposal passage.

5. A chemical analysis apparatus comprising a rotatably supported structural member, the structural member comprising a captor for capturing a specific chemical substance in a sample, and a plurality of reagent containers for carrying liquids to be passed through the captor,
   wherein carriers are provided individually for a sample solution and an eluting solution that have passed through the captor, wherein a ventilation

hole for an eluting solution carrier is opened after the sample solution passed through the captor, so that the eluting solution can pass through the captor.

6. A chemical analysis apparatus comprising a rotatably supported structural member, the structural member comprising a captor for capturing a specific chemical substance in a sample, and a plurality of reagent containers for carrying liquids to be passed through the captor, the apparatus further comprising:

   an eluting solution carrier for carrying an eluting solution after the specific chemical substance has been eluted from the captor; and
   a detection reagent container for supplying a detection reagent to the eluting solution carrier,

   wherein a detection reagent controller for controlling the flow of the detection reagent is located upstream of a detection reagent outlet for supplying the detection reagent to the eluting solution carrier, and an eluting solution disposal passage for disposing of part of the eluting solution from the eluting solution carrier is provided, wherein the detection reagent is caused to flow to the eluting solution carrier after part of the eluting solution has been disposed from the eluting solution disposal passage.

7. The chemical analysis apparatus according to claim 6, wherein the reagent controller comprises an openable ventilation hole and a hole-opening mechanism.

8. The chemical analysis apparatus according to claim 6, wherein the reagent controller is a reagent dispenser.

9. A chemical analysis apparatus comprising a rotatably supported structural member, the structural member comprising a captor for capturing a specific chemical substance in a sample, and a plurality of reagent containers for carrying liquids to be passed through the captor, the apparatus further comprising:

   an eluting solution carrier for carrying an eluting solution that has passed through the captor; and
   a waste liquids disposal passage for disposing of liquids other than the eluting solution from the eluting solution carrier,

   wherein a flow passage entrance connecting the eluting solution carrier and the flow passage is located more towards the center of rotation than a flow passage exit on another end of the flow passage,

   wherein after the reagent that has passed through the captor during the rotation of the rotary structural member flowed through the flow passage, the rotary structural member is stopped, rotated again, and then stopped again, and thereafter the eluting solution is caused to pass through the captor.

10. A genetic diagnostic apparatus comprising the chemical analysis apparatus according to claims 1-9.

FIG.1

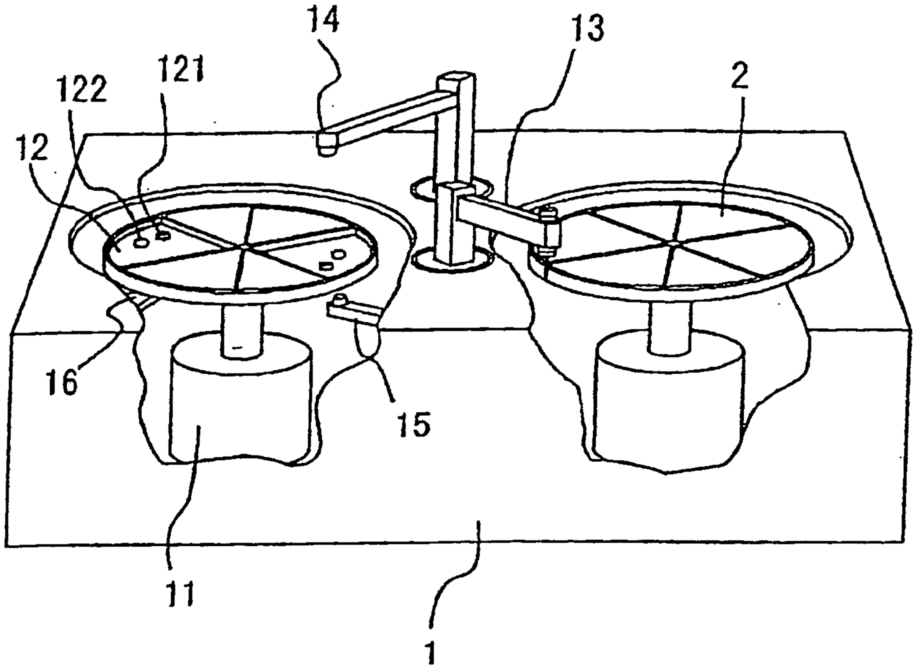


FIG.2

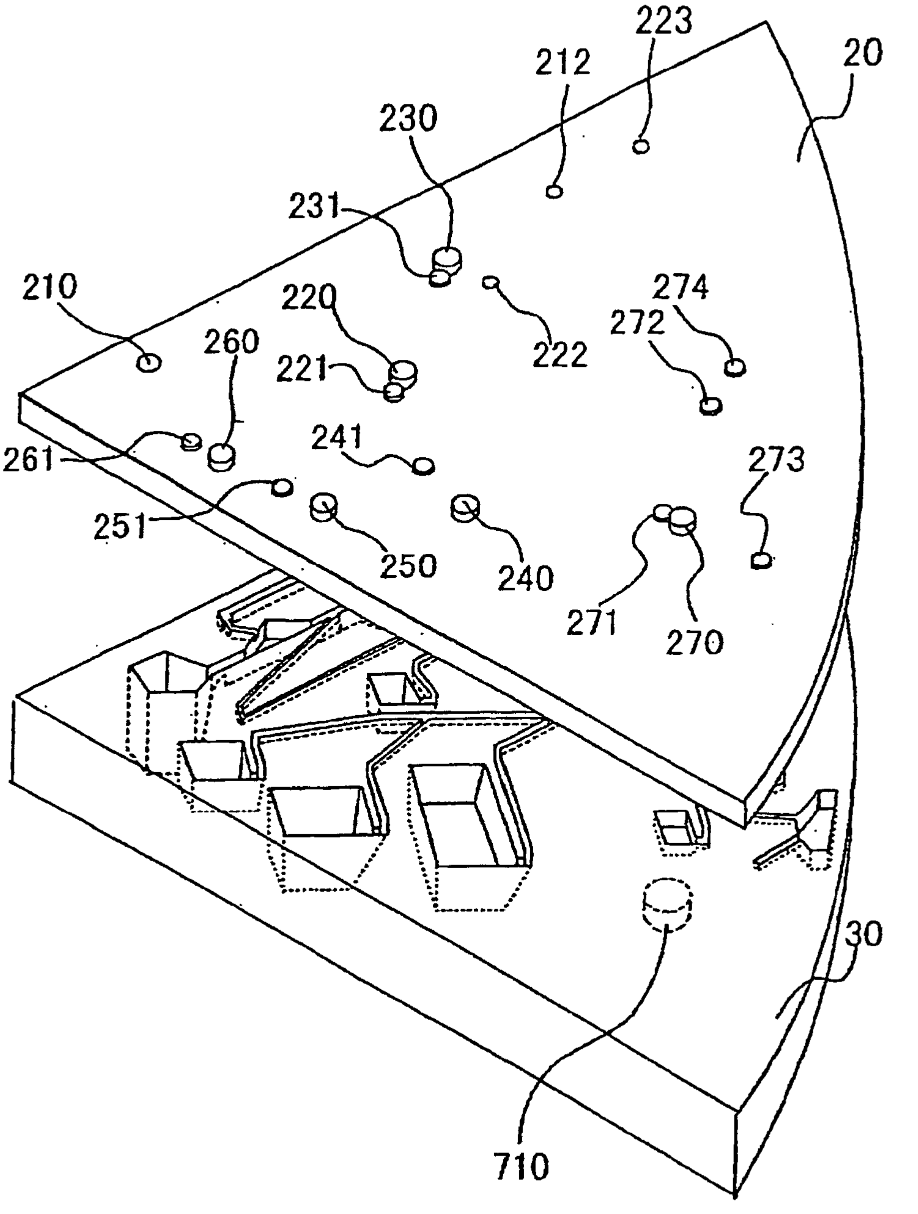

223
212
20
230
231
210
220
274
272
260
221
222
261
241
273
251
250
240
271
270
30
710

FIG.3

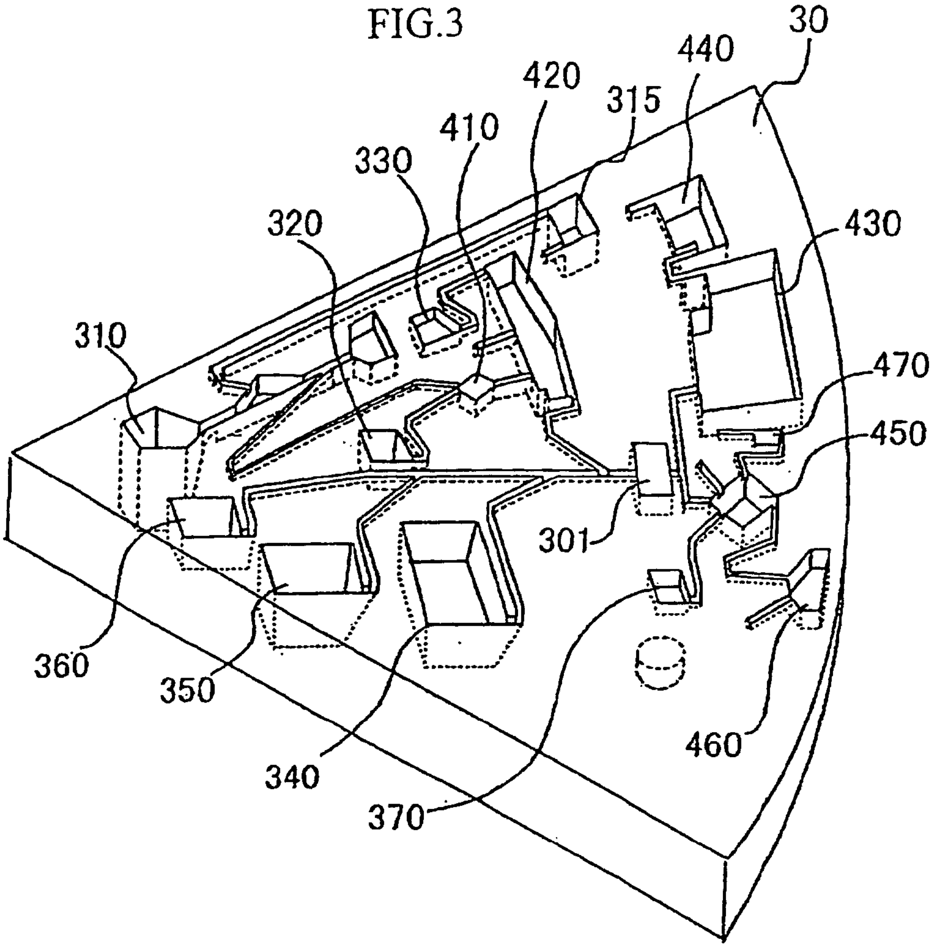

30
440
420
315
410
330
320
310
430
470
450
301
360
350
340
370
460

FIG.4

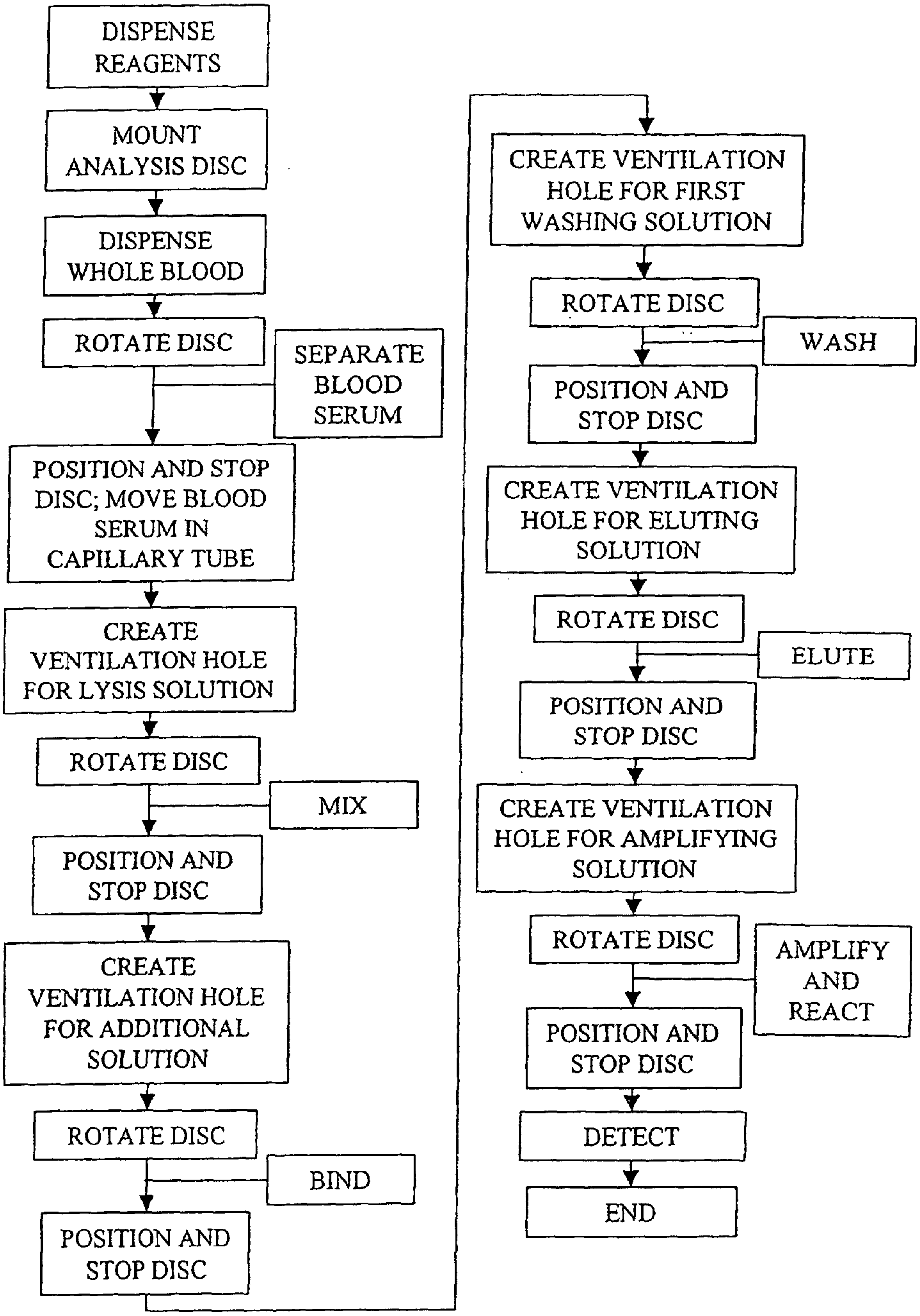

DISPENSE REAGENTS
MOUNT ANALYSIS DISC
DISPENSE WHOLE BLOOD
ROTATE DISC
SEPARATE BLOOD SERUM
POSITION AND STOP DISC; MOVE BLOOD SERUM IN CAPILLARY TUBE
CREATE VENTILATION HOLE FOR LYSIS SOLUTION
ROTATE DISC
MIX
POSITION AND STOP DISC
CREATE VENTILATION HOLE FOR ADDITIONAL SOLUTION
ROTATE DISC
BIND
POSITION AND STOP DISC
CREATE VENTILATION HOLE FOR FIRST WASHING SOLUTION
ROTATE DISC
WASH
POSITION AND STOP DISC
CREATE VENTILATION HOLE FOR ELUTING SOLUTION
ROTATE DISC
ELUTE
POSITION AND STOP DISC
CREATE VENTILATION HOLE FOR AMPLIFYING SOLUTION
ROTATE DISC
AMPLIFY AND REACT
POSITION AND STOP DISC
DETECT
END

FIG.5

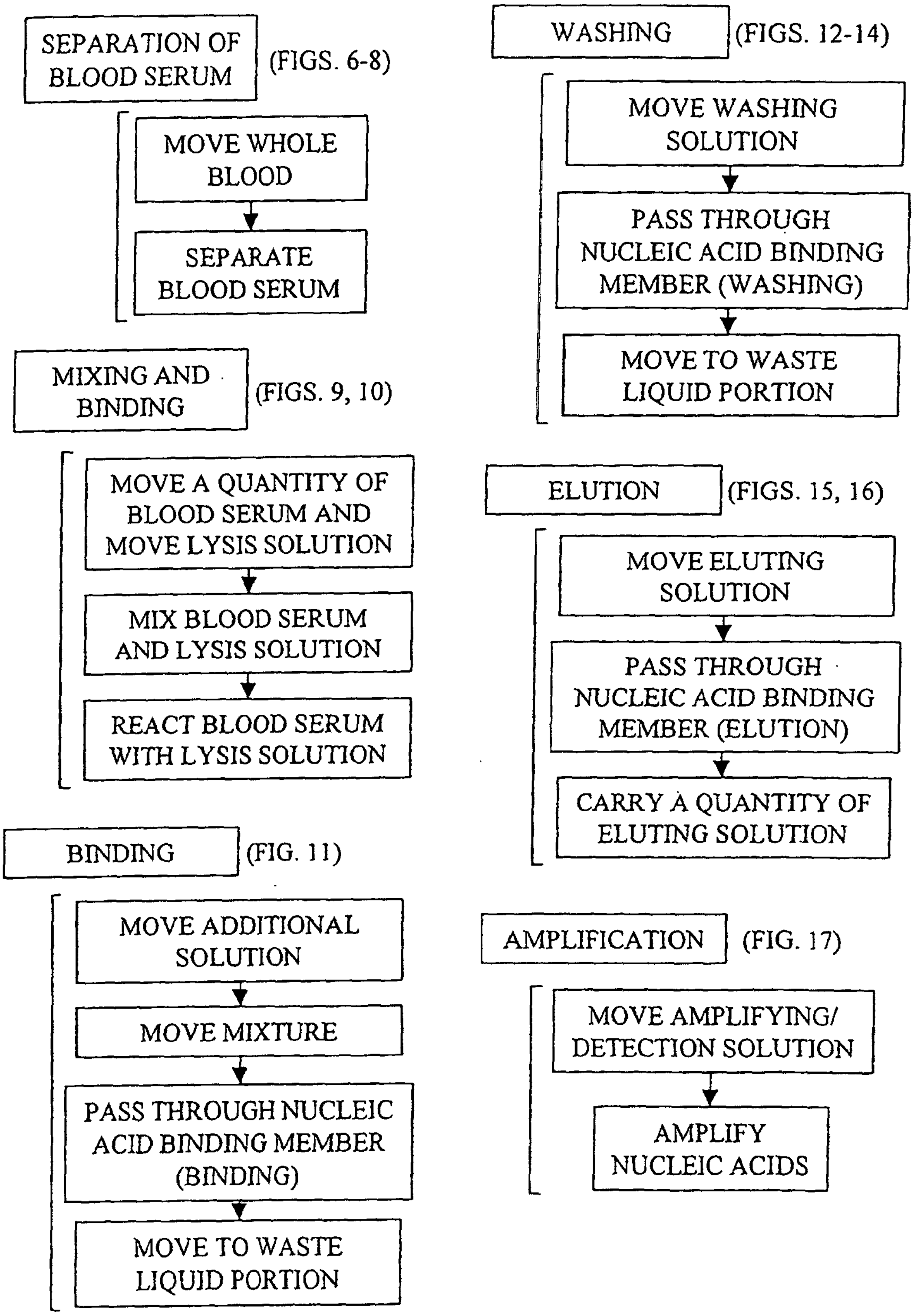

SEPARATION OF BLOOD SERUM
(FIGS. 6-8)
MOVE WHOLE BLOOD
SEPARATE BLOOD SERUM
MIXING AND BINDING
(FIGS. 9, 10)
MOVE A QUANTITY OF BLOOD SERUM AND MOVE LYSIS SOLUTION
MIX BLOOD SERUM AND LYSIS SOLUTION
REACT BLOOD SERUM WITH LYSIS SOLUTION
BINDING
(FIG. 11)
MOVE ADDITIONAL SOLUTION
MOVE MIXTURE
PASS THROUGH NUCLEIC ACID BINDING MEMBER (BINDING)
MOVE TO WASTE LIQUID PORTION
WASHING
(FIGS. 12-14)
MOVE WASHING SOLUTION
PASS THROUGH NUCLEIC ACID BINDING MEMBER (WASHING)
MOVE TO WASTE LIQUID PORTION
ELUTION
(FIGS. 15, 16)
MOVE ELUTING SOLUTION
PASS THROUGH NUCLEIC ACID BINDING MEMBER (ELUTION)
CARRY A QUANTITY OF ELUTING SOLUTION
AMPLIFICATION
(FIG. 17)
MOVE AMPLIFYING/ DETECTION SOLUTION
AMPLIFY NUCLEIC ACIDS

FIG.6

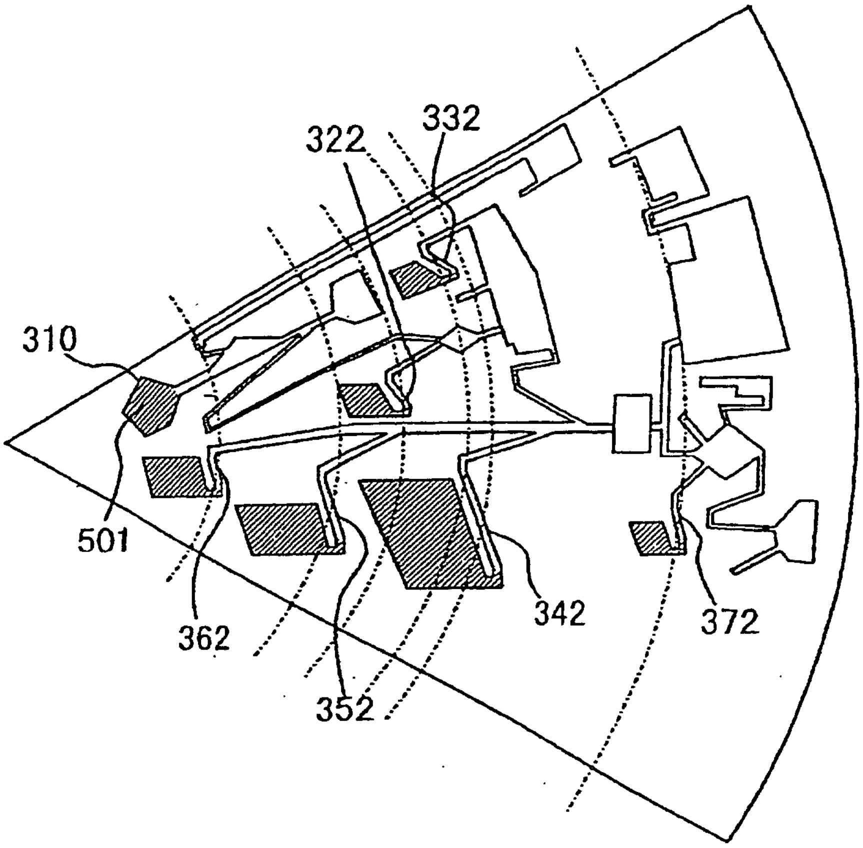


FIG.7

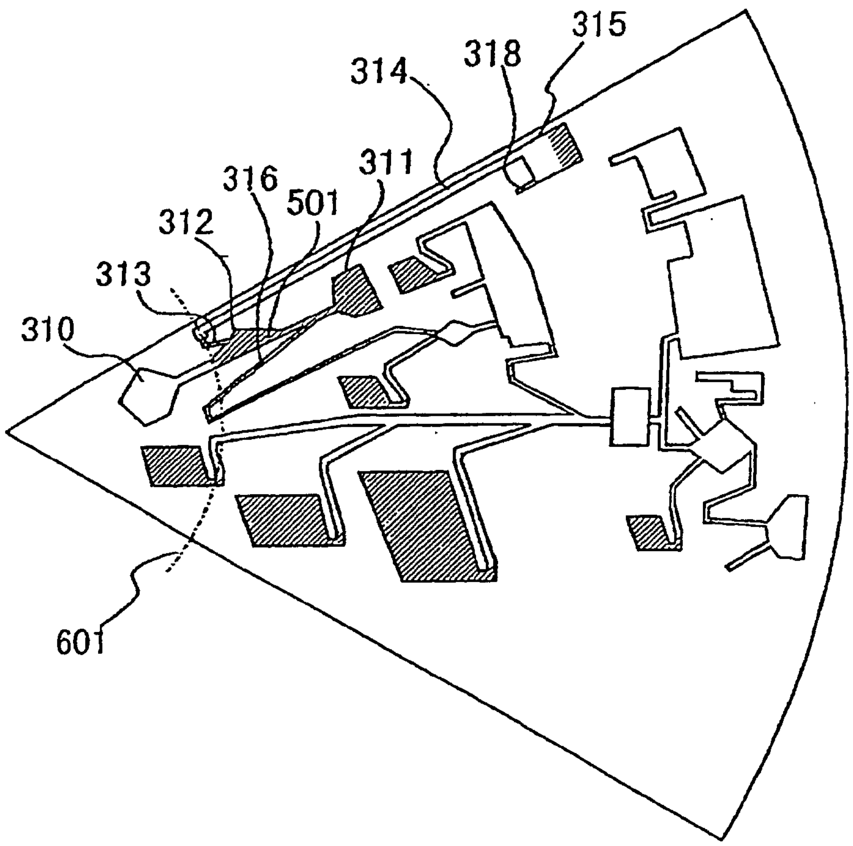


FIG.8

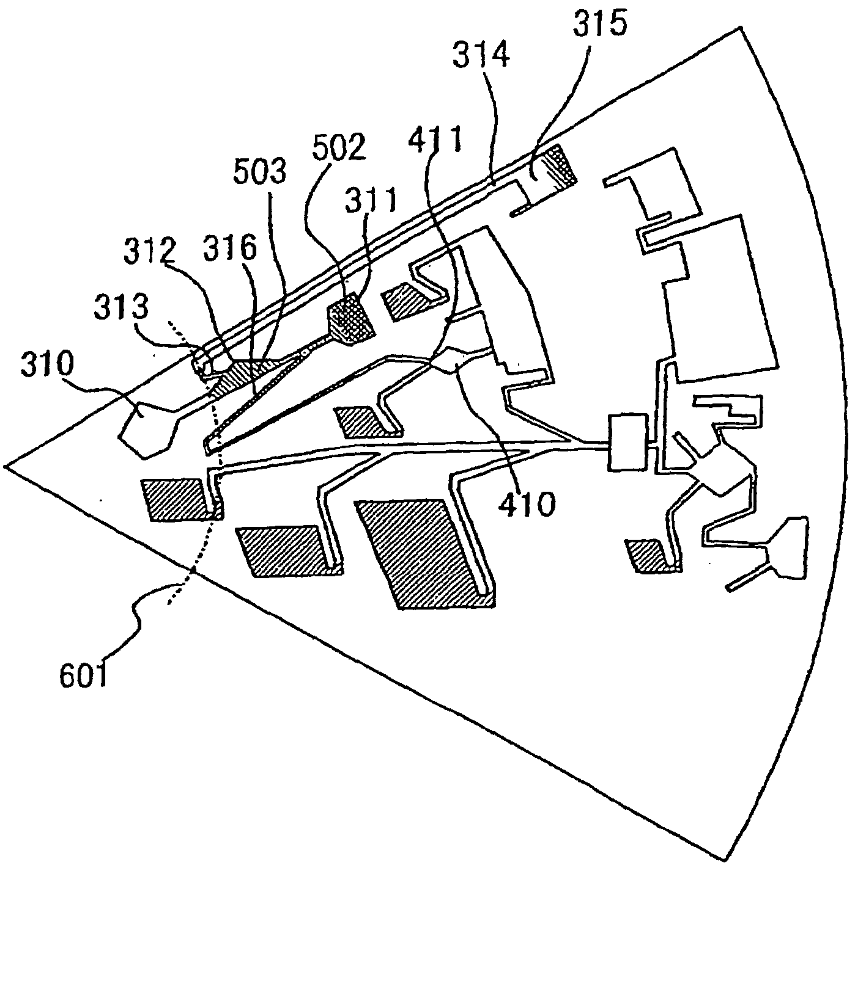


FIG.9

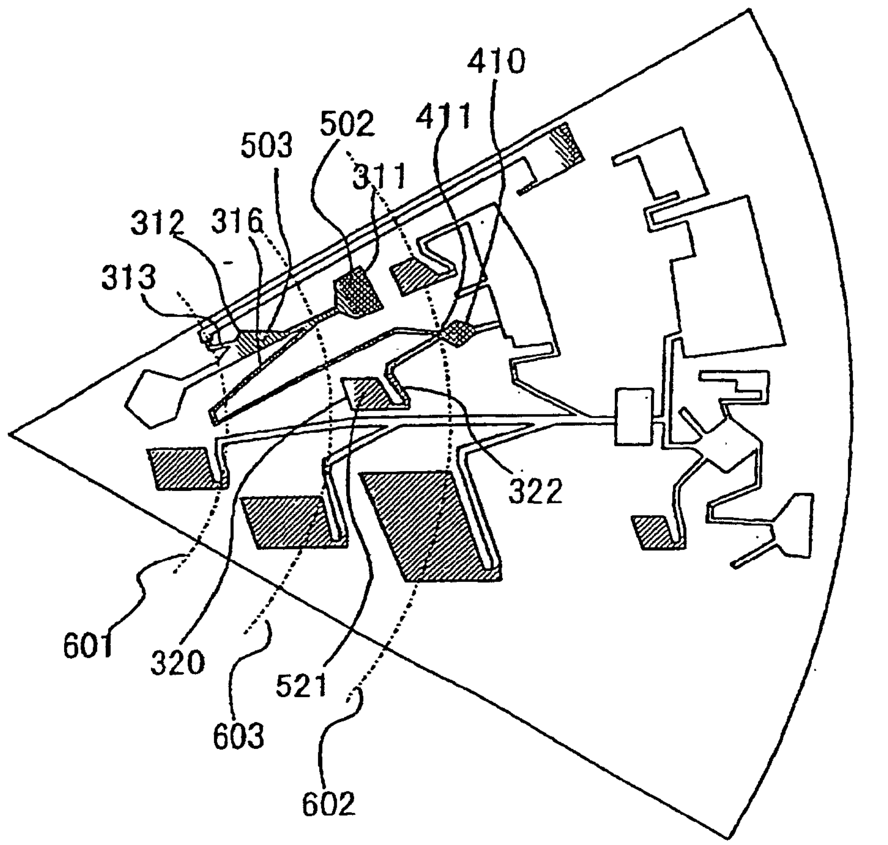


FIG.10

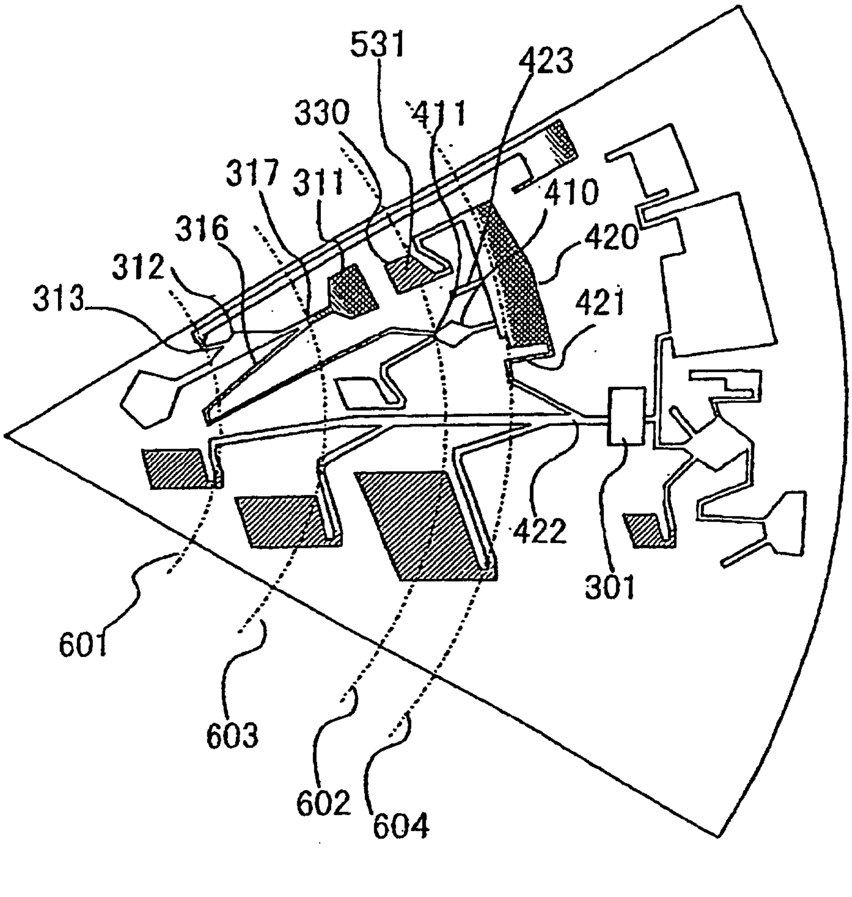

531
423
330
411
317
311
410
316
420
312
313
421
422
301
601
603
602
604

FIG.11

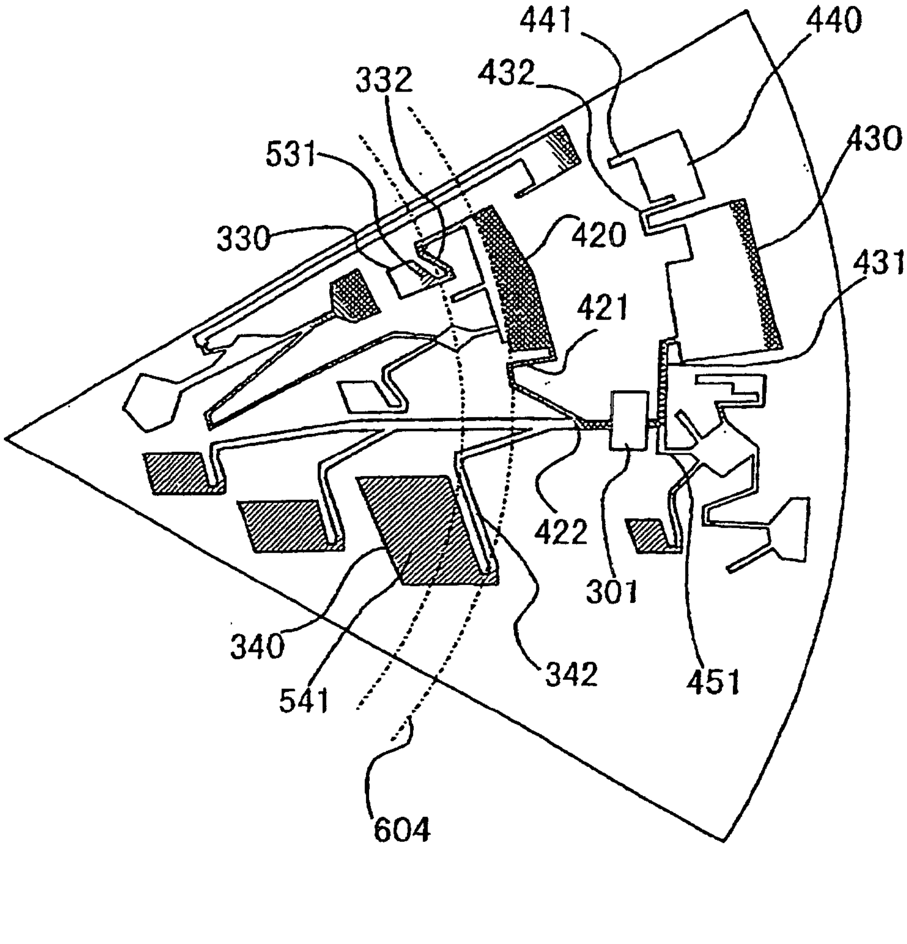

441
440
332
432
430
531
330
420
431
421
422
301
340
342
451
541
604

FIG.12

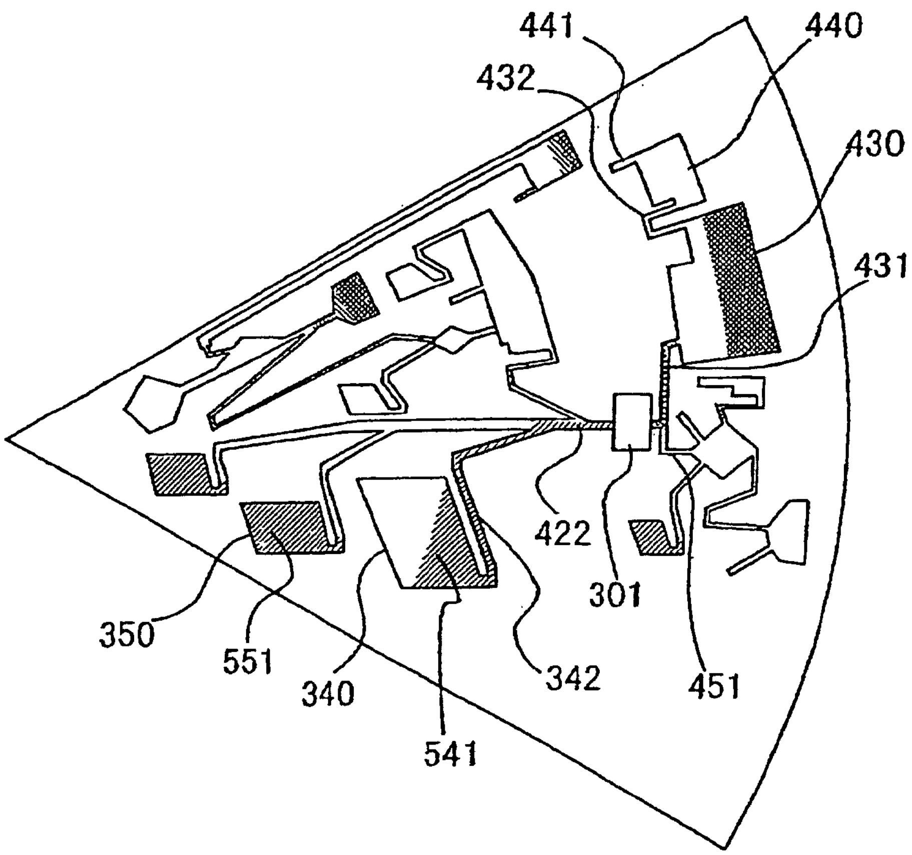


FIG.13

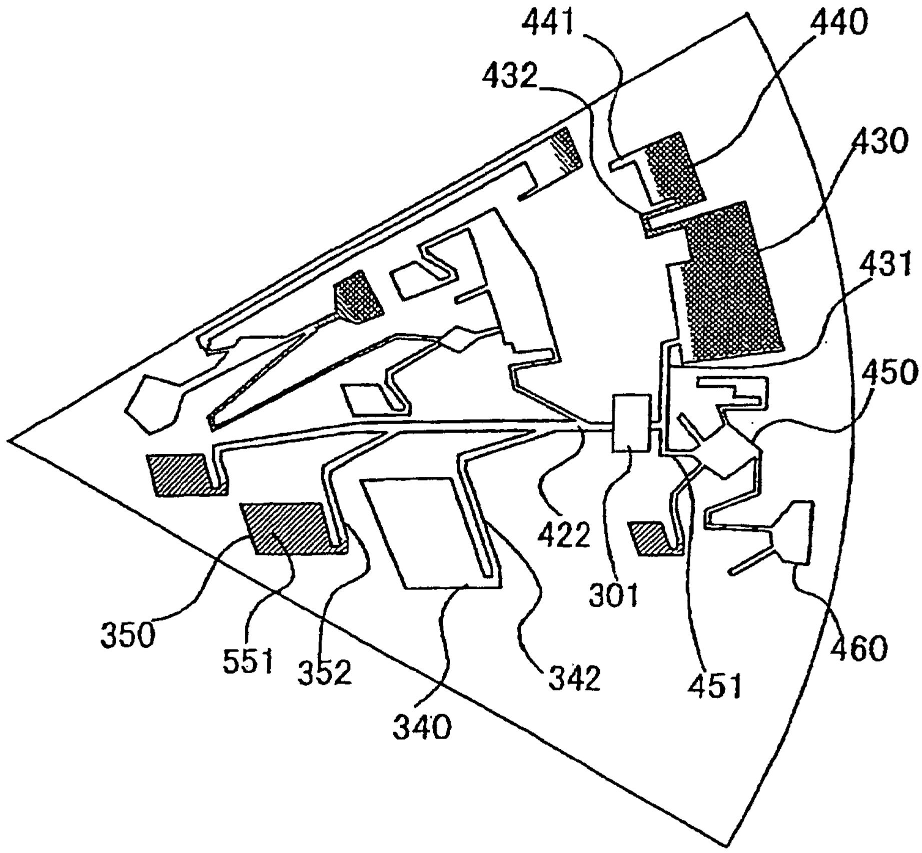


FIG.14

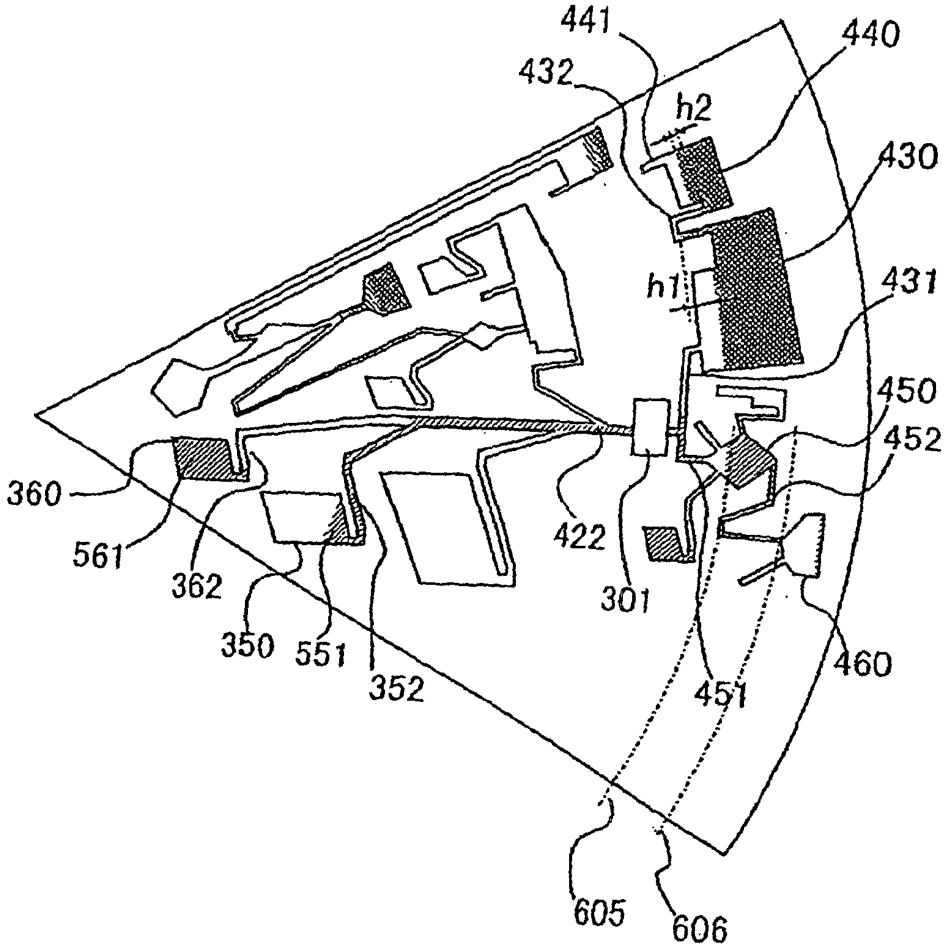

441
440
432
h2
430
h1
431
450
452
360
561
362
350
551
352
422
301
451
460
605
606

FIG.15

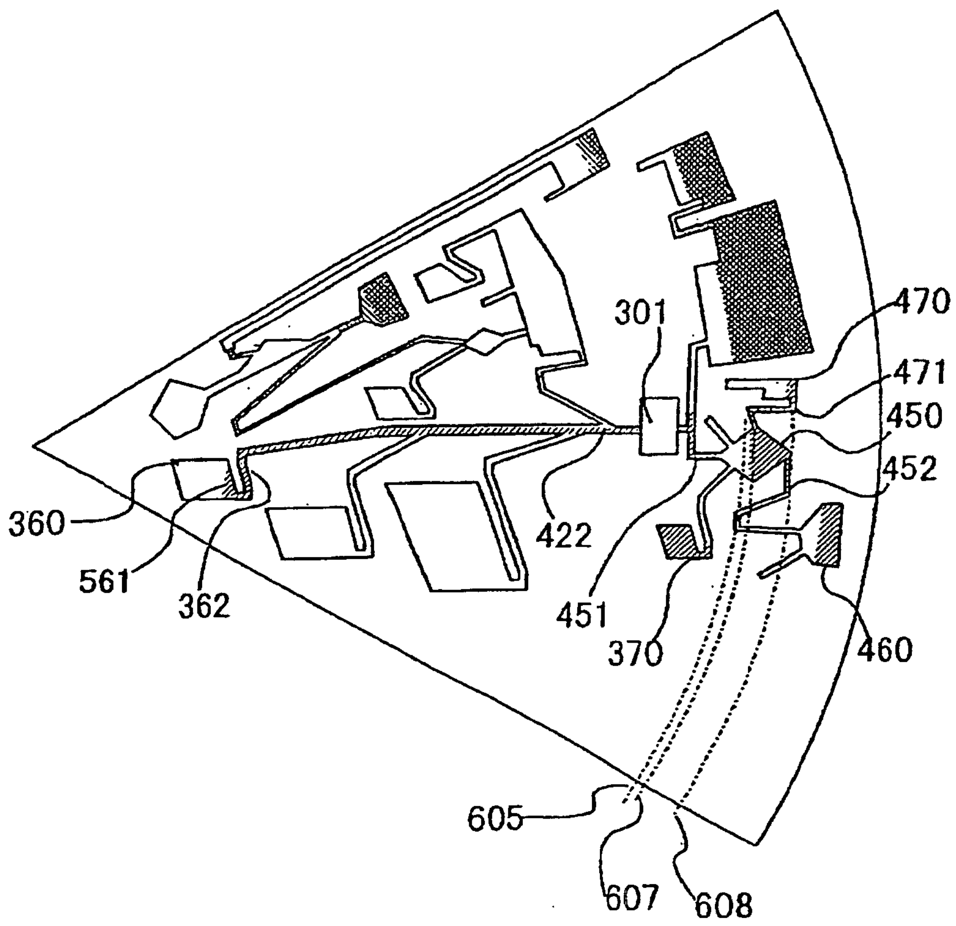


FIG.16

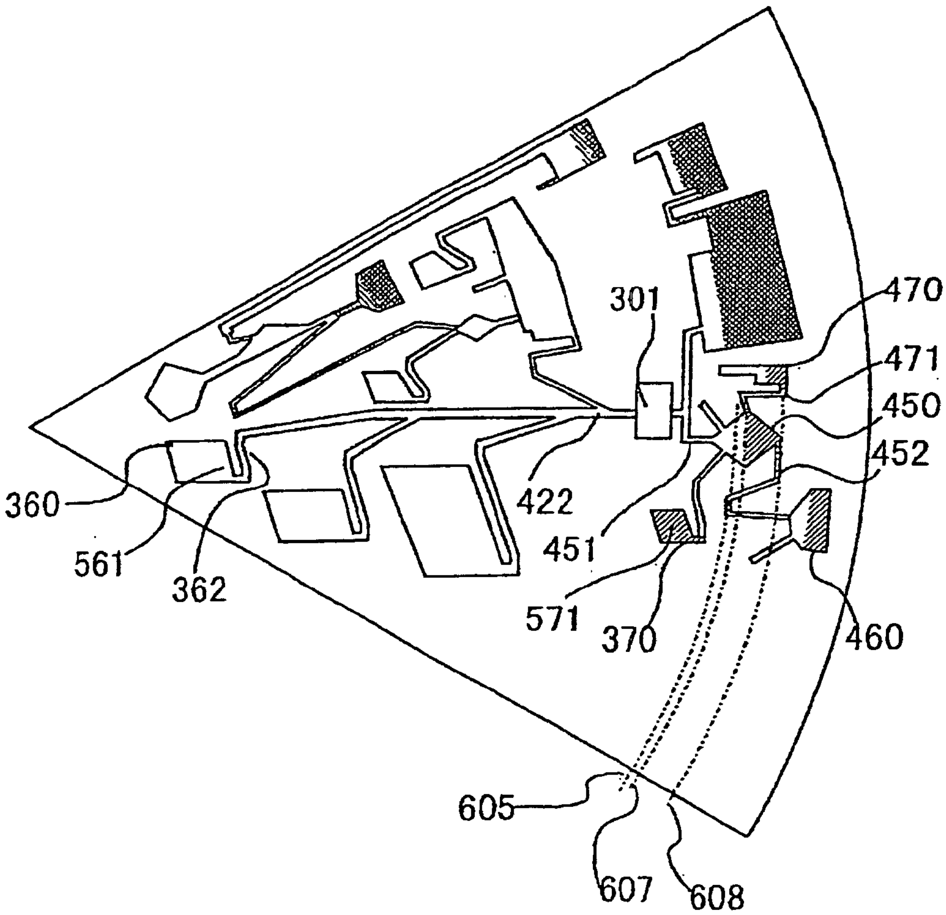


FIG.17

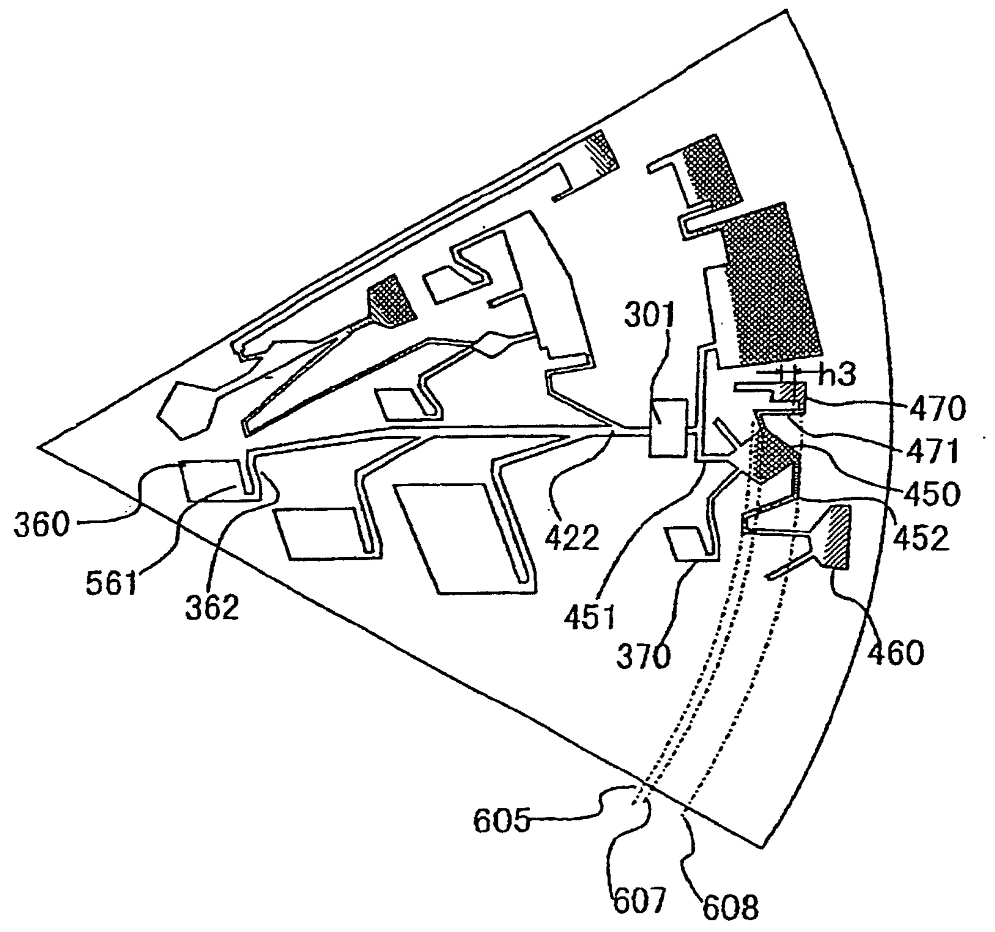


FIG.18A

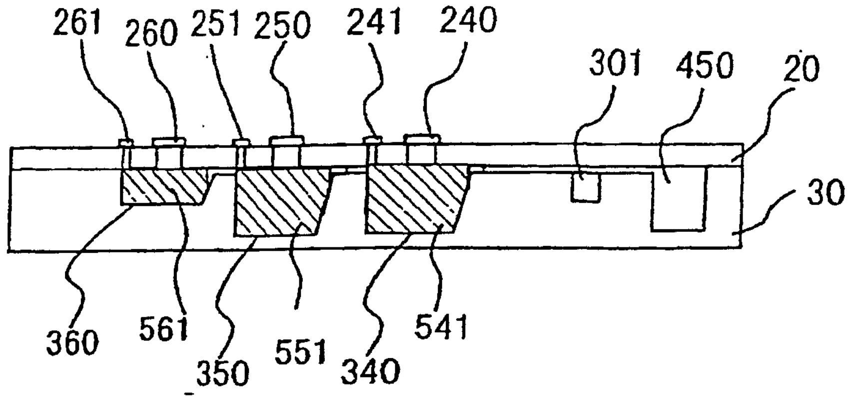


FIG.18B

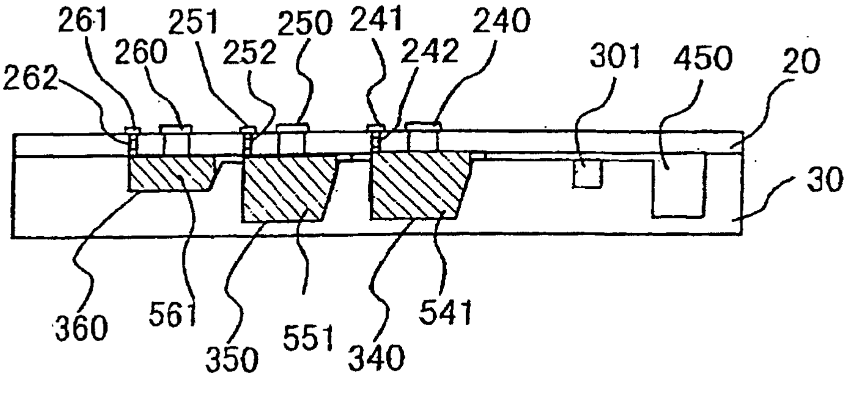


FIG.19

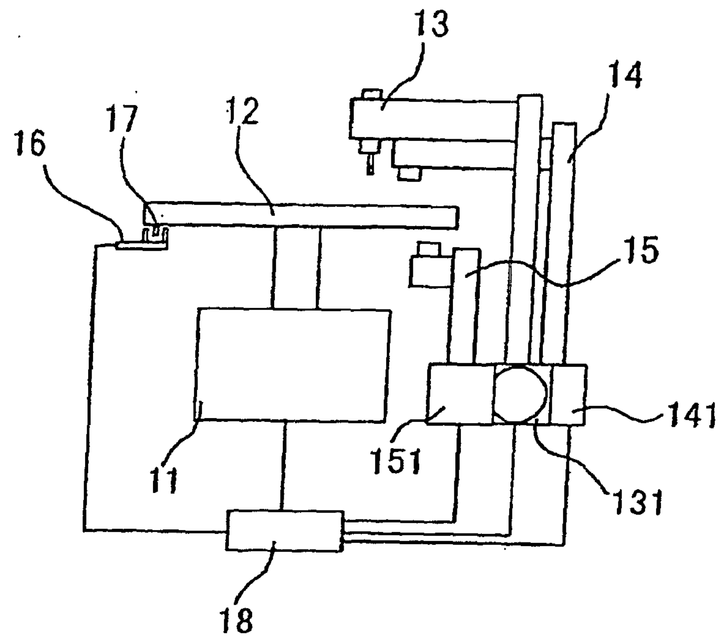

13
14
17
12
16
15
141
151
11
131
18

FIG.20

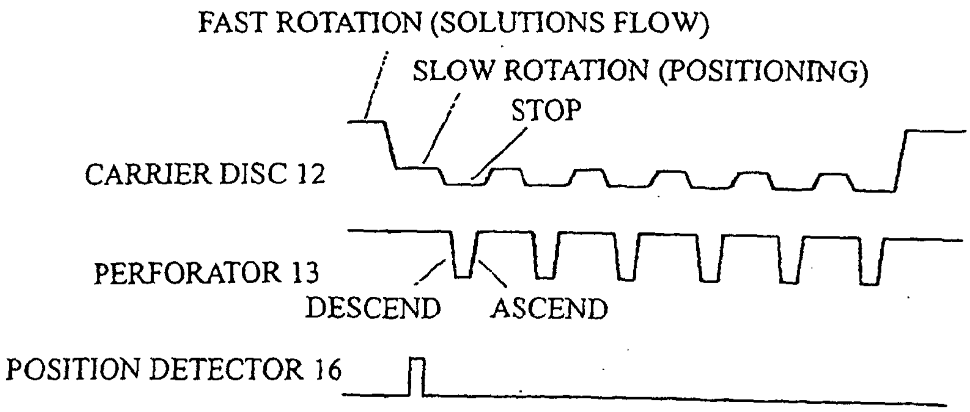

FAST ROTATION (SOLUTIONS FLOW)
SLOW ROTATION (POSITIONING)
STOP
CARRIER DISC 12
PERFORATOR 13
DESCEND
ASCEND
POSITION DETECTOR 16

FIG.21

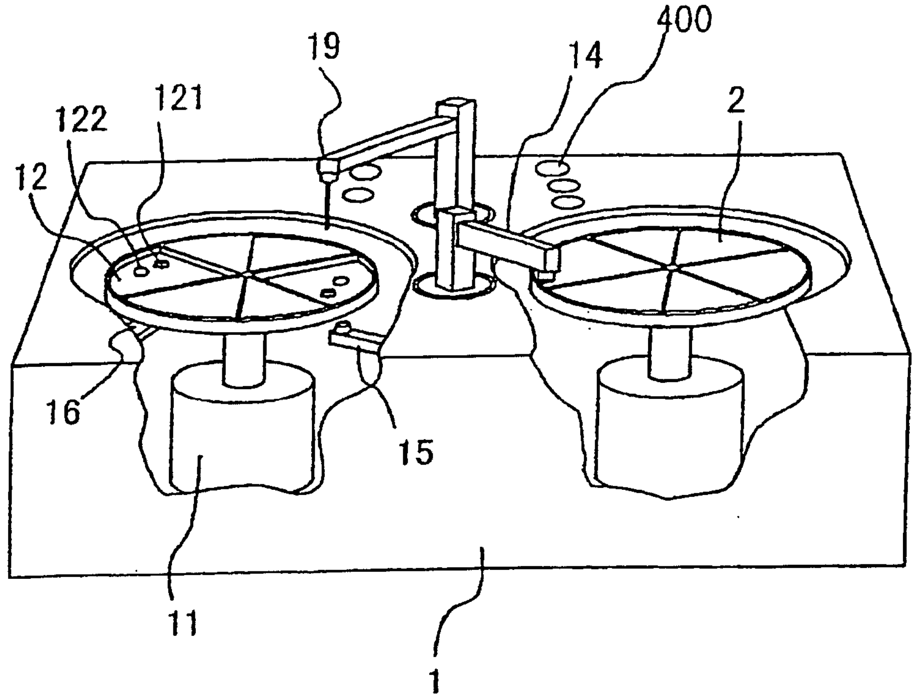
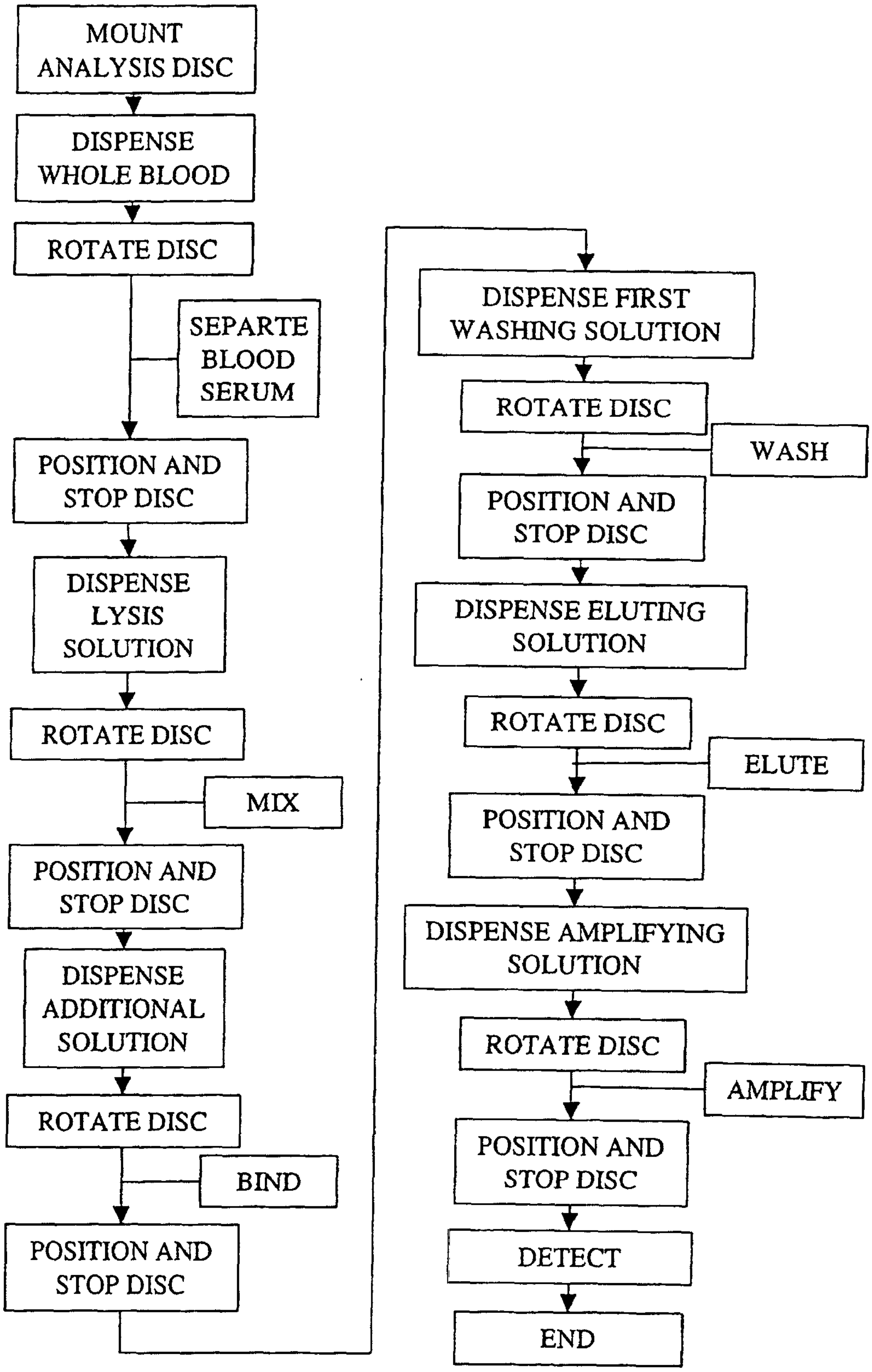


FIG.22


MOUNT ANALYSIS DISC
DISPENSE WHOLE BLOOD
ROTATE DISC
SEPARTE BLOOD SERUM
POSITION AND STOP DISC
DISPENSE LYSIS SOLUTION
ROTATE DISC
MIX
POSITION AND STOP DISC
DISPENSE ADDITIONAL SOLUTION
ROTATE DISC
BIND
POSITION AND STOP DISC
DISPENSE FIRST WASHING SOLUTION
ROTATE DISC
WASH
POSITION AND STOP DISC
DISPENSE ELUTING SOLUTION
ROTATE DISC
ELUTE
POSITION AND STOP DISC
DISPENSE AMPLIFYING SOLUTION
ROTATE DISC
AMPLIFY
POSITION AND STOP DISC
DETECT
END

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP02/04458

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] G01N30/00, G01N31/20, G01N33/53, G01N33/48, G01N37/00, G01N35/02

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)
Int.Cl[7] G01N30/00, G01N31/20, G01N33/53, G01N33/48, G01N37/00, G01N35/02

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

Jitsuyo Shinan Koho	1922-1996	Toroku Jitsuyo Shinan Koho	1994-2002
Kokai Jitsuyo Shinan Koho	1971-2002	Jitsuyo Shinan Toroku Koho	1996-2002

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 2000-514928 A (Gamera Bioscience Corp.), 07 November, 2000 (07.11.00), Full specification & AU 751998 A & WO 98/53311 A & AU 3662199 A & EP 983504 A & US 6063589 A & GB 2354789 A	1-10

☐ 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 document 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	Date of mailing of the international search report
05 June, 2002 (05.06.02)	18 June, 2002 (18.06.02)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1998) patsnap

专利名称(译)	化学分析仪和基因诊断仪		
公开(公告)号	EP1503209A4	公开(公告)日	2005-07-06
申请号	EP2002724714	申请日	2002-05-08
[标]申请(专利权)人(译)	株式会社日立高新技术		
申请(专利权)人(译)	日立高新技术公司		
当前申请(专利权)人(译)	日立高新技术公司		
[标]发明人	NAGAOKA YOSHIHIRO MECHANICAL ENG RES LAB WATANABE NARUO MECHANICAL ENG RES LAB TAKENAKA KEI MECHANICAL ENG RES LAB OHASHI TOMOKI MECHANICAL ENG RES LAB MIYAHARA YUJI C O DESIGN & MFG GRP		
发明人	NAGAOKA, YOSHIHIRO, MECHANICAL ENG. RES. LAB. WATANABE, NARUO, MECHANICAL ENG. RES. LAB. TAKENAKA, KEI, MECHANICAL ENG. RES. LAB. OHASHI, TOMOKI, MECHANICAL ENG. RES. LAB. MIYAHARA, YUJI, C/O DESIGN & MANUFACTURING GROUP		
IPC分类号	B01L3/00 G01N30/00 G01N31/20 G01N33/48 G01N33/53 G01N35/00 G01N35/02 G01N35/04 G01N37/00		
CPC分类号	G01N35/025 B01L3/50273 B01L3/502738 B01L3/502753 B01L2200/0621 B01L2200/10 B01L2300/0803 B01L2300/0861 B01L2400/0409 B01L2400/0694 G01N35/00069 G01N2035/00504 G01N2035/0436		
其他公开文献	EP1503209A1		
外部链接	Espacenet		

摘要(译)

在用于从含有多种化学物质的样品中提取特定物质（例如核酸）的化学分析装置中，防止了残留在阀门中的溶液，使得较早步骤中的试剂不会污染后续步骤。通过离心力携带和提供预定量的溶液，而不提供用于控制溶液流动的阀门。在将检测容器450和处理容器460和470与分析盘2连通的通风孔272,273,274的盖子穿孔后，旋转分析盘2以供给和携带预定量的样品。

FIG.3